Expression and activation of protein kinase C-ζ in eosinophils after allergen challenge

DAVID J. EVANS, MARK A. LINDSAY, BENJAMIN L. J. WEBB, HANNU KANKAANRANTA, MARK A. GIEMBYCZ, BRIAN J. O’CONNOR, AND PETER J. BARNES
Thoracic Medicine, National Heart and Lung Institute at the Imperial College
School of Medicine, London SW3 6LY, United Kingdom

Evans, David J., Mark A. Lindsay, Benjamin L. J. Webb, Hannu Kankaanranta, Mark A. Giembycz, Brian J. O’Connor, and Peter J. Barnes. Expression and activation of protein kinase C-ζ in eosinophils after allergen challenge. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L233–L239, 1999.—Protein kinase (PK) C is an increasingly diverse family of enzymes that has been implicated in a range of cellular functions within the eosinophil. Using isoform-specific polyclonal antibodies, we have explored the expression of PKC isozymes in circulating eosinophils. Initial studies demonstrated the presence of the α, βI, βII, and γ isoforms and the low-level expression of the δ, ε, η, ι, and ι isoforms in both normal and asthmatic subjects. There was no difference in the total protein expression between these two groups. Subsequent studies examined the expression and activation of PKC isozymes in circulating eosinophils from asthmatic patients before and 24 h after a late asthmatic response to an inhaled allergen. Cellular fractionation showed PKC-α and PKC-βII to be mainly located in the cytosol, whereas PKC-βII was constitutively more expressed in the membrane. No changes in expression or subcellular localization of these isoforms were seen after allergen challenge. In contrast, PKC-ζ expression was increased after allergen challenge, and we demonstrated a significant PKC-ζ translocation to the membrane, in keeping with activation of the enzyme. Our results suggest that 24 h after allergen exposure of asthmatic patients, there is increased expression and activation of eosinophil PKC-ζ that correlates with late asthmatic responses recorded between 4 and 10 h postallergen challenge.

Protein phosphorylation catalyzed by kinases is thought to play a central role in the mechanisms of signal transduction. Posttranslational modification of proteins by phosphorylation can activate or inhibit enzyme pathways and, in turn, modify cell function. Protein kinase (PK) C is an increasingly diverse family of enzymes that are believed to be important to signal transduction in multiple cell systems (24, 25) including eosinophils. Studies using the phorbol ester phorbol 12-myristate 13-acetate, an activator of PKC, suggests a role for this enzyme in the mechanism of eosinophil cell adhesion (8), degranulation (17), and NADPH oxidase activation (33). To date, eleven isoforms of PKC (α, βI, βII, δ, ε, γ, η, ι, ι, ι, µ, and ζ) have been identified. On the basis of molecular structure and biochemical properties, the PKC family can be divided into three groups. The conventional PKCs (cPKCs; α, βI, βII, and γ isoforms) are Ca²⁺ and phospholipid dependent. The novel PKCs (nPKCs; δ, ε, η, and ι isoforms) lack the Ca²⁺-binding region and are therefore Ca²⁺ independent, whereas the third group, atypical PKCs (aPKCs; ι, µ, and z isoforms) lack both the Ca²⁺- and diacylglycerol- or phorbol ester-binding sites (24, 25). At present, the biological significance of this heterogeneity as well as the function of the individual isoenzymes is largely unknown.

Using Western blot analysis, Bates et al. (2) have previously demonstrated the presence of the β isoform of PKC within human blood and BAL fluid eosinophils. This study also found that the Ca²⁺- and phospholipid-dependent PKC activity (i.e., cPKCs) was increased in low-density eosinophils. Because a decrease in the sedimentation density has been observed to correlate with increases in a number of functional activities, it was suggested that members of the PKC family may be important in the mechanism of eosinophil activation (2). To extend this earlier study, we used Western blot analysis to identify the expression and subcellular distribution (cytosolic and membrane fractions) of eleven PKC isoforms (α, βI, βII, δ, ε, γ, ι, µ, η, ι, and ζ) in eosinophils. Furthermore, we have examined a possible role for PKCs during in vivo eosinophil activation by investigating the effect of allergen challenge on PKC expression and activation in the circulating eosinophils of asthmatic patients who demonstrated a late asthmatic response (LAR). Evans et al. (12) have previously demonstrated that the LAR correlates with the activation of circulating eosinophils obtained 24 h after allergen challenge, resulting in the priming of NADPH oxidase activation and increased survival.
METHODS

Study protocol. We studied 14 steroid-naive asthmatic patients and 5 nonasthmatic control subjects. Each of the asthmatic patients underwent allergen challenge, and all showed an LAR. Levels of PKC isom form expression in eosinophils were examined in the normal subjects and asthmatic patients (n = 8) both before and after allergen challenge. In six other subjects, the subcellular distribution of PKC isoforms was determined. Eosinophils were purified from peripheral blood immediately before and 24 h after allergen challenge.

Subjects and baseline lung function. All asthmatic patients demonstrated clinical features of asthma and had a provocative concentration of histamine causing a 20% fall in forced expiratory volume in 1 s (FEV1) of <4 mg/ml. The mean baseline percent predicted FEV1 was 94.3 ± 1.4% (range 87–103%). All subjects had positive skin prick tests (>6 mm) to at least one of a number of common aeroallergens. None of the subjects had previously taken inhaled or oral glucocorticosteroids, and the only medication used by any of the group was albuterol on an “as required” basis (Table 1).

Allergen challenge. The subjects were admitted to the Royal Brompton Hospital (London, UK) Clinical Studies Unit for a 24-h period. On arrival, the subjects underwent clinical assessment before venesection. Allergen inhalation tests were performed with a nebulizer attached to a breath-activated dosimeter (dosimeter MB3, MEFAR Electromedical, Bovezzo, Italy). The nebulizer delivered particles with an aerodynamic mass median diameter of 3.5–4 µm at an output of 9 µl/breath. The nebulizer was set to nebulize for 1 s, with a pause time of 6 s, at a pressure of 22 psi. Freeze-dried allergen extracts (Aquagen SQ, Allergologisk Laboratium, Horsholm, Denmark) were used. Known dilutions of the allergen were made to give final concentrations of 200, 1,000, 2,500, 5,000, 12,500, 25,000, and 50,000 IU/ml. The initial dose for the allergen inhalation test was 200 IU/ml, and FEV1 was measured 5 and 10 min after each allergen dose. Serially increasing doses of allergen were inhaled, and the cumulative dosage resulting in a 15% reduction within 10 min was recorded and constituted an adequate challenge. The FEV1 was recorded every 15 min for the first hour and hourly thereafter. The LAR was defined as a fall in FEV1 of 15% from the postsaline FEV1 between 4 and 10 h. Twenty-four hours after allergen challenge, the subjects were venesected for a second time.

Chemicals and materials. Rabbit polyclonal antibodies to the PKC isoforms were obtained from Santa Cruz Biotechnology (Autogen Bioclear UK, Wilts, UK). Recombinant PKC was obtained from Calbiochem (Nottingham, UK), whereas donkey anti-rabbit horseradish peroxidase-linked IgG, rainbow molecular-weight markers, enhanced chemiluminescence (ECL) Western blotting detection agents, and [γ-32P]ATP were purchased from Amersham Life Sciences (Little Chalfont, UK). Polyclonal antibodies to α1(IV) collagen and α2(I) collagen were purchased from Bio-Rad Laboratories (Hemel Hempstead, UK). Percoll was supplied by Pharmacia Biotech (St. Albans, UK), and the CD16 immunomagnetic beads were supplied by Eurogentec (Teddington, UK). Orthophosphoric acid was purchased from British Drug House (Poole, UK), and phosphocellulose was purchased from Whatman (Maidstone, UK). Penicillin, streptomycin, and RPMI 1640 medium were supplied by Gibco BRL (Paisley, UK), and interleukin (IL)-5 was supplied by R&D Systems (Abingdon, UK). All other chemicals were obtained from Sigma (Poole, UK). Kodak X-OMAT-S film was supplied by Kodak-Pathé.

Eosinophil preparation. Eosinophils were purified with the method of Hansel et al. (15). Briefly, venous blood (50 ml) was collected into 10 ml of acid citrate-dextrose anticoagulant. The blood was diluted 1:1 with Hank’s balanced salt solution (HBSS), layered onto 1.082 g/l of Percoll, and centrifuged at 1,300 g for 25 min at 22°C. After centrifugation, the mononuclear cell layer was discarded, and the pellet containing the granulocytes and red blood cells was washed in HBSS. Contaminating red blood cells were lysed by hypotonic lysis. The granulocyte fraction was washed, counted, and then resuspended in 250 µl of HBSS containing 2% fetal calf serum (FCS) and 5 mM EDTA (HBSS-FCS-EDTA). The eosinophils were purified from the neutrophils with immunomagnetic anti-CD16 antibody-conjugated beads (1 µl beads/5 × 106 neutrophils). After addition of the beads, the cells were incubated at 4°C for 30 min before being resuspended in 10 ml of HBSS-FCS-EDTA. The mixture was loaded onto a separation column positioned within a magnetic field and eluted with 2 ml of HBSS-FCS-EDTA. The CD16+ eosinophils were retained on the column while the eluted eosinophils were collected, washed in HBSS, counted, and then resuspended at 107 cells/ml. Eosinophil purity was >99.9% as assessed with microscopic examination with Kimura stain.

Subcellular fractionation. Cytosolic and membrane fractions were prepared by ultracentrifugation. The eosinophils were resuspended at 2 × 107/ml in ice-cold lysing buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 10 mM diethyrtrol, 4 mM EGTA, 4 mM EDTA, 100 µM phenylmethylsulfonyl fluoride, 2 mM benzamidine, 100 µM leupeptin, 10 µg/ml of soybean trypsin inhibitor, and 100 µg/ml of bacitracin) and then lysed by sonication (4 × 5 s). Fresh rat brain and skeletal muscle tissues were chopped, frozen in liquid N2, and then homogenized in lysis buffer. All samples were then centrifuged at 100,000 g for 60 min. The supernatant (cytosolic fraction) was removed, and the pellet (membrane fraction) was resuspended in an equal volume of lysis buffer by sonication (3 × 10 s). Both fractions were boiled in Laemmlli buffer (15) to give an equivalent final concentration of 105 cells/ml and stored at −20°C for Western blotting. The preparation of the samples was carried out at 4°C throughout.

Western blotting analysis. PKC isoforms were identified and quantified by Western blot analysis. Protein samples containing the equivalent of 2.5 × 105 cells (25 µl) were run in...
parallel with rat brain or skeletal muscle protein extracts or recombinant protein, which served as positive controls for immunodetection of the PKC isoforms. For each isoform, to minimize error arising from interassay reproducibility, all samples for each individual (i.e., both pre- and postchallenge eosinophils) were run in parallel on the same gel. The samples were loaded onto individual lanes of a 10% acrylamide gel (Bio-Rad ready gel) and were separated by SDS-PAGE. After electrophoresis, the protein was transferred to nitrocellulose (Hybond-ECL, Amersham) for 2 h at 1,000 mA in transblotting buffer [183 mM glycine-HCl, 25 mM Tris base, and 20% (vol/vol) methanol]. To block nonspecific antibody binding, nitrocellulose was incubated for 1 h in 25 mM Tris base, 150 mM NaCl, and 0.05% Tween 20, pH 7.4 (Tris-buffered saline-Tween 20 (TBS-T)) containing 5% (wt/vol) nonfat dry milk. After this, the nitrocellulose membranes were incubated for 1 h in TBS-T containing 5% (wt/vol) nonfat dry milk, and the specific antibody against each PKC isoform (Santa Cruz Biotechnology) was used at a dilution of 1:500. The membranes were then washed with TBS-T (5 × 5 min) and incubated with a 1:7,000 dilution of horseradish peroxidase-linked anti-rabbit IgG in TBS-T-5% nonfat dry milk for 1 h at room temperature. The blots were washed in TBS-T (5 × 5 min) and developed with ECL Western blotting detection agents (Amersham) and Kodak X-OMAT-S film. All blots for each isoform were developed together to ensure identical exposure times. Quantification of the developed blots was performed with laser densitometry.

cPKC cytosolic enzyme activity. PKC activity was estimated by measuring the phosphorylation of histone III S. Assays were performed in duplicate at 30°C and initiated by the addition of 25 µl (2.5 × 10^6 cells) of the cytosolic fraction to 75 µl of a reaction cocktail containing 20 mM MOPS, 15 mM magnesium acetate, 10 µM ATP (supplemented with 100 counts·min⁻¹·pmol (γ-32P)ATP⁻¹), 2 mg/ml of BSA, and 1 mg/ml of histone III S in the presence of either 2 mM EGTA or 1.5 mM CaCl₂, 100 µg/ml of phosphatidylserine, and 500 nM 4-phorbol 12,13-dibutyrate. Reactions were terminated after 30 min by spotting 50-µl aliquots of the reaction mixture onto 2 × 2-cm P81 phosphocellulose paper squares that were left for 30 s and then immersed in 150 mM orthophosphoric acid. The paper squares were then extensively washed (4 × 5 min) with fresh orthophosphoric acid to displace any nonspecifically bound ATP and Pi, immersed in industrial methylated spirit (5 min) and diethyl ether (5 min), and allowed to dry. Bound radioactivity (representing phosphorylated substrate) was subsequently quantified by liquid scintillation counting in 4 ml of ACS II scintillant (Amersham International).

In vitro IL-5 incubation. Eosinophils (1 × 10^6 in 1 ml) were suspended in RPMI 1640 medium, 2% FCS, and 100 U of penicillin-100 µg/ml of streptomycin and cultured for 24 h in the presence of 10 pM IL-5. Cells obtained at baseline and after 24 h of culture with IL-5 were prepared for Western blot analysis as described in Western blotting analysis.

Statistics. All values for lung function are expressed as means ± SE. Analysis of the optical densities from the laser densitometry was performed with the Wilcoxon nonparametric test. A P value of <0.05 was taken to be significant.

RESULTS

Response to allergen challenge. All 14 asthmatic subjects (age 19–32 yr) had a well-defined dual response to allergen challenge. The mean maximum change in FEV₁ for the early response was 29 ± 2.5% (range 17–49%) and for the LAR 28 ± 2.0% (range 17–37%; Table 1). The values quoted represent changes compared with the postdiluent FEV₁.

Eosinophil PKC isoforms. After SDS-PAGE, Western blot analysis identified the expression of the α, β, βII, and γ isoforms of PKC in the circulating eosinophils of both normal and asthmatic subjects (Fig. 1). Prolonged ECL exposure also demonstrated the presence of all PKC isoforms; and recombinant protein (αI) was employed as positive controls. No significant difference was observed in the total protein expression of the α, β, βII, and γ isoforms of PKC between normal and asthmatic subjects (data not shown).

Effect of allergen challenge and IL-5 on PKC isoform expression. After allergen challenge, the levels of the α,
βI, and βII isoforms were unchanged compared with baseline measurements (Fig. 2). However, there was an increase in the level of detectable ζ isoform (mean band optical density 0.20 ± 0.04 preallergen and 0.33 ± 0.04 postallergen; \( P = 0.02 \)). Allergen challenge did not induce the expression of the γ, η, and ι isoforms (data not shown).

As a pilot to test for the ability of IL-5 to induce a similar change in PKC-ζ, we compared the level of PKC-ζ expression before (0 h) and 24 h after incubation with 10 pM IL-5. The studies showed there to be no significant change in the expression of the ζ isoform.

Subcellular localization of PKC isoforms and the effect of allergen challenge. After allergen challenge, the cytosolic and particulate distributions of the PKC isoforms in circulating eosinophils were compared (Fig. 3). The translocation of PKC from the cytosolic to the membrane fraction, where the enzyme becomes activated by phospholipids, was used as a measure of PKC activation (7, 16–18). Of the total isoform proteins detected, PKC-α and PKC-βII were predominantly located within the cytosolic fraction of preallergen eosinophils, with no change after allergen challenge (PKC-α: 66 ± 4% preallergen cytosol and 74 ± 6% postallergen cytosol; PKC-βII: 71 ± 10% preallergen cytosol and 68 ± 7% postallergen cytosol). For the βI isoform, there was constitutively more of the total protein present in the membrane at baseline, but there was no change in the relative amounts in the cytosolic and membrane fractions after allergen challenge (preallergen: 52 ± 10% cytosol, 48 ± 10% membrane; postallergen: 56 ± 6.0% cytosol, 44 ± 6% membrane). Preallergen, the ζ isoform was distributed equally between the cytosolic and membrane fractions. However, after allergen challenge, membrane PKC-ζ expression was found to be significantly increased after its redistribution from the cytosol to the membrane (preallergen: 56 ± 8% cytosol, 44 ± 8% membrane; postallergen: 39 ± 8% cytosol, 61 ± 8% membrane; \( P = 0.03 \)). As with the total cellular extracts, the sum of cytosolic and membrane PKC-ζ was increased in the six patients after allergen challenge, although this did not reach significance, whereas the expression of the α, βI, and βII isoforms was unchanged.

Cytosol enzyme activity. The activity of the cPKC enzymes present in the cytosolic fraction was measured before and 24 h after allergen challenge and showed that there was no significant change (cytosolic cPKC activity: 47 ± 13 pmol·min\(^{-1}\)·10\(^7\) cells\(^{-1}\) preallergen; 39 ± 8 pmol·min\(^{-1}\)·10\(^7\) cells\(^{-1}\) postallergen).
eosinophils. A previous report by Bates et al. (2) demonstrated platelet contamination of the eosinophil preparations. Electron micrograph images demonstrate platelets closely adherent to granulocytes, and, therefore, expression of PKC isoforms by platelets would influence our findings. In addition to these cPKCs, we identified the presence of the aPKCs ε, µ, and γ and the nPKCs δ and θ. We were unable to demonstrate expression of the other two members of the nPKC family, η and θ. Because the positive controls showed the effectiveness of the antibodies for the nPKC isoforms, it implies that if these isoforms are indeed expressed, it is at very low levels and probably have no role during cellular activation or metabolism.

In subsequent studies, we examined the expression and subcellular distribution of the α, βI, βII, and γ isoforms after allergen challenge. Investigation of the effect of challenge on the δ, ε, µ, γ, η, and θ isoforms was precluded by their low-level expression. Examination of the subcellular distribution of PKCs in allergic patients showed that the α and βII isoforms were predominantly associated with the cytosolic fraction, whereas the βI and γ isoforms were equally distributed between the cytosolic and membrane fractions. This distribution contrasts with that described in another study (2) but may result from nonspecific cellular activation during separation, such as a result of the hypotonic lysis of the red cells. Alternatively, it is possible that we are examining a preprimed population of cells because they were collected from a group of atopic asthmatic patients. The proportion of the βI and βII isoforms associated with the membrane fractions is considerably greater than that reported by Bates et al. (2), who demonstrated that only 7% of activity was associated with this fraction. This discrepancy between protein expression and enzyme activity may be related to the difficulty in solubilizing membrane-associated PKC during the determination of activity.

Allergen challenge had no effect on either the expression or subcellular distribution of the cPKC isoforms α, βI, and βII. The lack of change in cytosolic enzyme activity postallergen for cPKC supports this finding, i.e., that the α, βI, and βII isoforms do not translocate postallergen. However, both the expression of PKC-γ and its translocation from the cytosolic to the membrane fraction was increased after allergen challenge. Unfortunately, there is no definitive and reliable method to directly assess enzyme activity for PKC-γ to endorse our immunoblot findings. Although membrane translocation and subsequent binding of diacylglycerol is thought to be important to the activation of the cPKC and nPKC isoforms (24), its relevance for aPKCs (i.e., PKC-ε) is uncertain. However, there are data that show PKC-ε stimulation by membrane-associated phosphati-
dylinositol 3,4,5-trisphosphate (23) and ceramide (20), suggesting that translocation may also be important to PKC-ε activation. In addition, a recent study (21) has identified a family of membrane-associated receptors

---

**Fig. 3. Distribution of PKC isoforms in eosinophils before and after allergen challenge.**

**A** Distribution of α isoforms between cytosolic (solid bars) and membrane (open bars) fractions of eosinophils before and after allergen challenge in 6 asthmatic subjects.

**B** Distribution of βI isoforms between cytosolic (solid bars) and membrane (open bars) fractions of eosinophils before and after allergen challenge in 6 asthmatic subjects.

**C** Distribution of βII isoforms between cytosolic (solid bars) and membrane (open bars) fractions of eosinophils before and after allergen challenge in 6 asthmatic subjects.

**D** Distribution of γ isoforms between cytosolic (solid bars) and membrane (open bars) fractions of eosinophils before and after allergen challenge in 6 asthmatic subjects.

---

for this discrepancy is uncertain, although it could be related to differences in antibody sensitivity. Alternatively, there may be platelet contamination of the eosinophil preparations. Electron micrograph images demonstrate platelets closely adherent to granulocytes, and, therefore, expression of PKC isoforms by platelets would influence our findings. In addition to these cPKCs, we identified the presence of the aPKCs ε, µ, and γ, and the nPKCs δ and θ. We were unable to demonstrate expression of the other two members of the nPKC family, η and θ. Because the positive controls showed the effectiveness of the antibodies for the nPKC isoforms, it implies that if these isoforms are indeed expressed, it is at very low levels and probably have no role during cellular activation or metabolism.

In subsequent studies, we examined the expression and subcellular distribution of the α, βI, βII, and γ isoforms after allergen challenge. Investigation of the effect of challenge on the δ, ε, µ, γ, η, and θ isoforms was precluded by their low-level expression. Examination of the subcellular distribution of PKCs in allergic patients showed that the α and βII isoforms were predominantly associated with the cytosolic fraction, whereas the βI and γ isoforms were equally distributed between the cytosolic and membrane fractions. This distribution contrasts with that described in another study (2) but may result from nonspecific cellular activation during separation, such as a result of the hypotonic lysis of the red cells. Alternatively, it is possible that we are examining a preprimed population of cells because they were collected from a group of atopic asthmatic patients. The proportion of the βI and βII isoforms associated with the membrane fractions is considerably greater than that reported by Bates et al. (2), who demonstrated that only 7% of activity was associated with this fraction. This discrepancy between protein expression and enzyme activity may be related to the difficulty in solubilizing membrane-associated PKC during the determination of activity.

Allergen challenge had no effect on either the expression or subcellular distribution of the cPKC isoforms α, βI, and βII. The lack of change in cytosolic enzyme activity postallergen for cPKC supports this finding, i.e., that the α, βI, and βII isoforms do not translocate postallergen. However, both the expression of PKC-γ and its translocation from the cytosolic to the membrane fraction was increased after allergen challenge. Unfortunately, there is no definitive and reliable method to directly assess enzyme activity for PKC-γ to endorse our immunoblot findings. Although membrane translocation and subsequent binding of diacylglycerol is thought to be important to the activation of the cPKC and nPKC isoforms (24), its relevance for aPKCs (i.e., PKC-ε) is uncertain. However, there are data that show PKC-ε stimulation by membrane-associated phosphati-
dylinositol 3,4,5-trisphosphate (23) and ceramide (20), suggesting that translocation may also be important to PKC-ε activation. In addition, a recent study (21) has identified a family of membrane-associated receptors...
for activated C kinases (RACKs) that are thought to be important to PKC function. Furthermore, studies in neutrophils and renal mesangial cells have demonstrated PKC-ζ membrane translocation after stimulation with formyl-methionyl-leucyl-phenylalanine (4) and IL-1β (30), respectively. Given this evidence, the data from these experiments suggest that membrane translocation of PKC-ζ is probably related to enzyme activation and that this could play a role in the mechanism of eosinophil activation after the LAR.

The actual signaling mechanism responsible for increased expression and subsequent activation of eosinophil PKC-ζ after the LAR as well as its cellular function is presently unknown. Increased expression implies RNA transcription and protein synthesis. PKC-ζ can be activated in vitro by phosphatidylinositol 3,4,5-trisphosphate (23), phosphatidic acid (19), and ceramide (20), which are released after the activation of phosphatidylinositol 3-kinase, phospholipase D, and sphingomyelinase, respectively. In addition, the direct association of PKC-ζ with Ras has recently been demonstrated both in vitro and in vivo (7). Once activated, PKC-ζ has been demonstrated to stimulate nuclear factor-κB (6, 20) as well as RNA transport and processing via phosphorylation of heterogenous ribonucleoprotein A1 (22) and in this manner may influence eosinophil function by promoting the expression of genes coding for proinflammatory proteins.

IL-5 has been implicated as the predominant eosinophil-active cytokine during the LAR (26, 31). This cytokine has been demonstrated to regulate the growth, accumulation, and survival as well as the activation and priming of eosinophils (3, 10, 29). An inhibitor study (35) has shown that IL-5-induced survival is dependent on both RNA transcription and protein synthesis. Furthermore, IL-5 has been demonstrated to activate a number of intracellular messenger systems including the J anus kinase (Jak2)-signal transducer and activator of transcription (STAT1) (28) and the Ras-Raf-1-mitogen-activated protein kinase (MAPK) pathways (27, 34) as well as phosphatidylinositol 3-kinase (14). However, with our in vitro studies with IL-5, we have been unable to demonstrate the induction of PKC-ζ expression over a 24-h period. This implies that under in vitro conditions, 10 pM IL-5 was unable to replicate our in vivo observations and that an additional unidentified factor(s) may be required.

In conclusion, we have identified the presence of three conventional (α, β, and βII), three atypical (λ, μ, and ɛ), and two novel (δ and e) isoforms of PKC in circulating human eosinophils. In contrast to the α, β, and βII isoforms, we have shown increased expression and activation of PKC-ζ in patients producing an LAR to whole lung allergen challenge. Because, under identical experimental conditions, eosinophils have been shown to demonstrate priming of NADPH oxidase and increased survival, PKC-ζ may be involved in mediating these responses.

D. J. Evans and M. A. Lindsay contributed equally toward this work.

D. J. Evans was supported by a grant from Byk Gulden (Constance, Germany). M. A. Lindsay was supported by Wellcome Trust Grant 056814.

Address for reprints and other correspondence: M. A. Lindsay, Dept. of Thoracic Medicine, National Heart and Lung Institute, Imperial College School of Science, Technology and Medicine, Dovehouse St., London SW3 6LY, UK (E-mail: m.lindsay@ic.ac.uk).

Received 14 October 1997; accepted in final form 26 March 1999.

REFERENCES


