Brief communication

Characterization of PGE_2 receptor subtypes in human eosinophils

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Although previous pharmacologic studies have indicated that PGE receptors are expressed in human eosinophils, the exact distribution of the subtypes remains mostly unknown. By using a combination of genetic and conventional pharmacologic approaches, coexpression of mRNAs encoding the PGE receptor 2 (EP2) and EP4 was confirmed in eosinophils. Moreover, competitive PCR analysis of eosinophil RNA revealed that levels of the EP4 receptor mRNA were significantly higher than those of the EP2 receptor mRNA ($P = .04$). On the basis of the expression levels of mRNAs, an EP4 agonist, but not an EP2 agonist, was effective in inducing cyclic AMP production in eosinophils, suggesting that the EP4 receptor is of primary importance in eosinophil functions of PGE_2. (J Allergy Clin Immunol 2002;110:457-9.)

Key words: *Eosinophils, prostaglandin E, prostaglandin E receptors*

Cell-surface expression of multiple functionally distinct subtypes of PGE_2 receptor is a principle determinant of the diversity and specificity of cellular effects of PGE_2.[1] At least 4 subtypes of the PGE receptor (EP), which differ in tissue distribution, ligand-binding affinity, and coupling to intracellular signaling pathways, have been cloned to date. The EP2 and EP4 receptors activate adenylate cyclase through Gs and stimulate increases in the intracellular concentration of cyclic AMP (cAMP). Activation of the EP1 receptor is associated with increases in intracellular calcium concentration, and the EP3 receptor generally couples to Gi and inhibits increases in intracellular cAMP levels.

The inhibitory effects of PGE_2 on the production of leukotriene C_4 (LTC_4) in eosinophils have been recognized[2]; however, it is unknown what type of receptor binds PGE_2 and mediates the response. The objectives of our study were to characterize the expression of EP receptor mRNAs and to compare the expression levels of EP receptor mRNAs in human eosinophils and to identify which receptor is mainly responsible for the PGE_2-induced increase in cAMP production.

Abbreviations used
cAMP: Cyclic AMP
EP: PGE receptor
LTC_4: Leukotriene C_4

METHODS

After informed consent was obtained, peripheral blood samples were collected from patients with mild persistent asthma who did not take oral steroids. All were in clinically stable conditions at the time of the study. Blood was layered on top of 3 discontinuous gradients of Percoll solutions, as described previously.[3] After centrifugation, neutrophils were retrieved from the interface, with densities of between 1.082 and 1.094 g/mL. Granulocytes were collected from the Percoll layer with a density of 1.094 g/mL, and eosinophils were negatively selected with anti-CD16 immunomagnetic beads by using an MACS cell separation system (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), obtaining a greater than 99% eosinophil preparation.

After total RNA was extracted from approximately 1.4×10^6 eosinophils with an RNeasy mini-kit (Qiagen, Hilden, Germany), cDNA was synthesized in the presence of an oligo-dT primer. The primers used for RT-PCR were 5′-GCCAGCTTGTCGGTATCATG-3′ (sense) and 5′-CGCAGTAGGATGTACACCCA-3′ (antisense) for the EP1 receptor (resulting PCR product, 175 bp), 5′-TTCATCCGGCACGGGCGGACCGC-3′ (sense) and 5′-GTCAGCCTGTTTACTGGCAGCTG-3′ (antisense) for the EP2 receptor (510 bp), 5′-GTCCGTGGCCTTCCCGATCAC-3′ (sense) and 5′-GCGCTGGCGATGAACAACGAG-3′ (antisense) for the EP3 receptor (300 bp), and 5′-CGGTGACCATCGCCACGTACA-3′ (sense) and 5′-CGCCAATCGCTTGTCCACGTA-3′ (antisense) for the EP4 receptor (188 bp).

A heterologous competitor DNA was prepared with a competitive DNA construction kit (Takara, Shiga, Japan). The relative intensities of the target and competitor PCR products were quantified by using the Bioanalyzer (Agilent, Palo Alto, Calif) and calculated as described previously.[3] The amount of glyceraldehyde 3-phosphate dehydrogenase mRNA was also measured with an Amplifluor GAPDH kit (Intergen, Purchase, NY).

A suspension of eosinophils in 0.5 mL of Tyrode's buffer containing 0.1% human serum albumin was preincubated with 10^{-4} mol/L Ro-20-1724, a cAMP phosphodiesterase inhibitor, for 10 minutes at 37°C. Reactions were started by the addition of PGE_2 (10^{-8} to 10^{-6} mol/L) or other agonists, and the incubation was continued for 10 minutes at 37°C. Various concentrations of butaprost (10^{-8} to 10^{-6} mol/L, an EP2 agonist; Cayman, Ann Arbor, Mich) and ONO-AEI-329 (10^{-9} to 10^{-7} mol/L, an EP4 agonist; kindly supplied by ONO Pharmaceutical Co, Osaka, Japan)[4] were used in this study. The reaction was stopped by the addition of 0.5 mL of 20% trichloroacetic acid solution. The amounts of cAMP were quantified

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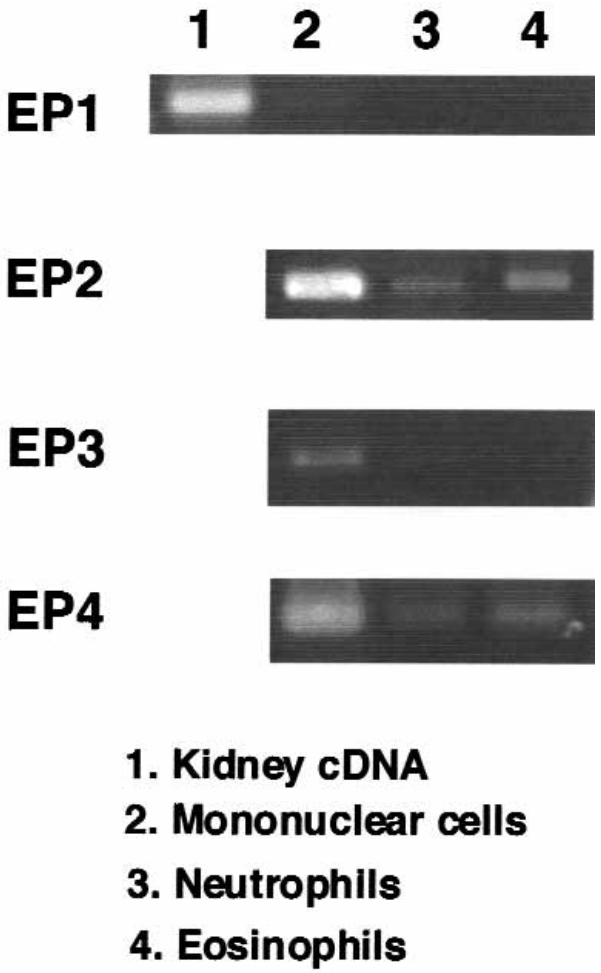

FIG 1. Expression of EP1, EP2, EP3, and EP4 receptor mRNAs in human mononuclear cells, neutrophils, and eosinophils. Human kidney cDNA was used as a positive control for determining EP1 mRNA expression levels.

by means of enzyme immunoassay (Cayman), according to the manufacturer's protocol.

The inhibitory effect of PGE_2 or ONO-AEI-329 on LTC_4 generation was assessed according to the method of Tenor et al.[2] Briefly, eosinophils were preincubated with indomethacin (10^{-5} mol/L) for a few minutes, followed by incubation with ONO-AEI-329 for 10 minutes. The cells were stimulated with C5a (10^{-7} mol/L) for 15 minutes. LTC_4 released in the supernatant was quantitated with an enzyme immunoassay kit (Cayman).

The data were analyzed with the Student *t* test and were considered statistically significant at a *P* value of less than .05.

RESULTS

RT-PCR analysis of cDNA isolated from human eosinophils revealed PCR products for the EP2 and the EP4 receptor subtypes. Data of PCR analysis of mononuclear cells and neutrophils are also shown in Fig 1 for comparison: neutrophils expressed the EP2 and EP4 receptor mRNAs, whereas mononuclear cells expressed the EP2, EP3, and EP4 receptor mRNAs. When normalized by the number of glyceraldehyde 3-phosphate dehydrogenase mRNAs, the expression level of the EP4 receptor mRNA was more than 10-fold higher than that of the EP2 receptor mRNA in eosinophils (0.25 ± 0.19 vs 3.43 ± 2.48, n = 7, $P = .04$).

Analysis of adenylate cyclase signaling revealed that both PGE_2 and ONO-AEI-329, but not butaprost, which works as a full agonist of the EP2 receptor,[1] evoked an increase in cAMP production in eosinophils, confirming the expression of the functional EP4 receptor (Fig 2, *A*). Thus the EP4 receptor is responsible for the PGE_2-induced increase in the intracellular cAMP level in eosinophils. A statistically significant difference in the extent of increase in the cAMP level induced by ONO-AEI-329 and that by PGE_2 has been observed, suggesting that the potency of ONO-AEI-329 is weaker than that of the natural ligand. Because it has been reported that ONO-AEI-329 is a full agonist for EP4, further studies will be needed to establish the function. ONO-AEI-329, but not butaprost, inhibited LTC_4 release from eosinophils with an inhibitory concentration of 50% of approximately 3×10^{-9} mol/L (Fig 2, *B*).

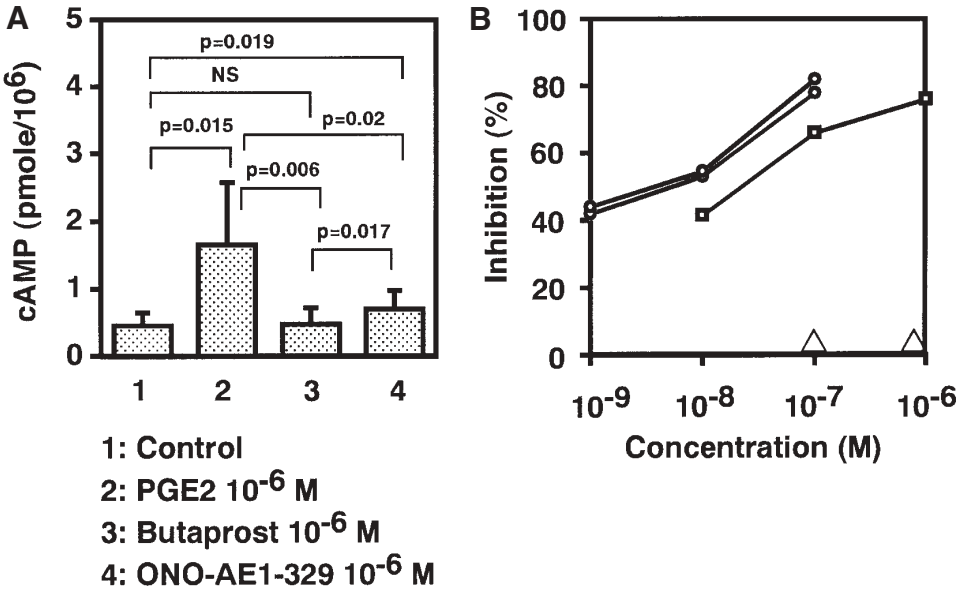

FIG 2. A, Effect of PGE_2, butaprost, and ONO-AEI-329 on the increase in the intracellular cAMP level in eosinophils. **B,** Effect of PGE_2 *(open squares)*, ONO-AEI-329 *(open circles)*, and butaprost *(open triangles)* on C5a-induced LTC_4 generation in human eosinophils.

DISCUSSION

Results of our study suggest the possibility that there are almost no functional EP2 receptors in eosinophils. It has been shown that the receptor subtype that is expressed in eosinophils is the EP4 receptor, which transmits signals from PGE_2 to the cells. Thus EP4 is the main, if not the only, receptor that is responsible for cAMP production in eosinophils. Peacock et al[5] reported that 11-deoxyPGE_1, which was considered as an EP2 agonist, inhibited the apoptosis of eosinophils. However, this compound is currently considered to be a nonselective agonist of the EP receptor,[1,6] and thus this does not negate our conclusion. Both the EP2 receptor and the EP4 receptor increase the production of cAMP; however, the EP4 receptor tends to be affected by desensitization. The transient action of PGE_2 is caused by the EP4 receptor, whereas its persistent action is caused by the EP2 receptor.[7] Because only the EP4 receptor is functional in eosinophils, the effects of PGE_2 on eosinophil functions might be dependent on the level of exposure to PGE_2.

PGE_2 inhibited the release of LTC_4 from eosinophils. However, as shown in the study by Peacock et al,[5] PGE_2 inhibited apoptosis of eosinophils, thus prolonging their survival and resulting in the retention of the effect of eosinophils. Taken together, PGE_2 might or might not have a beneficial role in the treatment of allergic inflammation.

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