

Phosphodiesterase 4B Gene Transcription Is Activated by Lipopolysaccharide and Inhibited by Interleukin-10 in Human Monocytes

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ABSTRACT

There are four different genes encoding the cAMP-specific phosphodiesterase (PDE4) isozymes (A, B, C, and D). cAMP has been the only agent known to induce PDE4 gene expression. In the present study, we demonstrate, for the first time, that lipopolysaccharide (LPS) significantly and selectively stimulated PDE4B mRNA production in human monocytes. The LPS stimulation occurred very rapidly (in 30–45 min) and in a dose-dependent manner (0.01–100 ng/ml). We also demonstrate that LPS induction of PDE4B mRNA expression was inhibited strongly by interleukin (IL)-10. The inhibition with IL-10 was dose-dependent (0.1–10 ng/ml). IL-4 also inhibited the LPS

induction, but to a lesser extent than IL-10. PDE4B mRNA expression was also stimulated by dibutyryl-cAMP. Interestingly, unlike LPS induction, the dibutyryl-cAMP induction of PDE4B mRNA expression was not inhibited by IL-10. By performing nuclear run-on and mRNA stability assays, we demonstrate further that IL-10 inhibited LPS-stimulated PDE4B mRNA synthesis by abolishing the gene transcription rather than by enhancing mRNA degradation. The present study suggests that PDE4B, as the only LPS-inducible PDE4 subtype, may be an appropriate target for discovering anti-inflammatory drugs.

The cyclic nucleotides, cAMP and cGMP, are intracellular second messengers that play key roles in mediating biological responses generated by a variety of extracellular signals, including hormones, autoids, and neurotransmitters. By catalyzing hydrolytic inactivation of these cyclic nucleotides, the cyclic nucleotide phosphodiesterases (PDE, E.C. 3.1.4.17) are important in regulating intracellular concentrations of the second messengers and, consequently, biological responses to these signal-transducing molecules (Beavo, 1995; Manganiello et al., 1995). PDE was initially purified and characterized more than 30 years ago (Butcher and Sutherland, 1962). Several PDEs, differing in their substrate specificity, kinetic properties, responsiveness to endogenous regulators, and susceptibility to inhibition by various compounds, have been isolated, purified, and characterized from various tissues. To date, this enzyme class is composed of at least seven structurally, biochemically, and pharmacologically distinct families, PDE 1-7, with a total of more than 15 genes. Most of the isozymes share a highly conserved catalytic domain located near the carboxyl termini of the proteins.

One of the families, PDE4, is characterized by its selective high affinity for cAMP over cGMP and its sensitivity to inhibition by the antidepressant drug rolipram. Four PDE4 genes (A, B, C, and D) have been isolated in humans (Livi et

al., 1990; Bolger et al., 1993; McLaughlin et al., 1993; Obernolte et al., 1993; Baecker et al., 1994; Engels et al., 1995) and rats (Swinnen et al., 1989), and their chromosomal localizations have been defined (Milatovich et al., 1994; Horton et al., 1995; Szpirer et al., 1995). The amino acid sequence analysis of PDE4s reveals three distinct, highly conserved regions: a catalytic domain and two upstream conserved regions (UCR1 and UCR2) (Bolger et al., 1993; Bolger, 1994). Northern blotting and reverse transcriptase-PCR studies demonstrate that transcripts of the four PDE4 subtypes are expressed differently among tissues (Muller et al., 1995). PDE4 is the predominant PDE isozyme in many leukocytes, including mast cells, basophils, neutrophils, eosinophils, and monocytes (Palfreyman and Souness, 1996; Torphy, 1998). These inflammatory cells are implicated in allergic and other inflammatory diseases. It has been demonstrated extensively that PDE4 plays a key role in the activation of these inflammatory cells. Recently, significant interest has been centered on selective inhibitors of PDE4 as a potential therapy for inflammatory diseases such as asthma. However, because PDE4 isozymes also are present in other tissues such as brain, PDE4-selective inhibitors may produce undesired side effects. Indeed, the side effect profile of PDE4 inhibitors is a significant issue (Torphy, 1998; Palfreyman and Souness, 1996). One of the approaches to reducing the side effect

ABBREVIATIONS: PDE, phosphodiesterase; LPS, lipopolysaccharide; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

potential is to discover subtype-specific inhibitors. It is, therefore, important to determine the expression and regulation of the various PDE4 subtypes in appropriate tissues and cells.

PDE4s can be regulated at the level of gene transcription. Conti and coworkers show a more than 100-fold increase in PDE4D mRNA level in Sertoli cells after prolonged stimulation by dibutyryl-cAMP (Swinnen et al., 1991). PDE4B gene transcription is also stimulated, albeit to a lesser extent. It is also reported that long-term increases in intracellular cAMP, in response to a *beta* agonist or rolipram, result in increased PDE4D gene expression in the human monocytic cell line U937 (Torphy et al., 1995). The cAMP stimulation of PDE4 gene expression may be due to activation of the transcription factor cAMP-responsive element-binding protein. On the other hand, at least some of the PDE4 gene products can be activated by cAMP-dependent phosphorylation. In FRTL-5 thyroid cells, thyroid-stimulating hormone activates PDE4D through cAMP-dependent phosphorylation (Sette et al., 1994). Phosphorylation and activation of recombinant PDE4D by the catalytic subunit of protein kinase A are demonstrated in a cell-free system (Alvarez et al., 1995; Sette and Conti, 1996). In the human monocytic cell line Mono Mac 6 it is shown that dibutyryl-cAMP transiently increases PDE4 enzyme activity 2- to 3-fold and then significantly stimulates the expression of PDE4A, B, and D mRNAs and proteins (Verghese et al., 1995). Presumably, the direct phosphorylation provides a short-term regulation, and the stimulation of gene transcription represents a longer-term activation.

However, thus far, cAMP has been the only agent known to induce the expression of PDE4 mRNAs. We have been investigating PDE4 gene expression regulation in leukocytes by various pathophysiologically relevant stimuli. In the present study, we demonstrate that lipopolysaccharide (LPS) specifically stimulates PDE4B gene transcription in human monocytes and that this activation is inhibited by interleukin (IL)-10. Mechanistic studies show that the IL-10 inhibition occurs at the level of gene transcription.

Materials and Methods

Reagents. RPMI 1640 medium, fetal bovine serum (heat-inactivated), penicillin-streptomycin, nonessential amino acids, L-glutamine, and TRIzol Reagent were obtained from GIBCO (Grand Island, NY). LPS, dibutyryl-cAMP, and actinomycin D were purchased from Sigma (St. Louis, MO). Recombinant human IL-10, IL-4, and transforming growth factor (TGF)- β 1 were from R&D Systems (Minneapolis, MN). Prehybridization solution (2 \times), 2 \times hybridization solution, salmon sperm DNA, and torula yeast RNA were from 5 Prime \rightarrow 3 Prime (Boulder, CO). cDNAs from various human tissues and β -actin cDNA probe were from Clontech (Palo Alto, CA). Duralon UV membrane was from Stratagene (La Jolla, CA). RNasin was from Promega (Madison, WI). [α - 32 P]UTP (specific activity: 3000 Ci/mmol) was from New England Nuclear (Boston, MA). Nytran membrane was from Schleicher & Schuell (Keene, NH).

Cell Preparation and Treatment. Human monocytes were prepared from fresh blood of healthy adult donors by elutriation (Wahl et al., 1994). The purity of the cell preparations was greater than 95% as judged by Wright's staining and by immunofluorescence assay using anti-CD14. Cells were suspended at a density of 1×10^6 cells/ml in RPMI 1640 medium, which was supplemented with 1% each of penicillin-streptomycin, nonessential amino acids, and L-glutamine and with 10% fetal bovine serum, and incubated at 37°C

in a humidified atmosphere of 5% CO₂/95% air for 1 h before each treatment. The cells (30 million cells per reaction) were treated with appropriate agents for periods of time as indicated.

Hybridization Probes. Four PCR fragments were used as probes for Northern blot hybridizations. The primers were designed as follows: PDE4A (GenBank accession number L20965): 5'-TCGAG-GAAGCTCTGGATGCAAC-3' and 5'-TCTCAGGAGGGACAAGAGGA-CAAG-3'; PDE4B (GenBank accession number L20966): 5'-TTGGAGT-CAGAAAGCAAGACCAG-3' and 5'-CAGGGGAAGGAAGTAAATG-TGG-3'; PDE4C (GenBank accession number L20968): 5'-ACACT-GAAGCTCTGTCCCCTGAAG-3' and 5'-GATGTGACTCAAGAGTGA-CCACTGG-3'; and PDE4D (GenBank accession number L20970): 5'-TCGTTCTCCTGACACGTAACAGTG-3' and 5'-TCCTCCTACTGGTA-ACAGATTCGTG-3'. The PCR fragments were generated using cDNAs from human testis (for PDE4A and C) or leukocyte (for PDE4B and D) as templates. The sizes of the probes were 546, 506, 410, and 479 bp, respectively. All the probes were corresponding to regions downstream of the catalytic domain and able to detect all known variants derived from each PDE4 gene.

Northern Blot Analysis. Total cellular RNA was extracted using TRIzol Reagent according to the manufacturer's instructions. Fifteen micrograms of total RNA was denatured and then loaded onto 1% agarose-formaldehyde gel. Fractionated RNA was then transferred to Duralon UV membrane with PosiBlot Pressure Blotter (Stratagene) and then crosslinked with UV Crosslinker (Stratagene). Prehybridization was performed at 37°C for 2 h in a solution containing 1 \times prehybridization solution [5 \times SSC, pH 7.0, 5 \times Denhardt's solution, 50 mM sodium phosphate, pH 6.8, 0.1% sodium dodecyl sulfate (SDS), 5 mM EDTA, and 10 μ g/ml poly(A)_n], 50% formamide, 50 μ g/ml denatured salmon sperm DNA, and 50 μ g/ml denatured torula yeast RNA. The hybridizations were performed at 37°C for 18 h in a solution of 1 \times hybridization solution [5 \times SSC, pH 7.0, 1 \times Denhardt's solution, 20 mM sodium phosphate, pH 6.8, 0.2% SDS, 5 mM EDTA, and 10 μ g/ml poly(A)_n], 50% formamide, 50 μ g/ml denatured salmon sperm DNA, 50 μ g/ml denatured torula yeast RNA, and various PDE4 probes labeled with 32 P by the random primer-labeling method. After the hybridization, the membranes were washed twice with 2 \times SSC/0.1% SDS at room temperature, each for 5 min, then twice with 0.1 \times SSC/0.1% SDS at 50°C, each for 20 min. For β -actin detection, the PDE4 probe on each membrane was stripped by boiling the membrane in a solution containing 2 mM EDTA and 0.1% SDS and washed twice with 2 \times SSC at room temperature, each for 20 min. Then, the membrane was hybridized with labeled β -actin cDNA probe.

Nuclear Run-On Assay. PDE4 gene transcription rates were measured by nuclear run-on assays as described previously (Wang et al., 1994a). Cells (30 million per sample) were cultured in the absence or presence of various agents as indicated at 37°C for 1 h. Then nuclear fraction was prepared. Nuclei from each sample were resuspended in 200 μ l of TEG buffer (50 mM Tris-HCl, pH 8.3, 5 mM MgCl₂, 0.1 mM EDTA, and 40% glycerol). Elongation of nascent RNA chains was initiated by mixing this nuclear suspension with 200 μ l of reaction buffer, which contained 10 mM Tris-HCl, pH 8.0, 5 mM MgCl₂, 0.3 M KCl, 10 μ l each of 100 mM ATP, CTP, and GTP, 5 μ l of 1 M dithiothreitol, 2 U of RNasin, and 0.1 mCi of [32 P]UTP. After incubation at 30°C for 30 min, 32 P-labeled RNA was isolated and then dissolved in 500 μ l of TES buffer (10 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.2% SDS, 0.6 M NaCl, and 5 \times Denhardt's solution). One microliter of the solution was used to measure 32 P incorporation. An equal amount of radioactivity from each sample, adjusted to 500 μ l with TES buffer, was used for hybridization. PDE4B and β -actin cDNA probes, 250 ng of each, were UV-crosslinked on Nytran membrane, and the membrane was prehybridized overnight at 42°C in a solution of 5 \times standard saline phosphate/EDTA (0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA, SSPE), 5 \times Denhardt's, 1% SDS, 50% formamide, and 100 μ g/ml denatured salmon sperm DNA. Labeled RNA was hybridized with the membranes containing immobilized cDNA probes at 60°C for 3 days. The membranes were washed

twice with $2\times$ SSPE/0.1% SDS at room temperature, each for 15 min, and then three times with $0.1\times$ SSPE/0.1% SDS at 65°C , each for 20 min.

mRNA Stability Assay. Cells (30 million per sample) were stimulated with LPS (100 ng/ml) at 37°C for 1 h and then incubated with actinomycin D (5 $\mu\text{g/ml}$) for 10 min to stop RNA synthesis. The cells subsequently were treated in either the absence or the presence of IL-10 (10 ng/ml) for indicated time periods. Total RNA was extracted and PDE4B mRNA was examined by Northern analysis, as described above. Then, PDE4B probe was stripped and the same membrane was hybridized with β -actin cDNA probe.

Data Presentation. In each of the figures, the raw data are from a representative experiment and the quantitative data were obtained by scanning the autoradiographic signals by DESKSCAN II (Hewlett-Packard, Sunnyvale, CA) followed by quantification using Scan Analysis (BioSoft, Ferguson, MO). The plotted values represent means \pm S.D. of the ratios of mRNA levels of appropriate PDE4s to β -actin of two to four different experiments.

Results

LPS Specifically Induces Expression of PDE4B mRNA in Human Monocytes. Thus far, cAMP has been the only agent known to have a stimulatory effect on the expression of PDE4 mRNAs (Swinnen et al., 1991; Torphy et al., 1995; Verghese et al., 1995). We examined effects of a variety of pathophysiologically relevant agents including LPS, IL-1, IL-6, tumor necrosis factor (TNF)- α , interferon- γ , and granulocyte/macrophage colony-stimulating factor on mRNA expression of the various PDE4 subtypes in human monocytes. As a result, we found that LPS specifically stimulated the expression of PDE4B mRNA, whereas none of the other agents affected any of the four subtypes. As shown in Fig. 1, in resting human monocytes, only PDE4A and B mRNAs could be detected. Dibutyryl-cAMP (0.5 mM), used as a positive control, strongly stimulated the expression of PDE4B and, albeit to lesser degrees, A and D mRNAs. This result is consistent with the previous data obtained in the human monocytic cell line Mono Mac 6 (Verghese et al., 1995)

and in human monocytes (Manning et al., 1996) (although in the latter study PDE4D could not be detected). Nevertheless, it was found that LPS at a concentration of 100 ng/ml strongly stimulated the expression of PDE4B mRNA but not of A, C, or D mRNA. The enhancement of PDE4A mRNA level by dibutyryl-cAMP, although small, was reproducible. However, LPS could not reproducibly enhance the level of PDE4A mRNA. Essentially identical results, that is, the predominant presence of PDE4A in resting cells, up-regulation of PDE4A, B, and D by dibutyryl-cAMP, and specific induction of PDE4B by LPS, were obtained from Mono Mac 6 cells (data not shown).

LPS potently stimulated PDE4B mRNA expression in human monocytes. At 0.01 ng/ml, LPS significantly enhanced the levels of PDE4B mRNA. In the LPS concentration range of 0.01 to 100 ng/ml, PDE4B mRNA expression increased in a dose-dependent manner. The LPS enhancement of PDE4B accumulation plateaued at 100 ng/ml (Fig. 2). The ED_{50} (50% effective dose) value was 0.04 ng/ml. This LPS dose-response profile was similar to that of cytokine production in human monocytes (Wang et al., 1994a).

The LPS stimulation of PDE4B mRNA expression occurred very rapidly. The accumulation of PDE4B mRNA induced by 100 ng/ml LPS reached a maximum level in about 45 min. The mRNA level then decreased gradually, but after 3 h it was still at a high level (Fig. 3). The stimulation of PDE4B mRNA expression was undetectable within 30 min after the addition of LPS, indicating that the LPS-stimulated production of PDE4 mRNA occurred between 30 and 45 min.

IL-10 Inhibits LPS-, but Not cAMP-, Stimulated Expression of PDE4B mRNA. Because IL-10 was shown to inhibit LPS-stimulated production of inflammatory cytokines including IL-1, IL-6, IL-8, and TNF- α (Wang et al., 1994a,b, 1995) and enzymes such as cyclooxygenase-2 (Niino et al., 1995) in human monocytes, we examined the effect of IL-10 on the LPS-stimulated PDE4B mRNA expression. As shown in Fig. 4, IL-10 at a concentration of 10 ng/ml blocked the

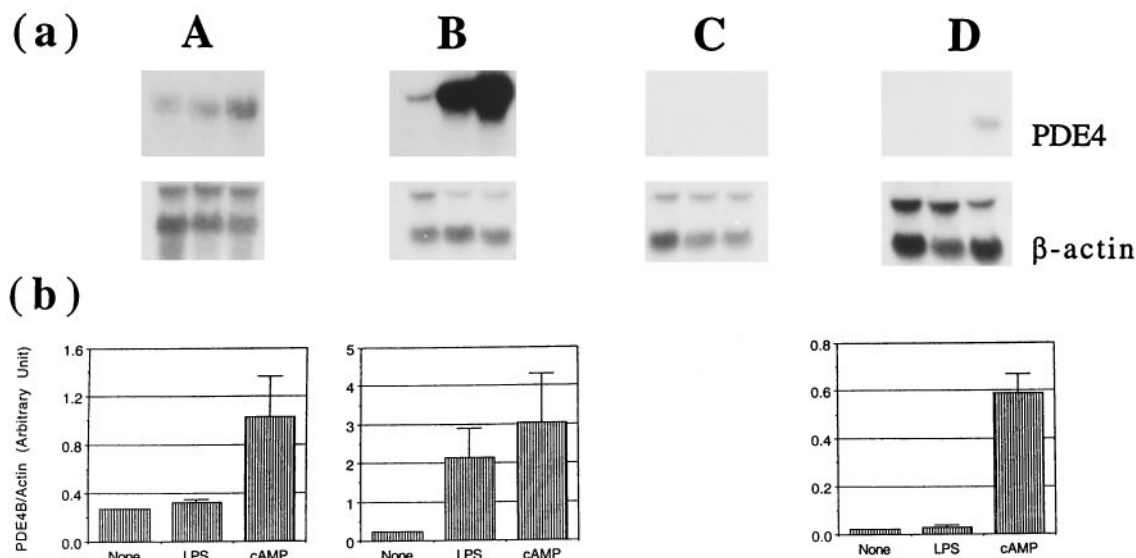


Fig. 1. Effects of LPS and cAMP on PDE4 mRNA production in human monocytes. a, cells were incubated in the absence (lane 1, from left to right) or presence of 100 ng/ml LPS (lane 2) or 0.5 mM dibutyryl-cAMP (lane 3) for 1.5 h. Total RNA was extracted and then subjected to Northern blot analysis to detect PDE4A, B, C, and D mRNAs, respectively. Each membrane was stripped and reprobed for β -actin as an internal control. b, the autoradiographic signals were scanned and quantified. The plotted values represent means \pm S.D. of the ratios of mRNA levels of PDE4A, B, or D to β -actin (arbitrary unit). Experimental details are described in *Materials and Methods*.

LPS-induced PDE4B mRNA expression. On the other hand, IL-10 only slightly inhibited the dibutyl- α -cAMP stimulation of PDE4B mRNA production (by about 17%). This result suggests that LPS and cAMP utilize different pathways to activate the expression of PDE4B gene, and that IL-10 has differential effects on these signal transduction pathways.

IL-10 inhibited the LPS-induced expression of PDE4B mRNA in a dose-dependent manner. IL-10 inhibited the LPS-stimulated PDE4B mRNA production slightly at 0.1 ng/ml but strongly at 1 ng/ml (Fig. 5). This dose-response profile of IL-10 for LPS-induced PDE4B mRNA expression was very similar to that for cytokine production in human monocytes (Wang et al., 1994a).

Because IL-4 and TGF- β also have been shown to inhibit LPS-stimulated cytokine synthesis in monocytes/macrophages (Wang et al., 1994a, 1995), we examined the effects of IL-4 and TGF- β on the LPS-induced expression of PDE4B mRNA. As shown in Fig. 6, at a concentration of 10 ng/ml, which was shown to be optimal for inhibiting cytokine synthesis in human monocytes (Wang et al., 1994a), IL-4 had a moderate inhibitory effect (about 56% inhibition) on the LPS-stimulated PDE4B mRNA production, whereas TGF- β only slightly inhibited the mRNA accumulation (about 19% inhi-

bition). The relative potencies of IL-10, IL-4, and TGF- β against PDE4B mRNA accumulation were, again, very similar to that for cytokine production in human monocytes (Wang et al., 1994a). Thus, although both IL-10 and IL-4 had an inhibitory effect, IL-10 was a more potent inhibitor for the LPS-stimulated PDE4B mRNA synthesis.

IL-10 Inhibition of LPS-Stimulated PDE4B mRNA Expression Occurs at the Level of Gene Transcription.

To determine mechanisms by which IL-10 inhibits LPS-stimulated PDE4B mRNA expression, mRNA stability analysis and nuclear run-on gene transcription assays were performed. In mRNA stability assay, human monocytes were stimulated with 100 ng/ml LPS for 1 h, and then RNA synthesis was stopped by the addition of 5 μ g/ml RNA synthesis inhibitor actinomycin D. The cells were incubated further in the presence or absence of 10 ng/ml IL-10 for various periods of time, and then PDE4 mRNA levels were analyzed by Northern blotting. As shown in Fig. 7, PDE4B mRNA level decreased with time after RNA synthesis was stopped, but IL-10 did not enhance the degradation of PDE4 mRNA at all. This assay was repeated three times, and none of the experiments showed a significant enhancing effect of IL-10 on PDE4B mRNA degradation.

On the other hand, in nuclear run-on transcription assay,

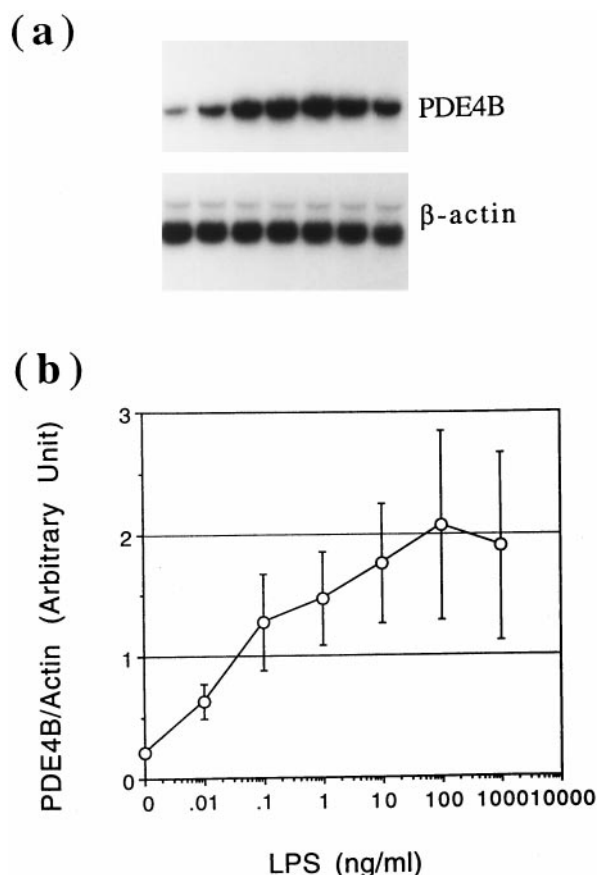


Fig. 2. LPS dose response for PDE4B mRNA production in human monocytes. a, cells were incubated in the absence (lane 1, from left to right) or presence of various concentrations of LPS (lanes 2–7: 0.01, 0.1, 1, 10, 100, and 1000 ng/ml, respectively) for 1.5 h. Total RNA was extracted and then subjected to Northern blot analysis to detect PDE4B mRNA. The membrane was stripped and reprobed for β -actin as an internal control. b, the autoradiographic signals were scanned and quantified. The plotted values represent means \pm S.D. of the ratios of mRNA levels of PDE4B to β -actin (arbitrary unit). Experimental details are described in *Materials and Methods*.

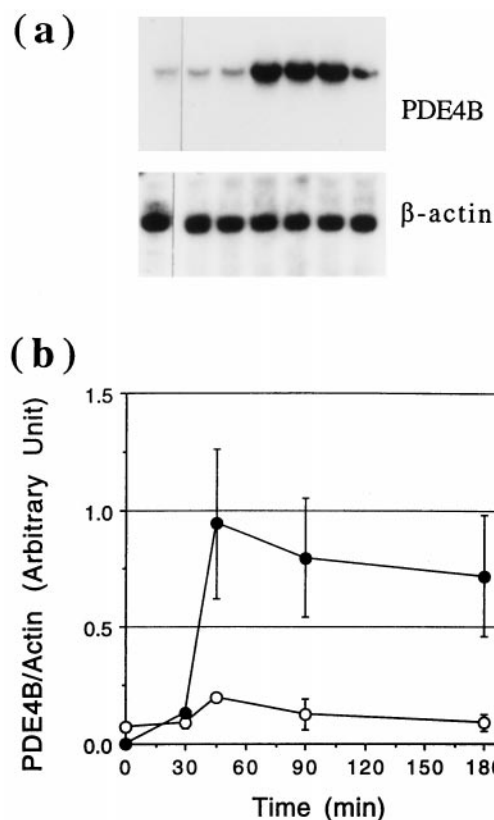


Fig. 3. Time course of LPS-stimulated PDE4B accumulation in human monocytes. a, cells were incubated in the absence (not shown) or presence of 100 ng/ml LPS for 0, 15, 30, 45, 60, 90, or 180 min (lanes 1–7 from left to right, respectively). Total RNA was extracted and then subjected to Northern blot analysis to detect PDE4B mRNA. The membrane was stripped and reprobed for β -actin as an internal control. b, the autoradiographic signals were scanned and quantified. The plotted values represent means \pm S.D. of the ratios of mRNA levels of PDE4B to β -actin (arbitrary unit) (○, without LPS; ●, with LPS). Experimental details are described in *Materials and Methods*.

the cells were stimulated with 100 ng/ml LPS in the absence or presence of 10 ng/ml IL-10 for 1 h, and then nuclei were isolated, followed by *in vitro* transcription reaction in the presence of ^{32}P -labeled UTP. The radiolabeled, newly synthesized PDE4B mRNA then was detected by hybridization with PDE4B cDNA probe. As shown in Fig. 8, LPS strongly stimulated PDE4B gene transcription, but this gene transcription was blocked by IL-10. Taken together, these data clearly demonstrate that IL-10 inhibits LPS-stimulated PDE4B mRNA synthesis by suppressing the gene transcription but not by enhancing mRNA degradation.

Discussion

Although it has been known for many years that PDE4 can be regulated at the level of gene expression, thus far cAMP has been the only agent known to be able to induce PDE4 gene expression (Swinnen et al., 1991; Torphy et al., 1995; Verghese et al., 1995). In the present study, we demonstrate in human monocytes that LPS stimulates PDE4B mRNA production. Nuclear run-on transcription assays clearly showed that LPS strongly stimulates the gene transcription

of PDE4B. The LPS induction occurs very rapidly (in about 30–45 min), which does not suggest involvement of newly synthesized proteins. Indeed, the LPS induction of PDE4B mRNA expression was not inhibited by the protein synthesis inhibitor cycloheximide (data not shown). Moreover, LPS is very potent for PDE4B mRNA expression, with an ED_{50} of 0.04 ng/ml. In these regards, the PDE4B gene bears striking similarities to that for the inflammatory cytokines IL-1, IL-6, IL-8, and TNF- α (Wang et al., 1994a,b, 1995) and the enzyme cyclooxygenase-2 (Niirio et al., 1995). Nevertheless, the LPS induction of PDE4B gene does not seem to be via these cytokines, because these cytokines are induced at the same time as, but not earlier than, PDE4B (Wang et al., 1994b) and also because these cytokines, when added to monocytes, could not induce PDE4B expression.

In the present study, we show that LPS-stimulated PDE4B mRNA expression is inhibited by IL-10. IL-10 is very potent against the LPS-induced PDE4B mRNA synthesis, with an IC_{50} (50% inhibiting dose) of 0.5 ng/ml (1 ng/ml = 56 pM). Mechanistic experiments clearly demonstrated that IL-10 inhibits LPS-stimulated PDE4B mRNA expression by suppressing the gene transcription but not by enhancing mRNA degradation. IL-4 and TGF- β , two other cytokines that also can inhibit LPS-stimulated cytokine synthesis in monocytes/

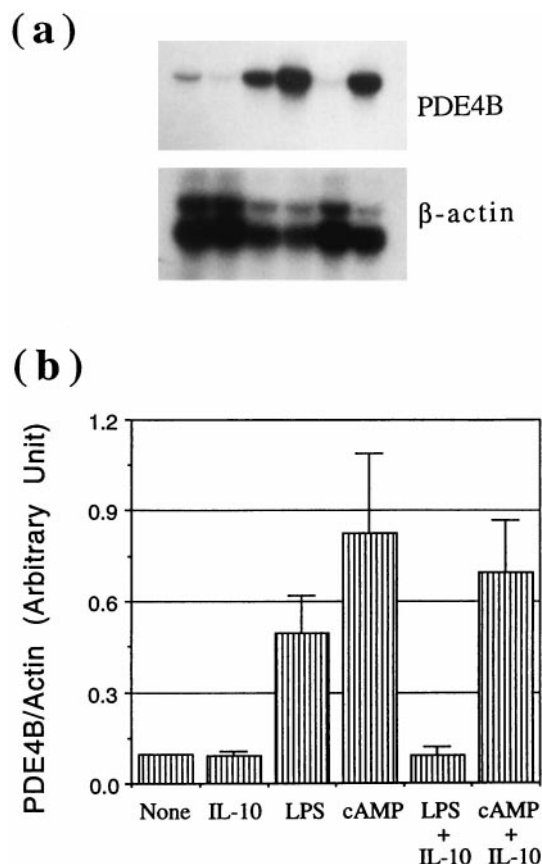


Fig. 4. Effects of IL-10 on LPS- or cAMP-stimulated PDE4B mRNA production in human monocytes. a, cells were cultured in the absence (lane 1, from left to right) or presence of 10 ng/ml IL-10 (lane 2), 100 ng/ml LPS (lane 3), 0.5 mM dibutyryl-cAMP (lane 4), IL-10 plus LPS (lane 5), or IL-10 plus dibutyryl cAMP (lane 6) for 2 h. In lanes 5 and 6, IL-10 was added 10 min before the addition of LPS or dibutyryl-cAMP. Total RNA was extracted and then subjected to Northern blot analysis to detect PDE4B mRNA. The membrane was stripped and reprobed for β -actin as an internal control. b, the autoradiographic signals were scanned and quantified. The plotted values represent means \pm S.D. of the ratios of mRNA levels of PDE4B to β -actin (arbitrary unit). Experimental details are described in *Materials and Methods*.

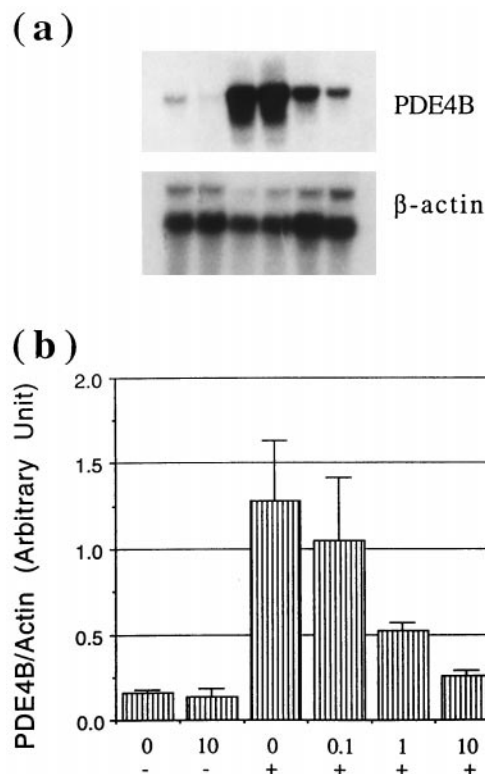


Fig. 5. IL-10 dose response for inhibition of LPS-stimulated PDE4B mRNA accumulation in human monocytes. a, cells were incubated in the absence (lane 1, from left to right) or presence of 10 ng/ml IL-10 (lane 2), 100 ng/ml LPS (lane 3), LPS plus 0.1 ng/ml IL-10 (lane 4), LPS plus 1.0 ng/ml IL-10 (lane 5), or LPS plus 10 ng/ml IL-10 (lane 6) for 2 h. In lanes 4 to 6, cells were incubated with IL-10 for 10 min before the addition of LPS. Total RNA was extracted and then subjected to Northern blot analysis to detect PDE4B mRNA. The membrane was stripped and reprobed for β -actin as an internal control. b, the autoradiographic signals were scanned and quantified. The plotted values represent means \pm S.D. of the ratios of mRNA levels of PDE4B to β -actin (arbitrary unit). Experimental details are described in *Materials and Methods*.

macrophages (Wang et al., 1994a, 1995), had moderate and marginal inhibitory effects, respectively, on the LPS-stimulated PDE4B mRNA expression. Again, PDE4B is very similar to the inflammatory cytokines (Wang et al., 1994a, 1995) and cyclooxygenase-2 (Niir et al., 1995). Thus, genes for PDE4B and the cytokines and cyclooxygenase-2 may be activated by a common, IL-10-inhibitable signal transduction pathway(s) upon LPS stimulation.

Interestingly, IL-10 can only slightly inhibit cAMP-stimulated PDE4B gene expression. The differential effects of IL-10 on LPS- and cAMP-stimulated PDE4B mRNA synthesis suggest that cAMP and LPS utilize different signal transduction pathways to activate PDE4B gene and that IL-10 has differential effects on these pathways. Although the PDE4B gene promoter is not characterized, extrapolating from established cAMP-regulated genes (Papavassiliou, 1994) it is possible that a cAMP-responsive element(s) may be present in the promoter. The transcription factor cAMP-responsive element-binding protein is activated by protein kinase A-catalyzed phosphorylation, then binds to cAMP-responsive elements in pro-

motor regions thereby activating cAMP-inducible genes. Presumably, cAMP induces the transcription of the PDE4B gene via the protein kinase A-cAMP-responsive element-

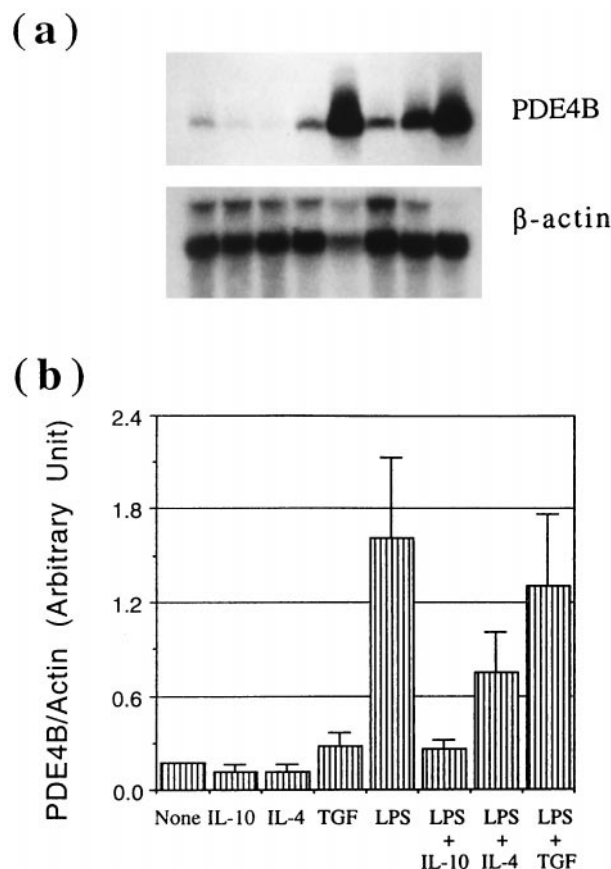


Fig. 6. Comparison of effects of IL-10, IL-4, and TGF- β on LPS-stimulated PDE4B mRNA production in human monocytes. a, cells were incubated in the absence (lane 1, from left to right) or presence of 10 ng/ml IL-10 (lane 2), 10 ng/ml IL-4 (lane 3), 10 ng/ml TGF- β (lane 4), 100 ng/ml LPS (lane 5), LPS plus IL-10 (lane 6), LPS plus IL-4 (lane 7), or LPS plus TGF- β (lane 8) for 2 h. In lanes 6 to 8, cells were incubated with IL-10, IL-4, or TGF- β for 10 min before the addition of LPS. Total RNA was extracted and then subjected to Northern blot analysis to detect PDE4B mRNA. The membrane was stripped and reprobed for β -actin as an internal control. b, the autoradiographic signals were scanned and quantified. The plotted values represent means \pm S.D. of the ratios of mRNA levels of PDE4B to β -actin (arbitrary unit). Experimental details are described in *Materials and Methods*.

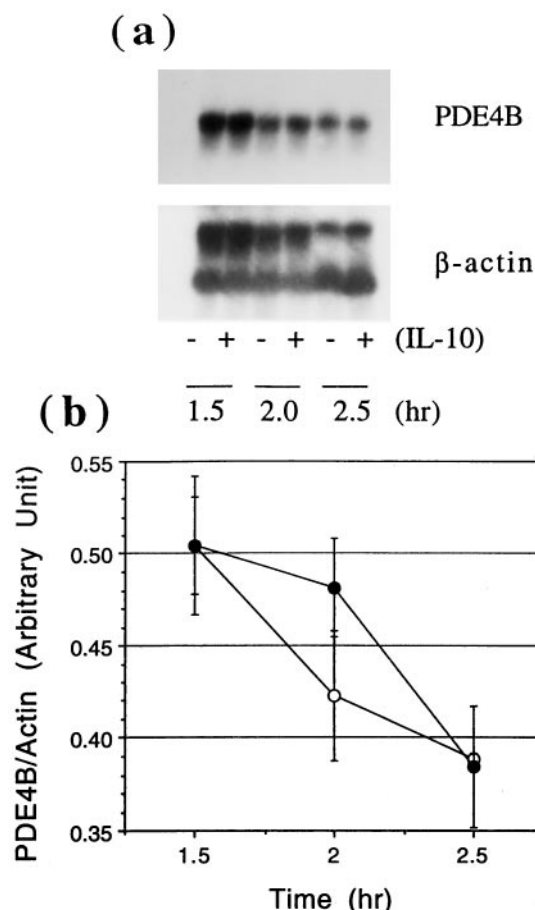


Fig. 7. Effect of IL-10 on degradation of LPS-stimulated PDE4B mRNA in human monocytes. a, cells were incubated with 100 ng/ml LPS for 1 h before adding 5 μ g/ml actinomycin D to stop RNA synthesis. Cells then were incubated in the absence (lanes 1, 3, and 5, from left to right) or presence (lanes 2, 4, and 6) of 10 ng/ml IL-10 for 1.5 h (lanes 1 and 2), 2 h (lanes 3 and 4), or 2.5 h (lanes 5 and 6). Total RNA was extracted and then subjected to Northern blot analysis to detect PDE4B mRNA. The membrane was stripped and reprobed for β -actin as an internal control. b, the autoradiographic signals were scanned and quantified. The plotted values represent means \pm S.D. of the ratios of mRNA levels of PDE4B to β -actin (arbitrary unit) (○, without IL-10; ●, with IL-10). Experimental details are described in *Materials and Methods*.

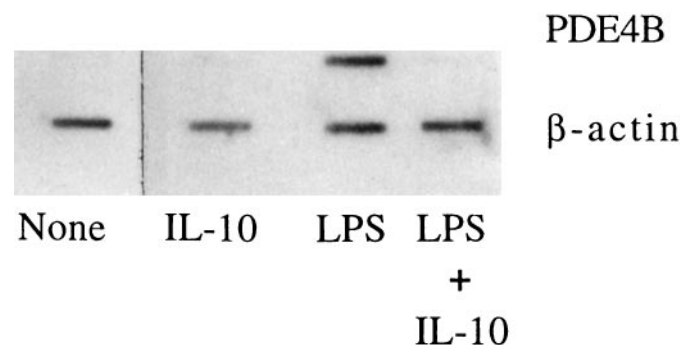


Fig. 8. Effect of IL-10 on LPS-stimulated transcription of PDE4B gene in human monocytes. Cells were incubated in the absence (lane 1, from left to right) or presence of 10 ng/ml IL-10 (lane 2), 100 ng/ml LPS (lane 3), or IL-10 plus LPS (lane 4) for 1 h. Nuclei were isolated and in vitro nuclear run-on assay was performed as described in *Materials and Methods*.

binding protein pathway. Thus far, there has been no evidence for IL-10 inhibition of this pathway. On the other hand, it is well known that LPS activates several transcription factors including nuclear factor- κ B, nuclear factor-IL6, and activator protein-1, which are involved in the induction of the inflammatory cytokines IL-1, IL-6, IL-8, and TNF- α (Drouet et al., 1991; Natsuka et al., 1992; Rhoades et al., 1992; Yasumoto et al., 1992; Kunsch and Rosen, 1993; Serkkola and Hurme, 1993; Zhang and Rom, 1993). Recent studies have shown that at least some of the transcription factors such as nuclear factor- κ B and activator protein-1 can be inhibited by IL-10 (Wang et al., 1995; Dokter et al., 1996). Thus, transcription factors such as these may be involved in the LPS stimulation of PDE4B gene transcription. Nevertheless, molecular mechanisms by which LPS activates PDE4B gene transcription and IL-10 inhibits the gene expression remain to be elucidated. Cloning and characterization of PDE4B gene promoter should greatly facilitate the elucidation of the molecular mechanisms.

Because the various PDE4 subtypes are expressed differentially between tissues and cells (Muller et al., 1995), it is very important to determine the relative role of each PDE4 subtype in a particular tissue or cell. mRNA distribution of various PDE4 subtypes in normal tissues and cells has been widely investigated (Conti et al., 1992; Muller et al., 1995). However, gene induction under various pathophysiological conditions may be more relevant for evaluating the relative importance of PDE4 subtypes. For instance, in resting human monocytes, PDE4A is a major PDE4 subtype (this study and Livi et al., 1990), whereas, in conditions with bacterial endotoxin, PDE4B may become the predominant form in monocytes.

In conclusion, the present study demonstrates, for the first time, that LPS specifically induces PDE4B gene expression in human monocytes. This finding suggests that, as with the LPS-inducible inflammatory cytokines (IL-1, IL-6, IL-8, and TNF- α) and cyclooxygenase-2, PDE4B may be an appropriate target for the discovery of anti-inflammatory drugs. In addition, the present study also demonstrates that the LPS-stimulated PDE4B gene expression is inhibited effectively by IL-10. IL-10 is currently under clinical development for a number of inflammatory diseases. The present study suggests that IL-10 may exert its anti-inflammatory effects via, in part, a cAMP pathway by inhibiting PDE4B gene expression.

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