Immunopharmacological Potential of Selective Phosphodiesterase Inhibition. I. Differential Regulation of Lipopolysaccharide-Mediated Proinflammatory Cytokine (Interleukin-6 and Tumor Necrosis Factor-α) Biosynthesis in Alveolar Epithelial Cells

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ABSTRACT

In an attempt to elaborate in vitro on a therapeutic strategy that counteracts an inflammatory signal, we previously reported a novel immunopharmacological potential of glutathione, an antioxidant thiol, in regulating inflammatory cytokines. In the present study, we investigated the hypothesis that selective regulation of phosphodiesterases (PDEs), a family of enzymes that controls intracellular cAMP/cGMP degradation, differentially regulates proinflammatory cytokines. Selective PDE1 inhibition (8-methoxymethyl-3-isobutyl-1-methylxanthine) blocked lipopolysaccharide-endotoxin (LPS)-mediated biosynthesis of interleukin (IL)-6, but this pathway had no inhibitory effect on tumor necrosis factor-α (TNF-α). Furthermore, inhibition of PDE3 (amrinone) abolished the effect of LPS on IL-6, but attenuated TNF-α production. Reversible competitive inhibition of PDE4 (rolipram) exhibited a potent inhibitory effect on IL-6 and a dual, biphasic (excitatory/inhibitory) effect on TNF-α secretion. Blockading PDE5 (4-[(3',4'-[methylenedioxy]benzyl amino)-6-methoxyquinazoline) showed a high potency in reducing IL-6 production, but in a manner similar to the inhibition of PDE4, exhibited a biphasic effect on TNF-α biosynthesis. Simultaneous inhibition of PDE5, 6, and 9 (zapriniast), purported to specifically elevate intracellular cGMP, reduced, in a dose-independent manner, IL-6 and TNF-α biosynthesis. Finally, nonselective inhibition of PDE by pentoxifylline suppressed LPS-mediated secretion of IL-6 and TNF-α. The involvement of specific PDE isoenzymes in differentially regulating LPS-mediated inflammatory cytokine biosynthesis indicates a novel approach to unravel the potential therapeutic targets that these isozymes constitute during the progression of inflammation within the respiratory epithelium.

The alveolar epithelium is recognized as a dynamic barrier that plays an important role in regulating the inflammatory and metabolic responses to oxidative stress, sepsis, endotoxemia, and other critical illnesses in the lung (Thompson et al., 1985; Freeman et al., 1993; Pittet et al., 1995; Matuschak and Lechner, 1996; Matthey et al., 2000; Haddad et al., 2001a,b). The respiratory epithelium, in particular, is a primary target of an inflammatory/infectious condition at the epithelial-blood interface, and it is itself capable of amplifying an inflammatory signal by recruiting inflammatory cells and producing inflammatory mediators (Thompson et al., 1985; Zeiher and Hornick, 1996; Laffon et al., 1999; Haddad et al., 2001a,c). Many of the side effects of lipopolysaccharide-endotoxin (LPS), derived from the cell wall of Gram-negative bacteria, are secondary to the overproduction of proinflam-
however, has yet to be ascertained and characterized in the alveolar epithelium. Subsequently, we designed a series of investigations to closely determine the role that selective and nonselective modulation of PDEs plays in regulating proinflammatory cytokines. We herein report the involvement of specific PDE isoenzymes that are differentially regulating LPS-mediated inflammatory cytokine biosynthesis, thereby indicating a novel approach to unravel the potential therapeutic targets that these isoforms constitute during the progression of inflammation within the respiratory epithelium.

Materials and Methods

All experimental procedures involving the use of live animals were reviewed and approved under the Animals Act legislation, 1986 (United Kingdom). Unless indicated otherwise, chemicals/reagents of the highest analytical grade were obtained from Sigma-Aldrich (Dorset, UK) and Calbiochem (Nottingham, UK).

Primary Cultures of Alveolar Epithelia. Fetal alveolar type II epithelial cells were isolated from lungs of rat fetuses on gestation day 19, essentially as described elsewhere (Haddad and Land, 2000a,b; Haddad et al., 2000). Briefly, fetal rats were removed from pregnant Sprague-Dawley rats by caesarian section at day 19 of gestation (term of 22 days), and the lungs were excised, teased free from heart and upper airway tissue, and finely minced then washed free of erythrocytes by using sterile, chilled Mg$^{2+}$- and Ca$^{2+}$-free Hanks’ balanced salt solution. The cleaned lung tissue was resuspended in 1 mℓfetus Hanks’ balanced salt solution containing 0.1 mg/ml trypsin, 0.06 mg/ml collagenase, and 0.012% (w/v) DNase I, and was agitated at 37°C for 20 min. The solution was then centrifuged at 100g for 2 min to remove undispersed tissue, the supernatant was saved to a fresh sterile tube, and an equal volume of Dulbecco’s modified Eagle’s medium (DMEM) with 10% (v/v) fetal bovine serum (FBS) was added to the supernatant. After passing the supernatant through a 120-μm pore size sterile mesh, the filtrate was centrifuged at 420g for 5 min, the pellet resuspended in 20 mL of DMEM/FCS, and the cells were placed into a T-150 culture flask for 1 h at 37°C to enable fibroblasts and nonepithelial cells to adhere. Unattached cells were washed three times by centrifugation at 420g for 5 min each and then seeded onto 24-mm-diameter Transwell-clear permeable supports (0.4-μm pore size; Costar, Cambridge, MA) at a density of 5 × 10⁶ cells/filter and were allowed to adhere overnight (pO₂ = 152 Torr ≈21% O₂, 5% CO₂). DMEM/FCS was exchanged for 4 mL of serum-free PC-1 media (BioWhittaker, Walkersville, MD) pre-equilibrated to pO₂ = 152 Torr and 37°C 24 h later and cells were maintained at this pO₂ until the experiment.

Inactivated and its progression is exquisitely sensitive to modulations in the steady-state levels of cyclic nucleotides, where target cells for their effects extend beyond immune cells to include accessory cells, such as airway smooth muscle, epithelial and endothelial cells, and neurons (Perry and Higgs, 1998; Essayan, 1999). Inactivation of cAMP/GMP is achieved by hydrolytic cleavage of the 3’-phosphodiester bond catalyzed by the cyclic-nucleotide-dependent phosphodiesterases (PDEs), resulting in the formation of the corresponding, inactive 5’-monophosphate (Perry and Higgs, 1998; Essayan, 1999). Of note, the inflammatory response and its progression is exquisitely sensitive to modulations in the steady-state levels of cyclic nucleotides, where target cells for their effects extend beyond immune cells to include accessory cells, such as airway smooth muscle, epithelial and endothelial cells, and neurons (Perry and Higgs, 1998; Essayan, 1999). In this respect, the emerging concept that selective modulation of intracellular cyclic nucleotides plays a major role in regulating the inflammatory milieu has recently evolved in targeting and ameliorating inflammatory/autoimmune responses. The cyclic nucleotide PDEs are a large, growing multigene family, comprising at least 10 families of PDE isoenzymes (Perry and Higgs, 1998; Essayan, 1999). The profile of selective and nonselective PDE inhibitors in vitro and in vivo, therefore, suggested a potential therapeutic utility as antidepressants, antiproliferative and immunomodulatory agents, tocolyties, inotropes/chronotropes, and cytoprotective agents (Pagani et al., 1992; Bolger et al., 1993; Tsuboi et al., 1996; Ekholm et al., 1997; Essayan, 1999).

The immunopharmacological potential of PDE inhibitors, however, has yet to be ascertained and characterized in the alveolar epithelium. Subsequently, we designed a series of investigations to closely determine the role that selective and nonselective modulation of PDEs plays in regulating proinflammatory cytokines. We herein report the involvement of specific PDE isoenzymes that are differentially regulating LPS-mediated inflammatory cytokine biosynthesis, thereby indicating a novel approach to unravel the potential therapeutic targets that these isoforms constitute during the progression of inflammation within the respiratory epithelium:

Enzyme-Linked Immunosorbent Assay (ELISA) Assessment of Proinflammatory Cytokine Profile. The bioactivity of extracellularly released cytokines was measured by a two-site, solid-phase, developed sandwich ELISA in cell-free supernatants (Haddad et al., 2001b,c). Immunofluorescence-purified polyclonal rabbit anti-rat IL-6 and TNF-α (2 μg/mL) primary antibodies were used to coat high-binding microtiter plates (MaxiSorp; Nunc, Paisley, Scotland, UK). Recombinant rat and biotinylated immunofluorescence-purified
sheep anti-rat cytokine (R & D Systems Europe, Oxford, UK) were used as standard and recognition antibodies, respectively. The color was developed incorporating streptavidin-poly-horseradish peroxidase-coupled reaction with the chromagen 3,3',5,5'-tetramethyl-benzidine dihydrochloride, and the optical density was measured at 450 nm against a filter background measuring at 595 nm. Inter- and intra-assay coefficients of variations were <10%, and the minimum detectable sensitivity for each cytokine is ≤2 pg/ml. Results interpolated from the linear regression of the standard curves were expressed as picograms per millilitre.

Analysis of Cytokine Secretion Exposed to LPS: Dose- and Time-Response Curves. Cells were treated with LPS (0–10 μg/ml) (from Escherichia coli, serotype 026:B6) for 24 h and cell-free supernatants (1-ml aliquots) were collected, snap frozen on liquid nitrogen, and subsequently stored at −70°C. For time-dependent assessment, optimum concentration of LPS (10 μg/ml) that caused maximum induction was subsequently used. Cells were challenged with LPS and sample aliquots were withdrawn as indicated (0–96 h) for analysis of cytokines. Cytokine release was subsequently assayed by ELISA.

Effects of Selective Phosphodiesterase Inhibitors (PDEIs) on LPS-Induced Cytokine Biosynthesis. All PDEIs were purchased from Calbiochem, with the exception of amrinone, which was purchased from Sigma-Aldrich. Confluent cells were exposed to LPS (10 μg/ml) for 24 h in the presence or absence of selective and nonselective PDE inhibitors. Cell-free supernatants were collected and analyzed for cytokines.

Statistical Analysis and Data Presentation. Data are the means and the error bars the S.E.M. of at least three independent cell cultures. Statistical evaluation was performed by one-way analysis of variance, followed by post hoc Tukey’s test, and the a priori level of significance at 95% confidence level was considered at P ≤ 0.05.

Results

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Dose-Response Analysis of Inflammatory Cytokines with Ascending LPS Concentrations. In screening for the optimum concentration for cytokine release with ascending LPS concentration, we studied a dose-response curve of up to 10,000 ng/ml LPS. As shown in Fig. 1A, LPS induced extracellular accumulation of IL-6 (24 h), determined in cell-free supernatants, at doses ≥100 ng/ml. IL-6 bioactivity increased exponentially with the highest secretion at 10,000 ng/ml (Fig. 1A). LPS doses ≤10 ng/ml were ineffective in inducing IL-6 secretion (Fig. 1A). PseudoVoigt curve analysis shows the exponential increase of IL-6 biosynthesis, in a dose-dependent manner (EC50 = 637.3 ± 72.8 ng/ml) (Fig. 1A). The dose-response curve for TNF-α secretion due to LPS administration is shown in Fig. 1B. LPS induced extracellular accumulation of TNF-α (24 h) at doses ≥100 ng/ml, with exponential increase to highest concentration used in this study (10,000 ng/ml) (Fig. 1B). LPS doses ≤10 ng/ml were ineffective in inducing TNF-α secretion (Fig. 1B). PseudoVoigt curve analysis shows the exponential elevation in TNF-α biosynthesis, in a dose-dependent manner (EC50 = 254.6 ± 27.3 ng/ml) (Fig. 1B).

Time-Response Analysis of Inflammatory Cytokines with LPS. The time-response curve for IL-6 secretion in the presence of LPS (10 μg/ml) is shown in Fig. 2A. IL-6 appeared in cell-free supernatants as early as 4 h postaddition of LPS, and continued to elevate to maximize at 24 h (Fig. 2A). The concentration of IL-6 remained significantly different from control (in the absence of LPS) up to 96 h, despite the observation that the elevation in comparison with the 24-h time point was relatively lower (Fig. 2A). The control supernatants showed no detectable levels of IL-6 in the absence of LPS across the same time-response curve (Fig. 2A). The time-response curve for TNF-α secretion in the presence of LPS (10 μg/ml) is shown in Fig. 2B. TNF-α appeared in cell-free supernatants as early as 2 h postaddition of LPS, and maximized around the 2- to 4-h time point (Fig. 2B). The concentration of TNF-α remained significantly different from control (in the absence of LPS) up to 48 h, despite the observation that the elevation in comparison with the 2- to 4-h time point was relatively lower, thereafter declining to become insignificantly different at 72 to 96 h (Fig. 2B). The control supernatants showed no detectable levels of TNF-α in
the absence of LPS across the same time-response curve (Fig. 2B).

**Regulatory Effect of Selective and Nonselective PDEIs on LPS-Mediated IL-6 Biosynthesis.** The effect of 8-methoxymethyl-3-isobutyl-1-methylxanthine (8-methoxymethyl-IBMX), a selective inhibitor of Ca^{2+}-calmodulin-dependent PDE1, is shown in Fig. 3A. 8-Methoxymethyl-IBMX reduced LPS-induced IL-6 biosynthesis at effective doses ≥1 μM (IC\textsubscript{50} = 7.08 ± 0.36 μM) (Fig. 3A). The inhibitory role of 5-amino-(3,4-bipyrindin)-6-[1H]-one (amrinone), a selective inhibitor of PDE3, is displayed in Fig. 3B. Amrinone reduced LPS-induced IL-6 secretion at effective doses ≥1 μM (IC\textsubscript{50} = 25.96 ± 2.18 μM) (Fig. 3B). The effect of 4-(3-(cyclopentyloxy)-4-methoxy-phenyl)-2-pyrrolidine (rolipram), a selective inhibitor of PDE4, is shown in Fig. 3C. Rolipram reduced LPS-induced IL-6 secretion at effective doses ≥10 μM (IC\textsubscript{50} = 24.25 ± 2.21 μM) (Fig. 3C). The inhibitory effect of 4-[[3',4'-((methylenedioxy)benzyl)amino]-6-methoxyquinazoline (MBMQ) is displayed in Fig. 3D. MBMQ, a specific inhibitor of PDE5, reduced LPS-induced IL-6 secretion at effective doses ≥1 μM (IC\textsubscript{50} = 2.81 ± 0.27 μM) (Fig. 3D). The role of [1,4-dihydro-5-(2-propoxyphenyl)-7H-1,2,3-triazolo[4, 5-d]pyrimidine-7-one] (zaprinast), a potent inhibitor of PDE5 with mild effect on PDEs 6 and 9, is shown in Fig. 3E. Zaprinast reduced LPS-induced IL-6 production at effective doses ≥1 μM (IC\textsubscript{50} = 0.82 ± 0.07 μM) (Fig. 3E). The inhibitory effect of 3',7-dihydro-3,7-dimethyl-1-(5-oxohexyl)-1H-purine-2,6-dione (pentoxifylline; Trental, Oxpentifylline), a nonspecific inhibitor, is displayed in Fig. 3F. Pentoxifylline reduced LPS-induced TNF-α secretion, showing an excitatory effect as early as 8 h, continuing to be significantly different from control up until 48 h. *P < 0.05; **P < 0.01; ***P < 0.001, compared with control (LPS = 0 ng/ml at different time points). n = 3 to 5, which represents the number of independent experiments performed in duplicate.
reduced LPS-induced IL-6 biosynthesis at effective doses $\geq 1 \mu M$ ($IC_{50} = 2.70 \pm 0.15 \mu M$) (Fig. 3F).

**Regulatory Effect of Selective and Nonselective PDEIs on LPS-Mediated TNF-α Biosynthesis.** The effect of 8-methoxymethyl-IBMX is shown in Fig. 4A. 8-Methoxymethyl-IBMX has no apparent inhibitory effect on LPS-induced TNF-α biosynthesis at all doses (Fig. 4A). The marginal inhibitory role of amrinone is displayed in Fig. 4B. Amrinone reduced LPS-induced TNF-α secretion at 100 $\mu M$ ($IC_{50} > 100 \mu M$) (Fig. 4B). The dual effect of rolipram is shown in Fig. 4C. Rolipram augmented LPS-induced TNF-α secretion at a dose of 10 $\mu M$, but reduced the effect of LPS at 100 $\mu M$, with no apparent excitatory/inhibitory effects at the lowest dose (1 $\mu M$) (Fig. 4C). The dual effect of MBMQ is displayed in Fig. 4D. MBMQ augmented LPS-induced TNF-α secretion at a dose of 10 $\mu M$, but reduced the effect of LPS at 100 $\mu M$, with no apparent excitatory/inhibitory effects at 1 $\mu M$ (Fig. 4D). The role of zaprinast is shown in Fig. 4E. Zaprinast reduced LPS-induced TNF-α production at effective doses $\geq 1 \mu M$ ($IC_{50} = 0.75 \pm 0.11 \mu M$) (Fig. 4E). The inhibitory effect of pentoxifylline is displayed in Fig. 4F. Pentoxifylline reduced LPS-induced TNF-α biosynthesis at effective doses $\geq 1 \mu M$ ($IC_{50} = 0.08 \pm 0.36 \mu M$) (Fig. 4F).

**Discussion**

Within a physiological environment, the lung is remarkable for its stability despite the possession of the potential for rapid proliferation and tissue remodeling (Kelley, 1990; Zeiher and Hornick, 1996; Rogers and Laurent, 1998). The major participants in the regulation of cellular/molecular composition of the lung are the cytokines, also known as peptide growth factors, biological response modifiers, and inflammatory mediators (Kelley, 1990; Nicod, 1993). Well known as extracellular signaling proteins transcribed and biosynthesized in response to various stimuli by specific effector cells, cytokines are crucial molecules in the initiation and/or progression of inflammatory disease and pulmonary dysfunction (Kelley, 1990; Nicod, 1993; Rogers and Laurent, 1998). Aberrant regulation of cytokines or their selective receptors may therefore contribute to the exacerbation of disease condition. Indeed, such dysregulation has been implicated in a number of human diseases, including sepsis syndrome (Beutler and Cerami, 1986), psoriasis (Elder et al., 1989), psoriatic arthritis (Bauer et al., 1986), adult respiratory distress syndrome (Bunnell and Pacht, 1993), idiopathic pulmonary fibrosis (Cantin et al., 1989), Crohn’s disease and ulcerative colitis (Reimund et al., 1998), allergic bronchopulmonary aspergillosis (Wark and Gibson, 2001), bronchopulmonary dysplasia (Saugstad, 1997), cystic fibrosis (Doring, 1996; Zeiher and Hornick, 1996), and rheumatoid arthritis (Feldmann and Maini, 2001).

Insight into the molecular mechanisms of the regulatory role of cytokines in cellular homeostasis as well as inflammatory/autoimmune/infectious diseases has begun to provide new approaches to design therapeutic strategies for pharmacological interventions (Zeiher and Hornick, 1996; Rogers and Laurent, 1998). One such novel approach is the chemotherapeutic potential of PDE isoenzyme blockade, which revealed a phenomenal diversity and complexity scheme for promising therapeutics across a broad spectrum of disease states (Perry and Higgs, 1998; Essayan, 1999). One mechanistic understanding of PDE inhibition is centered on the immunomodulatory properties of cyclic nucleotides (cAMP/cGMP), thereby paving a channel through which anti-inflammatory, therapeutic applications could be clearly demonstrated (Perry and Higgs, 1998; Essayan, 1999). The observations herein reported revealed the involvement of selective PDE isoenzymes in differentially modulating an inflammatory signal mediated by cytokines, for the moment specific to alveolar epithelial cells, recognized as major participants in the evolution and progression of inflammatory disease contracted and amplified from within the pulmonary, infiltrated milieu (Thompson et al., 1985; Kelley, 1990; Freeman et al., 1993; Pittet et al., 1995; Matuschak and Lechner, 1996; Zeiher and Hornick, 1996; Rogers and Laurent, 1998; Matthey et al., 2000; Haddad et al., 2001b,c).

There are three PDE1 isoforms, termed PDE1A, 1B, and 1C, respectively (Perry and Higgs, 1998; Essayan, 1999). The catalytic activity of this subtype of PDEs is regulated via calmodulin-binding domains, thereby allowing for control by intracellular Ca$^{2+}$. PDE1 isoforms hydrolyze both cAMP ($K_M = 1–30 \mu M$) and cGMP ($K_M = 3 \mu M$), although different isoenzymes inactivate either nucleotide with different preference (Perry and Higgs, 1998). The primary distribution of PDE1 isoenzymes includes a variety of tissues, especially the heart, brain, lung, and smooth muscle (Essayan, 1999). Despite the limitations of having selective inhibitors with preference to either of the aforementioned isoenzymes, vinpocetine and 8-methoxymethyl-IBMX have emerged as selective inhibitors of Ca$^{2+}$-calmodulin-dependent PDE1 (Demoliou-Mason, 1995; Perry and Higgs, 1998; Essayan, 1999). 8-Methoxymethyl-IBMX is a xanthine derivative whose structure-activity relationship revealed a selective potency against PDE1, despite its mild, nonselective inhibitory effect on PDE5 (Wells and Miller, 1988; Perry and Higgs, 1998; Essayan, 1999; Piaz and Giovanni, 2000). Although 8-methoxymethyl-IBMX efficiently reduced LPS-mediated IL-6 biosynthesis, it has no apparent effect whatsoever on TNF-α secretion. Although the molecular basis for this preferential discrepancy is not understood, it is possible that 8-methoxymethyl-IBMX selectively targets inflammatory cytokines. IL-6, also termed interferon-β, is involved in the induction of acute phase protein synthesis during the process of pulmonary inflammation (Kelley, 1990; Nicod, 1993). We have reported that alveolar epithelial cells strongly responded to exogenous irritants, such as LPS, by up-regulating IL-6 and other mediators (Haddad et al., 2001a,b,c,d) and that endogenous cytokines, such as TNF-α, regulate endogenous formation of pro- and anti-inflammatory cytokines by an autocrine mechanism (J. J. Haddad, N. E. Saadeé, B. Safieh-Garabedian, and S. C. Land, unpublished observations). Among those cytokines regulated by TNF-α, IL-6 is a likely candidate; however, whether 8-methoxymethyl-IBMX-mediated down-regulation of IL-6 is TNF-α-dependent cannot be excluded, but its inability to suppress LPS-mediated TNF-α biosynthesis rather suggested the involvement of a TNF-α-insensitive pathway mediating the inhibitory effect of PDE1 on IL-6 production.

PDE3A and PDE3B isoforms are products of different genes located on chromosomes 12 and 11, respectively (Perry and Higgs, 1998). Their catalytic domains contain an insert that distinguishes between the two isoenzymes, thereby of-
fering selectivity to bind with mutually competitive affinities to cAMP and cGMP ($K_M = 0.2$ and $0.3 \mu M$, respectively) (Degerman et al., 1997; Perry and Higgs, 1998). PDE3 isoforms are particularly distributed within heart, lung, liver, platelets, immunocytes, and adipose tissues (Perry and Higgs, 1998). PDE3, furthermore, is known for its cGMP-inhibited characteristics because some biological effects of endogenous cGMP may be mediated by inhibition of PDE3, which results in increased cAMP and activation of cAMP-dependent protein kinase (Degerman et al., 1997). Amrinone is one of the selective inhibitors of PDE3 and a plethora of other selective inhibitors have been already identified (Degerman et al., 1997; Perry and Higgs, 1998). Selective inhibition of PDE3 emerged with similar behavior to the effect of 8-methoxymethyl-IBMX, despite the observation that amrinone at 100 $\mu M$ partially reduced LPS-mediated secretion of TNF-$\alpha$, with strong inhibitory mechanics on IL-6 biosynthesis. This effect bears an interesting therapeutic approach, because type 3 PDEs have been reported to be active in the airway epithelium (Kelley et al., 1995), consistent with the notion that selective PDE3 blockade interfered with the progression of cystic fibrosis in vivo (Al-Nakkash and Hwang, 1999; Smith et al., 1999), reduced ischemia-reperfusion injury in the heart (Rechtman et al., 2000), and conferred cardiac protection under surgery (Butterworth et al., 1995).

PDE4 isoforms are cAMP-specific ($K_M = 4 \mu M$) and cGMP-insensitive ($K_M > 3000 \mu M$) phosphodiesterases, distributed in Sertoli cells, kidney, brain, liver, and immunocytes (Essayan, 1999). There are four PDE4 genes encoding distinct isoforms (A, B, C, and D) with additional diversity particularly arising from alternative initiation sites and/or alternative splicing (Perry and Higgs, 1998). The immunopharmacological potential of rolipram, a selective inhibitor of PDE4, is well documented. For instance, it was reported that, from a functional standpoint, the up-regulation of PDE4 activity resulted in a heterologous desensitization to the effect of 8-methoxymethyl-IBMX, despite the observation that amrinone at 100 $\mu M$ partially reduced LPS-mediated secretion of TNF-$\alpha$, with strong inhibitory mechanics on IL-6 biosynthesis. This effect bears an interesting therapeutic approach, because type 3 PDEs have been reported to be active in the airway epithelium (Kelley et al., 1995), consistent with the notion that selective PDE3 blockade interfered with the progression of cystic fibrosis in vivo (Al-Nakkash and Hwang, 1999; Smith et al., 1999), reduced ischemia-reperfusion injury in the heart (Rechtman et al., 2000), and conferred cardiac protection under surgery (Butterworth et al., 1995).

Contrary to PDE1 and 2, PDE5, which is reportedly present in lung tissue and platelets with more limited tissue distribution in comparison with other PDEs, catalyzes the hydrolysis of cGMP with absolute specificity (Perry and Higgs, 1998; Essayan, 1999). MBMQ is one such selective inhibitor of cGMP-specific PDE5. It elevates intracellular cGMP level ($K_M = 1 \mu M$) without causing any change in the cAMP level ($K_M = 150 \mu M$) (Essayan, 1999). On the other hand, zaprinast exhibits potential inhibitory effects of cGMP-specific PDE5, 6, and 9 ($K_M = 2000$ and $60 \mu M$ for cAMP and cGMP, respectively) (Perry and Higgs, 1998; Cobrin and Francis, 1999; Essayan, 1999). These potent inhibitors of PDE5 have been profiled extensively in preclinical models, especially those of cardiovascular disease (Eddahibi et al., 1998; Perry and Higgs, 1998; Dukarm et al., 1999). In a manner similar to rolipram, MBMQ exhibited a dual effect on TNF-$\alpha$, but blocked the production of IL-6. In contrast, zaprinast abolished the excitatory effect of LPS on both TNF-$\alpha$ and IL-6, suggesting a nonspecific inhibition that is not solely confined with PDE5 blockade, thereby implicating other PDEs. Despite the fact that the molecular basis for this dual discrepancy has yet to be ascertained, the mobility and efficiency of selective PDE inhibition may thus explain the involvement of one pathway or another mediating the effect of specific PDE isoenzymes in regulating a proinflammatory signal within the alveolar space.

Pentoxifylline is a rheologically active hemorheological agent for the treatment of peripheral vascular disease, cerebrovascular disease, and a number of other conditions involving a defective regional microcirculation (Ward and Clissold, 1987). In addition, pentoxifylline is a nonspecific PDE inhibitor, recognized as a modulator of immune functions with immunopharmacological efficiency. For instance, pentoxifylline is well known for its clinical implications in regulating proinflammatory cytokines (Edwards et al., 1992; van Furth et al., 1997; Poulakis et al., 1999; Marcikiewicz et al., 2000) and in the treatment of bronchopulmonary dysplasia (Lauterbach and Szymura-Oleksiak, 1999) and sepsis (Lauterbach and Zembala, 1996; Staudinger et al., 1996; Szymura-Oleksiak et al., 1997; Lauterbach et al., 1999). Equi-potent active, pentoxifylline reduced LPS-dependent biosynthesis of IL-6 and TNF-$\alpha$, with comparable mechanics. On the mechanism of action of this rather nonspecific inhibition, it has been previously reported that pentoxifylline may act as a nitric oxide and reactive nitrogen species (RNS) scavenger (Lauterbach et al., 1995; Gómez-Cambronero et al., 2000) and up-regulate a counter, anti-inflammatory loop via IL-10 (van Furth et al., 1997). Moreover, we have previously reported a key role for the compartmentalization of intracellular ROS in the induction of inflammatory cytokines (Haddad et al., 2001b,c). Whether the inhibitory effect of pentoxifylline on IL-6 and TNF-$\alpha$ production is secondary to its effect on intracellular ROS/RNS remains a possible mechanism. Besides, the likely involvement of an IL-10-sensitive pathway mediating the effect of pentoxifylline on proinflammatory cytokines is rather supported by equivocal evidence implicating IL-10 in regulating inflammatory mediators within the alveolar epithelium (Haddad et al., 2001d). Taken together, the potent anti-inflammatory potential of pentoxifylline points to a multifaceted mechanism of action: it is possible that this nonselective PDE inhibitor regulates an inflammatory signal by counteracting intracellular ROS/RNS and up-
regulating a feedforward/feedback loop via amplification of IL-10. This latter mechanism, nevertheless, despite its eligibility, remains to be identified as part of the mechanism of action of pentoxifylline. However, the possibility that pentoxifylline suppresses IL-6 via direct inhibition of TNF-α cannot be excluded (Staudinger et al., 1996; Luterbach et al., 1999).

The observations reported herein have revealed a novel anti-inflammatory action of selective PDE inhibition, a mechanism that may counteract the deleterious effects of over-boosting an inflammatory response originating from within, and spreading of, the respiratory epithelium. These results could be highlighted as follows: 1) PDE1 inhibition reduced LPS-mediated biosynthesis of IL-6, whereas it had no inhibitory effect on TNF-α; 2) inhibition of PDE3 abolished the effect of LPS on IL-6 but attenuated TNF-α production; 3) reversible inhibition of PDE4 exhibited a potent inhibitory effect on IL-6 and a dual (excitatory/inhibitory) effect on TNF-α; 4) inhibition of PDE5 showed a high potency in reducing IL-6 production, but exhibited a biphasic effect on TNF-α; 5) simultaneous inhibition of PDE5, 6, and 9 reduced IL-6 and TNF-α secretion; and 6) nonselective inhibition by pentoxifylline suppressed LPS-mediated secretion of IL-6 and TNF-α. The involvement of these novel PDE isoforms in differentially regulating inflammatory cytokines thereby indicates a novel therapeutic potential of these enzymes isozymes, the potential therapeutic targets that these isozymes constitute during the progression of inflammation.

References


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