Phosphodiesterase 4 and Its Inhibitors in Inflammatory Diseases

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Type 4 cyclic nucleotide phosphodiesterases (PDE4) are a family of low k_m 3',5'-cyclic adenosine monophosphate (cAMP)-specific phosphodiesterases including at least 20 isozymes encoded by four genes (*PDE4A, PDE4B, PDE4C,* and *PDE4D*) in mammals. Each PDE4 gene plays a special, nonredundant role in the control of cell function even though the four subfamilies share the highly conserved catalytic domain and upstream conserved region (UCR) 1 and UCR2 motifs of the regulatory domain. By their wide tissue distribution as well as differential expression and regulation among various cell types, PDE4s are viewed as critical regulators of intracellular cAMP levels, cAMP signaling, and signal compartmentalization. By increasing cAMP levels, PDE4 inhibitors show a broad spectrum of anti-inflammatory effects in almost all inflammatory cells. Many PDE4 inhibitors have been evaluated in clinical trials for various inflammatory conditions. Developed inhibitors, including the recently approved and marketed roflumilast, have considerable efficacy, but they also have adverse effects such as nausea and emesis which limit their dosing and subsequently their immunomodulatory activity. Thus, the development of PDE4 inhibitors with improved therapeutic indexes has been a major focus of pharmaceutical research for the treatment of chronic inflammatory diseases. Recent PDE4 gene knockout studies strongly suggest that PDE4 inhibitors with PDE4B selectivity may retain the anti-inflammatory effects while limiting side effects. Development of PDE4 inhibitors with different delivery routes, such as topical application and inhalation, is also a promising approach for the treatment of pulmonary inflammatory conditions and dermatitis. This review includes a brief overview of the domain structure and function of PDE4 isozymes, the role of PDE4s in inflammatory cell responses, and the potential therapeutic utility of PDE4 inhibitors in inflammatory diseases. *(Chang Gung Med J 2012;35:197-210)*

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Since Sutherland and Rall identified the enzymatic activity of cyclic nucleotide phosphodiesterases (PDEs) in 1958,[1] continuing efforts have been devoted to advancing our understanding of the cell biology and functions of these enzymes. These efforts include biochemical and structural characterization, pharmacological implication, molecular cloning and sequencing, interacting protein identification, intracellular targeting and compartmentalization. PDEs are a superfamily of enzymes catalyzing the hydrolysis of 3',5'-cyclic adenosine monophosphate (cAMP) and 3',5'-cyclic guanosine monophosphate (cGMP) to their inactive 5'-AMP and 5'-GMP. Cyclic nucleotides are known to play pivotal roles in a myriad of cellular functions including immune and inflammatory responses,[2] cardiac activities such as heart rate and contractility, smooth muscle relaxation, energy homeostasis, fluid homeostasis, visual excitation, depression, cognition, oocyte maturation, and apoptosis.[3] Thus, as a central regulator of intracellular concentrations of cyclic nucleotides, PDEs have been considered pharmocologic targets for various disease therapies, such as for congestive heart failure,[4-6] intermittent claudication,[7,8] erectile dysfunction,[9,10] chronic obstructive pulmonary disease (COPD),[11,12] asthma,[2,13] depression,[14] and schizophrenia.[15,16]

To date, a total of 21 PDE genes have been identified in mammals and are classified into eleven families, called PDE1-11 based on their substrate specificity and affinity, primary sequence homology, inhibitor selectivity, and regulation by specific activators.[17-20] Among these, PDEs 4, 7, and 8 are selective for cAMP, PDEs 5, 6, and 9 for cGMP, and the other PDE families for both cAMP and cGMP. PDEs 1, 3, 4, 6, 7 and 8 are encoded by more than one gene, while the other families are each encoded by only one gene. Most PDE genes code for multiple variants derived from alternative splicing and the use of different promoters,[21,22] and as a result, more than 100 distinct PDE transcripts are likely to be present in any mammalian species.[20,23]

The mammalian PDEs contain a highly conserved catalytic domain located near the carboxyl-terminal half of the protein and a divergent regulatory domain towards the amino-terminal portion of the protein. A $HN(X_2)HN(X_n)E/D/Q(X_{10})HD(X_2)H(X_{25})E$ motif conserved in the catalytic domain represents two consensus metal binding domains that are com-

monly found in proteases such as matrix metalloproteinases.[18,20] The regulatory domains are conserved within each family. The conserved regions include Ca^{2+}/calmodulin binding domains for PDE1, upstream conserved regions (UCR) for PDE4, cGMP-activated PDEs, adenylyl cyclase, and Fh1A (GAF) domains for PDE6 as well as in PDEs 2, 5, 10, and 11, the Per-Arnt-Sim domain for PDE8, phosphorylation sites for kinases, and protein-protein interaction domains.[3,22] The carboxyl-terminal sequence is divergent among the PDEs but phosphorylation[24-26] and posttranslational modifications[27] have been reported in this region.

In this review we will provide a brief overview of the molecular characteristics of PDE4 isozymes and the role of these enzymes in regulation of inflammatory cell responses. The potential therapeutic utility of PDE4 inhibitors in inflammatory diseases will also be discussed.

The PDE4 family

The PDE4 family in mammals is composed of four subfamilies, which are encoded by four paralog genes (*PDE4A, PDE4B, PDE4C* and *PDE4D*). More than 20 PDE4 variants are present in cells arising from alternative mRNA splicing or the use of different transcriptional units.[21,28] These PDE4 isozymes share a highly conserved catalytic domain of approximately 320-350 amino acids with more than 80% sequence identity between the members of the four isotypes.[29-31] The sequences of the N-terminus regulatory domains among the four subfamilies are divergent except for those in the two upstream conserved regions (UCR1 and UCR2), which are unique to the PDE4 proteins.[32] The four subfamily isozymes can be subgrouped into three forms: the 'long' forms which contain both UCR1 and UCR2, the 'short' forms which lack UCR1, and the 'super-short' forms which contain only the C-terminal portion of UCR2.[33] The C-terminal sequence of the PDE4 enzymes is divergent and their functional significance remains to be defined.

UCR1 and UCR2 are functional modules of approximately 60 and 80 residues, respectively.[29,32] UCR2 bears an autoinhibitory nature, a property inferred from observations that removal of a portion of this domain causes an increase in the catalytic activity of the enzyme.[24,30,34] UCR1 contains a protein kinase A (PKA) phosphorylation site. Elevation

of cAMP levels in cells induces phosphorylation of its serine residue by PKA, which then leads to a rapid activation of PDE4 as well as increases in the sensitivity of the enzyme to the PDE4 inhibitor rolipram, as demonstrated in PDE4D3.[35,36] Experiments have further indicated that PDE4 activation led by PKA phosphorylation in UCR1 may be the consequence of relieving the inhibitory constraint of UCR2 on the catalytic domain.[34] Moreover, a potentially electrostatic intramolecular interaction between UCR1 and UCR2 has been described in PDE4D3.[37] Phosphorylation of UCR1 appears to disrupt this interaction.[37]

Evidence also indicates that UCR1 and UCR2 in PDE4 long forms can interact intermolecularly, thus leading to PDE4 dimerization, whereas short forms lacking UCR1 are monomers.[38,39] PKA phosphorylation of UCR1 does not interfere with this interaction. The dimerization takes place between the C-terminal region of UCR1 and the N-terminal region of UCR2 as deletion of either part leads to failure of PDE4 dimerization. Disruption of dimerization abrogates the activation of PDE4D3 by PKA phosphorylation as well as reduces the sensitivity of the enzyme to rolipram.[38,39]

Expression of PDE4 in inflammatory cells

The PDE4 isozymes are widely distributed in mammalian cells and tissues, implicating the diverse biological function of these proteins. PDE4s are the predominant cAMP-degrading isozymes in most, if not all, immune and inflammatory cells, including T cells, B cells, eosinophils, neutrophils, dendritic cells, monocytes, and macrophages.[2] Three PDE4 subtypes, PDE4A, PDE4B and PDE4D, are expressed in these cells, while PDE4C is minimal or absent.[13,40] PDE3 and PDE7 are also detected in most of the inflammatory cells.[13,40] The expression levels of these PDE isozymes are differentially regulated by a variety of inflammatory stimuli. As demonstrated in Jurkat T-cells and human peripheral blood T-cells, 8-Bromo-cAMP or prostaglandin E2 evidently induces PDE3 and PDE4 enzyme activity, and this effect is associated with increased PDE3B, PDE4A4, PDE4A1, 4D2, and 4D3 mRNA expression.[41] Stimulation of human peripheral blood cluster of differentiation 4+ T (CD4+ T) cells with anti-CD3 and anti-CD28 antibodies also regulates the expression of PDE4 subtypes differentially, with PDE4A and

PDE4D mRNAs upregulated along with enzyme activity within 5 days but PDE4B mRNA upregulated transiently with highest levels at 24 h after stimulation.[42] In addition, lipopolysaccharide (LPS) has been shown to selectively induce PDE4B2 mRNA expression in human circulating monocytes.[43] This regulation of PDE4B expression has been confirmed in monocytes and peritoneal macrophages of PDE4 knockout mice.[44,45] Among 12 PDE4 isozymes tested, PDE4A4 and PDE4B2 were detected at higher levels in peripheral blood monocytes of smokers compared with nonsmokers.[46] Moreover, PDE4A4 transcripts were found significantly upregulated in alveolar macrophages from smokers with COPD compared with smokers without COPD.[46] Although the functional consequences of the PDE4 regulation remain to be determined, these PDE4 isozymes altered in pathophysiological processes may serve as potential therapeutic targets for a variety of inflammatory conditions.

PDE4 and inflammation

To date, our understanding of the cellular functions of PDE4 has been mostly derived from experiments involving PDE4 inhibitors. These small molecule compounds, including the prototype inhibitor rolipram and second-generation compounds such as roflumilast and cilomilast, have been shown to produce a wide range of pharmacological effects *in vitro* and *in vivo*. These include antiinflammatory and immunomodulatory effects,[2,13,47-50] antidepressant and antischizophrenia actions,[14,15,51] and cognitive enhancement,[52,53] clearly demonstrating a broad, critical role of PDE4 in cellular and physiological functions. Among these effects, the inflammatory aspect of PDE4 functions has been explored most extensively. In fact, PDE4 is the major family of PDE enzymes expressed in immune and inflammatory cells. Inhibition of PDE4 has been shown to suppress a diverse spectrum of inflammatory responses *in vitro* and *in vivo*.[2,13,40] More importantly, many PDE4 inhibitors in development are efficacious in animal models of various inflammatory disorders, such as asthma, COPD, psoriasis, inflammatory bowel diseases, and rheumatoid arthritis,[13,54] as well as in clinical trials for asthma and COPD (see below).[11,55,56] These data thus provide strong evidence that PDE4 is a valid, promising drug target for different inflammatory conditions.

The non-selective PDE inhibitor theophylline

The nonselective PDE inhibitor theophylline, a methylxanthine drug, has been used in therapy for respiratory diseases such as asthma and COPD for almost 90 years.[57-59] Although initially recognized as a PDE inhibitor, theophylline is also known as a potent adenosine receptor antagonist[60,61] and an activator of histone deacetylase 2 (HDAC2).[62] It is thought that the beneficial effects of theophylline on asthma and COPD are largely due to its antiinflammatory properties. Several mechanisms have been proposed for such effects, which include (1) increasing intracellular cAMP concentrations via inhibition of PDE (mainly PDE4),[2,63] (2) decreasing inflammatory gene expression through inducing HDAC activity,[62] and (3) reversal of corticosteroid resistance by inhibiting oxidative stress dependent phosphoinositide 3 kinase δ.[64,65] In clinical practice, theophylline is known to interact with a number of drugs, such as cimetidine and phenytoin, and have a narrow therapeutic window. It causes a myriad of side effects at higher doses including nausea, diarrhea, headache, insomnia, increased heart rate, and arrhythmias.[66,67] These disadvantages together with its relatively low efficacy compared with inhaled glucocorticoids or β_2-agonists have limited its usage in asthmatic patients. Because of its nonselectivity towards most of the PDEs expressed in body cells, the pharmaceutical industry has devoted massive efforts in developing inhibitors selective for PDE4s, the isozymes expressed predominantly in proinflammatory cells.[68,69] In fact, PDE4 inhibitors are considered promising therapeutic agents because of their prominent antiinflammatory effects (detailed below).

PDE4 inhibitors

Numerous PDE4 selective inhibitors have been patented in the past two decades, and some of them have been evaluated in clinical trials for several inflammatory conditions, such as asthma, COPD, atopic dermatitis, multiple sclerosis, and rheumatoid arthritis. The development of most of these compounds, however, has been discontinued because of narrow therapeutic windows. A major reason for their poor clinical results is the consequence of dosing limitation caused by side effects such as nausea and emesis. The PDE4 inhibitors explored in clinical trials have been mostly for asthma, likely because of the high prevalence and increasing morbidity of the

disease. However, no compounds have yet reached the market as asthma treatments. Nevertheless, the first orally active PDE4 inhibitor roflumilast was approved in June 2010 by the European Medicines Agency Committee for severe COPD associated with chronic bronchitis in adult patients. In March 2011, the United States Food and Drug Administration approved the drug for reducing COPD exacerbations.[70,71] Clinical studies have shown that roflumilast improves lung function and reduces the frequency of COPD exacerbations in patients with symptoms of chronic bronchitis.[71-74] Although the side effects were generally mild to moderate, nausea, diarrhea, headache, and weight loss are noted with roflumilast.[70]

In view of the side effect profile of second-generation PDE4 inhibitors, new strategies for the design of active and nonemetic compounds have been attempted to hopefully overcome the problems and to improve therapeutic ratios. It has been hypothesized that the side effects of the PDE4 inhibitors are probably a result of their nonselectivity to all four PDE4 subtypes, and thus generation of new PDE4 inhibitors with subtype selectivity may provide clinical benefits by maintaining therapeutic efficacy while decreasing the side effects.[75] This notion is supported by a series of studies where PDE4 gene-targeted mice were used to define the function of individual PDE4 subtypes.[76] For example, the data have shown that ablation of PDE4B, but not PDE4A or PDE4D, significantly suppresses LPS-induced tumor necrosis factor (TNF)-α production in circulating monocytes and peritoneal macrophages.[44,45] In addition, in a murine model of allergic asthma, Th2 cell functions including proliferation and cytokine production, were also disrupted in mice deficient in PDE4B, but not PDE4A or PDE4D.[77,78] In a separate study, reversing α_2-adrenoceptor-mediated anesthesia, a behavioral correlate of emesis in nonvomiting species such as rodents, was evaluated in xylazine/ketamine-treated mice and the results indicated that inhibition of PDE4D but not PDE4B may be responsible for the emetic effects of non-selective PDE4 inhibitors.[79] Taken together, these findings in PDE4 knockout mice suggest that an inhibitor with PDE4B selectivity should retain many beneficial antiinflammatory effects without the emetic effects.

In spite of the significant challenges of synthe-

sizing PDE4 subtype-selective inhibitors due to the highly conserved catalytic domain of PDE4 isozymes, generation of inhibitors with PDE4 subtype selectivity has recently been described.[80,81] A series of potent PDE4B inhibitors with more than 100-fold selectivity over PDE4D have been synthesized from lead 2-arylpyrimidine derivatives.[80] Biological evaluation of a selective PDE4B inhibitor revealed its potent antiinflammatory effects *in vitro* and *in vivo*. Investigation in ferrets also showed a significantly less emesis with the compound compared with the non-selective PDE4 inhibitor cilomilast.[80] In a separate report, small-molecule allosteric modulators of PDE4D have been generated using a nontraditional approach.[81] These modulators do not completely inhibit enzyme activity, yet show potent cognition enhancement in animal models. Interestingly, the results from the rodent model of a behavioral correlate of emesis indicated that PDE4D allosteric modulators have reduced potential to cause emesis whereas PDE4D full inhibitors are highly emetic.[81] The reason for this different emetic effect is probably because PDE4D allosteric modulators have less effect on cAMP levels, because of their lower potency of PDE4 inhibition compared with full inhibitors of PDE4, and thus are able to better maintain cAMP signaling spatially and temporally while reducing target-based toxicity.[81]

To avoid the problem of systemic side effects caused by oral administration, development of PDE4 inhibitors with alternative routes of delivery has been reported.[82] In general, when delivered by inhalation, the drug may have reduced systemic exposure and focused delivery to lung tissues, thus minimizing the potential of systemic side effects. GSK256066 is an inhaled PDE4 inhibitor which shows a protective effect on both early and late asthmatic responses to inhaled allergen in atopic asthmatics.[82] The drug was well tolerated with low systemic exposure, but larger studies are needed to establish the safety profile. Topical application of PDE4 inhibitors is another potential means to minimize systemic side effects. Benzylamine-substituted phthalazinones have recently been developed as potent topically active PDE4 inhibitors, and have shown anti-inflammatory effects in a mouse model of dermatitis.[83] Additional studies are required to evaluate the therapeutic index of the compounds. Although the majority of orally administered PDE4 inhibitors face the issue of side effects,

a number of oral compounds currently in development, such as apremilast for psoriasis[84] and the PDE4D allosteric modulators as described above,[81] are reported to be less emetic and have wider therapeutic ratios. The molecular mechanism of this tolerability has not been reported.

Effects of PDE4 inhibition in leukocytes

Cyclic AMP-elevating agents including PDE4 inhibitors are known to suppress a myriad of inflammatory responses in most immune and inflammatory cells. These effector responses include proliferation, chemotaxis, phagocytosis, and release of proinflammatory mediators such as lipid mediators, reactive oxygen species (ROS), hydrolytic enzymes, cytokines and chemokines.[2,40,85] The antiinflammatory effects derived from PDE4 inhibition or ablation in leukocytes are briefly reviewed below.

Monocytes and macrophages

Among the immune cells, circulating monocytes and tissue macrophages are key players in the innate immune responses. They are a major source of TNF-α, a proinflammatory cytokine that orchestrates the complex processes involved in immunity as well as inflammatory disease states, such as rheumatoid arthritis, Crohn's disease, and septic shock.[86-89] Through activation of toll-like receptor (TLR) signaling, the endotoxin LPS stimulates TNF-α production in monocytes and macrophages. The TNF-α release induced by LPS is markedly inhibited by PDE4 inhibitors in blood monocytes,[2,44,75] whereas the inhibition is less evident in tissue macrophages.[44,45] This discrepancy may be explained by the different PDE isozyme activity profiles in the two cell types, with monocytes containing predominantly PDE4 while PDEs 1, 3 and 4 are major isoforms in alveolar macrophages.[90] Despite the simultaneous expression of the three PDE4 genes (PDE4A, PDE4B, and PDE4D), LPS stimulation of TLR selectively induces PDE4B expression and activity in circulating monocytes[43,44] and peritoneal macrophages.[45] Functionally, ablation of PDE4B, but not PDE4A or PDE4D significantly reduces LPS-induced TNF-α release in these cells. In addition, the PDE4 inhibitors have no additional inhibitory effect on the TNF-α release in PDE4B-deficient macrophages while significantly suppressing the response in PDE4A- and PDE4D-null cells. These

data demonstrate that the pharmacological effects of PDE4 inhibitors on macrophage TNF-α production are mediated exclusively through inhibition of PDE4B.[44,45]

cAMP regulates multiple cellular processes through activation of at least three distinct signaling effectors, including PKA, exchange proteins directly activated by cAMP (Epac), and cyclic nucleotide-gated ion channels. A variety of inflammatory responses inhibited by cAMP are mediated by PKA or Epac. For example, activation of PKA, but not Epac, suppresses LPS-induced TNF-α production and ionophore A23187-stimulated leukotriene B4 (LTB4) production in alveolar and peritoneal macropages.[45,91] By contrast, activation of Epac, but not PKA, suppresses FcγR-mediated phagocytosis and LPS-induced interferon (IFN)-β production in macrophages.[91,92] Interestingly, in human monocyte-derived macrophages, cAMP has been demonstrated to induce, rather than decrease, the expression and secretion of several proinflammatory chemokines such as CXCL-7, CXCL-5, and CCL-2. This effect is mediated by activation of Epac but not PKA.[93]

T lymphocytes

In T lymphocytes, PDE4 inhibitors are shown to attenuate anti-CD3/CD28-, mitogen-, and specific antigen-stimulated T-cell activation, proliferation, and cytokine release such as interleukin (IL)-2, IL-5, and IFN-γ.[2,42,54] Some of these inhibitory effects have been demonstrated to be prevented by PKA inhibitors.[94] PDE3 inhibitors have little or no effect on these responses, but they can enhance the effects of PDE4 inhibitors.[95-98] Additionally, induction of PDE7 is demonstrated to be necessary for T cell activation and IL-2 production.[99] To define the functional role of each PDE4 subtype in CD4+ T cells, Peter and colleagues employed PDE4 subtype-specific siRNAs in human anti-CD3/CD28-stimulated T cells. The results indicate that PDE4B and PDE4D are involved in modulating early or "short-term" responses, such as IL-2 release, and PDE4D is a predominant subtype conducting "long-term" responses, such as IFN-γ and IL-5 release and proliferation.[42]

CD4+ helper T (TH) cells are classified into different functional subsets depending on their cytokine profiles. TH1 cells produce predominantly IFN-γ and lymphotoxin and are pivotal in macrophage activation. TH2 cells secrete predominantly IL-4, IL-5, and IL-13, and are important for immunoglobulin (Ig) E production as well as eosinophil differentiation and activation. Exaggerated TH1 responses may lead to autoimmune diseases, such as type 1 diabetes, rheumatoid arthritis, and multiple sclerosis, whereas TH2 cells are associated with allergic conditions such as asthma and anaphylaxis.[100-102] Reports on the effects of PDE4 inhibitors on proliferation and cytokine release in these cells are somewhat irreconcilable. Essayan and colleagues demonstrated that the proliferation of both TH1 and TH2 clonal cells are inhibited by rolipram, with TH2 cells being more sensitive to PDE4 inhibition.[98] PDE4 inhibitors are also shown to block the release of both TH1 and TH2 cytokines.[98,103] Contrarily, using TH1 and TH2 cells derived from ovalbumin-specific T-cell receptor transgenic mice, Claveau et al. reported that PDE4 inhibitors significantly inhibited TH1-mediated IFN-γ production, but had no significant effect on TH2-mediated IL-4 or IL-13 production.[104] On the other hand, studies of PDE4 knockout mice in a murine model of asthma revealed that airway-draining lymph node cells deficient in PDE4B, but not PDE4A or PDE4D, produced a marked reduction in T-cell proliferation and TH2 cytokine production, including IL-4, IL-5, and IL-13.[77,78] Conversely, release of the TH1 cytokine IFN-γ was not affected in PDE4B null cells.[78]

The subset TH17 cells are important in host defense against specific extracellular pathogens, and are also thought to be involved in the pathogenesis of autoimmune diseases.[105] These cells produce IL-17A and IL-17F, which upon ligation to their receptors induce secretion of several proinflammatory cytokines and chemokines to promote neutrophil recruitment, thus leading to tissue inflammation. A recent study demonstrated that PDE4 inhibitors also profoundly attenuate IL-17 production in anti-CD3/CD28-stimulated peripheral blood mononuclear cells, purified CD4+ T cells, and memory TH17 cells.[106]

Neutrophils

Neutrophils are a type of phagocyte that circulates in the blood awaiting the call from an infected site to become activated and recruited. Upon stimulation by inflammatory mediators such as the bacterial component N-formyl-methionyl-leucyl-phenylalanine (fMLP), the C5 complement fragment C5a, and

the chemokine IL-8, these cells are induced to express adhesion molecules, infiltrate into the inflammatory site, and subsequently undergo phagocytosis and produce inflammatory mediators, such as ROS, proteases, and LTB$_4$. Consistent with the predominant PDE4 expression in these cells, PDE4 inhibitors suppress a variety of neutrophil responses, including fMLP-induced generation of superoxide anion and LTB$_4$,[107-109] degranulation,[110,111] and expression of adhesion molecules.[111-113] Inhibition of PDE4 also reduces the ability of neutrophils to phagocytose zymosan particles.[114] To define the role of individual PDE4 subtypes in neutrophils, a study in an animal model of airway neutrophilia, a characteristic feature of COPD, was conducted in PDE4-deficient mice.[115] The data showed that ablation of PDE4D or PDE4B, but not PDE4A, had profound effects on neutrophil functions. These include a significant reduction in neutrophil recruitment to the lung of PDE4B and PDE4D null mice after exposure to inhaled LPS, and an associated decrease in the expression of the adhesion molecule β$_2$-integrin (CD18) in the neutrophils of these mice. Chemotaxis in response to IL-8 or macrophage inflammatory protein (MIP)-2 is also attenuated in the splenic neutrophils of PDE4B and PDE4D null mice.[115]

Eosinophils

Eosinophils are responsible for defending against helminths, worms, and other intestinal parasites. Along with activated mast cells, they also participate in the pathogenesis of allergic conditions such as asthma. Upon stimulation by cytokines such as IL-5, IL-3 and granulocyte macrophage colony-stimulating factor or inflammatory factors such as fMLP, C5a, and platelet-activating factor (PAF), eosinophils release a plethora of toxic substances and proinflammatory mediators, including ROS, cationic granule proteins, leukotrienes, and various cytokines, which are thought to cause airway damage in asthmatics.[54] Some of these responses, such as fMLP- and C5a-induced ROS formation[109] as well as C5a- and PAF-stimulated leukotriene C4 (LTC$_4$) production,[116] have been shown to be potently inhibited by PDE4 inhibitors. Moreover, inhibition of PDE4 also suppresses the expression of adhesion molecules and consequently decreases the chemotaxis of human eosinophils.[116,117] An earlier study indicated that PDE4 inhibitors are able to inhibit IL-5-induced sur-

vival of human eosinophils.[118] However, a recent report suggests that spontaneous eosinophil apoptosis is delayed by rolipram *in vitro*, and combining a PDE4 inhibitor with a β$_2$-agonist produces a further delay in apoptosis.[119]

Mast cells and basophils

Mast cells are resident in tissues throughout the body, particularly in the connective tissues underlying the mucosa of the respiratory and gastrointestinal tracts as well as the dermis of the skin. Mast cells and circulating basophils are important in mediating allergic disorders such as asthma and anaphylaxis. These cells become activated when the IgE molecules bound to high affinity IgE receptors FcεRI on their cell surface are cross-linked to antigens. This results in a rapid release of granule contents such as histamine and other inflammatory mediators. Limited information is available on how PDE4 inhibitors regulate inflammatory responses in mast cells. In a series of studies, Peachell and colleagues showed that PDE4 inhibitors can attenuate anti-IgE-induced release of histamine and LTC$_4$ from human basophils,[120,121] but are ineffective at inhibiting these responses in human lung mast cells.[121] Additionally, the IgE- and IL-3-mediated release of histamine, as well as IL-4 and IL-13, have also been shown to be inhibited by PDE4 inhibitors in basophils.[122]

B lymphocytes

Upon antigen recognition, B lymphocytes are activated, proliferate, and differentiate into Ig-producing plasma cells, which represent the key mediator of humoral immunity. The isotype class switch of B cells to IgE production is known to be crucial in the development of many allergic conditions including asthma. Th2 cytokines, IL-4 in particular, induce IgE production in B cells.[123] Mice deficient in IL-4 fail to produce measurable levels of allergen-specific IgE.[124] Several cAMP-elevating agents, such as PDE4 inhibitors, β$_2$-agonists, cAMP analogs, and E-series prostaglandins, are shown to enhance the IL-4-directed IgE production.[125] A separate report also indicates that B-cell proliferation induced by LPS plus IL-4 is augmented by rolipram;[126] however, this effect is not mimicked by PGE2 or forskolin. Interestingly, Paul-Eugene et al. have shown that IL-4-induced IgE production can be potentiated by cAMP only when B cells are stimulated at a subopti-

mal concentration of IL-4.[127] These data are consistent with the recent findings that ablation of PDE4B, and therefore a condition of increased cAMP, results in normal IgE production in spite of low IL-4 in PDE4B null mice.[78]

Dendritic cells

Dendritic cells (DC) are antigen presenting cells whose major function is to prime naive T cells and trigger T-cell responses. Human monocyte-derived DCs express predominantly PDE4, with PDE4A being the most abundant mRNA.[128] In immature DC, LPS- or CD40L-induced TNF-α and IL-12p70 production is reduced by PDE4 inhibitors. DCs matured in the presence of PDE4 inhibitors are still able to stimulate T cells; however, they show an increased expression of C-X-C chemokine receptor 4 and reduced TNF-α and IL-12p70 production in response to CD40L. Moreover, when these matured DCs are used to stimulate naive T cells, a reduction in IFN-γ-producing (T_H1) cells is observed.[128] This result is in contrast with the findings reported in mice deficient in PDE4B or PDE4A, where a normal IFN-γ response is produced in response to allergen stimulation.[78] A separate study using murine bone marrow-derived dendritic cells (BMDC) has shown that analogs of the lipid mediator prostaglandin I_2, such as iloprost, cicaprost, and treprostinil, suppress the LPS-induced production of several proinflammatory cytokines and chemokines, such as IL-12, TNF-α, IL-1α, IL-6, MIP-1α, and monocyte chemotactic protein-1, while increasing the antiinflammatory cytokine IL-10 production in these cells. This modulatory effect is associated with an upregulation of intracellular cAMP and downregulation of nuclear factor kappa B activity.[129] Moreover, the regulation of cytokine and chemokine production by cAMP is mediated by both Epac-1 and PKA activation in BMDC.[130] Little is known about how PDE4 inhibitors influence inflammatory responses in these cells.

Conclusion

Almost two decades have passed since targeting PDE4 became a focus in the development of novel therapeutics for pulmonary inflammatory diseases. The recent approval of roflumilast as a drug for COPD therapy provides proof that the PDE4 isozyme family can be a therapeutic target.

Nevertheless, this second-generation PDE4 inhibitor is still not without side effects. Several strategies have been proposed to minimize this problem, such as designing inhibitors as inhaled drugs or topically applied agents, as well as improving subtype selectivity. The development of PDE4 inhibitors with PDE4B selectivity has been considered a promising approach because much evidence demonstrates that ablation or inhibition of PDE4B produces a broad spectrum of antiinflammatory effects while minimizing unwanted side effects.[76,80] Nevertheless, the impact of PDE4B-selective inhibitors on inflammatory diseases awaits further clinical trials. Several PDE4B and PDE4D selective inhibitors have been designed and synthesized, and their effects on inflammation are under investigation. The development of additional PDE4 subtype-selective inhibitors based on their detailed crystal structures is also underway.

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環狀核苷酸磷酸雙酯酶4及其抑制劑於發炎疾病之影響

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　　第四家族環狀核苷酸磷酸雙酯酶 (PDE4) 是分解 cAMP 的酵素，在哺乳生物中由四個基因 (*PDE4A, PDE4B, PDE4C, PDE4D*) 所組成，可生成二十種以上 PDE4 異構酶。雖然該等酵素具有高度保留的催化區以及在調控區內序列相似的 UCR1 與 UCR2 區，但每個基因卻各有其特殊的生理功能。由於 PDE4 異構酶的組織分布甚廣，且在不同細胞內的表現與調節各不相同，因此這些酵素被視爲是細胞內 cAMP 濃度、cAMP 訊息傳導與 cAMP 訊息區域性的主要調控者。文獻顯示，PDE4 抑制劑可經由增加 cAMP 濃度進而抑制多種免疫細胞的發炎反應，目前許多 PDE4 抑制劑已針對不同的發炎疾病進行臨床試驗，這些抑制劑（包含近來被通過上市的 roflumilast）雖具有相當的療效，但均會產生副作用如噁心、嘔吐等，使施藥劑量受限以致降低抗發炎的功效。爲此，研發功效佳、低副作用的 PDE4 抑制劑成爲藥廠努力的重心。近年來研究報告指出，利用 PDE4B 選擇性抑制劑應可保有 PDE4 抑制劑的藥效且可降低其副作用，此外，研發吸入式或皮膚外用式 PDE4 抑制劑對治療呼吸道發炎疾病或皮膚炎亦是極具潛能的策略。本文主要綜述 PDE4 異構酶的蛋白質結構與功能關係、PDE4 對免疫細胞發炎反應的影響，以及 PDE4 抑制劑做爲治療發炎疾病的潛能。(長庚醫誌 2012;35:197-210)

關鍵詞：環狀核苷酸磷酸雙酯酶4，發炎作用，氣喘，慢性阻塞性肺病，cAMP 訊息傳導