Cyclic AMP

Master Regulator of Innate Immune Cell Function

Carlos H. Serezani¹, Megan N. Ballinger¹, David M. Aronoff², and Marc Peters-Golden¹

Divisions of ¹Pulmonary and Critical Care Medicine and ²Infectious Diseases, Department of Internal Medicine, University of Michigan Health System, Ann Arbor, Michigan

Cyclic adenosine monophosphate (cAMP) was the original “second messenger” to be discovered. Its formation is promoted by adenylyl cyclase activation after ligation of G protein–coupled receptors by ligands including hormones, autocoids, prostaglandins, and pharmacologic agents. Increases in intracellular cAMP generally suppress innate immune functions, including inflammatory mediator generation and the phagocytosis and killing of microbes. The importance of the host cAMP axis in regulating antimicrobial defense is underscored by the fact that microbes have evolved virulence-enhancing strategies that exploit it. Many clinical situations that predispose to infection are associated with increases in cAMP, and therapeutic strategies to interrupt cAMP generation or actions have immunostimulatory potential. This article reviews the anatomy of the cAMP axis, the mechanisms by which it controls phagocyte immune function, microbial strategies to dysregulate it, and its clinical relevance.

Keywords: phagocytes; host defense; G protein–coupled receptors; protein kinase A; exchange protein activated by cyclic AMP

The cyclic nucleotide cyclic adenosine monophosphate (cAMP)—the original member of the family of second messengers—was discovered by Dr. Earl W. Sutherland during his studies of the mechanisms of hormone action. Sutherland was awarded the 1971 Nobel Prize for this work, which would prove to be the first of five Nobel Prizes recognizing research on this molecule. cAMP is now recognized as a universal regulator of cellular function in organisms including amoebas, plants, and humans (1). Biological processes mediated by this second messenger include memory, metabolism, gene regulation, and immune function (2). This review will address cAMP regulation of innate immunity, with an emphasis on the lung. Although many contemporary reviews on innate immunity focus on pathogen recognition receptors and signaling pathways (3), the importance of cAMP as a controller derives from its ability to exert broad modulatory effects that are independent of the pathogen, the recognition receptor, or the signaling pathway in question.

ANATOMY OF THE SYSTEM

The generation of cAMP is initiated when an extracellular first messenger (neurotransmitter, hormone, chemokine, lipid mediator, or drug) binds to a seven transmembrane–spanning G

CLINICAL RELEVANCE

This review will provide clinicians with an overview of the cyclic AMP axis, its role as a down-regulator of host antimicrobial defense functions, and the clinical and translational relevance of such actions.

protein–coupled receptor (GPCR) that is coupled to a stimulatory G protein α subunit ($G_{\alpha s}$) (Figure 1). Ligand binding results in the exchange of GDP for GTP on the $G_{\alpha s}$ protein and its subsequent dissociation from the $\beta\gamma$ subunit complex (4). The free $G_{\alpha s}$ subunit stimulates the enzyme adenylyl cyclase (AC) to catalyze the cyclization of ATP to generate cAMP and pyrophosphate (5, 6). Some of the well-known $G_{\alpha s}$ -coupled GPCR ligands include epinephrine and norepinephrine, histamine, serotonin, and certain cyclooxygenase (COX)-derived prostaglandins (particularly prostaglandin [PG] E_2 and I_2 [also known as prostacyclin]) (4). By contrast, $G_{\alpha i}$ subunits inhibit AC and the production of cAMP; some well-known $G_{\alpha i}$ -coupled GPCR ligands include chemokines CCR1–10 and CXCR1–6 as well as leukotrienes B_4 , C_4 , and D_4 (4).

To date there are 10 known AC isoforms that are differentially expressed in various cell types (6). Intracellular levels of cAMP are tightly regulated, not only by AC but also by the enzyme phosphodiesterase (PDE) (Figure 1). PDEs oppose cAMP signaling by degrading intracellular cAMP (7). There are 11 distinct PDE gene families whose expression is likewise tissue-specific. Moreover, because both ACs and PDEs can be localized to different spatial compartments within the cell, the evanescent presence of cAMP can be further regulated at a subcellular level (*see below*) (8).

Diverse cAMP effector molecules also contribute to the complexity of this pathway. Binding of cAMP to the regulatory subunit of protein kinase A (PKA) leads to dissociation of its catalytic subunit, which is then free to phosphorylate specific Ser and Thr residues on numerous target proteins, including the transcription factor cAMP response element–binding protein (9). A-kinase–anchoring proteins serve to localize PKA as well as PDEs within specific cellular microdomains, thereby creating discrete subcellular pools of intracellular cAMP and its effector within a cell. Recently, alternative (PKA-independent) intracellular targets of cAMP, including cyclic nucleotide-gated channels and two isoforms of exchange proteins directly activated by cAMP (Epac), have been identified and their roles in mediating a variety of cellular functions are emerging (10). Previous work has shown that PKA and Epac may have redundant, independent, or even opposing effects within the same cell (11, 12) (discussed more below). cAMP-binding domains of PKA or Epac have been employed as biosensors to measure the spatially discrete pools of intracellular cAMP in live cells (13) using fluorescent resonance

(Received in final form February 29, 2008)

This work was supported by National Institutes of Health grants HL078727 and HL058897 to M.P.-G., a Hartwell Foundation fellowship (M.N.B.), and a Doris Duke Clinical Scholar Award (D.M.A.).

Correspondence and requests for reprints should be addressed to Marc Peters-Golden, M.D., 6301 MSRB III, 1150 W. Medical Center Drive, University of Michigan Health System, Ann Arbor, MI 48109-5642. E-mail: petersm@umich.edu

Am J Respir Cell Mol Biol Vol 39, pp 127–132, 2008

Originally Published in Press as DOI: 10.1165/rcmb.2008-0091TR on March 6, 2008

Internet address: www.atsjournals.org

Figure 1. The regulation of cyclic AMP (cAMP) levels and antimicrobial actions. The binding of an agonist to the G protein-coupled receptor (GPCR) induces a conformational change resulting in the liberation of the $G\alpha$ subunit from the $\beta\gamma$ subunit complex. The binding of the $G\alpha$ subunit to adenylyl cyclase (AC) either stimulates ($G\alpha_s$) or inhibits ($G\alpha_i$) the enzyme's generation of cAMP. The production of cAMP is also regulated by microbial pathogens. Pertussis toxin and cholera toxin cause elevated cAMP levels through ADP-ribosylation of either the $G\alpha_i$ subunit to prevent its inhibition of AC or of the $G\alpha_s$ subunit to constitutively activate AC, respectively. Phosphodiesterases (PDEs), which degrade cAMP to AMP, are another regulator of intracellular cAMP levels. PDE inhibitors prevent such degradation, resulting in accumulation of intracellular cAMP. The downstream signaling of cAMP is mediated by its interactions with effector molecules, protein kinase A (PKA) or exchange proteins directly activated by cAMP (Epac), which have been shown to modulate phagocyte functions. Depicted here is a pattern demonstrated for alveolar macrophages in which specific antimicrobial functions are differentially regulated by particular cAMP effectors.

energy transfer (14). Cellular functions are ultimately influenced by the ability of these cAMP effectors to regulate activation of transcription factors as well as signaling molecules such as protein kinases, calcium, and small GTPases (4, 15).

CAMP AND PHAGOCYTE EFFECTOR FUNCTIONS

Elevations in intracellular cAMP suppress innate immune functions of monocytes, macrophages (mφs), and neutrophils (PMNs) (collectively referred to as phagocytes) predominantly through the modulation of three key effector functions of these cells: generation of inflammatory mediators (e.g., cytokine, chemokine, and lipids), phagocytosis, and intracellular killing of ingested pathogens. The host defense function of dendritic cells, important cellular adaptors of innate and acquired immunity, are also highly susceptible to regulation by cAMP but are not discussed here (16). While many mediators of innate immune defense (e.g., chemokines and leukotrienes) act through $G\alpha_i$ -coupled

receptors, the extent to which their immunostimulatory effects depend on reductions in intracellular cAMP and the impairment of cAMP effector pathways is uncertain.

Modulation of Mediator Generation

Leukocytes respond to microbial invasion by producing a balance of pro-inflammatory and anti-inflammatory mediators. Increased intracellular cAMP suppresses the expression of pro-inflammatory cytokines such as tumor necrosis factor- α (16) and interleukin-12 (17); chemokines, including macrophage inflammatory protein-1 α and -1 β (16); and the pro-inflammatory lipid mediator leukotriene B₄ (18). In contrast, cAMP enhances the production of the anti-inflammatory cytokine interleukin-10 (16) and stimulates the expression of the suppressor of cytokine signaling-3 protein in peripheral blood mononuclear cells and PMNs (19). While the effects of cAMP on mφ inflammatory mediator generation were originally reported to be mediated by PKA rather than Epac-1 (16), Epac-1 has been implicated in the suppression of endotoxin-induced interferon- β production in a mφ cell line (20). It is likely that the roles of these two cAMP effectors vary depending on the cell type and the mediator under investigation.

Modulation of Phagocytosis

Phagocytosis involves a highly regulated sequence of signal transduction events that lead to cytoskeletal and membrane rearrangements and eventually particle engulfment (21). A variety of specific cell surface receptors recognize microbial pathogens. These include opsonin-dependent complement receptors (CR) and Fcγ receptors (FcR) as well as the opsonin-independent class A scavenger receptors, mannose receptors, and dectin receptors. Elevations in intracellular cAMP suppress CR-, FcR-, and scavenger receptor-mediated phagocytosis (22–24), while it remains unknown whether mannose and dectin receptor-mediated phagocytosis are influenced by cAMP.

The molecular pathways involved in the inhibition of phagocytosis by cAMP are not completely defined. cAMP has been shown to both inhibit (25) and promote (26) F-actin polymerization during FcR-mediated phagocytosis. cAMP may also down-regulate phagocytosis through the modulation of phagocytic receptor expression. For example, Nambu and coworkers reported that cAMP decreased expression of the stimulatory Fcγ receptor I and increased expression of the inhibitory Fcγ receptor IIb in the monocytic cell line U937 (22). In contrast, stimulatory Fcγ receptor IIa expression was increased as a result of PKA-CREB activation in the mφ-like cell PL8 (27).

It was recently demonstrated that Epac-1, but not PKA, was involved in the cAMP-dependent reduction of FcR phagocytosis by alveolar macrophages (AMs) (11). However, others have reported that both PKA and Epac-1 can counterregulate phagocytosis in different cell types. Makranz and colleagues (24) showed that both PKA and Epac-1 suppress myelin phagocytosis by microglia and peritoneal mφs. Bryn and coworkers (28) reported that PKA regulated FcR phagocytosis in circulating monocytes, while both Epac-1 and PKA inhibited this function of monocyte-derived mφs. Canetti and colleagues recently reported that Epac-1 effects on FcR phagocytosis in AMs are mediated by the activation of the tyrosine phosphatase SHIP-1, resulting in increased activity of the phosphatase and tensin homolog on chromosome 10 (29).

Modulation of Microbicidal Activity

cAMP also suppresses the microbicidal capacity of leukocytes toward bacteria (11, 30, 31), viruses (32), fungi (33), and eukaryotic parasites (34). The mechanisms by which it does so are not well understood. Potential targets of cAMP regulation

include the production and release of reactive oxygen intermediates (ROIs), reactive nitrogen intermediates, phagosomal acidification, and lysosomal enzyme release.

cAMP down-regulates ROI release in phagocytes stimulated with a myriad of agonists (31, 35, 36). This effect is associated with, and presumably involves, inhibition of two pivotal steps in NADPH oxidase activation, namely, the phosphorylation as well as the translocation of the cytosolic p47phox subunit to the cell membrane (31, 35–37). However, the molecular mechanisms underlying these effects are poorly understood. While it has been shown that cAMP-activated PKA inhibits ROI release in phagocytes stimulated with formyl peptides, phorbol esters, and IgG-coated targets (31, 35–37), our group has shown that activation of both PKA and Epac-1 can inhibit ROI generation in *Klebsiella pneumoniae*-infected AMs (11).

Data on the regulation of reactive nitrogen intermediate generation by cAMP are conflicting. On the one hand, cAMP was found to be pivotal in the enhancement of inducible nitric oxide (NO) synthase expression and NO production by a mechanism dependent on PKA activity (38, 39). In addition, cAMP increased the stability of inducible NO synthase protein in a mφ cell line (40). On the other hand, elevation of cellular cAMP levels inhibited inducible NO synthase activation in hepatic mφs (41).

Contradictory findings also apply to the regulatory role of cAMP on phagolysosome maturation. Kalamidas and coworkers (42) showed that increased cAMP levels decreased phagolysosome formation and acidification by a mechanism dependent on PKA activity, while Di and colleagues (43) demonstrated that cAMP and PKA activity were required for phagosomal acidification. It is of interest that machinery involved in cAMP formation and signaling has been localized to the phagosome. PDE4 was targeted to the forming phagosome in PMNs, where it was colocalized with the catalytic subunit of PKA (44). In addition, Brock and coworkers (45) found that both Epac-1 and its downstream effector, the small GTPase Rap1, associated with phagosomes containing IgG-opsonized targets in AMs. Epac-1, but not Rap1, appeared to accumulate on maturing phagosomes after cAMP stimulation.

UTILIZATION BY MICROBES OF THE HOST cAMP SYSTEM

Underscoring the importance of cAMP as a negative regulator of antimicrobial responses is the finding that several pathogenic microorganisms have evolved mechanisms to exploit host cell cAMP signaling as a virulence factor. Microbial pathogens can increase the intracellular cAMP production of host cells directly (reviewed below) or indirectly, through the elicitation of host autocrine and paracrine mediators that secondarily enhance cAMP generation (e.g., adenosine, PGE₂, histamine, etc.). Only the former, direct actions are discussed here. By using cAMP to disable host cell phagocytosis, intracellular killing, and inflammatory mediator generation, pathogens gain an upper hand in establishing infection.

The respiratory pathogen *Bordetella pertussis* (the cause of whooping cough) provides a fascinating example of how a bacterium can derail innate immune defenses simply by overwhelming the cAMP regulatory system of host cells. *B. pertussis* produces several toxins, including the well-described pertussis toxin (PT) and the less well known AC toxin, CyaA (46). Both PT and CyaA toxins increase host cell cAMP, but by distinct mechanisms. *B. pertussis* PT catalyzes the ADP ribosylation of the inhibitory Gα_i subunit, increasing the intracellular cAMP levels in target cells (47). The actions of PT impair mφ phagocytosis and ROI generation (48, 49) and suppress PMN recruitment to the lungs during infection (50). CyaA is a pore-forming toxin that, in addition

to penetrating host cells, possesses an AC motif that is activated by eukaryotic calmodulin and catalyzes the unregulated conversion of cellular ATP to cAMP (reviewed extensively in Ref. 46). *In vivo* studies indicate that CyaA primarily inhibits the host defense functions of myeloid phagocytes, such as AMs and PMNs (46). CyaA impairs superoxide production, chemotaxis, cytokine production, and phagocytosis (46, 51, 52). In addition to CyaA, three other calmodulin-dependent AC toxins have been identified, including the edema factor of *Bacillus anthracis*, the ExoY of *Pseudomonas aeruginosa*, and the AC of *Yersinia pestis* (reviewed extensively in Ref. 53).

Although not a respiratory pathogen, *Vibrio cholerae*, the causative agent of cholera, is perhaps the best known microbial pathogen that uses cAMP amplification as a virulence factor. The cholera toxin (CT) is a member of the superfamily of heterodimeric AB toxins that bind to cell surfaces with their B subunit to facilitate the translocation of the A subunit into the cell. Within the cytoplasm, the A subunit of CT catalyzes the ADP-ribosylation of the stimulatory Gα_s subunits of G proteins. After ADP-ribosylation, Gα_s binds to AC and constitutively activates it, leading to a sustained increase in intracellular cAMP (54). A structurally related AB toxin that works through a similar mechanism is the heat labile toxin (LT) of *Escherichia coli*. Although the primary target for LT and CT is the intestinal epithelial cell (the poisoning of which causes diarrhea), data suggest that these toxins also increase cAMP production in phagocytes, resulting in the suppression of innate immune defenses. Thus, LT and/or CT have been shown to inhibit phagocytosis (55), intracellular bacterial killing (30), cytokine production (56), and chemotaxis (57) by monocytes, mφs, and PMNs. A growing list of other pathogens are known to hijack host cAMP signaling to impair innate immunity and the reader is referred to other resources for more information (58–61).

CLINICAL AND TRANSLATIONAL RELEVANCE

Diseases in which cAMP Is Increased

In view of the multiple inhibitory effects of increased cAMP on innate immune function (discussed above), it is of substantial interest that a number of conditions predisposing to respiratory infection are characterized by increased levels of cAMP (Table 1). *In vitro* infection with HIV increases cAMP levels in T cells and accounts for impaired proliferative responses (62). HIV infection exerts a similar effect in mφs, which was associated with impaired phagocytosis via both CR (63) and FcR (64). Although HIV infection has been shown to increase mφ PGE₂ synthetic capacity (65, 66), the increased cAMP was not explained by this mechanism (63). It has recently been reported (67) that *in vitro* exposure of normal AMs to cigarette smoke impaired phagocytic capacity and increased cAMP levels, a finding that might explain reduced phagocytic function in AMs from subjects with chronic obstructive pulmonary disease (COPD) as well as from healthy smokers as compared with healthy control subjects.

Among substances that ligate Gα_s-coupled receptors and elevate intracellular cAMP, PGE₂ stands out because it is the major PG product of most organs and its synthesis is universally up-regulated during host responses to infection. As will be seen, conditions characterized by overproduction of PGE₂ provide clinically relevant examples in which cAMP is, or is expected to be, increased. Increased plasma PGE₂ levels have been reported in patients after bone marrow transplantation (68). In a murine bone marrow transplantation model, we have recently observed high PGE₂ levels in both lung and peritoneal lavage fluid, as well as overproduction of PGE₂ by multiple cell types including AMs, PMNs, and alveolar epithelial cells (69). Importantly, abrogation

of PGE₂ synthesis by the COX inhibitor indomethacin reversed both the *in vitro* phagocytic defects in AMs and PMNs as well as the *in vivo* defect in pulmonary bacterial clearance observed in these transplanted mice. Similarly, a bactericidal defect in PMNs from guinea pigs after thermal burn injury has been linked to increased intracellular cAMP and overproduction of PGE₂ (70), and was completely overcome by treatment with COX inhibitors (71). Interestingly, overproduction of PGE₂ has also been reported in a number of other conditions associated with increased susceptibility to infection, including protein-calorie malnutrition (72, 73), cancer (74), infancy (75), aging (76), and cystic fibrosis (77, 78). Several studies have demonstrated that high dose ibuprofen is able to blunt the decline in lung function in patients with cystic fibrosis (79, 80). While this is conventionally regarded as an “anti-inflammatory” strategy, an alternative possibility is that ibuprofen prevents overproduction of immunosuppressive PGE₂ and instead represents an “immunostimulatory” strategy.

Pharmacologic Agents that Alter cAMP Levels

Short- and long-acting β_2 -adrenergic agonists are mainstays of therapy in obstructive lung diseases such as asthma and COPD. These agents are employed primarily for their bronchodilator actions, which are clearly attributed to cAMP accumulation in airway smooth muscle cells. A recent study showed that inhaled β_2 agonists salbutamol and salmeterol impaired the clearance of nontypeable *Haemophilus influenzae* from the murine respiratory tract, an effect which was prevented by the β receptor antagonist propranolol (81). Although the possibility that β_2 agonists might impair antimicrobial responses in patients has received little attention, recent data showing that salmeterol *reduced* the number of exacerbations in patients with COPD (82) suggests that this may not be a clinically relevant action. PDE inhibitors are currently under development for obstructive lung diseases, and these were shown to enhance the suppressive effect of PGE₂ on AM phagocytosis (83).

Ligands that act via G α s-coupled receptors and increase cAMP are used therapeutically in a variety of conditions. For example, analogs of PGI₂ are commonly used for the treatment of pulmonary arterial hypertension. Interestingly, it was recently reported that patients receiving the PGI₂ analog treprostinil had a higher rate of bloodstream infections than did patients receiving the alternative analog epoprostenol (84). Treprostinil inhibited phagocytosis, bacterial killing, and cytokine generation in AMs to a much greater degree than other PGI₂ analogs, and this was due in part to the previously unknown ability of treprostinil to bind and activate the E prostanoide 2 receptor for PGE₂ (85), whose expression in AMs exceeds that of the IP receptor for PGI₂.

TABLE 1. CLINICAL STATES AND PHARMACOLOGIC AGENTS THAT ALTER cAMP

Clinical States (all $\uparrow$ cAMP)	Pharmacologic Agents
HIV infection	NSAIDs ($\downarrow$ cAMP)
Smoking	PGE ₂ analogs ($\uparrow$ cAMP)
Chronic Obstructive Pulmonary Disease	PGI ₂ analogs ($\uparrow$ cAMP)
Bone marrow transplant	B-Adrenergic agonists ($\uparrow$ cAMP)
Cancer	Phosphodiesterase inhibitors ($\uparrow$ cAMP)
Infancy	
Aging	
Burns	
Malnutrition	
Cystic Fibrosis	

Definition of abbreviations: cAMP, cyclic AMP; HIV, human immunodeficiency virus; NSAIDs, nonsteroidal anti-inflammatory drugs.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in clinical practice for their analgesic and anti-pyretic actions, which depend primarily on inhibition of COX and resultant inhibition of PGE₂ and other PGs. Beneficial effects of NSAIDs on bacterial clearance were mentioned above in animal models of bone marrow transplantation and burn injury, as well as in patients with cystic fibrosis. Additional studies demonstrate that administration of these agents enhanced microbial clearance and/or survival in murine models of infection with *Mycobacteria tuberculosis* (86), *Leishmania amazonensis* (87), and *Taenia cysticercosis* (88), and likewise enhanced AM phagocytosis of *Klebsiella pneumoniae* (83). Although not tested explicitly, we speculate that PG inhibition by NSAIDs in these studies leads to reductions in intracellular cAMP, which may account for the immunostimulatory effects of NSAIDs in these models.

CONCLUSIONS

Alterations in cAMP levels can profoundly influence the innate immune functions of phagocytes, with increased cAMP generally down-regulating inflammatory mediator generation, phagocytosis, and microbial killing. The immunosuppressive actions of cAMP are broad, applying to a variety of innate immune cell types and their interactions with the entire gamut of microbial organisms. The circumstances resulting in cAMP perturbations are myriad and common, reflecting the actions of host-derived molecules, pathogen-derived molecules, and pharmacologic agents. The molecular mechanisms and the clinical impact of such changes in cAMP on innate immune function remain incompletely defined. However, a better understanding of the cAMP axis is likely to provide new insights into the regulation of innate immunity that can be translated into therapeutic benefit.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

- Hofer AM, Lefkimmatis K. Extracellular calcium and cAMP: second messengers as “third messengers”? *Physiology (Bethesda)* 2007;22:320–327.
- Beavo JA, Brunton LL. Cyclic nucleotide research—still expanding after half a century. *Nat Rev Mol Cell Biol* 2002;3:710–718.
- Mizgerd JP. Acute lower respiratory tract infection. *N Engl J Med* 2008;358:716–727.
- Landry Y, Niederhoffer N, Sick E, Gies JP. Heptahelical and other G-protein-coupled receptors (GPCRs) signaling. *Curr Med Chem* 2006;13:51–63.
- Kamenetsky M, Middelhaufe S, Bank EM, Levin LR, Buck J, Steegborn C. Molecular details of cAMP generation in mammalian cells: a tale of two systems. *J Mol Biol* 2006;362:623–639.
- Sunahara RK, Taussig R. Isoforms of mammalian adenylyl cyclase: multiplicities of signaling. *Mol Interv* 2002;2:168–184.
- Omori K, Kotera J. Overview of PDEs and their regulation. *Circ Res* 2007;100:309–327.
- Baillie GS, Scott JD, Houslay MD. Compartmentalisation of phosphodiesterases and protein kinase A: opposites attract. *FEBS Lett* 2005;579:3264–3270.
- Chin KV, Yang WL, Ravatn R, Kita T, Reitman E, Vettori D, Cvijic ME, Shin M, Iacono L. Reinventing the wheel of cyclic AMP: novel mechanisms of cAMP signaling. *Ann N Y Acad Sci* 2002;968:49–64.
- Kopperud R, Krakstad C, Selheim F, Doskeland SO. cAMP effector mechanisms: novel twists for an ‘old’ signaling system. *FEBS Lett* 2003;546:121–126.
- Aronoff DM, Canetti C, Serezani CH, Luo M, Peters-Golden M. Cutting edge: macrophage inhibition by cyclic AMP (cAMP): differential roles of protein kinase A and exchange protein directly activated by cAMP-1. *J Immunol* 2005;174:595–599.
- Huang SK, Wettlaufer SH, Hogaboam CM, Flaherty KR, Martinez FJ, Myers JL, Colby TV, Travis WD, Toews GB, Peters-Golden M.

- Variable prostaglandin E2 resistance in fibroblasts from patients with usual interstitial pneumonia. *Am J Respir Crit Care Med* 2008;177:66–74.
13. Lissandron V, Zaccolo M. Compartmentalized cAMP/PKA signalling regulates cardiac excitation-contraction coupling. *J Muscle Res Cell Motil* 2006;27:399–403.
 14. Nikolaev VO, Lohse MJ. Monitoring of cAMP synthesis and degradation in living cells. *Physiology (Bethesda)* 2006;21:86–92.
 15. Bos JL. Epac proteins: multi-purpose cAMP targets. *Trends Biochem Sci* 2006;31:680–686.
 16. Aronoff DM, Carstens JK, Chen GH, Toews GB, Peters-Golden M. Short communication: differences between macrophages and dendritic cells in the cyclic AMP-dependent regulation of lipopolysaccharide-induced cytokine and chemokine synthesis. *J Interferon Cytokine Res* 2006;26:827–833.
 17. van der Pouw Kraan TC, Boeije LC, Smeenk RJ, Wijdenes J, Aarden LA. Prostaglandin-E2 is a potent inhibitor of human interleukin 12 production. *J Exp Med* 1995;181:775–779.
 18. Luo M, Jones SM, Phare SM, Coffey MJ, Peters-Golden M, Brock TG. Protein kinase A inhibits leukotriene synthesis by phosphorylation of 5-lipoxygenase on serine 523. *J Biol Chem* 2004;279:41512–41520.
 19. Gasperini S, Crepaldi L, Calzetti F, Gatto L, Berlatto C, Bazzoni F, Yoshimura A, Cassatella MA. Interleukin-10 and cAMP-elevating agents cooperate to induce suppressor of cytokine signaling-3 via a protein kinase A-independent signal. *Eur Cytokine Netw* 2002;13:47–53.
 20. Xu XJ, Reichner JS, Mastrofrancesco B, Henry WL Jr, Albina JE. Prostaglandin E2 suppresses lipopolysaccharide-stimulated IFN- β production. *J Immunol* 2008;180:2125–2131.
 21. Gordon S. The macrophage: past, present and future. *Eur J Immunol* 2007;37:S9–S17.
 22. Nambu M, Morita M, Watanabe H, Uenoyama Y, Kim KM, Tanaka M, Iwai Y, Kimata H, Mayumi M, Mikawa H. Regulation of Fc gamma receptor expression and phagocytosis of a human monoblast cell line U937: participation of cAMP and protein kinase C in the effects of IFN-gamma and phorbol ester. *J Immunol* 1989;143:4158–4165.
 23. Atkinson JP, Michael JM, Chaplin H Jr, Parker CW. Modulation of macrophage C3b receptor function by cytochalasin-sensitive structures. *J Immunol* 1977;118:1292–1299.
 24. Makranz C, Cohen G, Reichert F, Kodama T, Rotshenker S. cAMP cascade (PKA, Epac, adenylyl cyclase, Gi, and phosphodiesterases) regulates myelin phagocytosis mediated by complement receptor-3 and scavenger receptor-AI/II in microglia and macrophages. *Glia* 2006;53:441–448.
 25. Zalavary S, Bengtsson T. Adenosine inhibits actin dynamics in human neutrophils: evidence for the involvement of cAMP. *Eur J Cell Biol* 1998;75:128–139.
 26. Ydrenius L, Majeed M, Rasmuson BJ, Stendahl O, Sarndahl E. Activation of cAMP-dependent protein kinase is necessary for actin rearrangements in human neutrophils during phagocytosis. *J Leukoc Biol* 2000;67:520–528.
 27. Hazan-Eitan Z, Weinstein Y, Hadad N, Konforty A, Levy R. Induction of Fc gammaRIIA expression in myeloid PLB cells during differentiation depends on cytosolic phospholipase A2 activity and is regulated via activation of CREB by PGE2. *Blood* 2006;108:1758–1766.
 28. Bryn T, Mahic M, Enserink JM, Schwede F, Aandahl EM, Tasken K. The cyclic AMP-Epac1-Rap1 pathway is dissociated from regulation of effector functions in monocytes but acquires immunoregulatory function in mature macrophages. *J Immunol* 2006;176:7361–7370.
 29. Canetti C, Serezani CH, Atrasz RG, White ES, Aronoff DM, Peters-Golden M. Activation of phosphatase and tensin homolog on chromosome 10 mediates the inhibition of Fc gamma R phagocytosis by prostaglandin E2 in alveolar macrophages. *J Immunol* 2007;179:8350–8356.
 30. O'Dorisio MS, Vandenbark GR, LoBuglio AF. Human monocyte killing of *Staphylococcus aureus*: modulation by agonists of cyclic adenosine 3',5'-monophosphate and cyclic guanosine 3',5'-monophosphate. *Infect Immun* 1979;26:604–610.
 31. Serezani CH, Chung J, Ballinger MN, Moore BB, Aronoff DM, Peters-Golden M. Prostaglandin E2 suppresses bacterial killing in alveolar macrophages by inhibiting NADPH oxidase. *Am J Respir Cell Mol Biol* 2007;37:562–570.
 32. Nokta MA, Pollard RB. Human immunodeficiency virus replication: modulation by cellular levels of cAMP. *AIDS Res Hum Retroviruses* 1992;8:1255–1261.
 33. Fulop T Jr, Foris G, Worum I, Leovey A. Age-dependent alterations of Fc gamma receptor-mediated effector functions of human polymorphonuclear leukocytes. *Clin Exp Immunol* 1985;61:425–432.
 34. Wirth JJ, Kierszenbaum F. Macrophage mediation of the inhibitory effects of elevated intracellular levels of adenosine-3':5' cyclic monophosphate (cAMP) on macrophage-*Trypanosoma cruzi* association. *Int J Parasitol* 1984;14:401–404.
 35. Lin P, Welch EJ, Gao XP, Malik AB, Ye RD. Lysophosphatidylcholine modulates neutrophil oxidant production through elevation of cyclic AMP. *J Immunol* 2005;174:2981–2989.
 36. Bengis-Garber C, Gruener N. Protein kinase A downregulates the phosphorylation of p47 phox in human neutrophils: a possible pathway for inhibition of the respiratory burst. *Cell Signal* 1996;8:291–296.
 37. O'Dowd YM, El-Benna J, Perianin A, Newsholme P. Inhibition of formyl-methionyl-leucyl-phenylalanine-stimulated respiratory burst in human neutrophils by adrenaline: inhibition of Phospholipase A2 activity but not p47phox phosphorylation and translocation. *Biochem Pharmacol* 2004;67:183–190.
 38. Chang YC, Li PC, Chen BC, Chang MS, Wang JL, Chiu WT, Lin CH. Lipoteichoic acid-induced nitric oxide synthase expression in RAW 264.7 macrophages is mediated by cyclooxygenase-2, prostaglandin E2, protein kinase A, p38 MAPK, and nuclear factor-kappaB pathways. *Cell Signal* 2006;18:1235–1243.
 39. Chen CC, Chiu KT, Sun YT, Chen WC. Role of the cyclic AMP-protein kinase A pathway in lipopolysaccharide-induced nitric oxide synthase expression in RAW 264.7 macrophages: involvement of cyclooxygenase-2. *J Biol Chem* 1999;274:31559–31564.
 40. Won JS, Im YB, Singh AK, Singh I. Dual role of cAMP in iNOS expression in glial cells and macrophages is mediated by differential regulation of p38-MAPK/ATF-2 activation and iNOS stability. *Free Radic Biol Med* 2004;37:1834–1844.
 41. Mustafa SB, Olson MS. Expression of nitric-oxide synthase in rat Kupffer cells is regulated by cAMP. *J Biol Chem* 1998;273:5073–5080.
 42. Kalamidas SA, Kuehnelt MP, Peyron P, Rybin V, Rauch S, Kotoulas OB, Houslay M, Hemmings BA, Gutierrez MG, Anes E, et al. cAMP synthesis and degradation by phagosomes regulate actin assembly and fusion events: consequences for mycobacteria. *J Cell Sci* 2006;119:3686–3694.
 43. Di A, Brown ME, Deriy LV, Li C, Szeto FL, Chen Y, Huang P, Tong J, Naren AP, Bindokas V, et al. CFTR regulates phagosome acidification in macrophages and alters bactericidal activity. *Nat Cell Biol* 2006;8:933–944.
 44. Pryzwansky KB, Kidao S, Merricks EP. Compartmentalization of PDE-4 and cAMP-dependent protein kinase in neutrophils and macrophages during phagocytosis. *Cell Biochem Biophys* 1998;28:251–275.
 45. Brock TG, Serezani CH, Carstens JK, Peters-Golden M, Aronoff DM. Effects of prostaglandin E(2) on the subcellular localization of Epac-1 and Rap1 proteins during Fc gamma-receptor-mediated phagocytosis in alveolar macrophages. *Exp Cell Res* 2008;314:255–263.
 46. Vojtova J, Kamanova J, Sebo P. Bordetella adenylate cyclase toxin: a swift saboteur of host defense. *Curr Opin Microbiol* 2006;9:69–75.
 47. Katada T, Ui M. Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein. *Proc Natl Acad Sci USA* 1982;79:3129–3133.
 48. Hiemstra PS, Annema A, Schippers EF, van Furth R. Pertussis toxin partially inhibits phagocytosis of immunoglobulin G-opsonized *Staphylococcus aureus* by human granulocytes but does not affect intracellular killing. *Infect Immun* 1992;60:202–205.
 49. Schaeffer LM, Weiss AA. Pertussis toxin and lipopolysaccharide influence phagocytosis of *Bordetella pertussis* by human monocytes. *Infect Immun* 2001;69:7635–7641.
 50. Kirimanjeswara GS, Agosto LM, Kennett MJ, Bjornstad ON, Harvill ET. Pertussis toxin inhibits neutrophil recruitment to delay antibody-mediated clearance of *Bordetella pertussis*. *J Clin Invest* 2005;115:3594–3601.
 51. Confer DL, Eaton JW. Phagocyte impotence caused by an invasive bacterial adenylate cyclase. *Science* 1982;217:948–950.
 52. Pearson RD, Symes P, Conboy M, Weiss AA, Hewlett EL. Inhibition of monocyte oxidative responses by *Bordetella pertussis* adenylate cyclase toxin. *J Immunol* 1987;139:2749–2754.
 53. Ahuja N, Kumar P, Bhatnagar R. The adenylate cyclase toxins. *Crit Rev Microbiol* 2004;30:187–196.
 54. Vanden Broeck D, Horvath C, De Wolf MJ. *Vibrio cholerae*: cholera toxin. *Int J Biochem Cell Biol* 2007;39:1771–1775.
 55. Niemaltowski M, Klucinski W, Malicki K, de Faundez IS. Cholera toxin (cholera toxin)-polymorphonuclear leukocyte interactions: effect on migration in vitro and Fc gamma R-dependent phagocytic and bactericidal activity. *Microbiol Immunol* 1993;37:55–62.
 56. Braun MC, He J, Wu CY, Kelsall BL. Cholera toxin suppresses interleukin (IL)-12 production and IL-12 receptor beta1 and beta2 chain expression. *J Exp Med* 1999;189:541–552.

57. Bergman MJ, Guerrant RL, Murad F, Richardson SH, Weaver D, Mandell GL. Interaction of polymorphonuclear neutrophils with *Escherichia coli*. Effect of enterotoxin on phagocytosis, killing, chemotaxis, and cyclic AMP. *J Clin Invest* 1978;61:227-234.
58. Hosono K, Suzuki H. Morphological transformation of Chinese hamster cells by acylpeptides, inhibitors of cAMP phosphodiesterase, produced by *Bacillus subtilis*. *J Biol Chem* 1985;260:11252-11255.
59. Uchiya K, Groisman EA, Nikai T. Involvement of *Salmonella* pathogenicity island 2 in the up-regulation of interleukin-10 expression in macrophages: role of protein kinase A signal pathway. *Infect Immun* 2004;72:1964-1973.
60. Jimenez de Bagues MP, Dudal S, Dornand J, Gross A. Cellular bioterrorism: how *Brucella* corrupts macrophage physiology to promote invasion and proliferation. *Clin Immunol* 2005;114:227-238.
61. Oberholzer M, Marti G, Baresic M, Kunz S, Hemphill A, Seebeck T. The *Trypanosoma brucei* cAMP phosphodiesterases TbrPDEB1 and TbrPDEB2: flagellar enzymes that are essential for parasite virulence. *FASEB J* 2007;21:720-731.
62. Aandahl EM, Aukrust P, Skalleberg BS, Muller F, Froland SS, Hansson V, Tasken K. Protein kinase A type I antagonist restores immune responses of T cells from HIV-infected patients. *FASEB J* 1998;12:855-862.
63. Azzam R, Kedzierska K, Leeansyah E, Chan H, Doischer D, Gorry PR, Cunningham AL, Crowe SM, Jaworowski A. Impaired complement-mediated phagocytosis by HIV type-1-infected human monocyte-derived macrophages involves a cAMP-dependent mechanism. *AIDS Res Hum Retroviruses* 2006;22:619-629.
64. Thomas CA, Weinberger OK, Ziegler BL, Greenberg S, Schieren I, Silverstein SC, El Khoury J. Human immunodeficiency virus-1 env impairs Fc receptor-mediated phagocytosis via a cyclic adenosine monophosphate-dependent mechanism. *Blood* 1997;90:3760-3765.
65. Ramis I, Rosello-Catafau J, Gomez G, Zabay JM, Fernandez Cruz E, Gelpi E. Cyclooxygenase and lipoxygenase arachidonic acid metabolism by monocytes from human immune deficiency virus-infected drug users. *J Chromatogr* 1991;557:507-513.
66. Foley P, Kazazi F, Biti R, Sorrell TC, Cunningham AL. HIV infection of monocytes inhibits the T-lymphocyte proliferative response to recall antigens, via production of eicosanoids. *Immunology* 1992;75:391-397.
67. Hodge S, Hodge G, Ahern J, Jersmann H, Holmes M, Reynolds PN. Smoking alters alveolar macrophage recognition and phagocytic ability: implications in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2007;37:748-755.
68. Cayeux SJ, Beverley PC, Schulz R, Dorken B. Elevated plasma prostaglandin E2 levels found in 14 patients undergoing autologous bone marrow or stem cell transplantation. *Bone Marrow Transplant* 1993;12:603-608.
69. Ballinger MN, Aronoff DM, McMillan TR, Cooke KR, Olkiewicz K, Toews GB, Peters-Golden M, Moore BB. Critical role of prostaglandin E2 overproduction in impaired pulmonary host response following bone marrow transplantation. *J Immunol* 2006;177:5499-5508.
70. Bjornson AB, Knippenberg RW, Bjornson HS. Bactericidal defect of neutrophils in a guinea pig model of thermal injury is related to elevation of intracellular cyclic-3',5'-adenosine monophosphate. *J Immunol* 1989;143:2609-2616.
71. Bjornson AB, Knippenberg RW, Bjornson HS. Nonsteroidal anti-inflammatory drugs correct the bactericidal defect of polymorphonuclear leukocytes in a guinea pig model of thermal injury. *J Infect Dis* 1988;157:959-967.
72. Stapleton PP, Fujita J, Murphy EM, Naama HA, Daly JM. The influence of restricted calorie intake on peritoneal macrophage function. *Nutrition* 2001;17:41-45.
73. Redmond HP, Shou J, Kelly CJ, Schreiber S, Miller E, Leon P, Daly JM. Immunosuppressive mechanisms in protein-calorie malnutrition. *Surgery* 1991;110:311-317.
74. Starczewski M, Voigtman R, Peskar BA, Peskar BM. Plasma levels of 15-keto-13,14-dihydro-prostaglandin E2 in patients with bronchogenic carcinoma. *Prostaglandins Leukot Med* 1984;13:249-258.
75. Lu MC, Peters-Golden M, Hostetler DE, Robinson NE, Derksen FJ. Age-related enhancement of 5-lipoxygenase metabolic capacity in cattle alveolar macrophages. *Am J Physiol* 1996;271:L547-L554.
76. Hayek MG, Mura C, Wu D, Beharka AA, Han SN, Paulson KE, Hwang D, Meydani SN. Enhanced expression of inducible cyclooxygenase with age in murine macrophages. *J Immunol* 1997;159:2445-2451.
77. Strandvik B, Svensson E, Seyberth HW. Prostanoid biosynthesis in patients with cystic fibrosis. *Prostaglandins Leukot Essent Fatty Acids* 1996;55:419-425.
78. Medjane S, Raymond B, Wu Y, Touqui L. Impact of CFTR DeltaF508 mutation on prostaglandin E2 production and type IIA phospholipase A2 expression by pulmonary epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L816-L824.
79. Lands LC, Milner R, Cantin AM, Manson D, Corey M. High-dose ibuprofen in cystic fibrosis: Canadian safety and effectiveness trial. *J Pediatr* 2007;151:249-254.
80. Konstan MW, Schluchter MD, Xue W, Davis PB. Clinical use of ibuprofen is associated with slower FEV1 decline in children with cystic fibrosis. *Am J Respir Crit Care Med* 2007;176:1084-1089.
81. Maris NA, Florquin S, van't Veer C, de Vos AF, Buurman W, Jansen HM, van der Poll T. Inhalation of beta 2 agonists impairs the clearance of nontypable *Haemophilus influenzae* from the murine respiratory tract. *Respir Res* 2006;7:57.
82. Calverley PM, Rennard SI. What have we learned from large drug treatment trials in COPD? *Lancet* 2007;370:774-785.
83. Aronoff DM, Canetti C, Peters-Golden M. Prostaglandin E2 inhibits alveolar macrophage phagocytosis through an E-prostanoid 2 receptor-mediated increase in intracellular cyclic AMP. *J Immunol* 2004;173:559-565.
84. Bloodstream infections among patients treated with intravenous epo-prostenol or intravenous treprostinil for pulmonary arterial hypertension—seven sites, United States, 2003-2006. *MMWR Morb Mortal Wkly Rep* 2007;56:170-172.
85. Aronoff DM, Peres CM, Serezani CH, Ballinger MN, Carstens JK, Coleman N, Moore BB, Peebles RS, Faccioli LH, Peters-Golden M. Synthetic prostacyclin analogs differentially regulate macrophage function via distinct analog-receptor binding specificities. *J Immunol* 2007;178:1628-1634.
86. Rangel Moreno J, Estrada Garcia I, De La Luz Garcia Hernandez M, Aguilar Leon D, Marquez R, Hernandez Pando R. The role of prostaglandin E2 in the immunopathogenesis of experimental pulmonary tuberculosis. *Immunology* 2002;106:257-266.
87. Guimaraes ET, Santos LA, Ribeiro dos Santos R, Teixeira MM, dos Santos WL, Soares MB. Role of interleukin-4 and prostaglandin E2 in *Leishmania amazonensis* infection of BALB/c mice. *Microbes Infect* 2006;8:1219-1226.
88. Terrazas LI, Bojalil R, Rodriguez-Sosa M, Govezensky T, Larralde C. *Taenia crassiceps* cysticercosis: a role for prostaglandin E2 in susceptibility. *Parasitol Res* 1999;85:1025-1031.