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ORIGINAL ARTICLE

Phosphodiesterase 4B is Essential for Lipopolysaccharide-induced CC Chemokine Ligand 3 Production in Mouse Macrophages

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Background: Phosphodiesterase 4 (PDE4) inhibitors negatively modulate many inflammatory responses, and some of these pharmacological effects are mediated by inhibition of PDE4B in inflammatory cells. While inactivation of PDE4B, but not other PDE4 isotypes, is known to inhibit lipopolysaccharide (LPS)-induced tumor necrosis factor-α (TNF-α) production in macrophages, a cell type critical in mediating innate immunity, the impact of PDE4B on many other inflammatory responses in these cells remains largely unknown. **Materials and Methods:** To investigate whether PDE4B regulates additional inflammatory mediators other than TNF-α, in this study we initially used two-dimensional gel electrophoresis approach to screen the secreted proteins that are modulated by the PDE4 inhibitor rolipram in LPS-stimulated Raw 264.7 macrophages. **Results:** Three proteins were identified, of which the proinflammatory chemokine CC chemokine ligand 3 (CCL3) and cytokine TNF-α were downregulated and the antiinflammatory cytokine interleukin-1 receptor antagonist was upregulated. Further analysis on CCL3 production in mouse peritoneal macrophages revealed that the reduced CCL3 secretion was associated with a substantial decrease in CCL3 mRNA accumulation. The inhibitory effect of rolipram on CCL3 production was mimicked by the protein kinase A activator 6-Bnz-cAMP, but not the exchange protein directly activated by cAMP activator 8-pCPT-2'-O-Me-cAMP. Analysis of PDE4-deficient macrophages showed that ablation of only PDE4B reproduced the rolipram effect on CCL3 production. Moreover, PDE4 inhibitor potentially attenuates T-cell migration to CCL3 in inflammatory sites. **Conclusions:** These findings suggest that PDE4B may regulate the production of diverse inflammatory mediators in LPS-stimulated macrophages, and an inhibitor with PDE4B selectivity should retain the anti-inflammatory effects of nonselective PDE4 inhibitors in endotoxin-induced inflammatory conditions.

Key words: Phosphodiesterase 4B, CC chemokine ligand 3, macrophage inflammatory protein-1α, macrophage, lipopolysaccharide

INTRODUCTION

Macrophages are the key player of the innate immune system. These cells reside in body tissues, where they phagocytize and process pathogens, engulf dead cells, and produce a myriad of immune effector molecules. They can be activated by numerous external stimuli, among which lipopolysaccharide (LPS), one outer membrane component of the Gram-negative bacteria, is known to induce strong immune responses in these cells. By interaction with toll-like receptor 4, LPS activates both MyD88- and TRIF-dependent signal pathways,[1,2] leading to production of various inflammatory cytokines and chemokines.[3] Through actions of these effector molecules, macrophages can protect hosts from infectious diseases, but they also may cause detrimental effects such as sepsis and other inflammatory conditions.[4]

Elevation of the second messenger cyclic adenosine monophosphate (cAMP) in macrophages suppresses several inflammatory responses, including inflammatory mediator production and receptor-mediated phagocytosis.[5] The cAMP effectors protein kinase A (PKA) and exchange proteins directly activated by cAMP (Epac) are thought to mediate these inhibitory effects.[5] By increasing intracellular cAMP level, phosphodiesterase 4 (PDE4) inhibitors are being developed as anti-inflammatory agents for the treatment

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of chronic inflammatory disorders such as asthma, chronic obstructive pulmonary disease, and psoriasis.[6-8] While exerting clinical benefits, these agents have adverse effects (nausea, emesis, and diarrhea) that limit their dosing and therefore, clinical efficacy.[9] The PDE4 isozymes in mammals are encoded by four genes termed PDE4A-4D, and each gene has unique, nonredundant role in regulation of cell functions.[10,11] In monocytes and macrophages, it has been well documented that inhibition of PDE4 effectively suppresses LPS-induced tumor necrosis factor-α (TNF-α) production,[12-16] and this effect is mediated through inhibition of PDE4B.[17,18] Thus, it was hypothesized that targeting a single PDE4B subtype may retain efficacy but reduce the side effects of nonselective PDE4 inhibitors. To date, LPS is known to induce expression of many cytokines and chemokines in macrophages and some of them are regulated by PDE4 inhibitors.[19,20] However, the exact impact of individual PDE4 subtypes on the production of these inflammatory mediators remains largely unknown.

To investigate whether PDE4B regulates the expression of additional inflammatory mediators other than TNF-α and whether inhibition of PDE4B is sufficient to block these responses in macrophages, in this study we used two-dimensional (2D) gel electrophoresis to screen the secreted proteins that are modulated by the PDE4 inhibitor rolipram in LPS-stimulated Raw 264.7 cells. We found that in addition to TNF-α, the proinflammatory chemokine CC chemokine ligand 3 (CCL3) also was downregulated by rolipram whereas the anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1Ra) was upregulated. We further demonstrated that the inhibition of CCL3 production by rolipram was mediated by inhibition of PDE4B and activation of the PKA signal pathway in mouse peritoneal macrophages. Other PDE4 isoforms expressed in macrophages (PDE4A and PDE4D) were not involved in the regulation of this response.

MATERIALS AND METHODS

Reagents

The PDE4 inhibitor rolipram, dibutyryl-cAMP, and LPS were obtained from Sigma-Aldrich (MO, USA), and 6-Bnz-cAMP, 8-pCPT-2'-O-Me-cAMP, and Rp-8-CPT-cAMPS were from BioLog (Bremen, Germany). Fetal bovine serum (FBS) was purchased from Thermo Scientific/Hyclone (Utah, USA), and DMEM and RPMI 1640 medium were from Life Technologies (NY, USA).

Cell line

Raw 264.7, a murine macrophage cell line, was obtained from Bioresource Collection and Research Center (Taiwan). Cells were maintained at 37°C (5% CO_2) in DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FBS (complete medium). For 2D gel analysis, cells were plated at 1 × 10^6/ml in 10-cm petri dish, cultured in the complete medium overnight, and then treated with LPS (100 ng/ml) in the absence or presence of rolipram (10 μM) for 6 h in the medium without serum. For quantitative reverse transcription polymerase chain reaction (PCR) analysis on CCL3 mRNA expression, the same procedures were performed except that the cells were incubated with LPS and drugs for 3 h in the complete medium. To assess the effect of LPS on CCL3 release, Raw 264.7 cells were plated at 2.5 × 10^5/ml in 96-well plate, grown overnight, and then incubated with LPS for desired times. The medium supernatant was collected for CCL3 ELISA.

Mice

The generation of mice deficient in PDE4A, PDE4B, and PDE4D has been described previously.[17,21,22] The PDE4A and PDE4B null mice have been backcrossed from the mixed 129/Ola × C57Bl/6 to the pure C57BL/6 background for at least 12 generations, whereas the PDE4D mice were kept in the mixed background because the PDE4D null mice become embryonic lethal when the null allele is transferred to the pure C57Bl/6 background. Mice used were 2-5 months of age. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at the authors' institute.

Protein sample preparation and two-dimensional gel electrophoresis

Following the incubation of Raw 264.7 cells with LPS (100 ng/ml) in the absence or presence of the PDE4 inhibitor rolipram (10 μM) for 6 h, the culture media were harvested and centrifuged at 4°C, 150 ×*g* for 10 min. Proteins in the supernatants were precipitated in 10% trichloroacetic acid followed by solubilizing in a sample buffer for 2D gel analysis. Detail methods are given in Online Supplementary Information.

Protein identification

Any protein spot that showed an apparent difference in signal intensity on 2D gels between the conditions with or without rolipram treatment was selected for further protein identification. Proteins in the spots were in-gel digested, and peptide-mass fingerprints was obtained by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonic, MA, USA). Spots that could not be identified by the MALDI-TOF method were further analyzed by N-terminal peptide sequencing. Detail methods are given in Online Supplementary Information.

Isolation and purification of peritoneal macrophages

Peritoneal macrophages were isolated and purified from PDE4-deficient mice and their corresponding wild-type mice, and cultured in the RPMI 1640 complete medium as described previously.[18] Cells were plated at 5 × 10^5/ml in 96-well plates, cultured overnight, and then incubated with LPS in the absence or presence of rolipram and cAMP analogs. The medium supernatant was collected for CCL3 ELISA.

RNA isolation, cDNA synthesis, and quantitative polymerase chain reaction

For quantitative measurement of CCL3 expression, total RNA was extracted from Raw 264.7 cells with the TRIzol reagent (Invitrogen Life Technologies, CA, USA) following the manufacturer's instructions. First strand cDNA was synthesized from 0.5 to 1 μg of total RNA in the presence of random primer using M-MLV reverse transcriptase according to the manufacturer's protocol (Invitrogen Life Technologies, CA, USA). Quantitative PCR (qPCR) was performed on the iQ5 real-time PCR Detection System (Bio-Rad, CA, USA). Detail methods are given in Online Supplementary Information.

CC chemokine ligand 3 ELISA

Levels of CCL3 in macrophage culture supernatants were measured with commercially available ELISA kit (R&D Systems, MN, USA). The sensitivity of the assay was 7.8 pg/ml.

Splenic T-cell preparation and chemotaxis assay

Splenic T cells were isolated from C57Bl/6 mice as detailed in Online Supplementary Information. T cells migration was performed in 24-transwell plates by placing the T cells in the upper chamber and recombinant murine CCL3 in the lower chamber. Detail methods are given in Online Supplementary Information.

RESULTS

Identification of phosphodiesterase 4-modulated proteins secreted from lipopolysaccharide-stimulated Raw 264.7 macrophages

Phosphodiesterase 4 inhibitors such as roflumilast have been shown to inhibit the release of several inflammatory cytokines and chemokines in LPS-stimulated macrophages.[20] To more systematically study the impact of PDE4 on the production of inflammatory mediators, we used a proteomic approach to screen the secreted proteins in Raw 264.7 macrophages stimulated with LPS in the presence or absence of the PDE4 inhibitor rolipram. The secreted proteins in the culture medium were profiled using 2D gel electrophoresis. Five spots with disparate stain intensities in the paired gels were consistently observed even without the aid of a 2D gel analysis software [Figure 1]. Among them, three spots could be identified by MALDI-TOF mass spectrometry after in-gel digestion of the proteins with trypsin. These proteins are TNF-α (for both spots 1 and 2) and IL-1Ra (spot 3), which are downregulated and upregulated by rolipram, respectively. By N-terminal peptide sequencing, spot 4 was identified as CCL3 (also known as macrophage inflammatory protein-1α), which is downregulated by PDE4 inhibition. The identity of spot 5 could not be resolved by either of the two methods used.

Inhibition of phosphodiesterase 4 suppresses lipopolysaccharide-induced CC chemokine ligand 3 production in mouse macrophages

To determine the time and dose effects of LPS on CCL3 release, Raw 264.7 and mouse peritoneal macrophages were incubated with LPS at different concentrations and times, and the levels of CCL3 in the culture medium were measured by ELISA. As shown in Figure 2, in the presence of 100 ng/ml of LPS, CCL3 release was increased with time up to 24 h in Raw 264.7 cells [Figure 2a], whereas the increase in the primary macrophages reached a maximum in 8 h and declined thereafter [Figure 2b]. Additionally, both cells showed a dose-dependent release of CCL3 to LPS stimulation [Figure 2c-d].

To confirm the impact of PDE4 on CCL3 production, mouse peritoneal macrophages were stimulated with 100 ng/ml of LPS in the presence of rolipram for 8 h. The CCL3 secretion

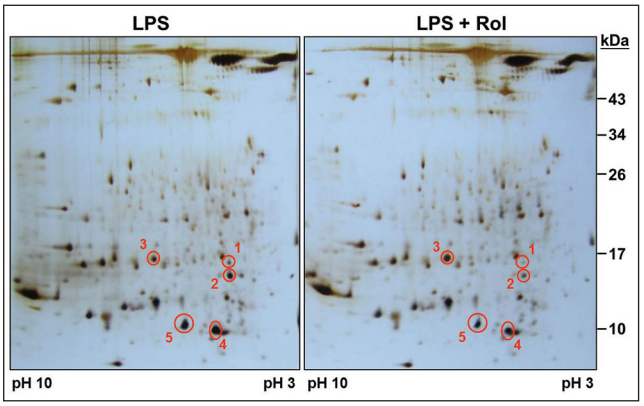

Figure 1. Representative two-dimensional-gel profiles of secreted proteins from lipopolysaccharide (LPS)-stimulated Raw 264.7 macrophages. Raw 264.7 cells were pretreated for 20 min with rolipram (10 μM) or vehicle (DMSO) before LPS (100 ng/ml) stimulation for 6 h. Medium proteins were separated by isoelectric focusing (pH 3-10) and then 15% SDS-PAGE followed by silver stain as described under Materials and Methods. The spots of up- or down-regulated proteins are denoted by numbered circles. One of three independent experiments is shown

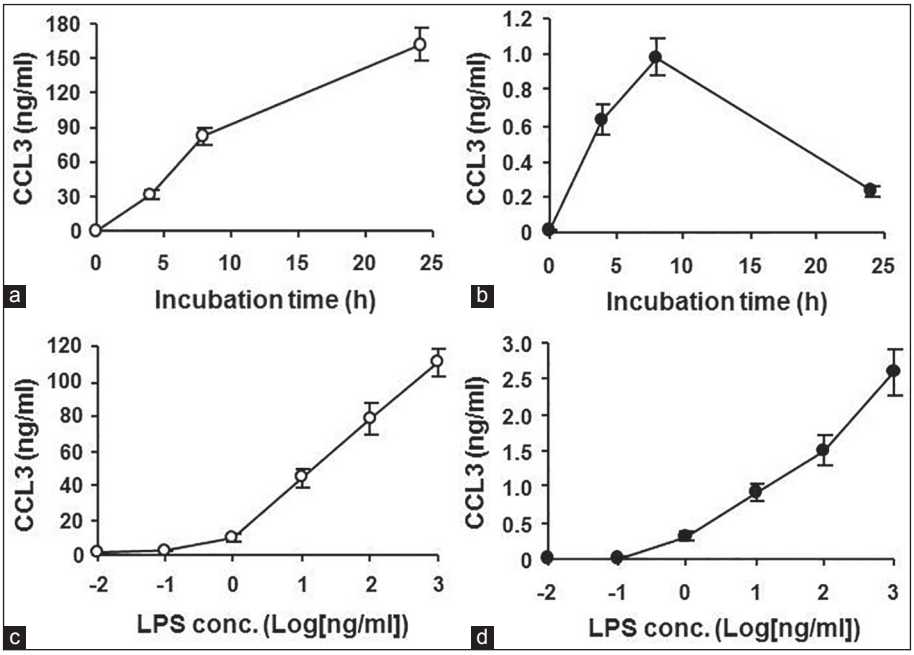

Figure 2. Time- and dose-dependent CC chemokine ligand 3 (CCL3) secretion in LPS-stimulated mouse macrophages. Raw 264.7 cells (a and c) and peritoneal macrophages from C57Bl/6 mice (b and d) were incubated with LPS (100 ng/ml) for the indicated times (a and b) or increasing concentrations of LPS for 8 h (c and d). Accumulation of CCL3 in the medium was measured by ELISA. Data are the mean ± SEM (n = 5-6 in a and c; n = 10-12 in b and d)

was dose-dependently inhibited by rolipram with the IC_{50} of approximately 0.1 μM [Figure 3a]. A similar IC_{50} value also was obtained for Raw 264.7 cells (data not shown). Further qPCR analysis of CCL3 mRNA level in Raw 264.7 cells revealed that these cells expressed limited amount of CCL3 mRNA unless they were stimulated with LPS. The mRNA induction by LPS was significantly decreased ($P < 0.01$) when the cells were preincubated with rolipram for 20 min, reaching approximately 75% inhibition [Figure 3b]. These results indicate that the decrease in CCL3 protein secretion by rolipram is associated with an altered steady state level of CCL3 mRNA and that the regulation of PDE4 on CCL3 production and secretion is mainly at the level of transcription or mRNA stability.

Ablation of phosphodiesterase 4B reduces lipopolysaccharide-induced CC chemokine ligand 3 production in macrophages

To date, most PDE4 inhibitors, including rolipram, are nonselective for PDE4 isotypes due to the presence of a highly conserved catalytic domain in these isozymes.[11] Thus, it is difficult to dissect the functional role of individual PDE4 subtypes by the pharmacological approach. Macrophages are known to express three PDE4 isoforms (PDE4A, 4B, and 4D) under a basal or LPS-stimulated condition.[18] To determine which PDE4 isoform impacts on LPS stimulation of CCL3 production, peritoneal macrophages isolated from PDE4A, 4B, and 4D null mice and their corresponding wild-type mice were incubated with 100 ng/ml of LPS for 8 h, and the levels of CCL3 in the medium were measured. As shown in Figure 4, LPS induced CCL3 release in all three wild-type macrophages and this induction was markedly inhibited by 10 μM of rolipram ($P < 0.001$). PDE4A$^{-/-}$ and PDE4D$^{-/-}$ macrophages responded to LPS stimulation as well as rolipram inhibition in a manner similar to their wild-type counterparts. Contrarily, LPS-induced CCL3 production was significantly decreased in PDE4B$^{-/-}$ macrophages ($P < 0.01$), exerting 62% decrease compared to the PDE4B$^{+/+}$ macrophages. Additionally, rolipram had no significant effects in the PDE4B$^{-/-}$ cells [Figure 4b], indicating that pharmacological inhibition of PDE4A and PDE4D in the PDE4B$^{-/-}$ macrophages has little or no effect on LPS-induced CCL3 production. These data also demonstrated that rolipram blocks CCL3 response is mediated mostly by inhibition of PDE4B in macrophages.

Cyclic adenosine monophosphate/protein kinase A signaling mediates the effect of phosphodiesterase 4 inhibition on lipopolysaccharide-induced CC chemokine ligand 3 production

The effect of cAMP on inflammatory cytokine and chemokine production can be mediated by activation of PKA or Epac.[5] To determine whether these signal pathways mediate the effect of PDE4 inhibitors or PDE4B ablation on CCL3 production, mouse peritoneal macrophages were stimulated

with LPS in the presence of the cAMP analog dibutyryl-cAMP (db-cAMP), the Epac activator 8-pCPT-2′-O-Me-cAMP or the PKA activator 6-Bnz-cAMP. Figure 5 shows that db-cAMP dose-dependently inhibited LPS-induced CCL3 secretion, and its inhibition at 500 μM reached to the level similar to that of 10 μM rolipram [Figure 5a]. A similar inhibition was also observed when 6-Bnz-cAMP, but not 8-pCPT-2′-O-Me-cAMP, was present [Figure 5b and c], suggesting that the inhibitory effect of rolipram or PDE4B ablation on the CCL3 release is mediated by cAMP-PKA but not cAMP-Epac pathway. Further analysis using the PKA inhibitor Rp-8-CPT-cAMPS revealed that the inhibitory effect of rolipram on LPS-induced CCL3 secretion was partially reversed, suggesting that the rolipram effect is not fully dependent on PKA activity [Figure 5d].

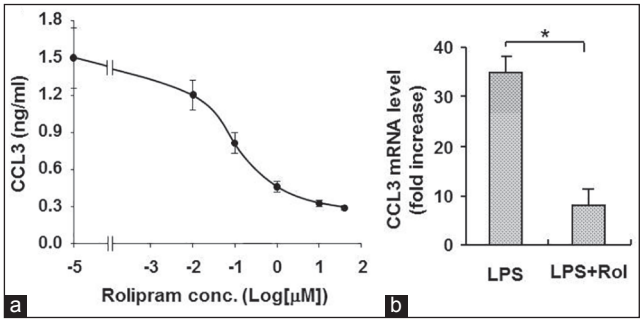

Figure 3. Effect of rolipram on lipopolysaccharide (LPS)-induced CC chemokine ligand 3 (CCL3) secretion and mRNA expression in mouse macrophages. Mouse peritoneal macrophages (a) and Raw 264.7 cells (b) were pretreated for 20 min with increasing concentrations of rolipram (a) or 10 μM rolipram (b) before LPS (100 ng/ml) stimulation for 8 h (a) or 3 h (b). CCL3 accumulation in the medium was measured by ELISA (a). CCL3 mRNA levels in the cells were determined by quantitative polymerase chain reaction and expressed as fold increase to the untreated cells (b). Data are the mean ± SEM (n = 9 in a; n = 3 in b). *P < 0.01

T-cell migration to CC chemokine ligand 3 is reduced by phosphodiesterase 4 inhibitor

CC chemokine ligand 3 attracts different immune cells, including T lymphocytes, to sites of inflammation through activation of the chemokine receptors CCR1 and CCR5.[23,24] In addition to the regulation of CCL3 production in macrophages, the role of PDE4 in T-cell chemotaxis to CCL3 was also assessed. Splenic T cells isolated from C57Bl/6 mice were tested in a Boyden two-chamber chemotactic assay. In the presence of CCL3, these T cells exhibited a concentration-dependent transmigration with an EC_{50} of approximately 30 ng/ml (data not shown). When the cells were preincubated with 10 μM rolipram for 20 min, the CCL3-induced migration was significantly decreased (P < 0.005) [Figure 6], indicating that PDE4 activity is necessary for efficient chemotaxis of T cells to CCL3.

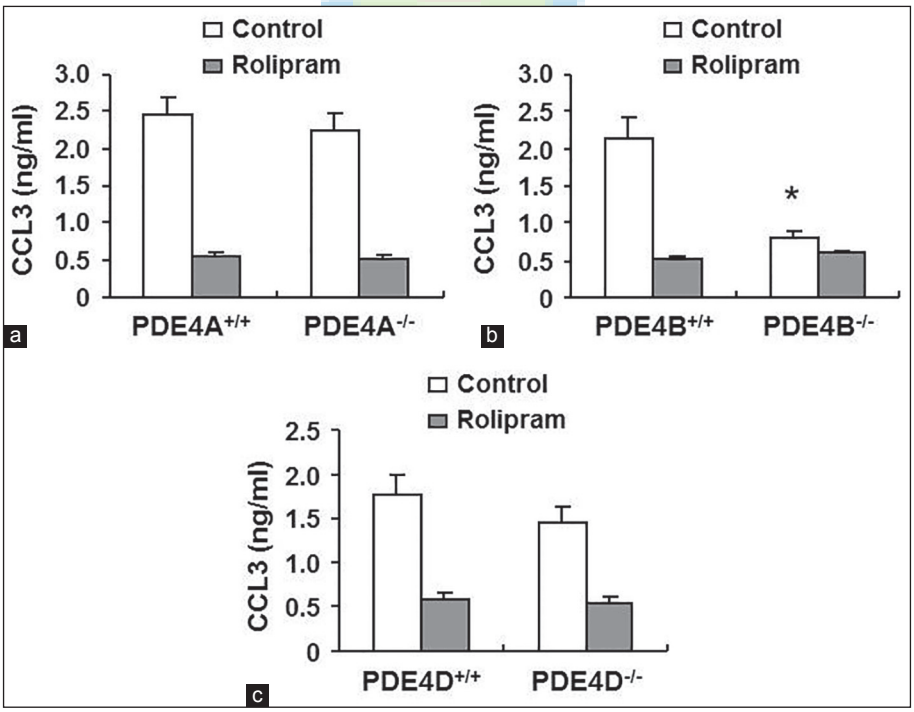

Figure 4. Effect of phosphodiesterase 4 (PDE4) inhibition on lipopolysaccharide (LPS)-induced CC chemokine ligand 3 (CCL3) production in PDE4-deficient macrophages. Peritoneal macrophages from PDE4A$^{-/-}$ (a), PDE4B$^{-/-}$ (b), and PDE4D$^{-/-}$ mice (c) and their wild-type counterparts were incubated with 10 μM rolipram or vehicle for 20 min before LPS (100 ng/ml) stimulation for 8 h. CCL3 accumulation in the medium was determined by ELISA. Data are the mean ± SEM (n = 6-9 mice/group). *P < 0.01, compared with PDE4B$^{+/+}$ control

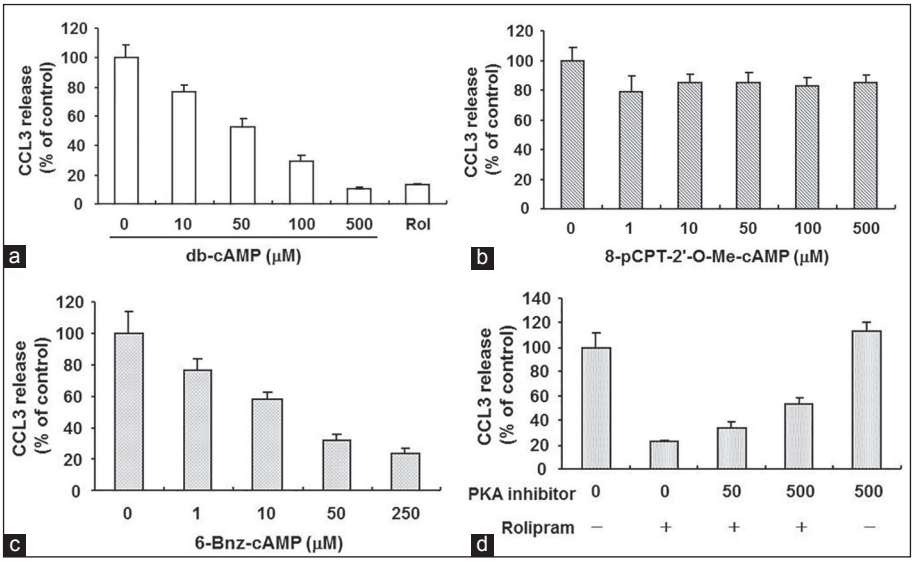

Figure 5. Effect of cyclic adenosine monophosphate (cAMP) signaling on lipopolysaccharide (LPS)-induced CC chemokine ligand 3 (CCL3) production in mouse macrophages. Peritoneal macrophages from C57Bl/6 mice were incubated for 20 min with 10 μM rolipram (a and d) or increasing concentrations of dibutyryl-cAMP (db-cAMP) (a), the exchange proteins directly activated by cAMP activator 8-pCPT-2′-O-Me-cAMP (b), the protein kinase A (PKA) activator 6-Bnz-cAMP (c), or the PKA inhibitor Rp-8-CPT-cAMPS (d) before LPS (100 ng/ml) stimulation for 8 h. CCL3 accumulation in the medium was measured by ELISA. Data are the mean ± SEM (n = 3-12)

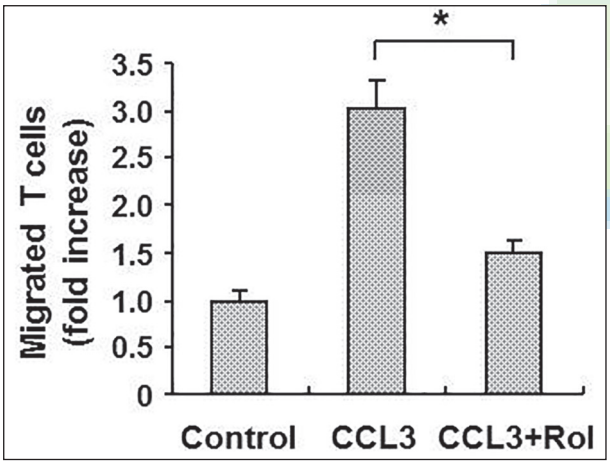

Figure 6. Phosphodiesterase 4 inhibitor attenuated CC chemokine ligand 3 (CCL3)-induced T-cell chemotaxis. Splenic T-cell from C57Bl/6 mice were treated with 10 μM rolipram or vehicle for 20 min before transferring to the upper chamber of the 24-transwell plate for chemotaxis assay. The assay was performed as described under materials and methods. The number of migrated T-cell was expressed as fold increase to the control where T-cell were not treated with 100 ng/ml CCL3. Data are the mean ± SEM (n = 7). *P < 0.01

DISCUSSION

In this study, we identified three secreted proteins that are regulated by inhibition of PDE4 in LPS-stimulated macrophages using a proteomics approach. Among them, the proinflammatory cytokine TNF-α and chemokine CCL3 were downregulated and the antiinflammatory cytokine IL-1Ra was upregulated. This decrease in proinflammatory cytokines and increase in the antiinflammatory cytokine once again point to the anti-inflammatory action of PDE4 inhibitors in inflammation. Of the three proteins identified, TNF-α regulation by PDE4B has been studied extensively,[12-16] whereas the information on CCL3 and IL-1Ra regulation by PDE4 subtypes remains largely unclear. In this study, we focused our efforts on understanding how PDE4, or more specifically PDE4B, affects CCL3 production and action in inflammatory cells. CCL3 is a member of the C-C subfamily chemokines. It is upregulated by inflammatory stimuli and induces leukocyte recruitment to inflamed tissues via interacting with two chemokine receptors CCR1 and CCR5 on inflammatory cell surface.[23,24]

The time course study of LPS-induced CCL3 production showed that CCL3 accumulation in the culture medium of Raw 264.7 cells was increased with time up to 24 h whereas the increase in the peritoneal macrophages only reached a maximum in 8 h and declined significantly thereafter [Figure 2a and b]. While it needs further investigation, the discrepancy between the two macrophages might be explained by the nature of continuing proliferation of Raw 264.7 cells, but not of primary cells, to increase cell number in contributing to CCL3 secretion and accumulation along the incubation time. Moreover, the low level of CCL3 accumulation observed in peritoneal macrophages after overnight LPS treatment is a phenomenon also noted previously by other groups.[25,26]

Using PDE4-deficient macrophages and their wild-type counterparts, we demonstrated that the pharmacological effect of rolipram on LPS-induced CCL3 production is exerted

mainly through inhibition of PDE4B, one of the three PDE4 subtypes expressed in macrophages.[18] Ablation of PDE4A or PDE4D has little or no effect on the CCL3 release. These results resemble our previous findings that of the three PDE4 subtypes, only PDE4B ablation suppresses LPS-induced TNF-α production,[18] suggesting that the expression of both TNF-α and CCL3 in macrophages is triggered by the same TLR signal pathways and modulated by PDE4B or cAMP signaling in a similar fashion.

Elevation of cAMP in leukocytes can activate the downstream targets PKA and/or Epac, leading to suppression of immune functions, such as phagocytosis, production of inflammatory cytokines and chemokines, and microbial killing.[5] The contribution of the PKA and Epac signaling in regulation of these functions varies by individual specific responses and by cell types.[26-28] In this study, we showed PKA mediates the cAMP-dependent suppression of CCL3 production in LPS-stimulated macrophages, in which Epac has no role. This is consistent with the cAMP regulation of LPS-induced TNF-α production, where the TNF-α inhibition by cAMP-elevating agents, including PDE4 inhibitors, is mediated solely by activation of PKA in primary macrophages as well as in macrophage cell lines.[29-31] Unlike CCL3 and TNF-α, the interferon-β production induced by LPS has been shown to be prevented by Epac rather than PKA in murine J774A.1 macrophages.[32] Moreover, a study on dendritic cells by Jing *et al*. indicated the LPS-induced CCL3 release is inhibited by prostaglandin E_2 via activation of the EP2 receptor-cAMP-Epac pathway.[33] This finding is opposite to our observations in macrophages, suggesting that cAMP-Epac-1 and cAMP-PKA differentially regulate CCL3 production in different cell types.

While PDE4 inhibitors are widely recognized as negative modulators in inflammatory cell responses, they have also been shown to enhance proinflammatory responses in inflammatory cells. A microarray study on GM-CSF-induced monocyte to macrophage differentiation by Hertz *et al*. showed that the cAMP agonists PGE_2 and forskolin cause a marked increase in the production of several pro-inflammatory chemokines, such as CXCL5, CXCL7, and CCL2 in the GM-CSF-induced macrophages, and that this effect was potentiated by PDE4 inhibition.[34] Moreover, such chemokine induction is mediated mostly by activation of Epac.[34] The CCL3 production in these macrophages was not up-or down-regulated by cAMP according to their microarray data.

Activation of CCR1 and CCR5 in inflammatory cells by CCL3 induces pro-inflammatory activities, such as chemotactic migration and inflammatory mediator generation.[24,35] CCL3 has been implicated in the recruitment of lymphocytes, monocytes, and neutrophils.[24,36] Our chemotaxis assay revealed that in the presence of rolipram CCL3-induced T-cell migration was impaired, indicating that PDE4 inhibitors exert their anti-inflammatory effects by attenuating both the CCL3 production and its receptor responses in target cells.

CONCLUSION

Like its impact on TNF-α production, ablation or inactivation of PDE4B also suppressed LPS-induced CCL3 production in macrophages. These data suggest that PDE4B may regulate production of diverse inflammatory mediators in LPS-stimulated macrophages, and an inhibitor with PDE4B selectivity should retain the anti-inflammatory effects of nonselective PDE4 inhibitors while devoid of the side effects.

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DISCLOSURE

The authors declared that this study has no conflict of interest.

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