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Role of Alveolar Macrophages in Productions of Prostaglandin D_2 and E_2 in the Inflamed Lung

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Our previous study showed that lungs infected by *Pseudomonas*, a gram-negative bacteria, produce prostaglandin D_2 (PGD_2) and prostaglandin E_2 (PGE_2), the two major prostanoids generated by cyclooxygenase-2 (COX-2), and that the ratio of PGD_2 and PGE_2 can affect the outcome of the bacterial lung infection. In this study, we sought to uncover the mechanism that determines the ratio of PGD_2 and PGE_2 produced in lung inflammation. When treated with lipopolysaccharide (LPS), primary alveolar macrophages, extracted from mouse lung, more PGE_2 was produced than PGD_2, whereas MH-S, a murine alveolar macrophage cell line, produced more PGD_2 than PGE_2 in a similar experiment. Western blot analyses showed that the kinetics of COX-2 expression in both cell types is similar and epigenetic silencing of COX-2 expression did not affect expressions of lipocalin-PGD synthase (L-PGDS) and PGE synthase (mPGES-1), major enzymes synthesizing PGD_2 and PGE_2 in inflammation, respectively, indicating no effect of COX-2 on expressions of the two enzymes. Expressions of L-PGDS and mPGES-1 were also similar in both cell types, suggesting no effect of the two key enzymes in determining the ratio of PGD_2 and PGE_2 in these cells. A single intraperitoneal injection of LPS to C57BL/6 mice induced COX-2 expression and, similar to alveolar macrophages, produced more PGE_2 than PGD_2 in the lung. These results suggest that the differential expressions of PGD_2 and PGE_2 in the lung reflect those in alveolar macrophages and may not be directly determined by the enzymes responsible for PGD_2 and PGE_2 synthesis.

Key words : Macrophages, prostaglandin D_2, prostaglandin E_2, lipopolysaccharide, cyclooxygenase-2, acute lung inflammation

Introduction

Alveolar macrophages play a key role in lung immunity, as evident in the experiment that treatment of mice with liposomal clodronate depletes macrophages in the lung, which results in impairment of mounting inflammatory response and of clearing bacteria from the lung [2,5,14]. Macrophages recognize bacterial pathogens via receptors for pathogen-activated molecular patterns, such as Toll-like receptors, and trigger a series of inflammatory responses by producing various cytokines that are essential for neutrophils recruitment and other immunologic effector functions of macrophages [9]. The inflammatory response normally confines bacterial infection.

Macrophages are the major cell type responding to lipopolysaccharide (LPS) [3], one of the major bacterial factors that trigger inflammation, and this feature is in part due to Toll like receptor 4 (TLR4) that is highly expressed in macrophages [15,16,18,27]. Binding of LPS to TLR4 initiates Toll/IL-1 receptor (TIR)-mediated signaling, which activates inhibitory κB kinase (IKK) and mitogen-activated protein kinases (MAPKs). Activation of these kinases increases transcriptional activities of NF-κB and AP-1, respectively, resulting in expression of various inflammatory genes including cyclooxygenase-2 (COX-2) [4,10]. COX is the rate-limiting enzyme in the biosynthesis of prostanoids [8].

Two isoforms of COX have been identified: COX-1 and COX-2. The former is constitutively expressed in most of cell types, and the latter is inducible by many pro-inflammatory stimuli. COX metabolizes arachidonic acid to produce prostaglandin H_2 (PGH_2), which is used as the common precursor for the synthesis of prostanoids including prostaglandin D_2 (PGD_2) and prostaglandin E_2 (PGE_2) [7]. Conversion of PGH_2 to PGD_2 is mediated by two distinctive enzymes, lipocalin-type prostaglandin D synthase (L-PGDS) and hematopoietic isotype (H-PGDS). In macrophages, H-PGDS is constitutively expressed but L-PGDS is inducible by inflammatory stimulus such as LPS [12]. On the other hand, conversion of PGH_2 to PGE2 is mediated by three iso-

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forms of PGE synthases: cytosolic PGE synthase and type 1 (mPGES-1) and type 2 membrane-bound PGE synthases (mPGES-2) [23-25]. While the expression of cytosolic PGE synthase is constant, expressions of mPGES-1 and mPGES-2 are inducible by various stimuli [6,22]. Among them, mPGES-1 is considered as the major enzyme responsible for PGE_2 produced in the inflammatory milieu [22].

It is well documented that prostanoids are involved in regulation of inflammation and host defense against bacterial and viral infections. For instance, we have shown that PGD_2 enhances clearance of *Pseudomonas* infected to the lung [12]. On the other hand, PGE_2 favors *Pseudomonas* infection by impairing the clearance of *Pseudomonas* from the lung [20]. Therefore, defining the mechanism of regulating the ratio of PGD_2 and PGE_2 could yield therapeutics that control acute and chronic bacterial infections. In this study, we sought to determine the mechanism that controls the ratio of the two prostanoids, PGD_2 and PGE_2. To examine the effects of enzymes synthesizing the two prostanoids on the production of the two molecules, we isolated primary macrophages from the lung, alveolar macrophages, and analyzed them in expressions of the enzymes and productions of PGD_2 and PGE_2 after LPS treatment, along with a well-established alveolar macrophage cell line, MH-S. Using an acute lung injury mouse model, we compared the profile of PGD_2 and PGE_2 in the lung with that of primary alveolar macrophages. Our results showed that the differential productions of PGD_2 and PGE_2 do not result from differences in the expression of the cognate enzymes, and suggest that alveolar macrophages play a key role in determining the ratio of PGD_2 and PGE_2 in the lung.

Materials and Methods

Reagents

TLR4-specific *Escherichia coli* LPS was purchased from Alexis Biochemical (San Diego, CA). Antibodies for COX-2, mPGES-1, and mPGES-2 antibodies were obtained from Cayman Chemical, and antibody for actin was purchased from Santa Cruz Biotechnology.

Animals

Male wild type mice (C57BL/6) weighing 20-28 g were used for experiment. Animal experiment was performed per the protocol approved by the Pusan National University Institutional Animal Care and Use Committee.

Lipopolysaccharide (LPS) administration

Gram-negative *E. coli* lipopolysaccharide (LPS serotype 055:B5; SIGMA) was suspended to 1 mg LPS in 10 ml sterile PBS. Mice received a single dose of 3 μg LPS per gram body weight administered by intraperitoneal (I.P.) injection.

Lung lavage, alveolar macrophages, and cell culture

Bronchoalveolar lavage (BAL) is performed by injecting 1 ml of cold PBS through trachea with a 20 gauge catheter. The effluent was centrifuged at 400 x g for 10 min, and the precipitates were suspended and plated in 10% FBS DMEM-LCM medium and cultured at 37°C in CO_2 incubator for an hour. The non-adherent cells were removed from the culture, and adherent cells were used for the experiment as primary alveolar macrophages. A murine alveolar macrophage cell line, MH-S cells, were obtained from the ATCC (American Type Culture Collection, Rockville, MD), and maintained as instructed by the manual of the cell supplier.

COX-2 siRNA

siRNA for murine COX-2 and controls were purchased from Santa Cruz Biotechnology. siRNA was delivered to RAW 264.7 cell by following the protocol suggested by the manufacturer.

Protein isolation and Western blot analysis

Total cell lysate was prepared by adding RIPA (radioimmunoprecipitation assay) cell lysis buffer (Invitrogen) to the cells. After determing the amount of proteins in the cell lysate by the Bradford assay (BioRad), proteins of interest fractionated by SDS-PAGE were transferred to PDVF membrane (Bio-Rad), which was incubated with appropriate antibodies. A specific immune complex was revealed by enhanced chemoluminescence (ECL plus, Amersham).

Prostanoids measurement

PGD_2 and PGE_2 in cell culture media and in lung homogenates were measured by a LC-ESI-MS-MS as previously described [13]. Liquid chromatographic (LC) separation was performed isocratically on a Phenomenex Luna 3 μm C18 5.0x0.2 cm column. The mass spectrometer (MS) was operated in positive-ion ESI mode. Detection of the analytes was accomplished by selected reaction monitoring, employing the following reactions: 370 → 317 (PGE2 and PGD2), 374 → 321 (PGD2-d_4). The Quantum was set to the following

parameters: capillary V=35 V; spray voltage=4.3 kV; capillary temperature=300°C; tube lens V=137 V; sheath gas=49 psi; auxiliary gas=25 (no units); CID pressure=1.0 mTorr. These values were observed to maximize the response of the SRM transitions employed. Collision energy was set to 13 eV for both reactions. Quantitation was accomplished by stable isotope dilution.

Total RNA and semi-quantitative RT-PCR

Total RNA from the lung was isolated by using QIAGEN RNeasy®mini kit (Qiagene, Hilden, Germany). Three micrograms of total RNA were reverse-transcribed by M-MLV reverse transcriptase (Promega, Madison, WI, USA), and single-stranded cDNA was amplified by PCR with a set of specific primers noted as follows: the forward and the reverse primers for COX-2 were 5′- CCCAGAGCTCCTTTTCA ACC-3′ and 5′- AATTGGCACATTTCTTCCCC- 3′, respectively; and the forward and reverse primers for GAPDH were 5′-GGAGCCAAAAGGGTCATCAT- 3′ and 5′- GTGATG GCATGGACTGTGGT- 3′, respectively. For PCR, TaqPCRx DNA polymerase (Invitrogen, Carlsbad, CA, USA) was used, and the reaction was carried out in an initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation for 40 sec at 95°C, annealing for 40 sec at 55°C, and extension for 50 sec at 72°C, along with a final extension for 7 min at 72°C. The resultant of PCR was analyzed by running on 1.5 % agarose gel, which was visualized under UV light.

Statistical analysis

For comparison among groups, paired or unpaired T tests and one-way analysis of variance (ANOVA) tests were used (with the assistance of InStat, Graphpad Software, Inc., San Diego, CA) (p values <0.05 are considered significant). All experiment was performed at least three times independently.

Results

Differential productions of PGD_2 and PGE_2 in alveolar macrophages

To understand the mechanism that determines differential productions of PGD_2 and PGE_2 in bacterial lung infection, we first examined the profiles of PGD_2 and PGE_2 in alveolar macrophages. For the study, we used primary alveolar macrophages extracted from the mouse lung and a well-studied, murine alveolar macrophage cell line, MH-S cell. These cells were treated with highly purified, TLR4 specific LPS to avoid stimulating other signaling cascades. At 16 hr after the treatment of MH-S cells with LPS (100 ng/ml), the cell culture media was collected to measure PGD_2 and PGE_2 excreted to the media. As shown in Fig. 1 A, LPS treatment induced productions of PGD_2 and PGE_2, in which the amount of PGD_2 was higher than PGE_2. To test whether this is also the case in primary alveolar macrophages, we obtained macrophages from the lung by performing bronchoalveolar lavage of the mice, and treated them with LPS similar to Fig. 1A. As shown in Fig. 1B, LPS treatment of the alveolar macrophages also induced productions of PGD_2 and PGE_2 but, unlike MH-S cells, more PGE_2 was produced than PGD_2. These results indicate that there was a difference in the ratio of PGD_2 and PGE_2 produced among alveolar macrophages.

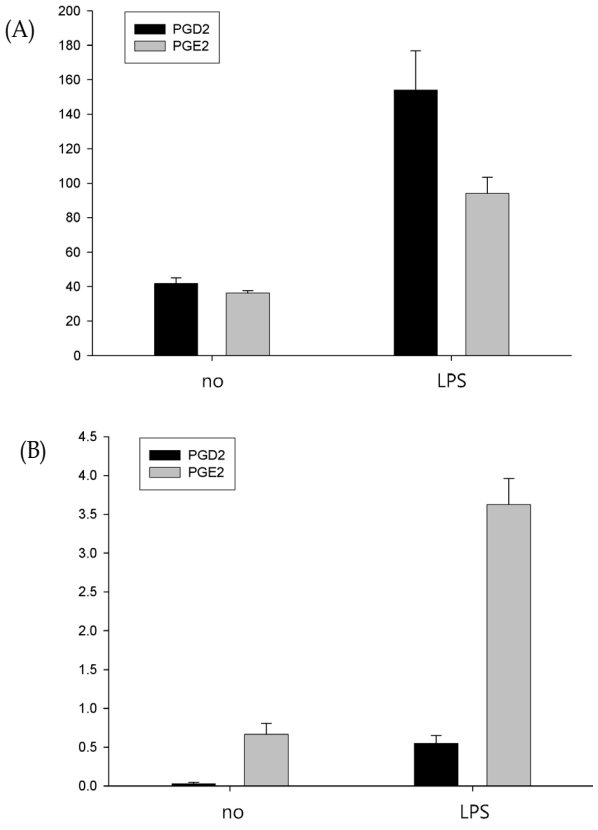

Fig. 1. Differential productions of PGD_2 and PGE_2 in alveolar macrophages. A murine alveolar macrophage cell line, MH-S cells, (A) and primary alveolar macrophages (B) were treated with TLR4 specific LPS (100 ng/ml) for 16 hr, and PGD_2 (dark bar) and PGE_2 (gray bar) in the cell culture media were measured as described in materials and methods. Each bar represents the mean value of three mice±SEM (* and **, $p<0.05$ compared with each controls).

Effects of COX-2 expression on the ratio of PGD_2 and PGE_2 in alveolar macrophages

Since COX-2 is the major enzyme responsible for the production of PGD_2 and PGE_2 in macrophages activated by inflammatory stimuli [8], we tested whether the differential expressions of PGD_2 and PGE_2 result from differences in induction of COX-2 expression. MH-S cells and primary alveolar macrophages were treated with LPS (100ng/ml) for various periods up to 12 hr, and the expression of COX-2 was measured by Western blot analysis. As shown in Fig. 2, LPS treatment of MH-S and primary alveolar macrophages induced COX-2 expression, and it appears that there was no significant time difference in the induction of COX-2.

Although no apparent differences in the kinetics of COX-2 induction between MH-S and primary alveolar macrophages were detected, it is possible that minor differences in the amount of COX-2 protein contribute to the differences in the amount of PGH_2, leading to the differential productions of L-PGDS and mPGES-1 and accordingly PGD_2 and PGE_2, respectively. To test this possibility, we suppressed the expression of COX-2 by using siRNA and tested whether the suppression of COX-2 affects the expressions of L-PGDS and mPGES-1 (Fig. 3). MH-S cells were transfected with either control or COX-2 specific siRNA, and the transfected cells were treated with LPS for 24h to induce COX-2 expression. Western blotting for COX-2 shows that siRNA effectively suppressed COX-2 expression (top panel). However, in similar analyses for L-PGDS and mPGES-1 in the COX-2 suppressed cells, we found no significant changes in expression levels of L-PGDS and mPGES-1 (middle and bottom panels,

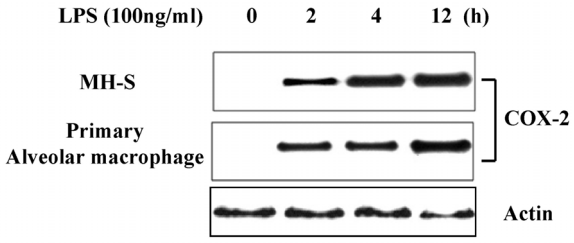

Fig. 2. COX-2 expression in alveolar macrophages. MH-S cells (top panel) and primary alveolar macrophages (middle panel) were treated with LPS (100 ng/ml) for various time points. At the indicated time point, the cells were harvested, and the total proteins were separated by SDS-PAGE and analyzed by Western blot for COX-2 expression. Actin was similarly analyzed for internal controls. Shown was actin in primary alveolar macrophages (bottom panel).

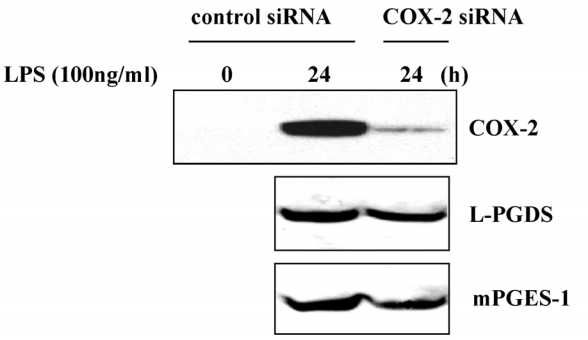

Fig. 3. Epigenetic silencing of COX-2 does not affect the expressions of L-PGDS and mPGES-1. MH-S cells were transfected with either control siRNA or COX-2 specific siRNA for 48 hr, and the transfected cells were treated with LPS for indicated periods. Suppression of COX-2 by the specific siRNA was shown in the top panel. The expressions of L-PGDS and mPGES-1 in control and silenced MH-S cells were examined by Western blot analysis (middle and bottom panel, respectively).

respectively). These data show that COX-2 protein and likely PGH_2 did not affect the expressions of L-PGDS and mPGES-1. Taken together, our results suggest that a difference in the amount of COX-2 does not affect the expression levels of L-PGDS and mPGES-1.

Effects of enzymes responsible for PGD_2 and PGE_2 production on the ratio of PGD_2 and PGE_2 in macrophages

Next, we tested the possibility that the enzymes directly responsible for productions of PGD_2 and PGE_2 are differentially expressed in MH-S and primary alveolar macrophages, resulting in differences in the ratio of PGD_2 and PGE_2. MH-S cells were treated with LPS for various periods, and expressions of L-PGDS and mPGES-1 were determined by Western blot analysis. As shown in Fig. 4A, LPS treatment induced L-PGDS, but did not affect the constitutive expression of mPGES-1 in MH-S cells. In experiment with primary alveolar macrophages, as shown in Fig. 4B, we obtained similar results to MH-S cells. It is unclear why alveolar macrophages used in this study expressed mPGES-1 constitutively, despite that the expression of mPGES-1 is known to be inducible by inflammatory stimuli. Nevertheless, combined with the results in Fig. 3, these data show that differential productions of PGD_2 and PGE_2 stemmed from the differences in neither COX-2 expression nor the enzymes synthesizing PGD_2 and PGE_2.

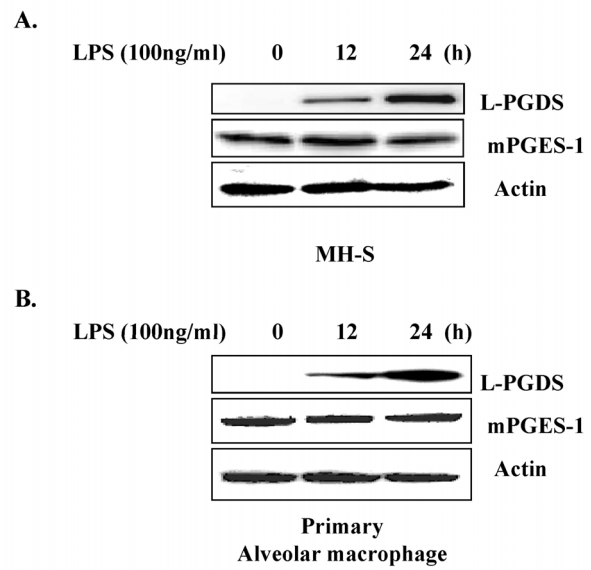

Fig. 4. L-PGDS and mPGES-1 expression in alveolar macrophages. (A) MH-S cells were treated with LPS (100 ng/ml) for indicated periods, and the expression of L-PGDS (top panel) and mPGES-1 (middle panel) was analyzed by Western blot. Similar experiment was performed to detect L-PGDS and mPGES-1 in primary alveolar macrophages (B).

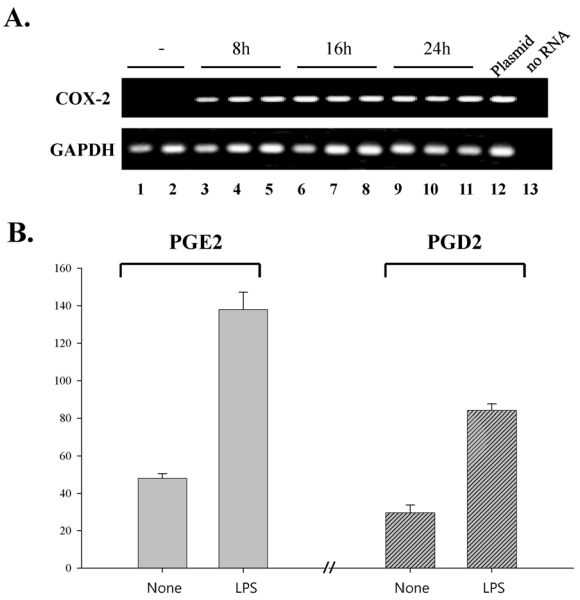

Fig. 5. Differential productions of PGD_2 and PGE_2 in lung inflammation. C57BL/6 mice received i.p. injection of LPS for indicated periods, and the lungs of the mice were harvested for COX-2 expression and the production of PGD_2 and PGE_2. (A) Except controls, three mice were used for the experimental group at each time point. Total RNA obtained from the lung tissue was analyzed for COX-2 expression by semi-quantitative RT-PCR. For RT control, PCR was performed without RT reaction (lane 13). For ensuring the PCR products are expected ones, plasmids encoding COX-2 was amplified by PCR (lane 12). As internal controls, GAPDH was similarly analyzed (bottom panel). (B) At 24 hr after LPS i.p. injection, mice lungs were harvested, and lung homogenates of the mice were analyzed for the production of PGD_2 and PGE_2. Each bar represents the mean value of three mice±SEM (* and **, $p<0.05$ compared with each controls).

Higher PGE_2 production in the lung inflamed by LPS

Finally, we examined the ratio of PGD_2 and PGE_2 in the lung inflamed by LPS. C57BL/6 mice received an I.P injection of LPS (3 μg/g), and at 24hr after LPS injection the mice were sacrificed and the lungs were extracted for the analysis of RNA and the measurement of prostanoids. At first, we determined whether LPS administration induces inflammation in the lung by measuring COX-2 expression. RT-PCR results, as shown in Fig. 5A, showed that LPS treatment of the mice elicited COX-2 expression in the lung, indicating that lung inflammation took place. Next, we measured PGD_2 and PGE_2 from the lung tissue. As shown in Fig. 5B, LPS treatment induced productions of PGD_2 (hatched bars) and PGE_2 (gray bars), and, similar to alveolar macrophages, more PGE_2 was produced than PGD_2. This imparity in production of PGD2 and PGE2 was also similarly observed at early time points, 8 hr and 12 hr (data not shown). Together with the results of Fig. 1B, these results show that there was a possible correlation between alveolar macrophages and the lung in productions and the ratio of PGD_2 and PGE_2.

Discussion

Prostaglandins are involved in regulation of inflammation, and PGD_2 and PGE_2 are the major prostanoids involved in acute lung inflammation and host defense against various bacterial challenges. However, the effects exerted by these prostanoids are not well understood because the effects are complex and dependent on the specific inflammatory milieu. Our previous studies, for instance, showed that while PGD_2 ameliorates *Pseudomonas* infection to the lung [12], PGE_2 exacerbates *Pseudomonas* infection [20]. These results suggest that understanding the mechanism that determines the differential productions of PGD_2 and PGE_2 by bacterial infection can lead to new therapeutics for the treatment of chronic *Pseudomonas* lung infection that is prevalent in cystic fibrosis patients. Therefore, we have interested in uncover-

ing the mechanism by which the ratio of PGD_2 and PGE_2 is regulated in the lung. In this study, we explored a possible mechanism by using TLR4-specific LPS as an inflammatory stimulus. Our results showed that the enzymes responsible for the synthesis of PGD_2 and PGE_2 do not directly affect the ratio of PGD_2 and PGE_2 in the macrophages and that, similar to higher production of PGE_2 in primary alveolar macrophages, the lung produces more PGE_2 over PGD_2. These results suggest that alveolar macrophages are important in determining the ratio of PGD_2 and PGE_2 in LPS-induced inflammation.

Pseudomonas, Gram-negative bacteria, has been recognized as a common pathogen associated with respiratory infections in various clinical settings. It is a common cause of noscomical infection in hospitalized and immune-compromised patients [11,17], and a major cause of *Pseudomonas* pneumonia in cystic fibrosis patient [26]. *Pseudomonas* infection sometime causes persistent infection in patients, in the state of which anti-bacterial treatments are no longer effective. The bacteria harbor various inflammatory molecules on their surface. Along with LPS, for instance, they have polar pili that are involved in attachment to host cells and stimulate TLR2 signaling [21]. *Pseudomonas* also contains flagella that enable them to move around, which induces TLR5 signaling [1]. Therefore, it is likely that the bacterial infection activates a battery of TLR signaling cascades simultaneously, causing a massive inflammation in the lung. It is, however, notable that *Pseudomonas* infection to the lung induces more PGD_2 over PGE_2, which makes the infection self-limited. [20]. This is likely associated with the fact that *Pseudomonas* contains various antigens that might activate various TLR signaling cascades at the same time [19]. Given our results showing that LPS induced the production of PGE_2 over PGD_2, it is possible that while LPS induces the production of PGE_2, other signaling cascades activated by *Pseudomonas* antigens combinatorially contribute to higher production of PGD_2 over PGE_2. It is conceivable that this is a part of defense strategies of the host immune system that diverts, or suppresses, an otherwise massive inflammation by tipping the balance of PGD_2 and PGE_2 to produce more anti-inflammatory PGD_2.

It is not clear why we could not find any difference in expressions of L-PGDS and mPGES-1 by LPS treatment between two alveolar macrophages, MH-S and primary alveolar macrophages, although the profiles of PGD_2 and PGE_2 in those cell types were quite different. One possibility is that differential levels of mRNA of L-PGDS and mPEGS-1 lead to the imparity in the amounts of PGD_2 and PGE_2. However, it is noteworthy that PGD_2 and PGE_2 are highly unstable and the amounts of these prostanoids at a given time are correlated with the activities of the enzymes that are responsible for the production of the prostanoids. Thus, it is likely that the amounts and activities, but not the level of mRNA, of the enzymes are determining factor for the levels of the prostanoids. Our study also ruled out the possibility that COX-2 expression and PGH_2 production affect the levels of L-PGDS and mPGES-1 and thereby determine the ratio of PGD_2 and PGE_2 because, as the results in Fig. 3, the absence of COX-2 and presumably a basal level of PGH_2 did not affect the expression of L-PGDS and mPGES-1. Although it is possible that COX-1, constitutively expressed in most cell types, affect the expression of L-PGDS and mPGES-1, a plethora of publications have consistently reported that COX-2 is the major COX enzyme in the inflammatory milieu. However, it is conceivable that, although having similar levels of L-PGDS and mPGES-1 proteins, MH-S and primary alveolar macrophages might have different expression profiles of enzymes catabolizing the prostanoids, by which a particular prostanoid was converted to its derivatives more rapidly and thus detected lower than the other prostanoid. While we do not understand the precise mechanism that governs the differential productions of PGD_2 and PGE_2, it is notable that the ratio of the two molecules in alveolar macrophages is similar to that of the lung. Thus, it is possible that alveolar macrophages isolated from a patient can be used as a parameter of whether or not infection by a type of bacteria will be self-limited or harmful to the patient.

In this study, we searched the mechanism that controls the ratio of PGD_2 and PGE_2 in the inflamed lung. We found that, unlike inflammation caused by *Pseudomonas* bacterial infection, lung inflammation induced by LPS produced more PGE_2 over PGD_2. Analysis of primary alveolar macrophages indicated that this was not due to differential expressions of L-PGDS and mPGES-1. Our results suggest that there is a potential correlation in the profiles of PGD_2 and PGE_2 between the lung and primary alveolar macrophages. Our results could provide a tool to predict whether a bacterial infection is self-limited or progressively inflammatory.

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초록 : 프로스타글란딘 D_2와 E_2의 생성에 대한 허파 마크로파이지의 역할

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프로스타글란딘 D2 (PGD2)와 E2 (PGE2)는 COX-2로부터 유래되는 주요 프로스타노이드로서, 슈도모나스에 의한 폐감염이 발생하였을 경우 폐에서 합성되어 슈도모나스 세균감염을 조절할 수 있음을 밝힌바 있음. 본 연구에서는 두 프로스타노이드의 생성 비율을 조절하는 기전을 연구하고자함. 마크로파아지에 의해 PGD2/PGE2 비율이 결정되는 지 조사하기 위해, 마우스의 허파로부터 마크로파아지를 분리하고 LPS로 처리할 경우, COX-2, PGD2합성 효소인 L-PGDS, PGE2의 합성효소인 mPGES-1 등의 발현이 두 프로스타노이드의 생성 비율에 미치는 영향을 조사하였음. 또한 이 효소들의 발현이 일차 허파 마크로파아지에 특이적인지의 여부를 조사하기 위해, 허파 마트로파이지 세포주인 MH-S와 비교 조사하였음. COX-2가 프로스타글린딘 비율에 미치는 영향을 조사하기 위해, COX-2 특이적 siRNA릉 이용하여 COX-2의 발현을 억제하고 L-PGDS, mPGES-1 등의 발현을 조사하였음. 결과에 따르면, 일차 허파 마트로파아지는 MH-S과는 달리 많은 양의 PGE2를 생성하나, 두 세포간 COX-2, L-PGDS, mPGES-1의 발현에는 큰 차이가 없었음. 이는 이들 효소 외에 다른 인자들이 두 프로스타노이드의 비율을 결정하는데 관여함을 제시함. LPS의 처리에 의해 폐염증을 발생시키고 허파에서의 PGD2/PGE2 비율을 조사한 결과, LPS에 의해 폐염증이 발생할 경우 LPS를 처리한 일차 허파 마크로파아지와 유사하게 PGE2의 발현이 PGD2에 비해 상당히 높았음. 결과적으로 본 연구의 결과는, 허파에서의 PGD2/PGE2 비율은, COX-2, L-PGDS, mPGES-1 등 PGD2나 PGE2의 합성에 직접적인 영향을 주는 효소에 의해 결정되지 않으며, 허파마크로파아지의 PGD2/PGE2 비율을 반영할 가능성을 제시함.