Elevated Prostaglandin E$_2$ Levels in Bronchoalveolar Lavage Fluid of Patients with Bronchogenic Carcinoma*

Ann LeFever, Ph.D.;† and Akira Funahashi, M.D., Ph.D., F.C.C.P.

Understanding local pulmonary immunoregulatory mechanism(s) in patients with carcinoma of the lung is an important step towards the development of innovative methods of treatment. Prostaglandin E$_2$ plays an integral role in immunoregulation. Therefore, we evaluated PGE$_2$ concentrations in BALF from 18 patients with bronchogenic carcinoma, compared to that from six patients with pulmonary diseases other than carcinoma and ten normal smokers of similar age. The level of PGE$_2$ in patients with lung carcinoma (158.1 ± 88.7 pg/ml) was significantly (p<0.001) higher than the other two groups (16.2 ± 6.9 and 4.4 ± 3.4 pg/ml). Levels of PGE$_2$ also varied among patients with carcinoma of different cell types. Patients with SQCA had significantly (p<0.001) higher levels of PGE$_2$ (242.7 ± 29.4 pg/ml) than patients with ADCA or SCCA (82.3 ± 27.9 and 66.3 ± 15.2 pg/ml, respectively). Furthermore, there was a marked difference in PGE$_2$ concentration between carcinomatous lung and clinically noninvolved lung in patients with SQCA and ADCA. Further study is warranted to determine the interactions between PGE$_2$ and other cytokines (interleukin-1, IL-2, and tumor necrosis factor), as well as the activity of cytolytic lymphocytes (LAK cells) in the lungs of patients with bronchogenic carcinoma.

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SQCA = squamous cell carcinoma; ADCA = adenocarcinoma; SCCA = small-cell carcinoma; LAK = lymphokine-activated killer; AM = alveolar macrophage; RIA = radioimmunoassay; PIE = pulmonary infiltrates with eosinophilia; NK = natural killer; IL-2 = interleukin-2

Prostaglandins are metabolites of arachidonic acid and are involved in the regulation of various biologic systems. Prostaglandin E$_2$ can enhance as well as suppress cell-mediated immune responses, including lymphocyte proliferation, cytotoxicity, and cytokine production. Prostaglandin E$_2$ is produced by macrophages, lymphocytes, and some tumors. The lung is one of the major sites of PGE$_2$ production, and the concentration of PGE$_2$ in BALF has been implicated in the immunoregulation of sarcoidosis.

Alveolar macrophages are the major cell population in pulmonary tissue and BALF; AM-suppressive functions are largely mediated through the production and release of PGE$_2$. Stimulated AM, obtained from patients with lung cancer, have been shown to release soluble factors which inhibit cell-mediated lytic activity against tumor cells. This suppression was blocked by indomethacin, suggesting a role for the immunosuppressive effects of prostaglandins during tumor progression.

*From the Departments of Pediatrics, Microbiology, and Medicine, Medical College of Wisconsin; the MACC Fund Research Center; and the Clement J. Zablocki Veterans Administration Medical Center, Milwaukee.


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†Assistant Professor of Pediatrics and Microbiology.

‡Professor of Medicine.

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Reprint requests: Dr. LeFever, Department of Pediatrics, Medical College of Wisconsin, Milwaukee 53226

The regulatory mechanism or mechanisms controlling local immune responses against the development and propagation of bronchogenic carcinoma are poorly understood. Since peripheral blood often does not reflect the immune processes involved locally in the lung, BAL has been used extensively to obtain immune effector cells and alveolar lining fluids from the lung; however, there is a paucity of information in regard to the immunoreactants in the BALF of patients with bronchogenic carcinoma. In the current study, we sought to determine whether the concentration of PGE$_2$ in BALF was altered by the presence of bronchogenic carcinoma and whether these levels correlated with the histologic classification of the tumor, the number of AMs recovered in BALF, or the size of the tumor. We evaluated PGE$_2$ activity in BALF from patients with primary bronchogenic carcinoma, compared to patients with diseases other than bronchogenic carcinoma, as well as age-matched, smoking-history-matched normal volunteers. The results of this study demonstrate that PGE$_2$ concentrations in BALF from patients with primary bronchogenic carcinoma are elevated, suggesting that PGE$_2$ may play a role in the disease's progression by suppressing local immune responsiveness.

Materials and Methods

Subjects

Bronchoalveolar lavage fluid was obtained from patients undergoing diagnostic bronchoscopy for suspected bronchogenic carcinoma at the Clement J. Zablocki VAMC, Milwaukee. Control volunteers were recruited from domiciliary residents of the VAMC.
Elevated Prostaglandin E₂ Levels in Bronchogenic Carcinoma (LaFevre, Funahashi)

The research protocol has been reviewed and approved by an institutional human research committee; informed consent was obtained prior to bronchoscopy.

We evaluated BALF and cell populations in 34 male subjects. There were 18 cases of primary bronchogenic carcinoma (nine with SQCA, four with ADCA, and five with SCCA); six cases of noncarcinomatous pulmonary disease, and ten normal smokers. The age, smoking history, forced expiratory volume, and clinical cancer staging (TNM system) of the patients who had bronchogenic carcinoma are shown in Table 1. The mean age of patients with carcinoma was 62 ± 10 years (range, 43 to 78 years), and the mean cigarette smoking history was 50.7 ± 16.3 pack-years. In normal control subjects, the mean age was 54 ± 5 years (range, 46 to 63 years), and the mean smoking history was 50.6 ± 15.3 pack-years.

In addition, there were six patients with diagnoses of pulmonary diseases other than carcinoma (one case of histoplasmosis, one infection with *Mycobacterium avium-intracellulare*, one pulmonary abscess, two with bacterial pneumonia, and one case of interstitial fibrosis). The mean age of these patients was 65 ± 5 years (range, 59 to 74 years), and the mean smoking history was 65.8 ± 16.4 pack-years. All patients had abnormal chest roentgenograms. Their FEV₁ varied from 20 to 94 percent of their predicted values (Table 1). Normal control subjects had normal chest roentgenograms and FEV₁.

** Bronchoscopy and BAL 

All but one subject (patients and controls) had pulmonary function studies and arterial blood gas determinations prior to bronchoscopy. Supplemental oxygen was given if arterial blood oxygen tension while breathing room air was less than 70 mm Hg. Patients whose arterial oxygen tension remained below 70 mm Hg with supplemental oxygen were excluded from the study. Bronchoscopy was performed with a fiberoptic bronchoscope (Olympus BF type 10) in the usual manner. If blood was noted in the airways upon introduction of the bronchoscope prior to BAL, the case was excluded from study. Subjects in whom endobronchial lesions were detected in the right or left main-stem bronchus or in the trachea were also excluded from the study. The BAL was performed prior to any diagnostic procedures such as brushing or biopsy. The location of the endobronchial lesion, if present, was determined first. In cases where there was no visible endobronchial lesion present, the location of the tumor (mass) was determined by chest roentgenograms supplemented with fluoroscopy at the time of the procedure. No lobe involved by tumor was lavaged. Instead, a segment of a lobe adjacent to the lobe which harbored the tumor was lavaged.

If the patient tolerated the initial lavage of the involved lung and showed no sign of excess coughing, then lavage of the noninvolved (contralateral) lung was performed. For lavage of a noninvolved lung, a segment of the right middle lobe or subsegment of a lingular segment was used. The BALF from different lungs was kept separate, and analysis was performed independently. The BAL was performed with aliquots of 30 ml of physiologic saline solution at room temperature. The volume of saline solution instilled into one segment was usually kept between 60 and 120 ml because of the concern that a larger volume would provoke coughing spells and interfere with subsequent diagnostic procedures. When both lungs were lavaged, the total amount of fluid instilled was kept below 150 ml. The total amount of solution used was left to the bronchoscopist's discretion. In none of the patients was the subsequent diagnostic procedure compromised by BAL.

**Preparation of BALF and BAL Cells**

The BALF was generally processed within one hour of the bronchoscopy. The volume of recovered fluid was noted and centrifuged at 2,000 rpm for ten minutes to separate cellular components. The supernatant was decanted and stored at -85°C until assayed for PGE₂ content. The cell pellet was resuspended in phosphate-buffered saline solution (without Mg⁺² or Ca⁺²). Differential analysis using cytospin slides and viability, as well as total cell counts, was done using a hemacytometer with trypan blue as the viability indicator. The viability of all BALF cell populations obtained during this study was greater than 92 percent.

**Prostaglandin E₂ Assay**

The concentration of PGE₂ in BALF was determined using an α²¹¹² thyroidmunoassay (DuPont) according to the procedures detailed in the RIA kit. Briefly, all assay reagents (ie, PGE₂ standards, PGE₂[α²¹¹²] tracer) were prepared immediately prior to the assay. All reactions were carried out in polypropylene test tubes to ensure against nonspecific loss of PGE₂ on the surface of the tube. Reagents were allowed to come to room temperature and mixed prior to use. Radiolabeled tracer solution (0.1 ml) was added to replicate tubes with 0.1 ml of BALF, diluted standard solution, or assay buffer (zero standard), respectively, and the contents gently mixed. Antiserum (0.1 ml) was added to all tubes, and each tube was vortexed thoroughly for two to five seconds, followed by incubation at 2°C to 8°C for 16 to 24 hours. After incubation the tubes were placed in an ice bath, and 1 ml of cold precipitating reagent was added. The contents were then vortexed thoroughly for two to five seconds, and the tubes were kept in the ice bath at 2°C to 8°C for 20 to 30 minutes. The tubes were then centrifuged at 4°C at 1,500 x g for 30 minutes. Supernatants were decanted, the tubes were counted in a gamma counter, and radioactivity was recorded as counts per minute. Determination of the percent bound (% B/B₀) for each standard and BALF sample was done using the following equation:

\[
\text{% B/B₀ = \frac{\text{net cpm of standard or sample}}{\text{net cpm of 0 standard}}} \times 100
\]

The amount of PGE₂ in each BALF sample was determined from a standard curve of % B/B₀ vs picograms of PGE₂ added and was

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** Table 1 — Clinical Features of Patients with Bronchogenic Carcinoma **

<table>
<thead>
<tr>
<th>Subject*</th>
<th>Age, yr</th>
<th>Cigarettes, pack-yr</th>
<th>FEV₁, L</th>
<th>Cell Type†</th>
<th>Stage</th>
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<tr>
<td>010</td>
<td>75</td>
<td>80</td>
<td>1.44 (45)</td>
<td>ADCA</td>
<td>T3N0M0</td>
</tr>
<tr>
<td>021</td>
<td>55</td>
<td>60</td>
<td>0.64 (20)</td>
<td>ADCA</td>
<td>T3N2M0</td>
</tr>
<tr>
<td>032</td>
<td>60</td>
<td>60</td>
<td>0.92 (20)</td>
<td>ADCA</td>
<td>T3N2M1</td>
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<tr>
<td>035</td>
<td>43</td>
<td>30</td>
<td>2.96 (82)</td>
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<td>T1N1M3</td>
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<tr>
<td>006</td>
<td>73</td>
<td>75</td>
<td>1.57 (56)</td>
<td>SCCA</td>
<td>T1N1M1</td>
</tr>
<tr>
<td>012</td>
<td>66</td>
<td>75</td>
<td>1.51 (50)</td>
<td>SCCA</td>
<td>T1N0M0</td>
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<tr>
<td>018</td>
<td>57</td>
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<td>1.78 (70)</td>
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<td>T3N2M0</td>
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<tr>
<td>024</td>
<td>46</td>
<td>40</td>
<td>2.20 (62)</td>
<td>SCCA</td>
<td>T3N1M0</td>
</tr>
<tr>
<td>026</td>
<td>51</td>
<td>45</td>
<td>2.10 (57)</td>
<td>SCCA</td>
<td>T2N1M1</td>
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<tr>
<td>008</td>
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<td>20</td>
<td>1.85 (66)</td>
<td>SQCA</td>
<td>T1N0M0</td>
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<td>017</td>
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<td>1.43 (55)</td>
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<td>T3N2M0</td>
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<td>35</td>
<td>2.29 (85)</td>
<td>SQCA</td>
<td>T3N0M0</td>
</tr>
</tbody>
</table>

* Each subject in this study was identified by unique assigned number.
† Bronchogenic carcinoma diagnosis by histologic type: ADCA, adenocarcinoma; SCCA, small-cell carcinoma; and SQCA, squamous cell carcinoma.
§ Not done.
‡ Percent predicted values given in ( ).
reported as picograms per milliliter of recovered BALF. The concentration of PGE\textsubscript{2} was also calculated as picograms of PGE\textsubscript{2} divided by micrograms of albumin and picograms of PGE\textsubscript{2} divided by milliliters of BALF after correcting for fluid recovery, but these values did not alter data interpretation or statistical relationships. All sample values reported in this study were evaluated in duplicate in two or three assays.

Statistical Analysis

Statistical comparisons of overall significant differences in the study’s population were done using the Kruskal-Wallis analysis-of-variance procedure. When differences were observed, comparisons of individual groups were done using a two-tailed Mann-Whitney U-test. Statistical determinations were done using the Epistat program.

Results

Bronchoalveolar Lavage Fluid and Cell Recovery

The mean recovery of lavage fluid was 26±11 percent (range, 6 to 50 percent) in patients with bronchogenic carcinoma, 28±10 percent (range, 13 to 43 percent) in patients with noncarcinomatous pulmonary disease, and 36±9 percent (range, 21 to 50 percent) in normal controls. These recoveries are comparable to those obtained by other investigators using similar BAL volumes.\textsuperscript{14-16} The mean recovery of BALF in the uninvolved lungs of patients with carcinoma or other pulmonary diseases (when done) was comparable to that obtained from the involved lungs (22±6 percent and 25±7 percent, respectively). The number of cells recovered by BAL in patients with cancer (3.3×10\textsuperscript{6}±2.1×10\textsuperscript{6} cells) was similar to the recovery in subjects with noncarcinomatous disease (5.5×10\textsuperscript{6}±2.7×10\textsuperscript{6} cells) but lower than in normal volunteers (2.6×10\textsuperscript{7}±0.5×10\textsuperscript{7} cells). The number of}

Study Population

![Study Population](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21622/)

Concentrations of PGE\textsubscript{2} in BALF

Average PGE\textsubscript{2} concentrations in subjects with carcinomatous and noncarcinomatous disease and in normal subjects were 158.1±88.7, 16.2±6.9, and 4.4±3.4 pg/ml of recovered BALF, respectively (Fig 1). The BALF from patients with carcinoma had significantly (p<0.001) higher levels of PGE\textsubscript{2} than that from patients with noncarcinomatous disease or normal subjects. No significant (p=0.19) difference was detected in normal subjects compared to patients with noncarcinomatous pulmonary disease. Data evaluation using picograms of PGE\textsubscript{2} per microgram of albumin and by correcting for BALF recovery did not alter the results (not shown).

Levels of PGE\textsubscript{2} in subgroups of patients, divided according to their histologic cell type, are shown in Figure 2. Levels in SQCA were significantly (p<0.001) higher (3X) than in the other histologic types; concentrations in BALF from ADCA and SCCA were similar (mean concentrations of 242.7±29.4, 82.3±27.9, and 66.3±15.2 pg/ml of recovered BALF, respectively).

In 11 subjects (three with noncarcinomatous disease, two with ADCA, three with SCCA, and three with SQCA), both involved and uninvolved lungs were lavaged (Fig 3). Although the number of subjects in each group is small, there is a marked difference between PGE\textsubscript{2} levels of involved and uninvolved lungs in ADCA and SQCA but not in SCCA or the noncar-
cinomatous groups, even though BALF recoveries were comparable. These results, together with those detailed previously, clearly demonstrate that PGE$_2$ levels in BALF are increased in patients with lung carcinoma and suggest that the carcinomatous lungs of patients with ADCA and SQCA have higher levels than the uninvolved lung.

**Relationship of Tumor Size and PGE$_2$ Levels**

Bronchogenic carcinoma cells can produce PGE$_2$, and the size of the tumor may influence the quantity of PGE$_2$ present or the production of PGE$_2$ by other local cell types. We sought to determine if there was a correlation between the tumor’s size and the level of PGE$_2$ found in BALF. The size of the tumor was determined using the standard posteroanterior chest roentgenogram by measuring the largest diameter of the tumor. If there was associated atelectasis or a large mediastinal mass or lesion(s) in the contralateral lung which could represent metastasis, the subject’s data were excluded from this analysis. There were 12 cases in which accurate measurement of the tumor size was possible. Linear regression analysis demonstrated that there was no correlation ($r^2=0.10$) between the concentration of PGE$_2$ in BALF and the size of the tumor mass (data not shown).

**Number of Recovered Cells and PGE$_2$ Levels**

Alveolar macrophages are an important local source of PGE$_2$, and thus we used linear regression analysis to determine if there was a correlation between the number of cells recovered from BALF and the concentration of PGE$_2$ in BALF. Statistical analysis failed to show any correlation between the number of recovered cells and the PGE$_2$ concentration in the patients with carcinomatous or noncarcinomatous disease or the normal controls ($r^2=0.18$, 0.12, and 0.19, respectively).

**Discussion**

Our data demonstrate that patients with primary bronchogenic carcinoma have significantly higher levels of PGE$_2$ in their BALF compared to BALF from patients with noncarcinomatous pulmonary disorders or normal volunteers. The PGE$_2$ in BALF may be the result of production by lymphocytes, macrophages, or tumor cells. Since AMs comprise 90 percent of the cells in BALF, it is likely that much of the PGE$_2$ found in BALF is of macrophage origin. In patients with bronchogenic carcinoma, tumor cells may contribute to the PGE$_2$ levels, as well as macrophages and lymphocytes that have been activated by the tumor’s presence. Patients diagnosed with SQCA and ADCA had higher levels of PGE$_2$ in BALF obtained from the clinically involved lung compared to that in the noninvolved lung. These data suggest that the tumor, either directly or indirectly, causes increased PGE$_2$ concentrations to be present. These increased levels of PGE$_2$ may be related to differences in the immune interactions which control PGE$_2$ production in the lung or to production of PGE$_2$ by the tumor. Bennett et al. studied PGE$_2$ in freshly resected pulmonary tissue from 133 patients who underwent surgery for bronchogenic carcinoma. These investigators compared PGE$_2$ levels from the tumor tissue and “normal tissues” from the same lung. Tumorous tissue contained a significantly higher concentration of PGE$_2$ than “normal” pulmonary tissue. These data also suggest the tumor’s contribution to PGE$_2$ levels in the lung; however, a direct contribution of PGE$_2$ produced by the tumor in the BALF in this study was probably minimal, because (1) we excluded cases in which the trachea or main-stream bronchus was involved with tumor; and (2) we did not lavage a lobe which harbored a carcinoma. Instead, we lavaged a lobe adjacent to an involved lobe.

Prostaglandin levels in BALF have been measured by several investigators in diseases other than lung cancer. Baughman et al. studied prostaglandins in BALF obtained from 11 patients with sarcoidosis and seven normal control subjects. These investigators failed to detect any differences between the two groups, and the PGE$_2$ concentrations reported were higher than those presented in this study. They were evaluating the concentration of total prostaglandin in BALF, rather than PGE$_2$, which explains the difference in the two studies. Ogushi and associates measured PGE$_2$ in patients with sarcoidosis, intersti-
tial fibrosis, summer hypersensitivity pneumonitis, and PIE. These investigators\textsuperscript{18} found increased levels of PGE\(_2\) in only two patients with PIE. Interestingly, their "controls" included ten patients with bronchogenic carcinoma. The details of these patients' disease status, such as cell types or stages of the disease, were not provided, and thus their results cannot be directly compared to those obtained with our patients; however, the concentration of PGE\(_2\) in BALF in their study was higher than what we have reported, which may reflect the different technical separations and assay procedures utilized.

Patients diagnosed with bronchogenic carcinoma generally have a poor prognosis, which in large part correlates with the extent and the rate at which metastasis occurs. The regulatory balance of local immune responses in the lung may well determine the rate of growth and metastasis. Natural killer cells and LAK cells can limit the progression of pulmonary metastases in mice with B16 metastatic melanoma.\textsuperscript{5,10} The presence and functional activity of NK cells in the lungs of patients with bronchogenic carcinoma have been reported,\textsuperscript{13,10,20} but the level of antitumor lytic activity reported has been highly variable. In preliminary studies, we have reported in situ LAK cell activity in BALF from six of 18 patients with bronchogenic carcinoma and one patient with metastatic lung carcinoma who had not yet undergone treatment for their disease.\textsuperscript{5,22} These results suggest that immune effector cells are present in the lungs of patients with bronchogenic carcinoma, but their effectiveness in improving a patient's prognosis may depend upon the balance of factors which enhance (ie, IL-2, interferon) or suppress (ie, PGE\(_2\), AMs) their activity. Prostaglandin E\(_2\) has been shown to prevent generation of LAK cells from PB-lymphocyte populations.\textsuperscript{23} PB-lymphocyte proliferation and T-lymphocyte cytotoxicity, as well as that of NK cells, can also be suppressed by PGE\(_2\).\textsuperscript{6,10,24} In addition, the immune response may also be regulated by PGE\(_2\)-mediated activation of suppressor T-cell populations.\textsuperscript{25}

Our data further demonstrate that the highest levels of PGE\(_2\) can be detected in the BALF of patients with SQCA, with moderate amounts in the BALF of patients with ADCA and less in those with SCCA, although all concentrations were significantly higher than those detected in the BALF from normal controls. These results are of particular interest, since of the six patients we have reported with in situ BAL LAK cell activity, four had SQCA, and two had ADCA.\textsuperscript{22} The LAK activity was observed only when BALF contained high levels of IL-2 and, most importantly, was detected even when PGE\(_2\) levels were 40 to 50 times higher than in normal volunteers. Thus, the mere presence of AMs or their products (ie, PGE\(_2\)) does not preclude the ability of the lung to generate antitumor cytolytic responses.

The higher PGE\(_2\) levels in the carcinomatous lung compared to the clinically uninvolved lung of patients with SQCA and ADCA in this study were probably related to the tumor presence. The absence of this difference in patients with SCCA is intriguing. If this observation is confirmed by a larger number of cases, it might indicate that there are micrometastases, not evident by the chest roentgenogram, since SCCA is known to have a higher incidence of early distant metastasis.

In conclusion, this study demonstrated that there was an increased amount of PGE\(_2\) in BALF from patients with primary bronchogenic carcinoma. The level of PGE\(_2\) varied due to tumor cell types. The highest level of PGE\(_2\) was seen in patients with SQCA, followed by ADCA and SCCA. There was also a difference in PGE\(_2\) concentrations between clinically involved lungs and noninvolved lungs in patients who had SQCA and ADCA. In patients with SCCA, this difference was not observed. The level of PGE\(_2\) in BALF was not related to the tumor mass present in the lung as measured by the chest roentgenogram or to the number of cells recovered from BALF. Although the role of elevated PGE\(_2\) in overall immunoregulation in lung carcinoma has yet to be defined, the measurement of PGE\(_2\) in BALF may prove to be clinically useful, since this elevation of PGE\(_2\) was not detected in conditions other than lung carcinoma. Clearly, further investigation is warranted to clarify the significance of PGE\(_2\) in BALF.

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REFERENCES


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