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ORIGINAL ARTICLE

The role of soluble and insoluble gastric fluid components in the pathogenesis of obliterative bronchiolitis in rat lung allografts

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Summary

Repetitive gastric fluid aspirations have been shown to lead to obliterans bronchiolitis (OB), but the component or components of gastric fluid that are responsible are unknown. This study investigates the role of particulates and, separately, soluble material in gastric fluid during the development of OB. Whole gastric fluid (WGF) was collected from male Fischer 344 (F344) rats and separated by centrifugation into particle reduced gastric fluid (PRGF) and particulate components resuspended in normal saline (PNS). Orthotopic left lung transplants from male Wistar–Kyoto rats into F344 rats were performed using a modification of the nonsuture external cuff technique with prolonged cold ischemia. Rats were subjected to weekly aspiration of 0.5 ml/kg of WGF (n = 9), PRGF (n = 10), PNS (n = 9), or normal saline (control, NS; n = 9) for 8 weeks following transplantation. Lung allografts treated with WGF, PRGF, or PNS developed a significantly greater percentage of OB-like lesions compared with the control. No statistical difference was observed when comparing the fibrosis grades or the percentage of OB lesions of WGF, PRGF, and PNS groups, suggesting that both soluble and insoluble components of gastric fluid can promote the development of aspiration-induced OB and fibrosis in lung allografts.

Introduction

For patients with end-stage lung disease, lung transplantation often provides a drastic improvement in quality of life and prolongs survival. However, despite advancements in surgical techniques, immunosuppressant therapies, and organ allocation, lung transplant recipients have a median survival of only 5.6 years post-transplantation [1]. Survivability after lung transplantation is limited most significantly by the onset of chronic allograft rejection known as obliterans bronchiolitis (OB), a pathology characterized by fibroproliferative narrowing and occlusion of graft bronchioles [2].

Understanding of the risk factors for the development of OB is progressing. Alloimmune and nonalloimmune factors such as lymphocytic bronchiolitis, acute cellular rejection, donor HLA antibodies and donor-specific non-HLA antibodies, cytomegalovirus infection, bronchial hypoxia, and gastroesophageal reflux disease (GERD) have been associated with OB or its clinical correlate, bronchiolitis obliterans syndrome [3–8]. Examining the association between GERD and OB, we have recently shown that

chronic aspiration of whole gastric fluid (WGF) promotes the development of OB in rat allografts [9]. We further showed that neutralizing gastric fluid aspirate does not reduce the occurrence of OB lesions after chronic aspirations in rats [10], suggesting that another component of gastric fluid, aside from acidity, is responsible for promoting the development of the OB lesions.

The histological consequences of aspirating gastric fluid or its components have been investigated in both native and transplanted lungs. In native lungs, a single aspiration of gastric fluid and gastric food particles leads to acute alveolar injury [11,12]. A study by Knight *et al.* [12] showed that in rats, a single aspiration of 40 mg/ml of gastric food particles suspended in a saline/HCl solution (pH = 5.3) produced acute inflammatory lung injury characterized by alveolar capillary leakage that peaked around 6 h postaspiration before decreasing by 24 h postaspiration. Interestingly, in this study, a single aspiration with 40 mg/ml of glass particles similar in size to gastric food particles in a suspension of saline/HCl (pH = 5.3) did not produce a significant change in alveolar capillary leakage compared with aspirating particle-free saline. These findings suggest that food particles uniquely activate an inflammatory response within the lungs. While a single, acute aspiration of gastric fluid or food particles causes transient lung injury, chronic aspiration of these materials results in persistent lung injury. Chronic aspiration of gastric fluid or food particles results in pneumonitis, while chronic aspiration of bile leads to mild pulmonary edema [13]. Downing *et al.* [13] showed that, in a rat model, repetitive aspiration of gastric fluid over 9 weeks leads to pneumonitis characterized by giant cell and granuloma formation with perivascular infiltrates, while repetitive aspiration of rodent chow food particles in a 10% suspension contributed to a more severe pneumonitis compared with aspiration of gastric fluid. The intensity of granulomatous tissue formation that results from chronic aspiration of food particles naturally present in gastric fluid is unclear because the food suspension used for aspirations contained a 10-fold greater concentration of food particles relative to the WGF used in the study. In native rat lungs, aspiration of gastric fluid and food particles acutely injure the lung and, when subjected to additional aspiration, leads to prefibrotic tissue formation within the lung. In the setting of lung transplantation, chronic aspiration of gastric fluid and its components contributes to a severe fibrotic pathology. In the transplanted lung, chronic aspiration of WGF leads to OB pathology rather than a pneumonitis, as seen in native lungs under similar conditions [9]. Currently, the exact component or components of gastric fluid that leads to OB pathology have not yet been isolated.

As the initial stages of OB involve lymphocytic infiltration in the submucosa of the bronchioles, any gastric fluid component that leads to inflammation is a potential contributor to the development of OB. In this study, we evaluate the effect of insoluble particulates in gastric fluid aspirates and, independently, the soluble component of gastric fluid on the development of chronic aspiration-induced OB in a rat lung transplantation model. We assess whether chronic aspiration of washed gastric particulates can promote OB, and whether depletion of particulates in WGF has an influence on the frequency of OB lesions.

Materials and methods

Animals

Male Wistar–Kyoto (WKY) and Fischer F344 (F344) rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN, USA). Rats weighed approximately 300 g at the time of transplantation. At least nine rats per experimental group were utilized. All rats were housed in specific pathogen-free conditions in the animal care facilities at Duke University Medical Center in accordance with institutional guidelines. All animal care and procedures were approved by the Duke Institutional Animal Care and Use Committee. Results from some of the animals in the control groups ($n = 9$ from the WGF group and $n = 4$ from the NS group) were previously reported [14] and selected for inclusion in this study if the transplants were conducted during the same period of time and by the same surgeon as other transplants described in this study. Those animals from previously reported studies were transplanted, aspirated and sacrificed in exactly the same manner as those in this study.

Orthotopic single lung transplantation

Donor WKY left lungs were orthotopically transplanted into F344 recipients using a cuff anastomotic technique as previously described [10]. Cold ischemic time was defined as the time between the start of perfusion with Perfadex (Vitrolife, Kungsbacka, Sweden) at 4 °C to the beginning of warm ischemic time. For all operations, cold ischemic time was maintained at 300 min. The warm ischemic time, defined as the time after the donor graft was removed from ice to reperfusion in the recipient, was maintained at 60 min. OB lesions have been consistently observed in aspirated rats that received left lung transplantations under these conditions [14,15].

Postoperative analgesia included several drops of bupivacaine (0.25%) (Hospira, Inc., Lake Forest, IL, USA) along the incision at time of closure, ketoprofen (5 mg/kg SC) once daily for 3 days, and buprenorphine (0.05 mg/kg SC) twice daily for 2 days after transplantation as per protocol. Transplanted rats received 5 mg/kg of subcutaneous

cyclosporine immediately postoperatively, and three times a week until the animals were euthanized 9–10 weeks after transplantation.

Preparation of gastric fluid components and Aspiration procedure

Gastric fluid from multiple F344 rats was collected, pooled, and filtered using a 70-μm strainer (BD Biosciences, Bedford, MA, USA) as previously described [10]. Pooled WGF was aliquoted, flash frozen with liquid nitrogen, and stored at −80 °C until needed.

To separate particulate matter from WGF, WGF was centrifuged at 8161 *g* for 15 min at 4 °C. The supernatant was removed and labeled as particle reduced gastric fluid (PRGF). The sediment was washed three times with normal saline (NS). After each wash, the sediment was centrifuged at 16 000 *g* for 20 min. After three washes, the resulting sediment was resuspended to the original volume of the WGF with normal saline and labeled as particulates in normal saline (PNS).

Transplanted rats were assigned into groups defined by the type of aspirate received: normal saline (control, NS; $n = 9$), WGF ($n = 9$), PRGF ($n = 10$), and PNS ($n = 9$). Aspirations were performed once weekly as previously described [10]. Briefly, transplanted rats were induced, intubated, and ventilated for 5–10 s and placed in left lateral decubitus position with reverse Trendelenburg at a 35–45° angle. A silastic catheter was inserted into the distal trachea through which 0.5 ml/kg of assigned aspirate was introduced into the left lung. Aspirations were started 1 week after transplantation and concluded after eight aspirations were given.

Characterization of gastric fluid components

To quantitate the total number of particulates, intact bacteria, bacterial debris, and fiber particles (other debris) in each of the samples used during the aspiration procedure, flow cytometry was performed as previously described [16].

To ascertain the percentage of viable bacteria removed from the PRGF component and reconstituted in the PNS component, twelve samples of neutralized WGF were inoculated with *Escherichia coli* (strain MG1655). The bacterial strain utilized in the present study is a derivative of *E. coli* K-12 (strain MG1655) that constitutively expresses the type 1 pilus and has been described previously [17]. Twenty microliters of *E. coli* was thawed, transferred into 10 ml of culture medium [500 ml EMEM (Sigma, St. Louis, MO, USA), 5 ml nonessential amino acids (GIBCO, Grand Island, NY, USA), 5 ml 100 mM sodium pyruvate (GIBCO), and 5 ml HEPES (GIBCO)], and left to grow overnight on a shaker at 38 °C. Samples of WGF ($n = 12$) were neutralized with NaOH to pH 7.4 and spiked with 2 μl of the mixture of containing approximately 9.4×10^5 CFU of *E. coli*. The *E. coli*-enriched, neutralized gastric fluid was used to prepare PRGF and particles suspended in normal saline as described above. Serial dilutions of WGF, PRGF, and PNS were plated onto agar to determine the percentage recovery of bacteria in these suspensions. Colony forming units were read after 20 h of incubation at 38 °C. Agar plates were prepared under sterile conditions using 24.8 g low salt LB broth media (Teknova, Hollister, CA, USA) and 15 g agar granulate, in a total volume of 1 l H_2O. *E. coli*-enriched gastric fluid samples were solely utilized to evaluate the efficacy of separation of viable bacteria into the PNS component and to confirm the flow cytometry findings. (At no time did transplanted animals receive aspiration with *E. coli*-enriched gastric fluid.)

Procurement, compliance measurement, and histology preparation

Lung allografts were procured as previously described [9,14]. In brief, rats ($n = 37$) were sacrificed 1 week after the eighth aspiration, at 10 weeks post-transplantation. Rats were placed supine and a midline sternolaparotomy was performed. Heart and lungs were harvested en bloc. A custom-made pressure volume device consisting of a WIKA low-pressure gauge (Valworx Inc., Cornelius, NC, USA) connected via a three-way valve to a syringe and a 14G angiocatheter was used to measure the compliance of the harvested lung allografts. The volume obtained following every 50 mm H_2O rise in pressure was recorded up to 350 mm H_2O, and one additional measurement was taken at 380 mm H_2O.

The left lung was divided into upper and lower segments, fixed in 10% neutral buffered formalin for at least 24 h, and then processed into paraffin blocks. Tissues were then sectioned at 5 μm thickness, mounted onto positively charged microslides (Erie Scientific Company, Portsmouth, NH, USA), and stained using hematoxylin and eosin stain.

Histological grading

All histological sections were assessed in a blinded fashion. For each tissue section, five fields viewed under 10× objective lens magnification were graded for fibrosis on a numerical scale (from 0 to 8) as previously described [18]. Each tissue section was systematically scanned and every bronchiole in each section was evaluated for evidence of OB. After all histological sections had been graded, the histological sections were unblinded and scores of all fields for each left lung were averaged to give an average fibrosis score. The percentage of bronchioles affected by OB lesions

for each harvested left lung allograft was calculated by dividing the total number of OB lesions in sections from the upper and lower lung segments by the total number of bronchioles in the same sections. OB lesions were characterized by bronchioles that showed evidence of fibroproliferation of the submucosa, leading to partial or complete occlusion [19].

Statistics

Overall and pairwise differences in expansion/consolidation of lung allografts between groups were examined using the Mantel–Haenszel test for ordinal categorical data. Wilcoxon's rank-sum tests were used to compare histological variables between groups. Linear regression was used to fit compliance curves. Correlation analysis was used to examine the degree of fibrosis and percentage of bronchioles affected by OB lesions. A prespecified significance level of alpha = 0.05 was used for all statistical tests. Statistical analyses were performed using SAS, version 9.4 (Cary, NC, USA) or GraphPad Prism 5.01 (La Jolla, CA, USA).

Results

Characterization of aspirate

To determine the recovery of viable bacteria from PRGF and particles suspended in normal saline (PNS), 2 ml samples of neutralized WGF were spiked with 2 µl of a mixture containing approximately 9.4×10^5 CFU of *E. coli* and were separated into PRGF and PNS components as described in the Materials and methods. The procedure consistently separated the viable bacteria into the PNS component (average of 103.2 ± 4.9% of original WGF sample), leaving a minute proportion in the PRGF component (average of 0.010 ± 0.003% of original WGF sample) (Fig. 1). The method used to separate particles from the WGF and recovery of the particles in the PNS were also successful based on direct assessment of the preparations by flow cytometry (Fig. 2); PRGF samples contained an average of 5.9% of total particles, 0.9% of bacteria, 1.4% of bacterial debris, and 14.7% of fiber and debris from the original WGF samples as assessed by flow cytometry. The amount of fiber and debris in the PNS component was increased slightly (by an average of 13.7%) compared with WGF (Fig. 2d), a phenomenon that might have resulted from clumping of bacteria with debris due to centrifugation.

Gross histology and lung compliance

Thirty-seven orthotopic left lung transplants were performed. Thirteen rats developed hemoptysis postoperatively (35% mortality rate occurring prior to aspirations) and were euthanized and excluded from analysis. This morbidity rate was consistent with that observed previously under similar conditions and is strictly related to prolonged ischemic time [14].

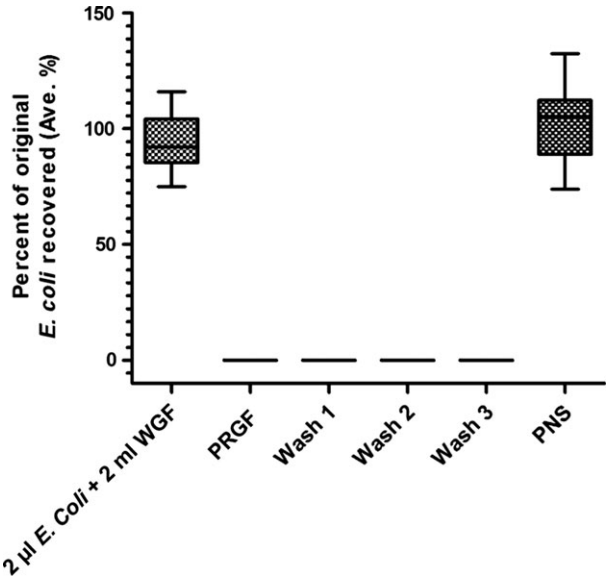

Figure 1 Percentage of *Escherichia coli* recovered at different steps in the procedure to isolate components of gastric fluid from samples (n = 12) of whole gastric fluid (WGF) spiked with 9.4×10^5 CFU of *E. coli*. The percentage of original *E. coli* recovered (mean ± SEM) for WGF, particle reduced gastric fluid, Wash 1, Wash 2, Wash 3, and particulates in normal saline were 94.2 ± 3.5%, 0.010 ± 0.003%, 0.004 ± 0.001%, 0.005 ± 0.002%, 0.004 ± 0.003%, and 103.2 ± 4.9%, respectively.

Most lung allografts treated with normal saline were completely expandable and significantly more expandable than lungs from the groups treated with WGF, PRGF, and PNS (Fig. 3). Eight of nine allografts treated with NS were completely expandable, while two of nine allografts treated with WGF, one of 10 allografts treated with PRGF, and three of nine allografts treated with PNS were completely expandable. An overall association was seen among the portion of allografts categorized as completely expandable, <50% consolidated, >50% consolidated, and completely consolidated and the type of aspirate (P = 0.006). Pairwise differences were seen between WGF and NS (P = 0.006), and PRGF and NS (P = 0.006) only. Consistent with the gross histological findings, the average compliances of lungs from the WGF and PRGF groups were lower than that of the NS group, whereas compliance of the lungs from the PNS group appeared similar to the NS group (Fig. 4). The difference in compliance among all groups was not statistically significant. The loss in compliance could be due to parenchymal fibrosis or the development of OB lesions that cause bronchiolar narrowing and obliteration.

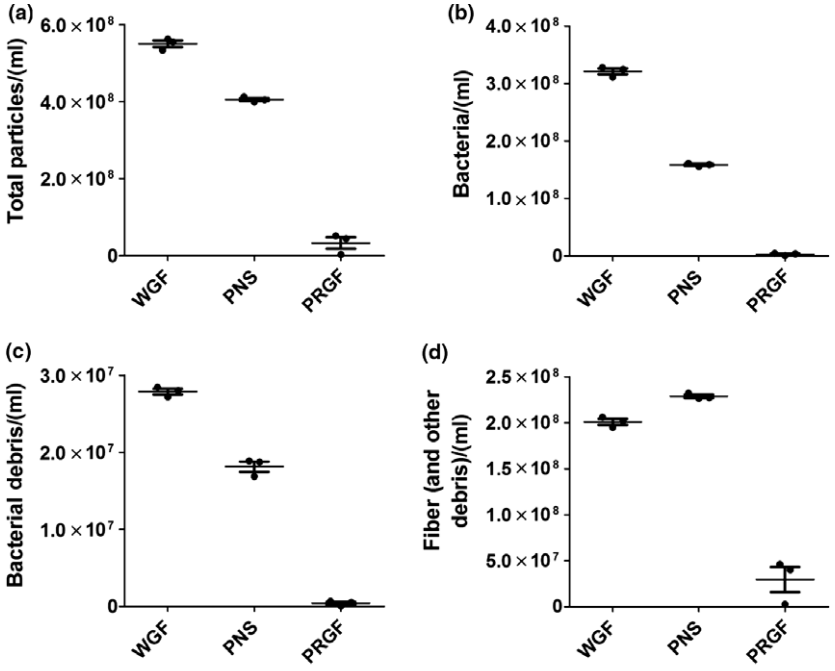

Figure 2 Number of (a) total particles, (b) bacteria, (c) bacterial debris, and (d) fiber and debris in whole gastric fluid, particle reduced gastric fluid, and particulates in normal saline as determined by flow cytometry. Flow cytometry was performed as described in the Materials and methods.

OB lesions

In total, 414 bronchioles were examined for the presence of OB from 74 sections of the left lungs of 37 rats. Characteristic OB lesions were observed frequently in this study, as seen in the histological findings shown in Fig. 5a–d. OB lesions developed in a significant proportion of the allografts that had been chronically exposed to gastric fluid or its components. The greatest percentage of bronchioles affected by OB lesions was observed among lungs in the WGF group (37.4 ± 8.7%), followed by PRGF (35.0 ± 7.0%), PNS (28.0 ± 5.5%) and NS (11.2 ± 5.3%). A significant difference in the percentage of bronchioles affected by OB lesions was observed between the NS group and the WGF ($P = 0.02$), PRGF ($P = 0.02$), and PNS ($P = 0.04$) groups (Fig. 6a). However, no statistical difference was observed in the percentage of bronchioles affected by OB lesions among the WGF, PRGF, and PNS groups.

Pulmonary fibrosis

In total, 370 sections from 37 rats were evaluated for the degree of pulmonary fibrosis. Representative histology is shown in Fig. 5e and f. Collagen deposition and parenchymal fibrosis were evident in the majority of fields from grafts aspirated with WGF or its components, while most lungs aspirated with NS displayed mild to no alveolar wall thickening. The fibrosis scores of lungs from WGF, PRGF, and PNS groups (Fig. 6b) were 6.2 ± 0.91, 6.4 ± 0.5, and 5.7 ± 0.6, respectively, and were significantly greater than that of the NS group (2.6 ± 0.9). No statistical difference was observed when comparing the fibrosis grades of lungs from WGF, PRGF and PNS groups.

A linear correlation analysis between the degree of fibrosis and the percentage of bronchioles affected by OB lesions was performed with the combined data from all four experimental groups (Fig. 7). There is a positive correlation between fibrosis and the percentage of bronchioles affected by OB lesions ($P < 0.0001$); however, the coefficient of determination ($r^2 = 0.41$) suggests that parenchymal fibrosis and the percentage of bronchioles affected by OB lesions is not strictly codependent.

Discussion

The exact component or components of gastric fluid that lead to aspiration-associated OB pathology has not yet been isolated. Previous studies have shown that chronic aspiration of WGF in lung allografts promotes the development of OB [9], and later studies showed that neutralizing the pH of gastric fluid does not change the percentage

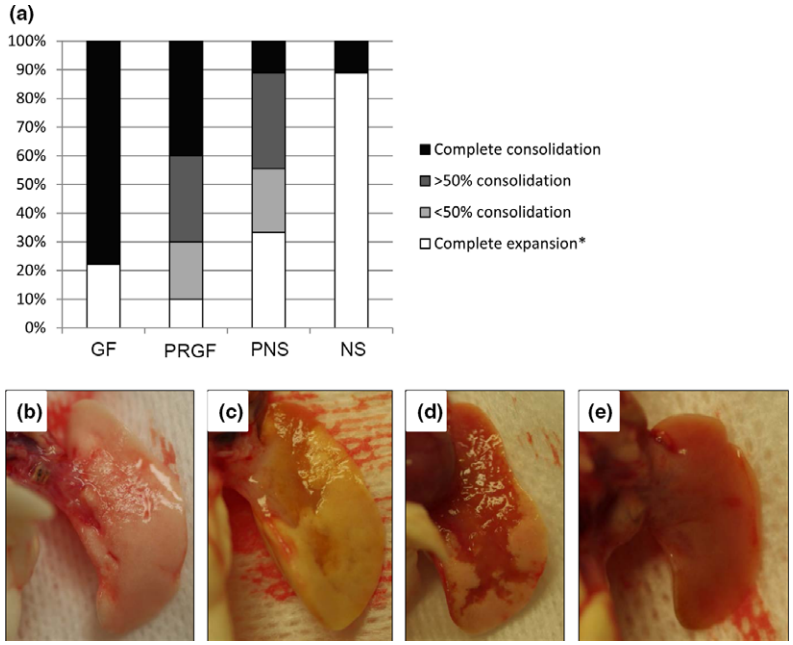

Figure 3 Gross histology of left lung allografts after 8 weeks of aspiration (10 weeks post-transplant). (a) An overall association was seen among the portion of allografts categorized as completely expandable, <50% consolidated, >50% consolidated, and completely consolidated and the type of aspirate ($P = 0.006$). Pairwise differences were seen between whole gastric fluid (WGF) and NS ($P = 0.006$), and particle reduced gastric fluid (PRGF) and NS ($P = 0.006$) only. In the WGF group, seven of nine allografts showed complete consolidation, whereas the rest were completely expanded. In the PRGF group, four of 10 allografts showed complete consolidation, three of 10 allografts showed >50% consolidation, two of 10 allografts showed <50% consolidation, and one of 10 allografts showed complete expansion. In the particulates in normal saline (PNS) group, three of nine allografts showed complete expansion, two of nine allografts showed <50% consolidation, three of nine allografts showed >50% consolidation, and one of nine allografts showed complete consolidation. In the NS group, eight of nine allografts showed complete expansion, whereas the remainder showed complete consolidation. (b) Complete expansion for a rat in the NS group. (c) <50% consolidation for a rat in the PRGF group. (d) >50% consolidation for a rat in the PNS. (e) Complete consolidation for a rat in the PRGF group. In the PNS group, one lung sample demonstrated near complete expansion*.

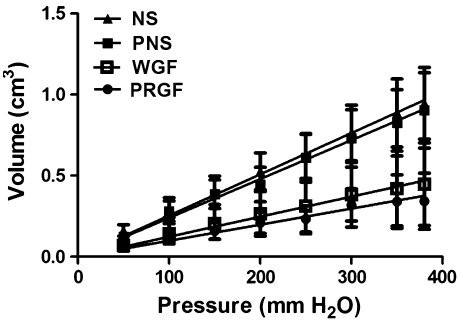

Figure 4 Compliance of left lung allografts as measured by volume of inflation/unit pressure. The average compliance of lungs in the whole gastric fluid (WGF) and particle reduced gastric fluid groups were lower than that of the NS group, whereas compliance of the lungs from the particulates in normal saline group appeared similar to the NS, although the difference in lung compliance among all groups was not statistically different.

of bronchioles affected by OB lesions observed after chronic aspiration [10]. Here, we observe that the effects of chronic aspiration on the development of OB are not significantly lessened by the depletion of particulate matter. Furthermore, this study shows that washed particulates from gastric fluid can independently lead to OB pathology.

In this study, allografts aspirated with gastric fluid or its components were significantly more fibrotic and had a greater percentage of bronchioles affected by OB lesions compared with those aspirated with normal saline. Unexpectedly, the degree of fibrosis and the percentage of OB lesions observed among the groups aspirated with WGF, PNS, or PRGF were not statistically significant; although allografts aspirated with WGF or PDGF had a greater mean fibrosis score and greater mean percentage of OB compared with allografts aspirated with PNS. Similarly, allografts aspirated with WGF or PDGF had a worse compliance curve compared with allografts aspirated with PNS, although these findings were not statistically significant. Given these findings, at least three potential explanations for the observed results are evident: either (i) very low amounts of particulate matter might provide enough stimulus to instigate the

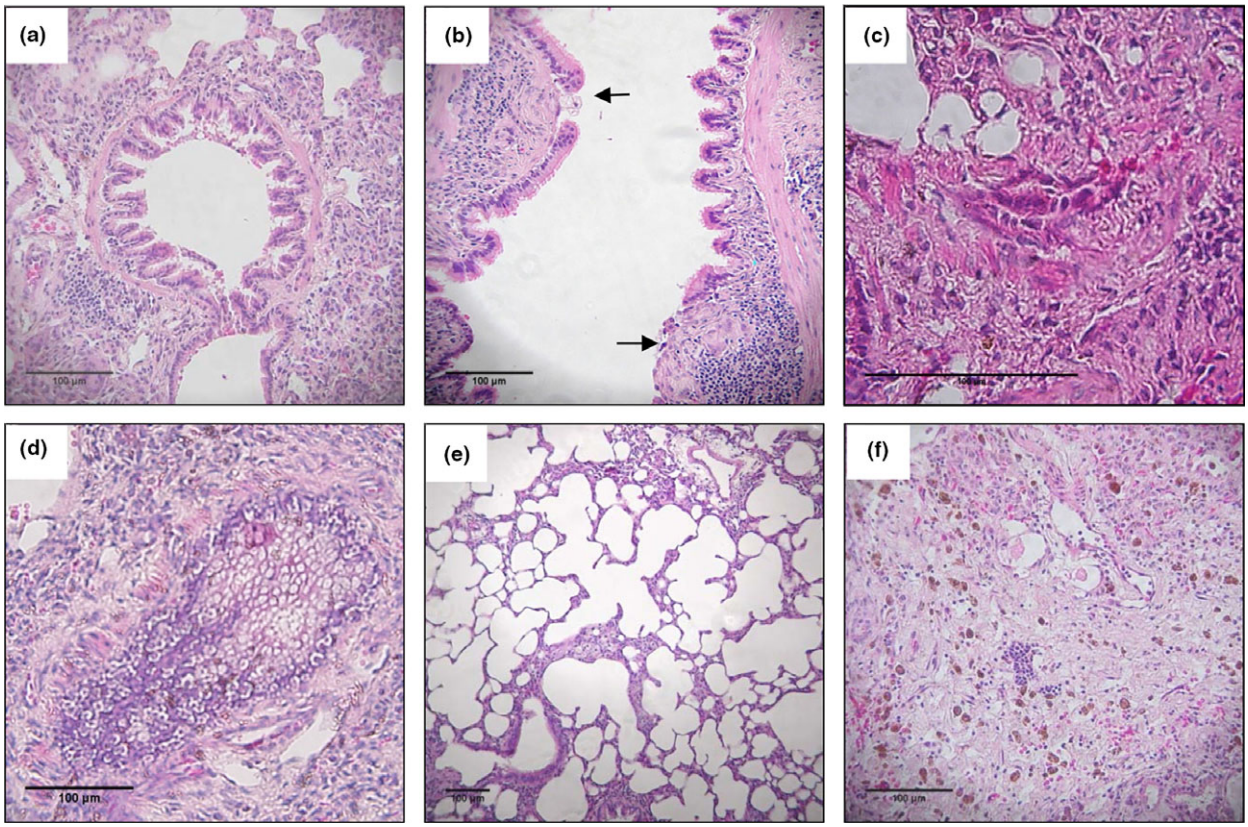

Figure 5 H&E fields showing representative histological features observed in this study. (a) A normal bronchiole is shown. Note that the bronchiole is normal, despite the surrounding pneumonitis. Lymphocytic infiltrates and giant cells are also present on this field. (b) Two foci suggestive of OB are shown here (arrows), involving epithelial damage, submucosa lymphocytic infiltration and fibrous deposition. (c) Complete obliteration of a bronchiole with only the surrounding smooth muscle remaining. (d) Complete obliteration of a bronchiole. The ring of smooth muscle cell helps to identify the location of the otherwise obliterated bronchiole. (e) Mild thickening of alveolar walls suggestive of grade 3 fibrosis. (f) Complete grade 8 fibrosis of the parenchyma. Hemosiderin-laden macrophages are abundant in this field. Bar = 100 µm (a–f).

immune response that leads to OB pathology, or (ii) the soluble fraction of gastric fluid contains a factor(s) that activates the pathway to OB with similar intensity as the insoluble fraction, or (iii) both soluble and insoluble factors can induce a cascade leading to OB. Flow cytometry of the aspirate samples demonstrated that centrifugation dramatically reduced the amount of total particles in PRGF samples, leaving substantially less bacteria, fiber, and debris than in the WGF. Further, evaluation of GF samples spiked with an *E. coli* mixture confirmed that centrifugation consistently separated viable bacteria out of the PRGF component; an average of 0.010% of *E. coli* from the original WGF sample remained. Given the effectiveness of removal of intact bacteria from the PRGF and the lack of reduction in OB lesions compared with other groups, more investigation is needed to determine if a threshold exposure to particulates, especially bacteria content, exists to initiate the pathway to OB lesions.

With the present data, it is unclear which of the three above hypotheses better explains the lack of reduction in bronchiole affected by OB lesions in the PRGF group compared with the other groups. Nevertheless, this study suggests the possibility that soluble components of gastric fluid can promote the development of OB with similar intensity as the insoluble component of gastric fluid. The soluble portion of gastric fluid contains proteolytic enzymes such as trypsin and pepsin, other bioactive molecules such as bile and soluble bacterial components that may stimulate an immune response within the lung [13]. However, as the centrifugation process was not entirely efficient, notably leaving about 15% of the fiber and debris, insoluble components could have led to the pathology associated with aspiration of PRGF. However, as the percentage of bronchioles affected by OB lesions and fibrosis scores were not significantly reduced despite dramatically reducing the amount of fibers, debris and bacteria in the PRGF, it seems unlikely that soluble

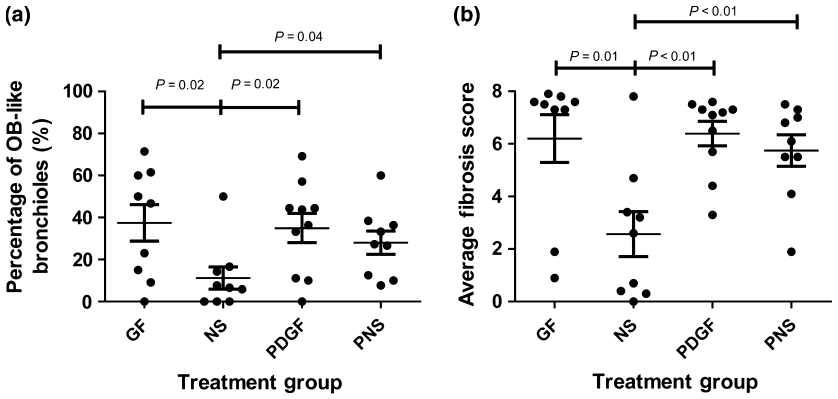

Figure 6 Quantitative assessment of histological findings. (a) The percentage of bronchioles associated with OB lesions is shown. OB lesions observed among the whole gastric fluid (WGF), NS, particle reduced gastric fluid (PRGF), and particulates in normal saline (PNS) groups were 37.4 ± 8.7 (mean ± SEM), 11.2 ± 5.3, 35.0 ± 7.0, 28.0 ± 5.5 respectively. A statistically significant difference in the percentage of OB lesions was observed among rats aspirated with WGF and NS ($P = 0.02$), PRGF and NS ($P = 0.02$), PNS and NS ($P = 0.04$). There were no statistically significant differences between the OB lesions observed in the bronchioles of rats among the PRGF, PNS and WGF groups. (b) Fibrosis scores for WGF, NS, PRGF, and PNS groups were 6.2 ± 0.9 (mean ± SEM), 2.6 ± 0.9, 6.4 ± 0.5, 5.7 ± 0.6, respectively. A statistically significant difference in fibrosis score was observed among rats aspirated with WGF and NS ($P = 0.01$), PRGF and NS ($P = 0.001$), and PNS and NS ($P = 0.01$). There were no statistically significant differences between the fibrosis score among the PRGF, PNS, and WGF groups.

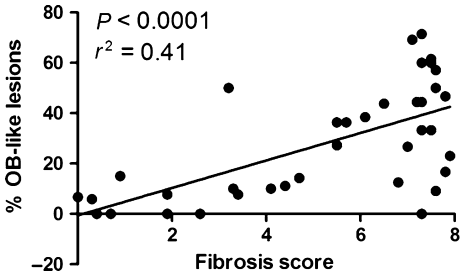

Figure 7 Correlation plot of the average percentage of bronchioles affected by OB lesions to the respective fibrosis scores. Although a positive correlation exists, the coefficient of determination (r^2) is 0.41, and some samples with very high fibrosis scores and few or even no OB lesions were found. Similarly, one sample with about 50% of the bronchioles affected by OB had a fibrosis score of <4.

components are responsible, at least in large part, for the degree of OB and fibrosis in the lungs of the PRGF group. More investigation is needed to discern if there is a threshold of insoluble gastric material that leads to OB pathology, and to discern the influence of the composition of insoluble and soluble material from the gastric fluid on the pathogenesis of OB. One potential approach might be to determine a dose–response curve for soluble and insoluble components, which may provide additional clues regarding the respective roles of these two fractions in the development of aspiration-associated OB.

In conclusion, this study addresses the impact of soluble and particulate matter in gastric fluid aspiration on OB lesion formation in lung allografts. Our study demonstrates that gastric fluid depleted of particles (PRGF) and gastric particles resuspended in normal saline (PNS) can both promote the development of aspiration-induced OB and fibrosis in lung allografts. These studies may suggest that multiple pathways may be involved in the aspiration-associated development of OB. Further studies are necessary to elucidate immunologic mechanisms that lead to OB associated with chronic aspiration of gastric particulates as well as chronic aspiration of the soluble portion of gastric fluid.

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Authorship

JHL and JCC: performed the study. SMB and SMT: analyzed the data and wrote the manuscript. ZEH and MLE: collected the data and performed the experiments. ZEH: proofread the manuscript. WP, RDD and SSL: provided funding, designed the study and proofread the manuscript.

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