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Rattus Model Utilizing Selective Pulmonary Ischemia Induces Bronchiolitis Obliterans Organizing Pneumonia

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Abstract

Background—Bronchiolitis obliterans organizing pneumonia (BOOP), a morbid condition when associated with lung transplant and chronic lung disease, is believed to be a complication of ischemia. Our goal was to develop a simple and reliable model of lung ischemia in the Sprague-Dawley rat that would produce BOOP.

Methods—Unilateral ischemia without airway occlusion was produced by an occlusive slipknot placed around the left main pulmonary artery. Studies were performed 7 days later. Relative pulmonary and systemic flow to each lung was measured by injection of ^{99m}Tc-macroaggregated albumin. Histological sections were examined for structure and necrosis and scored for BOOP. Apoptosis was detected by immunohistochemistry with an antibody against cleaved caspase-3.

Results—Pulmonary artery blood flow to left lungs was <0.1% of the cardiac output, and bronchial artery circulation was ~2% of aortic artery flow. Histological sections from ischemic left lungs consistently showed Masson bodies, inflammation and young fibroblasts filling the distal airways and alveoli, consistent with BOOP. Quantitative evaluation of BOOP using epithelial changes, inflammation and fibrosis were higher in ischemic left lungs than right or sham-operated left lungs. Apoptosis was increased in areas exhibiting histological BOOP, but there was no histological evidence of necrosis. TLR4 expression was increased in ischemic left lungs over right.

Conclusions—An occlusive slipknot around the main left PA in rats produces BOOP, providing direct evidence that ischemia without immunomodulation or coinfection is sufficient to initiate this injury. It also affords an excellent model to study signaling and genetic mechanisms underlying BOOP.

Keywords

Lung transplant; pulmonary artery; graft loss; reperfusion; TLR4

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Conflicts of Interest John C. Densmore, MD, Paul M. Jeziorczak, MD, Anne V. Clough, PhD, Kirkwood A. Pritchard Jr., PhD, Breana Cummens BS, Meetha Medhora, PhD, Arjun Rao, MD, and Elizabeth R. Jacobs, MD, derived no personal support and have not been involved with any organizations with financial interest in the conduct of this research. None of the authors have any conflict of interest in completion of this work.

Introduction

Bronchiolitis obliterans organizing pneumonia (BOOP) is an uncommon spontaneous cause of respiratory failure, but often occurs in the setting of underlying respiratory disease where it can be a therapeutic challenge with significant impact on long-term function and mortality. BOOP is defined as an organized polypoid granulation tissue proliferation in the distal airways extending into the alveolar ducts and alveoli. Affected lungs lack interstitial fibrosis, honeycombing, and traction bronchiectasis.(1) The incidence of BOOP in first world nations is 1-2 per 100,000 as established by Icelandic survey in 2006, but incidence following adjuvant chest radiation and lung transplant has been reported as high as 2.4% and 50% respectively.(1-5) In lung transplant recipients, BOOP occurs with greatest incidence at postoperative weeks 4-6 and may be a consequence of immunosuppression.(4) In the first two years posttransplant, 50% of organs will biopsy positive for BOOP.(4)

Recent large retrospective surveys in lung allograft recipients have shown a direct association of BOOP with subsequent interstitial fibrosis and bronchiolitis obliterans (BO), increasing odds ratios of these conditions to 2.9 and 1.8, respectively.(4, 5) This is a devastating consequence, as bronchiolitis obliterans is the leading cause of lung allograft dysfunction following transplant, affecting 43-80% of grafts by 5 years.(6) The strong association between BOOP and subsequent BO in lung allografts suggests an unfavorable initial milieu, which favors the development of long term graft failure.

Studies to identify mechanisms underlying BOOP as a first step in graft salvage are limited by imperfect and complex animal models.(7) Therefore, we sought to develop a simple and reliable model a model of BOOP via unilateral ischemia. This was ultimately accomplished via placement of a removable slipknot around the left main pulmonary artery. Such a method avoided manipulation of the airway. Reports of left pulmonary artery (LPA) ligation in animal models paint a rich history of lung pathophysiology.(8-15). The majority of these reports examine the histologic changes with respect to angiogenesis, bronchial artery dilation, and postobstructive pulmonary vasculopathy described as early as 1878.(16) In this report, we build upon these foundations to describe the modified surgical procedure and resulting pathology with respect to development of BOOP lesions. To further characterize this model in light of recent implication of the TLR4 pathway in BOOP pathogenesis, we sought evidence of increases in TLR4 after seven days of ischemia in our slip knot model of injury producing BOOP.(17, 18)

Materials and Methods

Animal Model

All studies were performed under approval of the Medical College of Wisconsin Institutional Animal Care and Use Committee review boards and in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Male Sprague Dawley rats (200-400 gm) were used for the experiments. Anesthesia was induced with 5% isoflurane then maintained with 1.5% to 2.5% of this anesthetic agent throughout the experiment. Body temperature was maintained with a warming table. Rats were intubated and ventilated with a FiO_2 of 0.6 and tidal volume of 7 ml/kg at a rate of ~75 breaths per minute adjusted to maintain eucapnea. They were given atropine sulfate (0.4 mg/kg, intraperitoneal (IP)) to prevent arrhythmias, heparin (500 units, IP; APP Pharmaceuticals, LLC # 504011), and 0.9% sodium chloride (1.5 mL, IP every hour).

Using sterile precautions with betadine and alcohol preparation, a left anterior thoracotomy was completed sharply at the level of the axilla. An Alms self-retaining retractor was

inserted in the intercostal space and widened to provide exposure. The superior-most segment of the left lung was grasped and retracted caudally. Using Dumont #7 forceps (Fine Science Tools #11271-30), the left main pulmonary artery was identified on the superior aspect of the left mainstem bronchus and was dissected free from the bronchus. A 7-0 PDS suture was then passed around the left main pulmonary artery and tied with a single throw followed by a slipknot.(9) The tail end of the slipknot was exteriorized through the thoracotomy incision. The skin was approximated with a second 3-O Vicryl running closure. Pneumothorax was evacuated with a temporary tube thoracostomy connected to suction. The ribs and skin were approximated around this tube, and the tube was removed with a purse string suture secured immediately upon removal. Isoflurane anesthesia was discontinued and the rats were ventilated until they regained consciousness, then extubated. Intraperitoneal carprofen (5 mg/kg) was administered postoperatively and as needed daily through postoperative day two for analgesia.

Sham rats received identical preoperative treatment. An anterior thoracotomy was made, the LPA mobilized, the chest wall closed, and the pneumothorax evacuated. Postoperative treatment was the same as that of rats treated with left pulmonary artery ischemia.

Perfusion Measurements

^{99m}Tc–macroaggregated albumin (MAA) particles 10-40 microns in diameter were used as a pulmonary perfusion marker because they lodge in perfused pulmonary capillaries in proportion to flow. After pentobarbital anesthesia, an injection of ^{99m}Tc-MAA (1 mCi) was made through the femoral vein to elucidate pulmonary artery flow. In order to visualize lung perfusion, a planar image was acquired by positioning the rat supine on a Plexiglas plate directly on the face of a parallel-hole collimator attached to a high-sensitivity modular gamma camera.(19, 20) To assure inflation of the left lung, micro-CT of the thorax was also performed. In a separate set of animals, ^{99m}Tc–MAA was infused in a retrograde manner via carotid artery cannulation in order to determine the contribution of the bronchial circulation to lung perfusion. In both cases, after images were obtained, the rat was sacrificed. To quantify pulmonary perfusion, lungs were harvested from the thorax, separated, and counted using an automated gamma counter. SPECT experiments were performed in rats 24, 48 hours and 7 days after surgery to determine if pulmonary flows changed over this time frame.

Histology

After 7 days, rats were exsanguinated and the heart and lungs removed *en bloc* under isoflurane anesthesia. For all studies, lungs were fixed with paraformaldehyde in the inflated state. Four-micron thick slices of paraffin-embedded, fixed whole mount sections of left and right lung were stained for hematoxylin and eosin. High-resolution JPEG images of the whole mount sections were used for quantification of BOOP (see section below). Cleaved caspase-3, a general marker of apoptosis, (Asp175)(5A1) was detected in paraffin embedded sections using Rabbit mAb to the large fragment (17/19 kDa) of activated caspase-3 resulting from cleavage adjacent to Asp175 (Cell Signaling Technology #9661). This antibody does not recognize full-length caspase-3 or other cleaved caspases. Sections treated with the blocking peptide (Cell Signaling Technology #1050) served as negative controls.

BO Morphometrics

To quantify BOOP, we used a modification of a scale initially developed for quantification of BO (19). This scale includes grading of epithelial injury, fibrosis, and inflammatory changes (EFI), each on a zero to three scale and an estimate of the percentage area of the lung exhibiting BOOP (Table 1).(21) Images were graded by two investigators blinded to the treatment group.

Western blots of TLR4

Whole lung homogenates in a buffer supplemented with protease inhibitor cocktail were centrifuged for 10 min at 20,000 *g*. Western blot analysis was performed as described previously.(22) Blots were developed with a primary antibody to TLR4 (R&D Systems, # AF1478) and β-actin (Sigma #A2228), matched secondary antibodies conjugated to horseradish peroxidase then visualized using ECL detection reagent (Pierce # 32106). The relative densities of protein bands were compared in images scanned with an Alpha Image 220 Analysis System, with β-actin used to correct for protein loading.

Statistical analysis

Ratings between two observers were assessed for interobserver agreement by Pearson Product Moment correlation. For comparisons of more than 2 groups (e.g. left lung scores for EFI in control, sham, and operated rats) we used ANOVA with post-hoc Mann-Whitney or Dunnett's tests when permitted. All grouped data shown in the figures and tables are presented as mean ± standard deviation if normally distributed or median ± 25% for non-normally distributed data.

Results

A total of 18 rats were used for these surgeries: 14 were treated with thoracotomy and slipknot LPA occlusion and 4 with thoracotomy alone (sham). There were no fatalities in either group. Rats lost an average of 8.13 ± 1.9 g (n=14; ~3% body weight) in the first 24 hours after surgery, but recovered their pre-surgical weight gain pattern thereafter.

Gross appearance

Right lungs appeared grossly normal at harvest seven days after surgery (Figure 1a). Left lungs exhibited variable appearance, ranging from near normal with minimally mottled and pale appearing upper lobe to these abnormalities extending throughout the entire left lung.

Perfusion and CT studies

A representative planar image of labeled MAA in control and slipknot operated rats appears in Figure 1b. Pulmonary artery flow to the left lung was quantified as the percent counts of ^{99m}Tc-MAA in the left lung compared to total lung counts following the femoral vein injection. In sham-operated animals left lung flow was 34 ± 1% (n=4), modestly but statistically significantly different from that of normal controls 40 ± 2% (n=6) (Table 2). In contrast, pulmonary artery flow to the left lungs in five rats studied 7 days after LPA slipknot occlusion was < 0.1% of total cardiac output ($p < 0.001$; n=5). In five additional rats studied at 24 or 48 hours, left pulmonary artery flow was also not detectable above background ($p<0.001\%$). These data confirm stability of severe ischemia over 7 days.

Systemic flow to both lungs (through the bronchial circulation) in slipknot-operated rats upon harvest at 7 days was ~3% of aortic artery flow (n=4; Table 3), consistent with our previous report of that in control rats.(20) Of the systemic flow to the lungs, 65% was directed to the left, with 35% to the right. These data support a similar supply of systemic blood to the left lung in control rats (2.5 ± 1.2%) compared to our slipknot operated rats 7 days after ischemia (1.8% ± 0.5% systemic blood supply to the left lung; $p = 0.31$, n.s.).(20)

Histologic changes

Representative images of BOOP lesions appear in Figure 2. Of fourteen left lungs treated with slipknot occlusion of the LPA, all showed extensive Masson bodies and admixed inflammatory cells filling the alveolar air spaces and terminal bronchioles when harvested at

7 days. In some sections, the fibroblasts were confined largely to Masson bodies, but in others they were also present in the interstitium. There was no traction bronchiectasis or isolated interstitial fibrosis, consistent with BOOP changes following seven days of ischemia. Mixed interstitial inflammation, was also commonly observed, sometimes localized to the peribronchial and perivascular spaces, and often throughout the parenchyma. Sections showed no evidence of necrosis/infarction, such as alveolar ghosts or karyorrhectic/pyknotic nuclei. BOOP lesions were generally localized to the left upper lung zones, but small foci with similar lesions were observed throughout the left lung in most rats.

Right lungs exhibited none of the histological abnormalities detailed above, but 4 of 8 sections examined blindly had evidence of thickened pulmonary artery walls scattered throughout the lungs. No pulmonary vascular lesions were noted in left lungs.

Morphometric Analysis

Two independent raters including a trained pulmonary pathologist and pulmonary medicine clinician investigator scored 10 images from sections selected to represent a range of pathologies for EFI endpoints (a total of 30 scores). These investigators were blinded to the source of the images and scores of the other rater. Inter-observer agreement in these scores based upon Pearson correlation was 0.8, $p < 0.001$.

Whole mount images from left and right lungs of 8 rats were rated by two investigators blinded to the source of the tissue for EFI endpoints. Percent of lung exhibiting BOOP pathology and presence or absence of pulmonary vascular lesions consistent with pulmonary hypertension were recorded. These data appear in Table 4. BOOP lesions covered a mean of 24% of the left lung (median 20% per table). Of rats undergoing the operation, 14 of 14 exhibited BOOP involving 10 to 50% of the left lung. Scores for epithelial, fibrotic and inflammatory endpoints were higher in left than right lungs. In data not shown, the left lungs of 2 rats receiving sham surgeries were grossly and histologically normal.

Caspase-3 immunostains

Apoptosis as detected by cleaved caspase-3 immunostaining was observed very infrequently (less than 1 positive cell per 10 high power fields) in right lungs and in left lungs that were histologically unaffected by BOOP, and both lungs of control rats. In contrast, areas in left lung regions exhibiting BOOP consistently demonstrated increased apoptosis with 1-5+ cells per high power field exhibiting the characteristic brown coloration of cleaved caspase-3 positivity (Figure 2e). Per trained pulmonary pathologist (author AR), the cleaved caspase-3 positive cells were epithelial in origin.

TLR4 expression

TLR4 protein was increased in homogenates of ischemic left lungs at seven days compared to that of right lungs ($p<0.05$; figure 3a and 3b).

Discussion

Our data demonstrate conclusively that ischemia alone in a single lung in Sprague Dawley rats, without manipulation of the airway or primary perturbations of the immune system, reliably yields histopathologic lesions of BOOP. In our model, less than 0.1% of the cardiac output is delivered to the left lung one, two, or seven days after ligation of the PA; 2% of the systemic circulation is directed to these lungs after seven days. Our previous studies have demonstrated that with occlusion of the left PA beyond 10 days, the bronchial artery circulation undergoes angiogenesis (20); in fact it is almost certain that this process is underway by seven days when our flow studies were performed. There is no evidence of

necrosis; in contrast, caspase-3 immunostains show colocalization of apoptotic cells within BOOP lesions, reflecting an organized remodeling of the lung parenchyma. Necrosis is not a feature of our rodent model of ischemia, consistent with our previous report of PA ligation in rats.(20) However ischemia secondary to pulmonary emboli in humans results in necrosis 10-15% of the time (61, 62), particularly when occluded PAs are < 3 mm in diameter. We speculate that necrosis results in conditions of limited collateral circulation through bronchial arteries or anastomosing pulmonary arteries and near complete and sustained occlusion of a peripheral pulmonary artery in humans. We cannot exclude species or blood supply differences accounting for necrosis with pulmonary emboli in humans but no necrosis observed in our model.

Modeling BOOP is important, as early detection and treatment may prove beneficial to avoid consequential BO.(23, 24) This is particularly true for lung transplant patients whose greatest long-term risk of graft failure is BO. The pathogenesis of BOOP and subsequent BO is not well understood and is limited by variable and complex animal models.(7) These models include orthotopic and heterotopic lung transplant rodent models, a chimeric T cell mouse model, heterotopic tracheal allografts, inhalational injury, viral infection, bone marrow transplant, and tracheal transplant.(9, 23, 25-60) Only a few models have implicated ischemia as a mechanism for BOOP in either via tracheal transplant, inhaled papaverine, or interruption of circulation.(7, 48, 52) Such models have systemic influence and affect both lungs simultaneously. Others parallel clinical BO in their evolution of disease states, but are quite complex in evaluation of early BOOP mechanisms. For example, whole lung orthotopic lung transplant in rats is a powerful model for studying acute rejection, but subjects do not consistently develop BOOP pathology. Like our model, rodent lung transplant is rarely associated with necrosis. While F344-to-WKY transplantation does exhibit some BO, the combination has limited clinical relevance since death due to rejection develops rapidly.(31) Viral infections including CMV can accelerate the development of BO lesions in the setting of chronic rejection in rodent lung transplant, but neither insult alone is sufficient. Heterotopic tracheal transplantation in mice or rats results in obliterative airway lesions, but these tissues are not in their native environment. Orthotopic tracheal transplants do not yield histological lesions of chronic rejection. Therefore, a rodent model consistently reproducing BOOP is highly desirable in order to understand mechanisms underlying the disorder, as well as to test interventions to prevent or treat it.(7).

Further implicating ischemia, alteration in nitric oxide levels and key oxidative enzyme function (e.g. heme oxygenase, nitric oxide synthase) that regulate microvascular dilation and participate in the oxygen burst of reperfusion injury have been shown to induce BOOP changes.(43, 56) These reports imply that the simplest system for studying the development of BOOP may not require alteration of the immune response, but controlled ischemia. The ideal model would reproduce BO reliably, avoid alteration of immune tolerance, affect only one lung, and leave the airways patent.

While EFI and ISHLT criteria have been used primarily for grading BO, in our morphometric analysis, epithelial cell proliferation, inflammation and proliferation of young fibroblasts (though not collagen deposition or dense fibrosis) describe well the key pathophysiological features of the lesions in our model. Our findings underscore the importance of gas diffusion through the alveoli and/or oxygen carried by the bronchial circulation in maintaining tissue viability. Interestingly, only a few centers have utilized bronchial artery revascularization in human lung transplantation. Early reports from a few centers with small numbers of such transplants do reflect a decreased overall BO rate from 40 to 25% at 2.5 years.(63)

Lung reperfusion following ischemia activates components of the innate immune system including complement and Toll like receptors, particularly TLR4 (18). Engagement of TLR4 receptors activates NF-κB, phosphorylation of p38, ERK and JNK, and signaling pathways that result in non-cardiogenic pulmonary edema and inflammation in part due to cytokine release.(18, 22, 64). We have previously reported increased expression of TLR4 protein in the lungs of SD rats subjected to 1 hour ischemia followed by 2 hours of reperfusion *in vivo.* (22) Our new results support activation of TLR4 in a different model (ischemia alone) seven days as opposed to hours after ischemia. The pathophysiological significance of increased TRL4 expression in this model remains to be determined.

This selective ischemia model reproduces BOOP reliably, avoids alteration of immune tolerance, affects only one lung, and avoids manipulation or interruption of the airways. In addition to meeting these standards, it provides the opportunity to study carefully the time course of ischemic injury relative to BOOP development, since the slipknot can be released without additional surgery or anesthesia at any point after the initial operation. It offers a unique opportunity to investigate time course, signaling pathways, and alterations in gene expression profiles underlying the development of this disorder. Our description is meant to afford an opportunity for further mechanistic studies elucidating BOOP pathogenesis.

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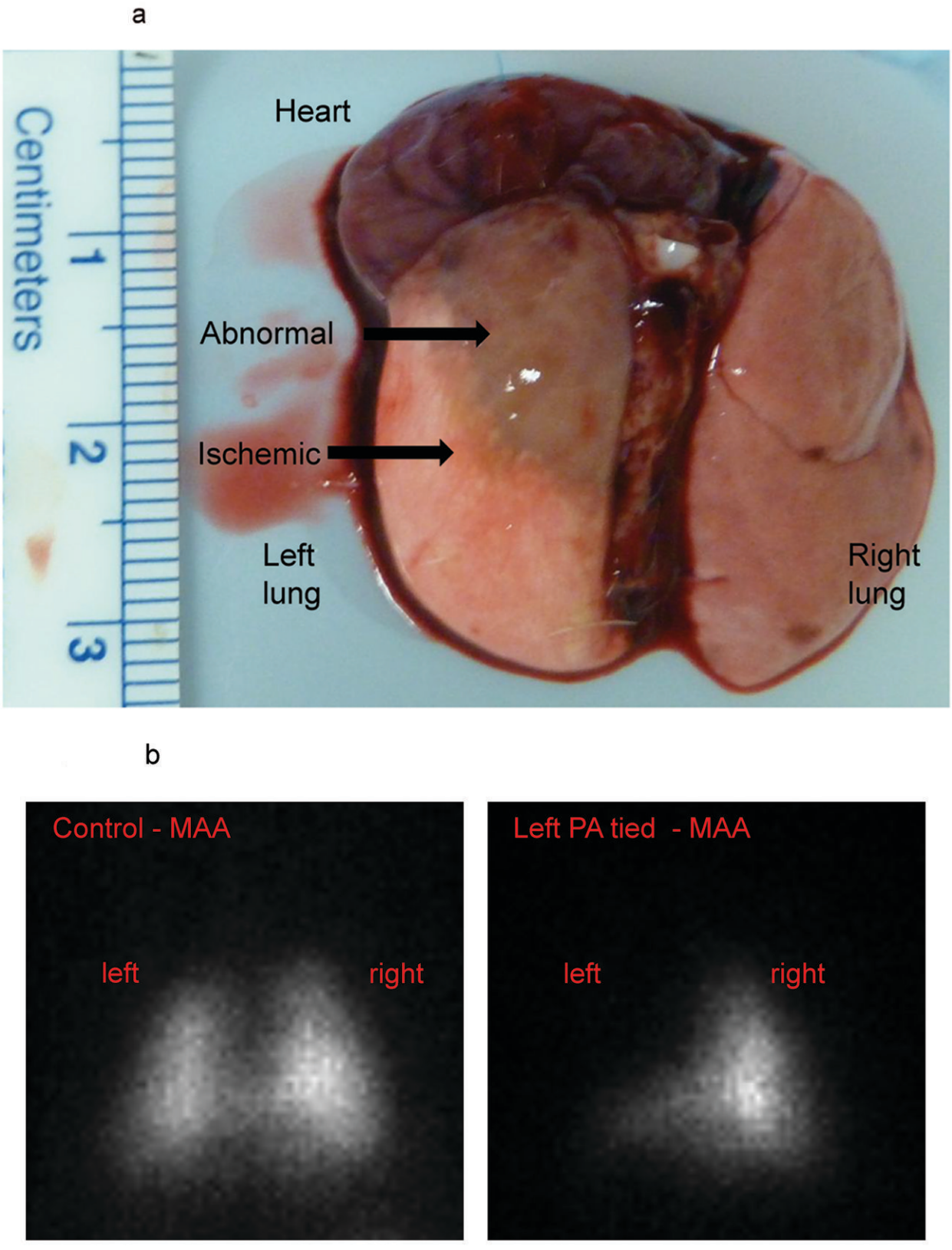
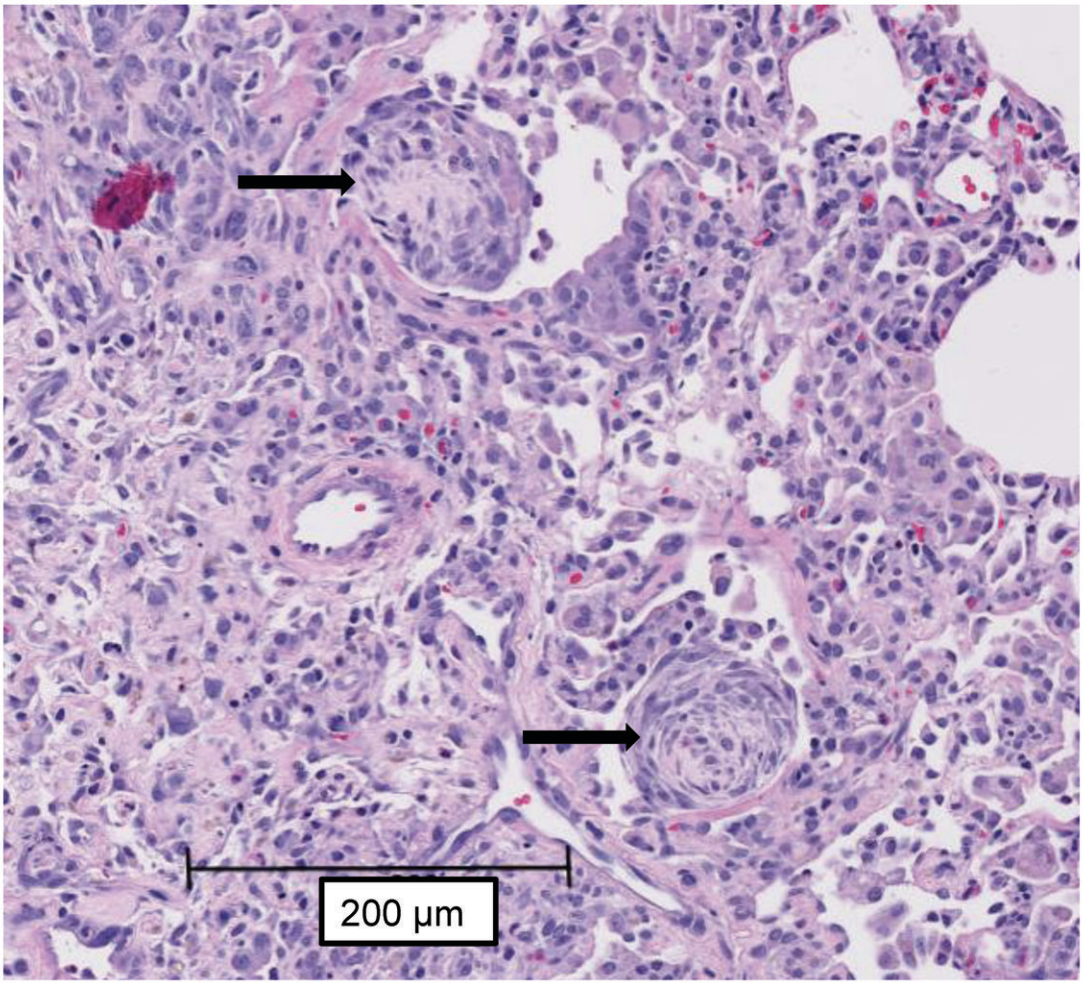


Figure 1.

a. A representative image shows the gross appearance of lungs at harvest seven days after ischemia. The heart, left and right lungs are labeled for orientation. The right lung appears pink and smooth. Portions of the left lung, particularly in the lower lobes look similar to the right lungs, though clearly more pale consistent with limited PA blood supply. The apical portion of the left lung typically looked pale or grey/white in color relative to the right lungs and was more firm in texture. In some cases, the majority of the left lung exhibited the appearance of the upper lobe in the representative figure, *and* scattered areas on the pleural surface of the lower lobes, which appeared similar. The left lungs of sham-operated animals (not shown) had the gross appearance of those from control rats.
b. Representative planar SPECT images demonstrating the distribution of ^{99m}Tc-macroaggregated albumin (MAA) (1 mCi) injected through the femoral vein of a control rat (on the left) and a rat 7 days post operatively from a slipknot surgery (on the right). The uptake in the left lung region is absent in the operated rat.

a



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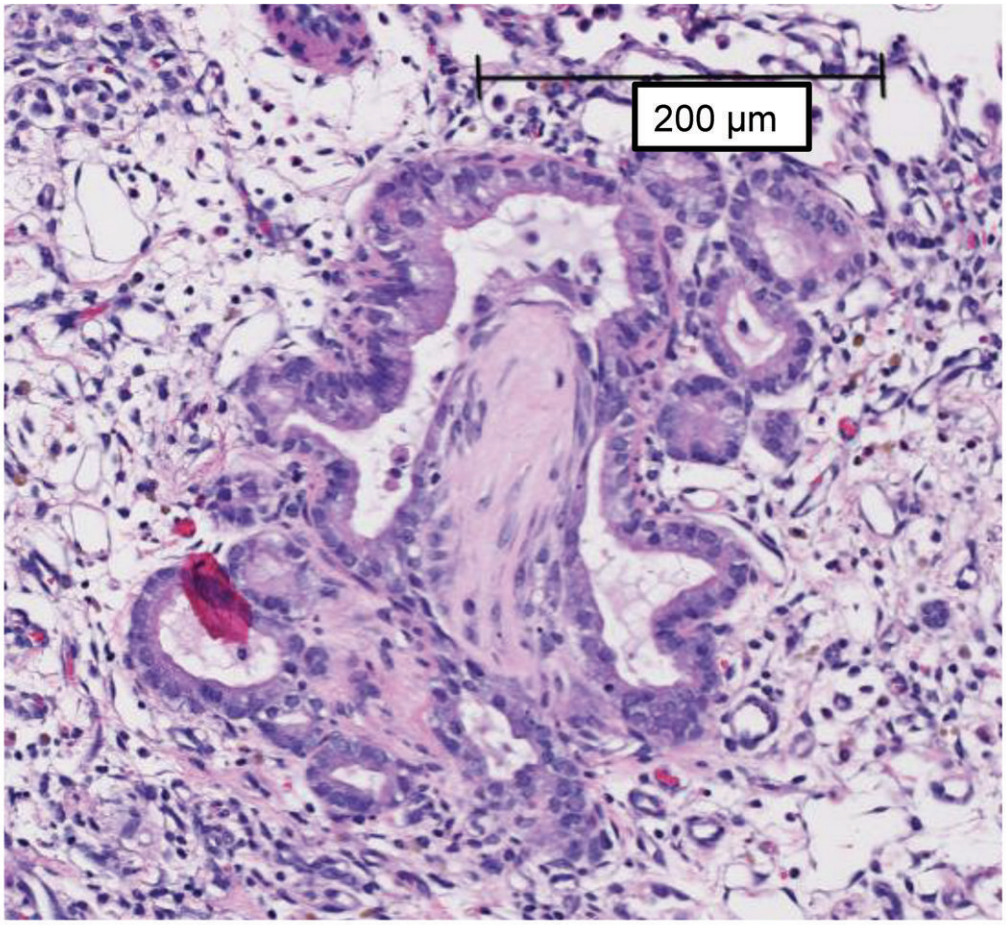

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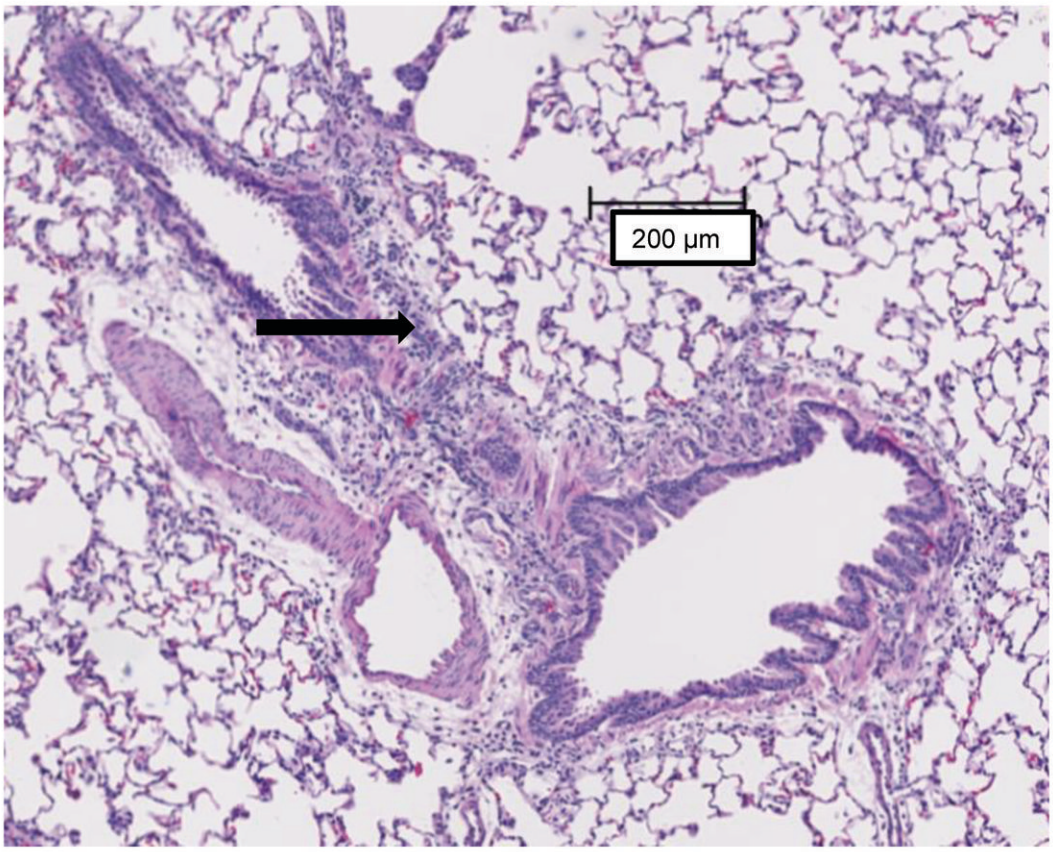

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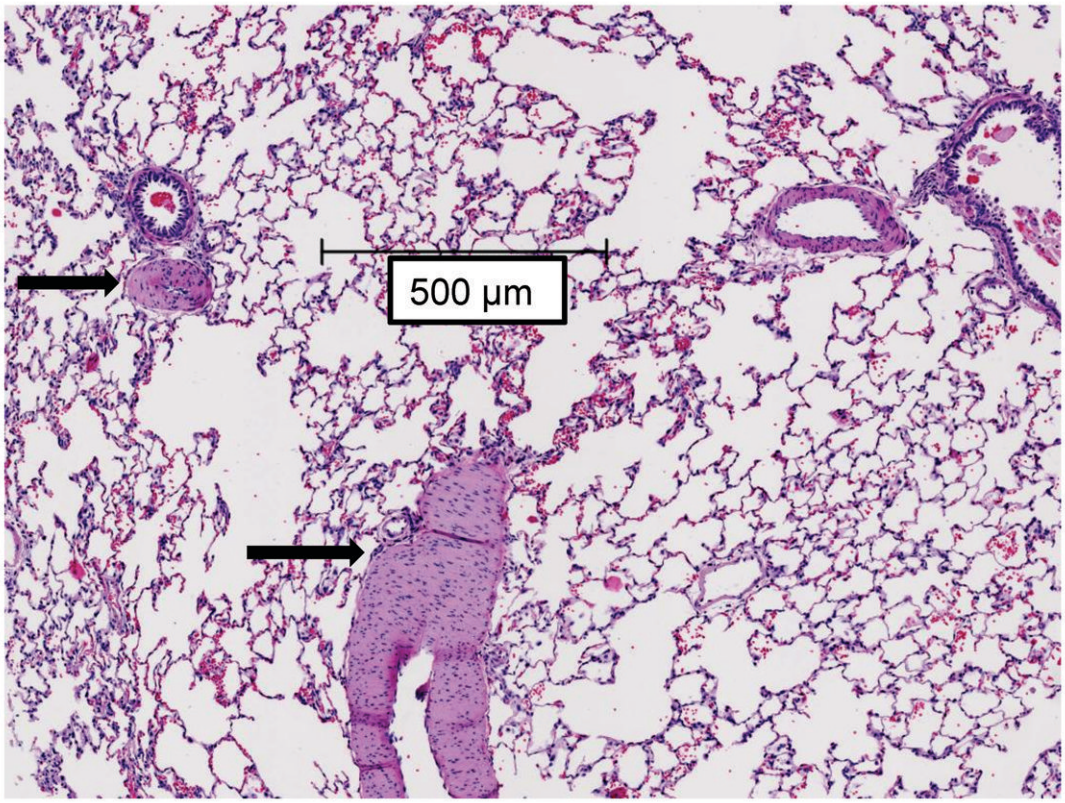

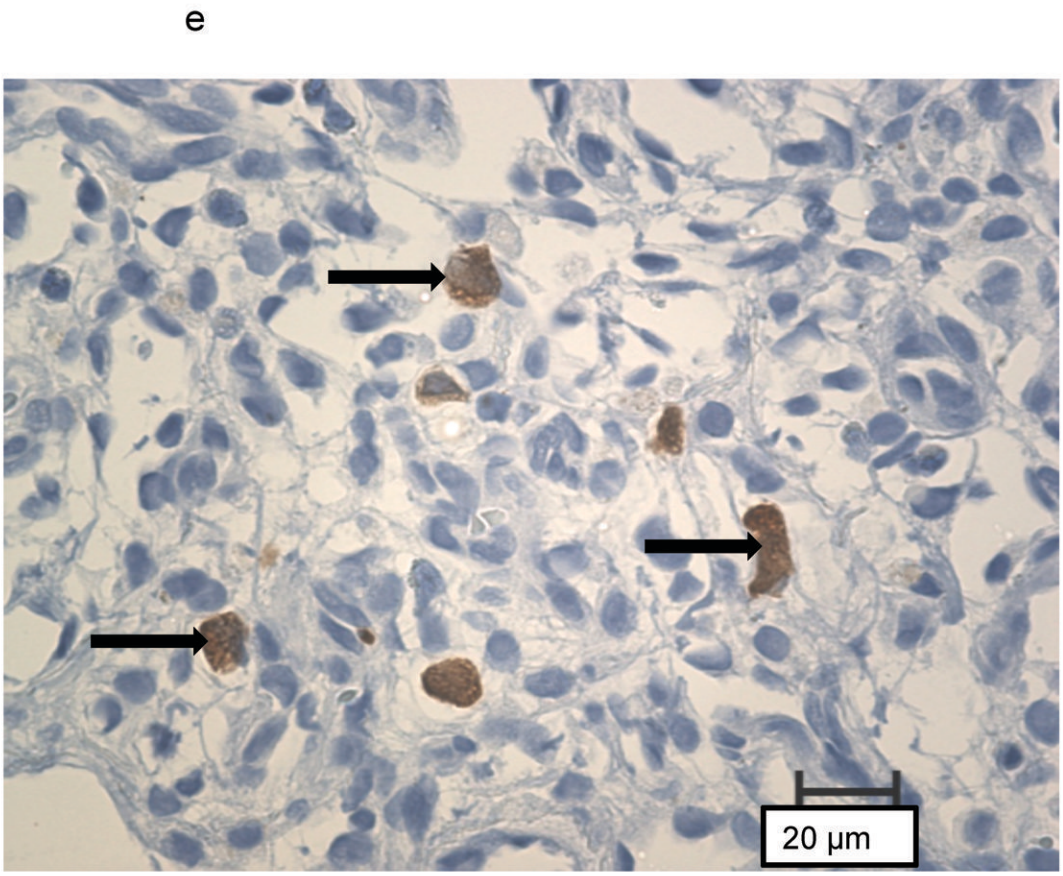

Figure 2.

Representative images of the histological findings in left lungs consistent with BOOP.
(a) The cells filling the terminal airways are an admixture of fibroblasts embedded in pale, myxoid stroma. The two arrows denote circular structures of fibroblastic foci with extracellular matrices (Masson bodies shown with two arrows).
(b) A bronchiole exhibits intraluminal filling with stroma and fibroblasts, a bronchiolar intraluminal polyp. The epithelia appear modestly reactive in this image.
(c) Inflammatory cells (blue in color) in the peribronchiolar and perivascular spaces are evident in this image. There was no evidence of necrosis: alveolar ghosts, pyknotic nuclei, debris in the intra-alveolar spaces and nuclear degeneration in histopathologic sections examined were absent.
(d) This image from a right lung shows thickening of the walls of pulmonary arteries, in the case of the small artery significantly limiting the luminal opening. These lesions were present in ~50% of the right lungs examined.
(e) Representative caspase-3 immunohistology images show organizing pneumonia and cleaved caspase-3 positive cells. Throughout the left lung unaffected by BOOP and all right lungs as well as lungs from control rats, cleaved caspase-3 positive cells were very sparse, with less than one per several high power fields (data not shown). In contrast, in areas affected by BOOP, cleaved caspase-3 positive cells were frequent, with consistently more than one and up to eight positive cells per high power field. The arrows in this image point to cells that have brown cytoplasm. Serial sections treated with the antibody and blocking peptide showed no cleaved caspase-3 positive cells.

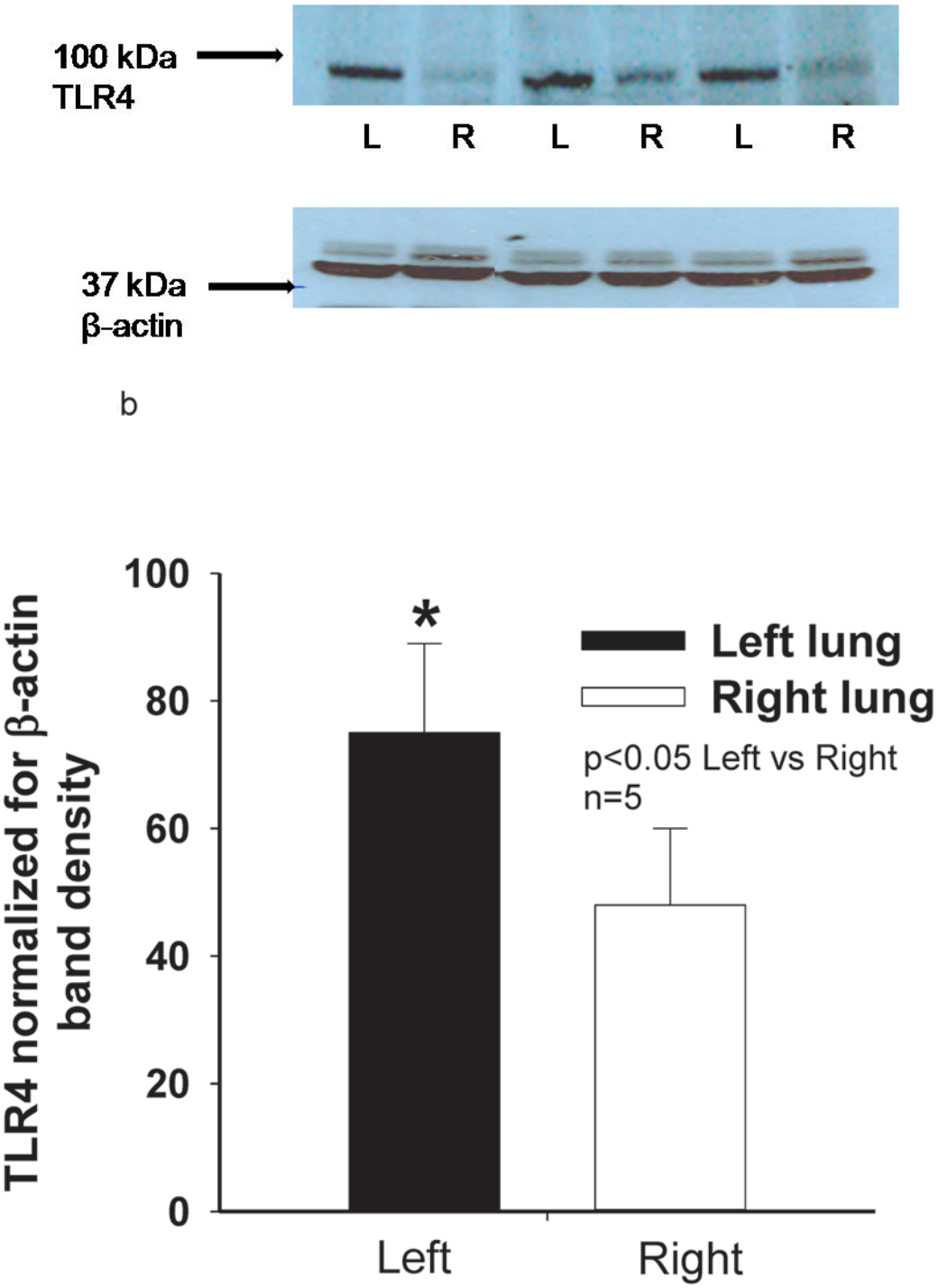

Figure 3.

(a) Representative images of western blots probed with a primary antibody to TLR4 show increased band density in samples from ischemic left lung compared to right lungs. β-actin bands from the same samples are shown for normalization (b) Graph depicts average densities and standard error bars of TLR4 normalized for β-actin. Asterisks indicate $p<0.05$ (one-way ANOVA followed by Holm-Sidak's post hoc test) relative to right lung of rats receiving slip knot surgery.

Table 1

Our injury grading scale, quantifying epithelial, fibrotic and inflammatory changes (EFI) on a 0 to 3 scale, is shown. We modified a scale from the International Society of Heart and Lung Transplantation used to grade BO in transplant patients and used by Martinu.(21, 65) In addition to these qualitative endpoints, the percentage area of a whole mount slide taken through the middle of the lung exhibiting characteristic BOOP lesions was estimated to the nearest 10%.

BO injury grading scale	**0**	**1**	**2**	**3**
Epithelial	Normal	Reactive epithelium ± intermittent disruption of epithelial layer	Markedly reactive epithelium ± epithelial disruption	Epithelium largely denuded
Fibrosis	Normal	Scattered young fibroblasts	Intraluminal fibromyxoid filling	Dense fibrosis
Inflammation	Normal	Perivascular or peribronchiolar cuffing with mixed sparse inflammatory cells/sparse chronic inflammation	Moderate mixed inflammatory infiltration throughout the lungs	Severe infiltration of mixed inflammatory cells

Percent area of whole mount rounded to nearest 10% exhibiting BO

Table 2

This table shows flows as estimated by ^{99m}Tc-MAA counts in the left and right lungs. The differences in the 3 groups were determined by Dunnetts and appear in the upper left hand side of the table. In control rats, the flow to the smaller left lung was 40% with the remaining 60% going to the right. In sham-operated rats, this number was decreased to 34%, and less than 0.1% in slipknot operated hosts.

Group name (Dunnett's between groups p<0.001)	N	Mean % flow through PA to the left lung	STD	P value
Control	6	40.1	1.9	
Sham	4	34.1	1.0	0.001 vs control
Slip knot	5	0.09	1.9	0.001 vs control

Table 3

This table shows flows to the left and right lungs through the systemic (bronchial) circulation as estimated by ^{99m}Tc-MAA injected retrograde through the carotid arteries of rats 7 days after a slipknot was placed around the left pulmonary artery. Of the MAA injected, 2.9 ± 0.8% (Mean ± STDV) localized to the lungs, with 65 ± 14% of the bronchial artery flow going to the left lung with the remainder to the right. These numbers are not statistically different than those of control rats reported by our group (2.5 ± 1.2% to the left lung), indicating that BOOP develops in this model prior to substantial remodeling of the bronchial circulation.(20)

Bronchial artery flow	N	Mean % flow through PA to the left lung	STD
% systemic flow to the lung	4	2.9	0.8
% bronchial artery flow Left/Right	4	65	13.8

Table 4

Grading of lesions from left and right lungs based upon EFI criteria as well as percent area of the whole mount involved with BOOP lesions based upon a scale adopted from Martinu *et al*, 2006.(21) Images were evaluated and scored by investigators blinded to the treatment group. No lesions consistent with BOOP were observed in right lungs, whereas no left lung had less than 10% of the whole mount area exhibiting lesions consistent with this histopathology. The median and 25% values as well as the range of values are given in the table. Scores for each of the 3 endpoints (epithelial changes, fibrosis and inflammation) in left and right lungs were compared by Mann-Whitney Rank Sum tests. Scores for 2 control rats and 2 sham-operated rats do not appear in the table as they exhibited no lesions consistent with BOOP in left or right lungs.

BO scores: median ± 25% (range) n=8 pairs	**Epithelial**	**Fibrotic**	**Inflammatory**	**% area**
Left lung	2.0 ± 1.5 (1.5 to 2.0)	2.0 ± 1.6 (1.0 to 2.5)	2.0 ± 1.1 (1.0 to 2.5)	20 ± 11.2 (10-50)
Right lung	0 ± 0	0 ± 0	0 ± 0	0 ± 0
p value L vs R Mann Whitney	<0.001	<0.001	<0.001	<0.001