Published in final edited form as:

Magn Reson Med. 2015 May ; 73(5): 1970–1978. doi:10.1002/mrm.25313.

Imaging mouse lung allograft rejection with ^1H MRI

Jinbang Guo^{1,5}, Howard J. Huang², Xingan Wang³, Wei Wang⁵, Henry Ellison⁵, Robert P. Thomen^{1,5}, Andrew E. Gelman^{3,4}, and Jason C. Woods^{1,5,*}

¹Center for Pulmonary Imaging Research, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA

²Department of Internal Medicine, Washington University in St. Louis, Missouri, USA

³Department of Surgery, Washington University in St. Louis, Missouri, USA

⁴Department of Pathology & Immunology, Washington University in St. Louis, Missouri, USA

⁵Department of Physics, Washington University in St. Louis, St. Louis, Missouri, USA

Abstract

Purpose—To demonstrate that longitudinal, non-invasive monitoring via MRI can characterize acute cellular rejection (ACR) in mouse orthotopic lung allografts.

Methods—Nineteen Balb/c donor to C57BL/6 recipient orthotopic left lung transplants were performed, further divided into control-Ig vs anti-CD4/anti-CD8 treated groups. A two-dimensional multi-slice gradient-echo pulse sequence synchronized with ventilation was used on a small-animal MR scanner to acquire proton images of lung at post-operative days 3, 7 and 14, just before sacrifice. Lung volume and parenchymal signal were measured, and lung compliance was calculated as volume change per pressure difference between high and low pressures.

Results—Normalized parenchymal signal in the control-Ig allograft increased over time, with statistical significance between day 14 and day 3 post transplantation ($0.046 \rightarrow 0.789$, $P < 0.05$), despite large inter-mouse variations; this was consistent with histopathologic evidence of rejection. Compliance of the control-Ig allograft decreased significantly over time ($0.013 \rightarrow 0.003$, $P < 0.05$), but remained constant in mice treated with anti-CD4/anti-CD8 antibodies.

Conclusion—Lung allograft rejection in individual mice can be monitored by lung parenchymal signal changes and by lung compliance through MRI. Longitudinal imaging can help us better understand the time course of individual lung allograft rejection and response to treatment.

Keywords

lung transplant; acute cellular rejection; compliance; MRI

*Correspondence to: Jason C. Woods, Ph.D., Center for Pulmonary Imaging Research, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave., Cincinnati, OH 45229. Jason.Woods@cchmc.org, Tel: 513-803-4463, Fax: 513-636-3754.

Introduction

Lung transplantation is a life-saving treatment for patients with end-stage lung disease (1). Despite improvements in short-term outcomes, graft failure due to chronic rejection remains the major barrier to long-term survival in lung recipients (2-4). Although the pathogenesis of chronic lung allograft rejection is poorly understood, clinical studies have consistently identified acute cellular rejection (ACR) as a major risk factor (5). Even minimal, clinically silent episodes of ACR are associated with increased risk for developing chronic rejection (6, 7). Accordingly, most centers perform routine surveillance biopsies to facilitate early detection and treatment of ACR (8). However, invasive transbronchial biopsies are subject to sampling heterogeneity, grading variability, and potentially life-threatening complications (9-11). ^1H MRI is a non-invasive imaging modality that may serve as a useful clinical adjunct for detecting ACR and assessing treatment response. We aim to demonstrate the feasibility of this technique using a mouse model of orthotopic lung transplantation (OLTx).

Mouse OLTx was first reported by Okazaki and colleagues in 2007 (12). This technique facilitates hypothesis-driven studies of lung transplant immunobiology in a small animal model that physiologically approximates an aerated, vascularized human lung allograft. Accordingly, investigators have utilized the model to study various aspects of lung transplant immunobiology including ischemia-reperfusion injury (13, 14), immune cell trafficking and molecular mechanisms underlying the pathogenesis of ACR and bronchiolitis obliterans (15-18). Fully mismatched allografts (major histocompatibility complex) develop edema, impaired oxygenation, and perivascular and interstitial mononuclear infiltrates on histology by post-operative day (POD) 7 (17, 19). Without immunosuppression, ACR progresses and the allograft become completely consolidated and atelectatic with diffuse hemorrhage and necrosis by POD28. Importantly, established ACR in this model is largely T-cell mediated and can be ameliorated by treating recipients with T-cell depleting antibodies (20).

The three main tomographic imaging modalities used for the lung are x-ray computed tomography (CT), positron emission tomography (PET) and magnetic resonance imaging (MRI). PET is particularly useful for acquiring molecular functional information, but specialized and expensive radio tracers must be developed for each functional abnormality (21). CT has become a gold standard for lung imaging but has received much attention recently because of increased cancer risk with ionizing radiation (22, 23), particularly for longitudinal study. MRI has now seen widespread success as an imaging modality due to increased resolution, information “richness” and its non-ionizing nature, but MRI of the lung has been challenging because of lower proton density and very short T_2^* in the lung. Hyperpolarized gases such as ^3He and ^{129}Xe have piqued significant interest in lung MRI in the last 15 years and have allowed monitoring of regional ventilation (24, 25), alveolar microstructure, and oxygenation in mice and humans (26-33).

Our goal in this study was to use ^1H MRI methods available on most clinical scanners (short echo time [TE], but not ultra-short TE [UTE]) and ventilation-synchronization to monitor lung rejection in a mouse model of lung transplantation. Since each mouse is unilaterally transplanted, the right lung serves as a control for each animal; the two groups are intended

to show allograft rejection (control-Ig treated) and non-rejection (anti-CD4/anti-CD8 treated). By monitoring individual animals longitudinally and utilizing new techniques for calculating respiratory compliance, we were able to quantify morphologic and physiologic changes over time. Imaging individual mice with unilaterally transplanted lungs in two treatment groups (rejecting & non-rejecting) demonstrates the feasibility of ^1H MRI at quantifying ACR via increased parenchymal signal and decreased regional compliance. These techniques can be translated to the clinic, since similar pulse sequences and ventilation synchronization exist on most clinical MR scanners.

Methods

Animal groups and OLTx

This prospective study was approved by the Washington University School of Medicine Animal Studies Committee; transplantation and imaging were performed at Washington University, with subsequent analysis performed in Cincinnati. The experimental plan is shown in Figure 1. Male Balb/c and C57 BL/6 mice (8-10 weeks old) were used as lung donors and recipients, respectively. OLTx were performed as previously described (12). Recipient mice were started on antibiotic water 24 hours before transplant and maintained on antibiotic water for the duration of the experiment. Nineteen Balb/c (H2^{d}) $\rightarrow$ BL/6 (H2^{b}) allogeneic lung transplants were performed. All recipients were treated with buprenorphine for analgesia for 2 days post surgery. Eight graft recipients in the control cohort received intraperitoneal injection of 500 μg of hamster isotype control antibody (control-Ig) at POD 3. Eleven graft recipients in the experiment cohort received intraperitoneal injection of 250 μg of anti-CD4 (GK1.5) and 250 μg of anti-CD8 (YTS169.4) monoclonal antibody (Bio X Cell, West Lebanon, NH 03784-1671, USA), also at POD 3. Lung recipients were serially imaged at POD 3, 7 and 14. All surviving recipients were sacrificed at POD 14 for lung tissue histological analysis.

Ventilation for imaging

Allograft recipients were anesthetized by intraperitoneal injection of a mixture of ketamine/xylazine and orally intubated with a 20-gauge angiocatheter. Each mouse was then ventilated with isoflurane at 120 breaths per minute and approximate tidal volume of 0.25 mL (at constant peak pressure of 15 cm H_2O and positive end-expiratory pressure of 3 cm H_2O). A schematic of the ventilation waveform (30) is shown in Figure 2. Each respiratory cycle consisted of a brief breath hold for imaging, an inhalation of O_2 , a second brief breath hold, followed by further inhalation of pure N_2 , then a third brief breath hold for imaging, and finally passive exhalation. Gas flow and airway pressure (at the breathing valve) were closely monitored by flow meters and an electronic pressure transducer, respectively. Approximately 25% of the total gas inhaled each breath was pure oxygen (for only 70 ms of each 500-ms breathing period), only slightly elevated from the normal oxygen content in air (34). We expect no physiological changes from this split-breath maneuver, compared to a simple breath of air. Airway pressure waveforms were monitored in real time by oscilloscopes in the control room before, during, and after imaging.

MRI

MR imaging was performed on a Varian 4.7 T horizontal-bore magnet (40 cm magnet bore and 12 cm gradient bore; Agilent Technologies, Santa Clara, CA). A custom-built solenoid coil covered the entire lungs with high transverse homogeneity. A trigger signal from the ventilator allowed the scanner to begin the short (gated) data acquisition at peak inspiration (“high pressure”) or end expiration (“low pressure”). A standard 2D multi-slice gradient-echo sequence with an appropriate flip angle pulse (flip angle=20°) was used for 1H MR imaging, for simplicity and potential translation. For each mouse, 9 transverse slices (thickness = 2.0 mm, gap = 0.2 mm) were acquired to cover the whole mouse lung with in-plane field of view of 30 mm × 30 mm, and spatial resolution of 0.3 mm × 0.3 mm (96 × 96). One line of k-space was scanned for each of the 9 slices, per breath, with echo time TE = 0.95 ms, repetition time TR = 2.46 ms, sweep width = 0.21 MHz, and readout duration = 0.46 ms; image sets were acquired with both 1 and 4 averages. Effective TR between radiofrequency (RF) pulses in the same slice was 500 ms. Thus, the number of respiratory cycles during imaging was equal to the number of total phase encoding steps times the number of averages. This respiratory-triggered scanning minimized motion artifacts and allowed multiple sets of images to be acquired (high and low pressures, 1 and 4 averages). One scan (1 average) took 48 seconds to acquire all slices, at a respiration rate of 120 breaths/min.

Lung tissue histology

Surviving lung allograft recipients were sacrificed at POD 14 for lung tissue histology. Heart and lung tissue were resected en bloc. Lung blocks were inflated and fixed in 10% neutral buffered formalin overnight. Left transplanted lungs (allograft) and right untransplanted lungs (native lung) were divided and submitted for paraffin embedding, sectioning, and H&E slide preparation.

Data analysis

A commercial graphic processing software package (Amira, FEI Company, Hillsboro, OR) was used to semi-manually segment the lung from other soft tissues; the major bronchi and large blood vessels were excluded for analysis. The left lung (allograft) and right lung (native) were also separated from each other with particular care taken to separate the accessory lobe from the left lung (Figure 1 in the online supplement). Then the total number of pixels (for volume calculation) and parenchymal signal were measured in all slices.

While RF field homogeneity was high in the transverse direction (perpendicular to the solenoidal axis), homogeneity varied in the slice direction (Figure 2 in the online supplement). The raw image data were thus normalized by the average soft-tissue signal of each slice (excluding the lung). This method was found to be more robust than a reference to a single organ or region. Parenchymal signal was measured specifically in regions of interest near the lung periphery to avoid the effects of blood vessels. Since images were acquired over many heart cycles, perfusion effects were averaged (35). Compliance was obtained by dividing the change in lung volumes at high and low pressures by the change in pressure measured at the breathing valve. Since allograft and native lung volumes can be measured separately, this allows the allograft and native-lung compliances to be measured separately,

with total lung compliance being the sum of the two. The volume-percentage of high intensity signal ($3\times$ of mean value of native lung parenchyma at POD 3) in the allografts was also measured and compared to allograft compliance (Figure 7). In order to quantify the stability of ventilation over time, the 1-average and 4-average image sets were analyzed separately.

Paired student *t* tests with an alpha level of 0.05 were used to compare lung parenchymal signal intensity and lung compliance between time points and between different groups. One tailed tests were used in cases where lung density was known to increase. Mean and standard deviation (SD) of lung parenchymal signal intensity and lung compliance were calculated.

Results

Ventilation in our apparatus was precise and stable (3.8% average percentage difference in volume per mouse between 1 and 4 averages at the same peak pressure). Three mice of the total 19 died as a result of surgery, intubation, anesthesia, and mechanical ventilation. Two mice were not imaged at POD 3 and POD 7 because of scheduling difficulties, thus were not included in the data. Images were of high quality at both high and low volumes/pressures (Figure 3). Signal-to-noise ratio (SNR) in the parenchyma (away from airways & vessels) was about 6 for 4 averages in the native lung; in the normalized images this corresponded to about 5% of the average soft-tissue SNR. Figure 3 presents representative axial images from a control-Ig treated mouse and an anti-CD4/anti-CD8 treated mouse at all 3 time points along with histology at POD 14. No obvious rejection (visually or quantitatively) was seen in the allograft at POD 3 in either group. At POD 7, little signal change was seen in most anti-CD4/anti-CD8 treated mice, while large increases were seen in most control-Ig treated mice; parenchymal signal was still near normal in most anti-CD4/anti-CD8 treated mice at POD 14. Parenchymal signal as percent of mean soft-tissue signal is shown in Figure 4 and Table 1. At POD 3 there is no statistically significant difference between the allograft and native lung ($P > 0.05$). Allograft parenchymal signal demonstrates large variations for both control-Ig treated and anti-CD4/anti-CD8 treated groups, reflecting both temporal change and different responses to treatment. For example, a suspected pneumonia in mouse 219 at POD 7 was resolved at POD 14; varying degrees of ACR were observed in most mice in the control-Ig treated group (all confirmed by histology). Because the true inter-mouse variation was so large, standard deviation of the measured values was concomitantly large. Mean parenchymal signal in the allograft for control-Ig treated and anti-CD4/anti-CD8 treated mice at POD 7 increased by 1109% ($P = 0.06$) and 290% ($P = 0.13$), respectively, over POD 3. Allograft mean parenchymal signal of control-Ig treated mice saw an increase by 42% at POD 14 over POD 7 ($P = 0.12$), but was near-constant for anti-CD4/anti-CD8 treated mice (7% decrease, $P = 0.48$). However, allograft mean parenchymal signal of control-Ig treated mice at POD 14 increased significantly compared to POD 3 (increase by 1615%, $P < 0.05$). As was expected, the right native lung parenchymal signal for all mice remained nearly constant (11% increase from POD3 to POD7 and 5% decrease from POD7 to POD 14, $P > 0.05$).

Respiratory compliance is presented in Figure 5 and Table 2. Allograft compliance of control-Ig treated mice at POD 14 and POD 7 decreases with statistical significance compared to POD 3 (by 77% [$P < 0.001$] and 69% [$P = 0.01$] respectively). There is no statistically significant change for native lung compliance ($P > 0.05$), but the mean compliance of native lung at POD 14 increases by 11%. Allograft compliance of anti-CD4/anti-CD8 treated mice remains constant ($P > 0.05$), while there is slight increase (25%) in native lung compliance at POD 14. Allograft compliance at both POD 7 and POD 14 shows a statistically significant difference between the control-Ig and anti-CD4/anti-CD8 treated mice ($P < 0.05$). Histological analysis demonstrated severe ACR in control-Ig treated mice; no evidence of ACR was seen in most anti-CD4/anti-CD8 treated mice or in the control (native) lungs (Figure 6). Quantification of the high-intensity volume of allografts showed negative correlation between volume percentage of high intensity signal and allograft compliance (Figure 7).

Discussion

We have demonstrated with MR imaging an ability to serially monitor lung ACR in individual mice. Importantly, ACR is dependent on T cells (12); accordingly, we observed sharp differences in MR measurements between recipients of lungs that received anti-CD4/anti-CD8 compared to control-Ig treated lung recipients. This opens the door to a much better understanding of the efficacy of the new treatment strategies through the ability to visualize and longitudinally monitor changes in individual lungs. For example, among the 9 anti-CD4/anti-CD8 treated mice, one mouse demonstrated signs of pneumonia at POD 14 (mouse 278), and one of the control-Ig treated mice recovered from what appeared to be pneumonia or atelectasis at POD 7 (mouse 219). In the future we anticipate that serial MR monitoring after murine lung transplantation will allow for better understanding of individual immune responses to treatment and reduce the number of animals used in pre-clinical studies. Moreover, this technique may be useful as a clinical adjunct for detecting ACR and monitoring response to treatment.

Since the mean difference in total lung volume between 1-average and 4-average images for all mice at high pressure is small, we expect little variation due to ventilation of the mice. Variation of parenchymal signal due to between-slice RF inhomogeneity (Figure 2 in the online supplement) was an initial concern, which was overcome by normalizing each slice separately. Time variation due to perfusion was averaged over many heart cycles. The fact that we see little difference (both visually and statistically) in mean parenchymal signal between control-Ig treated and anti-CD4/anti-CD8 treated native lungs at POD 3 is evidence that the ventilation and normalization schemes are robust. Very small longitudinal variation in native-lung signal also speaks to the robustness of the measurement technique.

Compliance is a standard physiological measurement for characterization of lung mechanics. Here we have introduced regional compliance, only possible because we can measure allograft and native lung volumes and volume changes separately (with pressure monitoring). Segmentation of the accessory lobe of the right lung (native) from the left lung (allograft) required expertise and care; we suspect this sometimes-invisible boundary contributed to some variation or error in the compliance measurement. Compliance of the

native lung, however, was quite stable between time-points. It is also important to note that the total respiratory compliance here is the sum of the allograft and native lungs ($V_L/P + V_R/P$), not the volume-average. A size-independent measure might be specific compliance, where each compliance is normalized by the volume at high pressure. We have chosen to cite absolute compliance here, for clarity. In control-Ig treated mice, compliance of the allograft decreased with time, due to lung rejection; compliance of the native lung increased slightly from POD7 to POD14, due to left-lung shrinkage with rejection (36, 37). In anti-CD4/anti-CD8 treated mice, compliance of the allograft remained unchanged, as expected, though compliance of the native lung increased slightly. Total compliance at POD 3 (all mice combined) was 0.036 ± 0.006 cc/cm H₂O, which is relatively consistent with measurements via FlexiVent (0.022-0.033 cc/cm H₂O) by other groups (38, 39). As expected, negative correlation was seen between allograft compliance and volume percentage of high-intensity signal in the allografts (Figure 7). We note that our approach to compliance is quite simple compared to that measured by the FlexiVent, (regarded as a standard for mouse physiology), with the advantage being that our measurements are within or close to the normal tidal-volume range, and the near-linear portion of the pressure-volume curve. This allows our technique to be easily repeated (not a terminal procedure, as the FlexiVent is often regarded), yet does not allow the full range of robust measurements or ventilation like the commercial device.

¹H MRI signal density at zero echo time (and long effective TR) is a direct reflection of proton density in tissue. For the more practical case of nonzero TEs, as is the case here, the signal is significantly lowered in lung due to the effects of magnetic susceptibility gradients arising from the multiple interfaces between lung tissue and alveolar airspaces. Since we have normalized lung signal to the average in-slice soft-tissue signal (with much longer T₂^{*}, but roughly the same T₁), we can estimate the expected signal of lung tissue in our imaging regime. The parenchymal density of the native lung in our study at peak pressure (15 cm H₂O) is expected to be 33% of the average soft-tissue (mainly heart) density (near 1 g/cm³) (40, 41). Taking into account T₂^{*} = 0.46 ms of C57BL/6 mouse lung at 4.7 T (40, 42), the expected parenchymal signal of native lung is 4.2%, which is consistent with our result of 5.3% at POD 3. In future studies, we expect to minimize the effects of short T₂^{*} of lung by using techniques that allow shorter or near-zero TE (13, 14, 43-50), but note that such non-Cartesian techniques will increase total image-acquisition time, for similar image resolution.

It is notable that some control-Ig treated mice may have experienced slower lung rejection (mouse 170 & 336 from POD 3 to POD 7); serial imaging allows close monitoring of the rejection in individual animals by the parenchymal signal intensity increase and by the compliance decrease. This can improve accuracy and reduce the number of mice needed in a study, thus increasing rapidity and accuracy of information about an experimental treatment. When a mouse model of chronic lung rejection is fully developed, imaging can serve an important role in understanding spatial variation and time course of that rejection for more accurate biological targeting. By utilizing MRI instead of CT, we are preparing the technique for translation to imaging in humans, without ionizing radiation (particularly important in vulnerable populations, like children). The fact that we are able to easily measure lung signal and lung compliance in vivo shows the potential for translation to the clinic for longitudinal monitoring of the allograft.

The increased parenchymal signal intensity and decreased lung compliance are consistent with histological findings of severe ACR at POD 14 in each of the control-Ig treated mice. The histological analysis also demonstrates no ACR in the anti-CD4/anti-CD8 treated mice (Figure 6). These results are consistent with our analysis of MR images.

^1H MRI has somewhat low SNR due to low proton density of lung parenchyma and short T_2^* . The main limitation of the technique's ability to monitor rejection, however, is that we cannot easily distinguish pulmonary edema or atelectasis that will resolve from consolidated pneumonia (e.g. resolution of abnormalities in mouse 217 at POD 14) demonstrated in Figure 6, yet we note that the longitudinal imaging allows repeated measures in the same animal. The anti-CD4/anti-CD8 allografts that demonstrated ephemeral increased parenchymal signal intensity did not show significant ACR, but the POD 14 histological results match the POD 14 imaging results, even in these more ambiguous cases. Residual edema and cellular consolidation are seen, for example, in mouse 278 allograft, no edema is seen in the 217 allograft and complete cellular consolidation is seen in mouse 170 allograft as shown. Some small segmentation errors in volume ($< 5\%$) result from our inability to distinguish the accessory lobe of the native lung from the allograft. Also, this particular system did not allow UTE (which would lead to higher SNR), but the gradient echo sequence had the advantage of being reliable and robust.

We have demonstrated the ability to acquire quantitative ^1H MR images of murine lung at reasonable resolution and SNR in a longitudinal study of unilateral lung transplantation and ACR. By repeated imaging in the same animals, we were able to measure clear parenchymal signal increases in the rejecting lung and statistically-significant decreases in lung compliance, even in small groups ($N = 5, 9$). The same measurements in the native lungs were near-constant at all timepoints. Monitoring lung rejection in the mouse is straightforward using standard pulse sequences, without the need for hyperpolarization equipment; the techniques used here can be easily translated to clinical studies of lung-transplant recipients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported in part by NIH grants R01090806 (JCW), 1R01HL113436-01A1 and 1R01HL094601-01 (AEG) and K08 HL107677 (HJH).

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Fig. 1. Experimental plan

Fig. 2. Schematic of ventilation and MRI acquisition

Fig. 3.

(a) Representative axial ^1H MR images ($\text{TE} = 0.954$ ms, flip angle = 20°) of a control-Ig treated mouse at POD 3, POD 7 and POD 14 (in-slice allograft [left lung] lung signal: 0.043, 0.077 and 0.701 respectively; allograft compliance: 0.010, 0.005 and 0.001 in cc/cm H_2O respectively), and one corresponding histological slice (H & E, 200 $\times$) of the allograft at POD 14. (b) Corresponding ^1H MR images and histological slice (H & E, 200 $\times$) of an anti-CD4/anti-CD8 treated mouse (allograft signal: 0.045, 0.049 and 0.042 respectively; allograft compliance: 0.015, 0.017 and 0.016 in cc/cm H_2O respectively). Rejection is visually apparent in the control-Ig treated allograft at POD 14, though signal increases slightly at POD 7.

Fig. 4.

Mean parenchymal signal (mean $\pm$ SD) of control-Ig treated and anti-CD4/anti-CD8 treated mice as percent of mean soft-tissue signal at POD 3, POD 7 and POD 14. We note that the anti-CD4/anti-CD8 treated allograft signal increases at POD 7 & POD 14, which we believe results from edema and/or atelectasis in the transplanted lung in mouse 217 at POD 7 and mouse 278 at POD 14.

^a $P = 0.04$ compared to POD 3, allograft, control-Ig.

^b $P = 0.04$ compared to POD 14, allograft, control-Ig.

Fig. 5.

Compliance (mean $\pm$ SD) of control-Ig treated and anti-CD4/anti-CD8 treated mice at 3 timepoints.

^a $P < 0.05$ compared to POD 3, allograft, control-Ig.

^b $P = 0.04$ compared to POD 3, native lung, anti-CD4/anti-CD8.

^c $P < 0.05$ compared to allograft, anti-CD4/anti-CD8, at POD 7 and POD 14 respectively.

Fig. 6.

An array of histological slides from 2 anti-CD4/anti-CD8 treated and 1 control-Ig treated mice demonstrating (a) some residual edema and cellular consolidation, (c) resolution of POD 7 edema, (e) near-complete consolidation & rejection, and (b, d, f) corresponding control, native lungs (H & E, 100x).

Fig. 7.
Negative correlation between the volume percentage of high-intensity signal in the allografts and allograft compliance.

Table 1

Normalized Lung Parenchymal Signal

Group	Mouse #	Signal, POD 3		Signal, POD 7		Signal, POD 14	
		Allograft	Native lung	Allograft	Native lung	Allograft	Native lung
Control-Ig treated	<u>170</u>	0.043	0.056	0.152	0.048	1.144	0.056
	<u>219</u>	0.050	0.056	0.170	0.049	0.047	0.060
	<u>336</u>	0.043	0.050	0.077	0.057	0.701	0.058
	<u>D</u>	0.054	0.048	1.259	0.055	1.262	0.055
	<u>196</u>	0.042	0.057	1.124	0.044	a	a
	mean	0.046	0.053	0.556	0.051	0.789 ^b	0.057 ^c
	SD	0.005	0.004	0.583	0.005	0.550	0.002
Anti-CD4/anti-CD8 treated	<u>171</u>	0.045	0.047	0.049	0.050	0.042	0.053
	<u>217</u>	0.045	0.056	1.300	0.055	0.058	0.054
	<u>218</u>	0.051	0.057	0.042	0.132	0.115	0.061
	<u>278</u>	0.051	0.052	0.054	0.051	1.320	0.062
	<u>279</u>	0.042	0.045	0.054	0.066	0.050	0.054
	<u>335</u>	0.053	0.056	0.039	0.044	0.062	0.062
	<u>337</u>	0.151	0.066	0.046	0.068	0.053	0.047
	<u>C</u>	0.044	0.044	0.050	0.048	0.044	0.050
	<u>199</u>	0.057	0.048	0.473	0.054	a	a
	mean	0.060	0.052	0.234	0.063	0.218	0.055
	SD	0.035	0.007	0.424	0.027	0.446	0.006

Five control-Ig treated mice (170, 219, 336, D, 196) and nine anti-CD4/anti-CD8 treated mice (171, 217, 218, 278, 279, 335, 337, C, 199) at high pressure/volume are presented here. Data are expressed as a fraction of the mean in-slice soft-tissue signal (excluding lung).

^aNo data available.

^b $P = 0.04$ compared to POD 3, allograft.

^c $P = 0.04$ compared to POD 14, allograft.

Table 2

Image-determined Lung Compliance (cc/cm H₂O)

Group	Mouse #	Compliance, POD 3		Compliance, POD 7		Compliance, POD 14	
		Allograft	Native lung	Allograft	Native lung	Allograft	Native lung
Control-Ig treated	170	0.011	0.025	0.007	0.030	0.000	0.035
	219	0.016	0.033	0.002	0.028	0.007	0.025
	336	0.010	0.027	0.005	0.025	0.001	0.033
	D	0.012	0.025	0.003	0.019	0.002	0.028
	196	0.015	0.024	0.002	0.030	a	a
	mean	0.013	0.027	0.004 ^b	0.027	0.003 ^c	0.030
Anti-CD4/anti-CD8 treated	SD	0.003	0.004	0.002	0.005	0.003	0.005
	171	0.015	0.031	0.017	0.035	0.016	0.024
	217	0.011	0.024	0.000	0.024	0.009	0.025
	218	0.011	0.024	0.014	0.015	0.006	0.035
	278	0.009	0.022	0.010	0.021	0.001	0.031
	279	0.009	0.023	0.009	0.022	0.014	0.032
	335	0.011	0.018	0.013	0.019	0.012	0.029
	337	0.005	0.029	0.007	0.019	0.013	0.039
	C	0.006	0.021	0.016	0.034	0.005	0.023
	199	0.009	0.026	0.000	0.031	a	a
	mean	0.009	0.024	0.010 ^e	0.024	0.010 ^e	0.030 ^d
	SD	0.003	0.004	0.006	0.007	0.005	0.006

Five control-Ig treated mice (170, 219, 336, D, 196) and nine anti-CD4/anti-CD8 treated mice (171, 217, 218, 278, 279, 335, 337, C, 199) are presented here.

^aNo data available.

^b $P = 0.01$ compared to POD 3, allograft.

^c $P < 0.001$ compared to POD 3, allograft.

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$p_e = 0.04$ compared to POD 3, native lung.
 $p_e < 0.05$ compared to control-Ig treated mice, allograft, at POD 7 and POD 14 respectively.