Imaging chronic rejection in mouse lung allografts with ^{1}H MRI

Jinbang Guo[1,2], Xingan Wang[3], Anne K. Perl[4], Zackary I. Cleveland[1], Randy Giaquinto[5], Andrew E. Gelman[3], and Jason C. Woods[1,2]

[1]Center for Pulmonary Imaging Research, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, [2]Department of Physics, Washington University in St. Louis, St. Louis, MO, United States, [3]Department of Surgery, Washington University in St. Louis, St. Louis, MO, United States, [4]Division of Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, [5]Imaging Research Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

Target audience: Researchers in the fields of lung imaging and preclinical MRI.

Purpose: Lung transplantation (Tx) is an effective treatment to many end-stage lung diseases[1]. Despite improvements in short-term outcomes, graft failure due to chronic allograft rejection—mainly in the form of obliterative bronchiolitis—remains the major obstacle to the long-term survival of lung transplant recipients[2,3]. Because the underlying causes of chronic allograft rejection are poorly understood, it is necessary to develop a small animal model that mimic the pathological changes observed in human lung transplant rejection. To this end, we have extended a previously developed mouse model of orthotopic lung transplantation[4], using a triple transgenic mouse (Scgb1a1/DT-A) as lung donors that spontaneously develops upper-epithelial injury when exposed to doxycycline. This injury induces multiple rejection events in the transplanted lung that is similar to that seen in human lung allografts. Here we report the use of longitudinal ^{1}H MRI to monitor transplant rejection and relate individual outcomes with traditional histological examination, thus providing insights into the mechanisms and time course of transplant rejection.

Methods: Twelve mouse-lung allografts were performed with Institutional Animal Care and Use Committee approval. Six triple transgenic (Scgb1a1/DT-A or "DTA") and 6 non-triple transgenic (control) mice were used as donors. Left lungs were transplanted into C57BL/6 recipients. Recipients were treated with MR1/anti-CD40L on post-operative day (POD) 0 and CTLA4_Ig on POD 2 to suppress acute allograft rejection. On POD 30, doxycycline was fed for 48 hours to induce Clara cell depletion by activating DTA expression; this induces an upper-epithelial injury similar to a human epithelial injury caused by environmental exposure or infection often seen in patients. MR images were acquired on a Bruker 7T scanner at end expiration from free-breathing mice on POD 28, POD 33, POD 35, and POD 42. After POD 42, mice were sacrificed for histology or FACS. A 2D gradient echo (GRE) sequence was used to acquire both low resolution and high resolution ^{1}H images with field of view = 24 mm × 24 mm, pixel size = 0.25 mm × 0.25 mm or 0.16 mm × 0.16 mm, slice thickness = 2.0 mm or 0.59 mm, echo time (TE) = 0.64 ms or 0.93 ms, flip angle = 30°, and effective TR = 0.5 second (breath period), with a home-built bird-cage coil. Low resolution images were used for signal intensity analysis. Images were semi-manually segmented using the Amira software package (FEI Company, Hillsboro, OR) to differentiate lung from other soft tissues and exclude major bronchi and large blood vessels for further analysis. Additionally, the left lung and right lung were also separated, taking care to separate the accessory lobe from the left lung via the fissure visible on high resolution images. Raw image data were normalized by the average soft-tissue signal of each slice (excluding the lung). Mean parenchymal signal intensity and the percent of pixels above normalized signal intensity of 0.4 were measured.

Results and Discussion: The signal-to-noise ratio in right-lung parenchyma for low resolution ^{1}H MR images (TE = 0.64 ms) is around 9, allowing the quantification of longitudinal changes. Representative single-slice high resolution ^{1}H MR images of three Tx-DTA recipients and one Tx-control recipient are shown in Figure 1 to demonstrate the longitudinal variation that would not have been detected without imaging. In Figure 1 (a), rejection didn't appear in the left lung of this control mouse; in Figure 1 (b), regional rejection appeared even before scheduled injury was induced and remained through all time points; in Figure 1 (c), severe rejection appeared at scheduled injury and remained through POD 42; in Figure 1(d), rejection on POD 33 was resolved on POD 35 and POD 42. The right lung mean normalized parenchymal signal intensity is shown in Figure 2; this is an approximate measure of proton density of tissue (attenuated by T_2^* relaxation). The parenchymal signal intensities in right lungs of Tx-DTA and Tx-control mice are consistent among mice and groups longitudinally. The percent of pixels above normalized signal intensity of 0.4 is shown in Figure 3; this percentage of Tx-DTA left lung is larger than that of Tx-control left lung at all time points, indicative of rejection in Tx-DTA mice; large standard deviations indicate large variation in rejection severity between individual mice, confirmed by left lung histology (Figure 1). Signal in the Tx-control left lungs is much larger than that in the right lung because of cellular infiltration and/or inflammation.

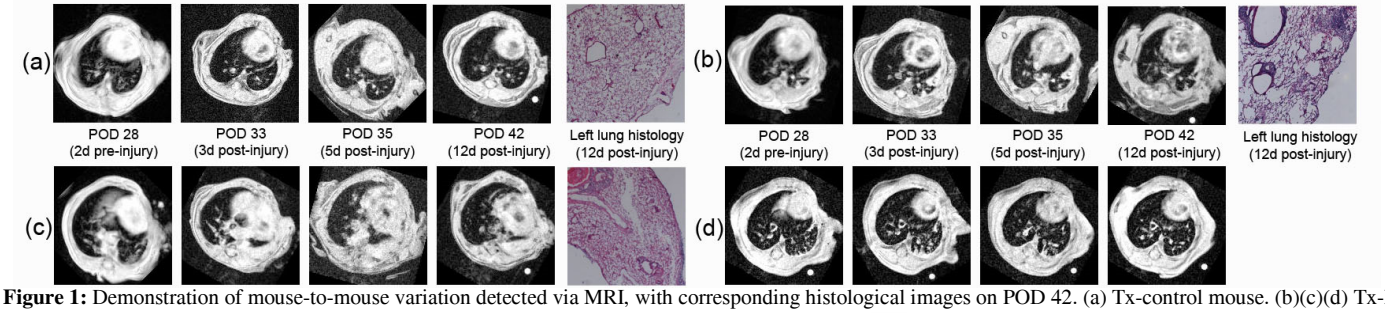

Figure 1: Demonstration of mouse-to-mouse variation detected via MRI, with corresponding histological images on POD 42. (a) Tx-control mouse. (b)(c)(d) Tx-DTA mice.

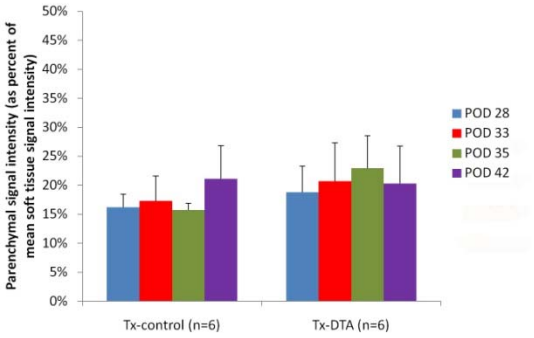

Figure 2: Low and constant right lung mean parenchymal signal intensity.

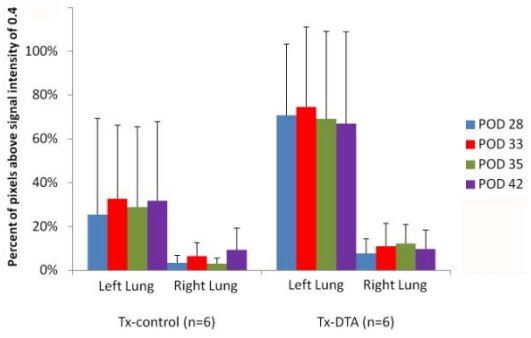

Figure 3: Percent of pixels above normalized signal intensity of 0.4.

Conclusions: Lung parenchyma can be quantified even at high field, with a fast GRE sequence. We demonstrate the ability to monitor lung rejection and longitudinal changes in individual mice via proton signal in the allograft. This technique may be translatable to the clinic to monitor the outcomes of human lung transplantation.

References: [1] Kotloff RM et al., Am J Respir Crit Care Med 2011;184:159–171. [2] Christie JD et al., J Heart Lung Transplant 2012;31:1073–1086. [3] Belperio JA et al., Proc Am Thorac Soc 2009;6:108–121. [4] Okazaki M et al., Am J Transplant 2007;7:1672–1679.