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Assessment of mustard vesicant lung injury and anti-TNF- α efficacy in rodents using live-animal imaging

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Abstract

Nitrogen mustard (NM) causes acute lung injury which progresses to fibrosis. This is associated with a macrophage dominant inflammatory response and the production of pro-inflammatory/profibrotic mediators including tumor necrosis factor alpha (TNF- α). Herein, we refined MRI and CT imaging methodologies to track the progression of NM-induced lung injury in rodents and assess the efficacy of anti-TNF- α antibody in mitigating toxicity. Anti-TNF- α antibody was administered to rats (15 mg/kg, every 8 days, intravenously) beginning 30 min after PBS control or NM (0.125 mg/kg, intratracheally). Animals were imaged by MRI and CT prior to exposure and 1–28 days post exposure. Using MRI, we characterized acute lung injury and fibrosis by quantifying high signal lung volume, which represents edema, inflammation, and tissue consolidation; these pathologies were found to persist for 28 days following NM exposure. CT scans were used to assess structural components of the lung and to register changes in tissue radiodensities. CT scans showed that in control animals, total lung volume increased with time. Treatment of rats with NM caused a prominent loss of lung volume; anti-TNF- α antibody mitigated this decrement. These studies demonstrate that MRI and CT can be used to monitor lung disease and the impact of therapeutic intervention.

Graphical Abstract

In the present studies we used MRI and CT imaging technology to follow the progression of lung injury induced by NM in live animals. By refining analytical methodology, pathologic changes were quantified, and the impact of anti-TNF- α antibody assessed. Our results demonstrate that

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Author contributions

A.M., J.A., A.V., D.A., E.Y., and D.L.L. designed and performed the experiments. A.M., A.J.G., J.A. analyzed the data. A.M., A.J.G., A.V., J.A., R.M., D.A., E.Y., J.D.L., and D.L.L. wrote and approved the final version of the manuscript.

Competing Interests

The authors declare no competing interests.

noninvasive MRI and CT imaging methodologies provide a more robust assessment of disease progression and resolution from repeated measurements in the same animals.

Keywords

mustard vesicants; lung; acute lung injury; MRI; CT; TNF- α

Introduction

Sulfur mustard (SM) and nitrogen mustard (NM) are chemical threat agents known to cause progressive damage to the respiratory tract. Pulmonary injury induced by mustards is the major cause of morbidity and mortality. Currently, there are no approved treatment options for acute lung injury and fibrosis caused by mustard exposure.¹

Pulmonary toxicity induced by mustards is associated with a prominent macrophage dominant inflammatory response. In previous studies, we characterized these cells as pro-inflammatory/cytotoxic and anti-inflammatory/profibrotic subpopulations.^{2–4} The fact that these macrophage subpopulations sequentially accumulate in the lung, coordinate with acute injury and fibrosis, suggests that they play a role in the pathogenic response to mustard lung toxicity. This is supported by our earlier findings that suppressing the activity of M1 macrophages reduces mustard lung toxicity, while blocking M2 macrophages blunts fibrosis.⁵ Evidence suggests that the pathologic actions of macrophages are due to excessive production of inflammatory proteins. One macrophage-derived mediator of particular interest is tumor necrosis factor alpha (TNF- α), which is known to exert both pro-inflammatory and profibrotic activity.⁶ TNF- α has been implicated in many acute and chronic pulmonary inflammatory diseases including asthma, chronic bronchitis, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome, and fibrosis, all of which are associated with mustard poisoning in humans.^{7–10} In earlier work we demonstrated that mice lacking TNF receptor-1 (TNFR1) were protected from half-mustard (2-chloroethyl ethyl sulfide) induced lung injury; additionally, treatment of animals with pentoxifylline, an inhibitor of TNF- α synthesis or anti-TNF- α antibody blunted NM toxicity.^{11–13} These data suggest that targeting TNF- α may be an efficacious approach to mitigating mustard vesicant toxicity in humans.

Traditional assessment of lung injury and fibrosis in rodents following mustard exposure includes analysis of bronchoalveolar lavage (BAL) fluid, lung inflammatory cells, and histology, and evaluation of pulmonary function^{3,4,14} Although informative, these are terminal procedures that limit assessment of heterogeneous time-related dynamic changes associated with mustard exposure. In humans, high definition imaging techniques are being increasingly utilized to perform repeated measurements in patients with lung injury caused by smoke, asbestos, asthma, idiopathic pulmonary fibrosis and chemical exposure, with successful results.^{15–18} In line with this, small size imagers have been optimized for use in rodents for experimental analysis of lung injury. In particular, magnetic resonance imaging (MRI) and computed tomography (CT) have been used to visualize and monitor lung diseases such as cancer and fibrosis in animal models.^{19–23} The goal of the present studies

was to evaluate the utility of MRI and CT imaging modalities to track pathological changes in the rat lung following exposure to mustard vesicants.

MRI depicts regional distribution of protons in tissue that form the nuclei of hydrogen atoms in water.²⁴ In nonpathological states, the hydrogen proton content of the lung is relatively low since it is filled with air. Following NM-induced lung injury, hydrogen proton content increases due to edema and tissue consolidation (e.g., fibrosis), making MRI particularly useful for evaluation of these pathologies.^{25–27} CT is an X-ray based technology that generates three-dimensional images based on differential radiodensities of tissues. Radiodensity is a 3-dimensional voxel-by-voxel measure of X-ray attenuation within the CT image. Relative radiodensities are typically expressed using the unitless Hounsfield scale. A Hounsfield unit (HU) is based on the ability of different tissues to attenuate or absorb x-ray energy. On this scale, the HU of water is zero (0), while air is –1000 HU.^{28,29} In healthy rats, the distribution of voxels within the lung for normal aeration ranges from –1000 to approximately –400 HU, while higher HU correspond to blood and tissue. Shifts in these distributions are used to identify structural alterations associated with histopathology (i.e., emphysema, edema, fibrosis).^{30,31} CT imaging is also useful for assessing total lung volume and alterations in tissue densities.^{27,32,33}

In the present studies we used MRI and CT imaging technology to follow the progression of lung injury induced by NM in live animals. By refining analytical methodology, pathologic changes were quantified, and the impact of anti-TNF- α antibody assessed. Our results demonstrate that noninvasive MRI and CT imaging methodologies provide a more robust assessment of disease progression and resolution from repeated measurements in the same animals.

Materials and methods

Animals and treatments

Male Wistar rats (175–199 g; 6–7 weeks old; $n = 6–8$ rats/treatment group) were purchased from Envigo (Somerset, New Jersey) and maintained in an AALAC approved animal care facility. Animals were housed in filter top microisolation cages and provided food and water *ad libitum*. Animals received humane care in compliance with the guidelines outlined in the *Guide for the Care and Use of Laboratory Animals*, published by the National Institutes of Health. Rats were treated with sterile phosphate-buffered saline (PBS) control or 0.125 mg/kg freshly prepared NM (mechlorethamine hydrochloride, Sigma Aldrich, St Louis, Missouri) by intratracheal instillation as previously described.¹⁴ Briefly, rats were anesthetized with 4% isoflurane and then placed on a tilted Rodent WorkStand (Hallowell EMC, Pittsfield, Massachusetts) in a supine position and restrained using an incisor loop. The tongue was extruded with a cotton tip applicator to allow for visualization of the larynx using a 4-mm speculum attached to an otoscope. NM or control (0.1 ml) was administered using Clay Adams Intramedic PE-60 (I.D. 0.76 mm, O.D. 1.22 mm) polyethylene tubing attached to a 20-gauge hypodermic needle and a 1 ml syringe. The tubing and speculum were immediately withdrawn following instillation. Animals were removed from the work stand and placed in a vertical position until normal respiration was observed (~1 min) in a filter top microisolation cage lined with ALPHA-dri® bedding placed on a heating pad.

Beginning 30 min post exposure, and then once every 8 days thereafter for 28 days, rats were treated intravenously with vehicle (PBS) or 15 mg/kg recombinant mouse IgG2 α monoclonal anti-rat TNF- α antibody (Janssen Research and Development, Spring House, Pennsylvania).¹² Double gloves, safety glasses, a mask, isolation gown, apron, and sleeves were used for preparation and instillation of NM, which was performed in a designated room under a chemical hood following Rutgers University Environmental Health and Safety guidelines.¹⁴

MRI image acquisition and analysis

MRI was performed on freely breathing animals that were anesthetized with 2.5% isoflurane (1 L/min). Animals were imaged one day prior to and then at day 1, 3, 7, 14, 21, and 28 post exposure to NM or PBS control. MRI images were acquired using T1-weighted gradient echo (GRE) and T2-weighted fast spin echo (FSE) on an Aspect M2TM Compact High-Performance MRI (Aspect Imaging, Shoham, Israel) using a 60 mm M2 rat-specific RF coil, field strength 1T. Scan settings are shown in Table 1. Lung injury was not detectable one day prior to exposure (data not shown).

MRI images were analyzed using VivoQuantTM 2.0 imaging software. Lung volume was calculated by defining a region of interest (ROI) within the thoracic cavity that incorporated the entire lung and larger conducting airways. FSE and GRE imaging were performed at the same location, and the images were overlaid and analyzed using the VivoQuantTM software. Areas of lung injury defined as edema and tissue consolidation in MRI images were identified by comparing signals in GRE images to signals in FSE images. ROIs in FSE images not correlated to signals in GRE images for each animal were manually selected and removed. As a higher signal threshold was detectable in FSE images when compared to GRE images, FSE images were used to identify areas of lung injury. Lung volume and volume of lung injury were calculated as a 2D area for each section image using a slice thickness of 1.5 mm. Using VivoQuantTM software, the sections were compiled into a 3D image, depicting lung volume and volume of lung injury. The volume of lung injury (mm³) was included as part of the total lung volume (mm³).

CT image acquisition and analysis

CT imaging was performed immediately after MRI using an Albira[®] PET/CT (Bruker, Billerica, Massachusetts). The following instrument settings were used: high definition scan setting (400 slices, ~10 min acquisition time), field of view (60 mm²) with tube current and voltage adjusted to 200 μ A and 45 kV, respectively. The respiration rate of the animals was monitored throughout the imaging session. Arterial oxygen saturation, heart and respiration rates, and pulse distention were assessed immediately following image acquisition using a MouseOx[®] Plus (Starr Life Sciences Corp., Oakmont, PA). CT scanning of rats for ~10 min resulted in ~110 mGy radiation dose per scan. Excluding the last scan conducted on the day of euthanasia (28 d), each rat received a cumulative deep-dose equivalent of ~660 mGy radiation. Previous studies showed that more frequent CT scanning of rodents (e.g., 3 times/week for 6-weeks) with a cumulative dose of >5 Gy did not significantly affect aerated lung volume, lung tissue volume, total lung volume, or gross histopathological endpoints.³⁴

Similarly, in our studies which involved only 6 scans and less total radiation exposure, no significant lung changes were observed (data not shown).

CT image reconstruction was conducted using a filtered back-projection (FBP) algorithm from the Albira® Reconstruction Software Suite (Bruker, Billerica, Massachusetts). Images generated from CT scans were analyzed using VivoQuant™ 2.0 imaging software. For CT analysis, the majority of the body with the exception of the tracheal bifurcation and the lung were selected via the connected threshold tool for each animal and each time point; this excluded the heart, but included the left and right bronchi. The selected non-lung tissue was then manually deleted, leaving an isolated CT scan of the lung. HU histograms were produced using 300 bins describing a range of -999 to -70 HU. Patterns of HU distribution were evaluated for all animals at all time points. According to HU, voxels were grouped and segmented based on the distribution of HU into three regions of radiodensity; low, intermediate, and high. Using these regions, thresholds were set to identify air (-999 to -400 HU), tissue (-399.9 to -250 HU), and fluid (-249.9 to -70 HU). Total lung volume was determined by summarizing the volume as area under the curve (AUC) for the total amount of air, tissue, and fluid. Fluid accumulation in the lung is reported as a percentage of total lung volume. As two independent experiments were performed, data were normalized by averaging pre-exposure total lung volumes. Change in total lung volume was calculated by subtracting the averaged total lung volume from the individual animal's total lung volume 1, 3, 7, and 28 days post exposure.

Lung collection and histology

Animals were euthanized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) 28 days after administration of PBS or NM. The lung was removed, fixed overnight following intratracheal instillation of 10 ml of 3% paraformaldehyde at a constant pressure (25 cm H₂O), and paraffin embedded. Sections (5 µm) were then prepared and stained with hematoxylin and eosin. Images were acquired using an Olympus VS-120 scanner (Olympus Corporation, Center Valley, Pennsylvania).

Statistical analysis

Data were analyzed using linear regression, Wilcoxon-Pratt signed-rank test, or Kruskal-Wallis with Dunn's post hoc test; $p < 0.05$ was considered statistically significant. Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, California).

Results

Consistent with our previous findings^{12,35}, treatment of rats with NM resulted in rapid (1–3 d) multifocal inflammatory lesions in the lung characterized by perivascular and peribronchial edema, inflammatory cell infiltrates including macrophages and neutrophils, blood vessel hemorrhage, and luminal accumulation of cellular debris and fibrin (data not shown). Patchy mild thickening of alveolar septa and increased numbers of mononuclear cells, neutrophils, and macrophages were also noted. At 3 days post NM, bronchiolization, an ingrowth of cuboidal cells lining adjacent bronchioles to alveoli, was also observed.

These changes in the lung are generally progressive in nature as the inflammatory lesions increase in number and size with time. By 7 days post NM exposure, prominent changes in the lung also included appearance of foamy macrophages in the alveoli, dilation and inflammation of the bronchi, squamous cell metaplasia, mesothelial cell proliferation, and emphysema. At 28 days post NM exposure, neutrophils, eosinophils, fibroblasts and lymphocytes were evident within the thickened alveolar walls while enlarged foamy macrophages were present occluding alveoli (Fig. 1). Additionally, multiple areas of frank fibrosis containing collagen fibers were observed around airways and bronchioles as determined by trichrome staining of the lung.^{12,35} Treatment of rats with anti-TNF- α antibody blunted these pathologic responses; thus, NM-induced acute and long-term inflammatory lesions were reduced in size, and intensity, and fibrotic alterations in the lung were attenuated (Fig. 1).

In further studies we used MRI to track and quantify the pathologic response to NM in live animals and the effects of anti-TNF- α antibody. Whereas signals in T1-weighted GRE scans were found to be efficient in detecting blood vessels and organs, signals in T2-weighted FSE scans specifically identified fluid accumulating in the lung after NM exposure. By overlapping these scans, we developed a robust method to measure extravasated fluids in the lung (Fig. 2). Using this analytical approach, we were able to accurately visualize progressive lung injury at all post exposure time points from 1 day to 28 days (Fig. 3A). We next quantified total lung volume and regions of lung injury in the MRI images. Treatment of rats with NM resulted in lung injury that persisted for 28 days. The greatest percentage of lung injury relative to total lung volume was observed 1 day post exposure (Fig. 3B). With time following NM administration, the percentage of injury appeared to decline, most likely a consequence of the fact that the rats were growing and gaining weight. Anti-TNF- α antibody was found to reduce the percentage of injured lung at 1-day post-NM, with no significant effects at the other time points.

We next used CT imaging to assess NM-induced pathology in the lung. To quantitate lung pathology, we developed a method for isolating different regions of the lung based on HU frequency and distribution. Initially, we identified all HU associated with the lung, and then deleted those that corresponded to background signals and other organs. Based on HU, we next segmented the lung into three distinct categories of tissue radiodensity: air, tissue, and fluid (Fig. 4). Signals of radiodensity corresponding to these different regions were confirmed by comparing MRI data and histopathologic changes at day 28 post NM exposure. Based on this comparison, we calculated the slope of the least squares line of best fit (2.48) and the correlation coefficient (0.15) between the percentage of injured lung derived from MRI images and fluid component derived from CT scans; this analysis confirmed that the area defined as fluid, indicative of lung injury in CT scans was correct (Fig. 5). While a similar correlation and slope were observed at day 14 and day 21 post-NM, at earlier time points (1, 3, and 7 days post-NM), the correlation was weaker and there was greater slope variation (data not shown). Using the Wilcoxon-Pratt signed-rank test, we determined if the extent of NM-induced lung injury assessed by MRI was linked to fluid accumulation (edema and tissue consolidation) measured by CT. We found a statistically significant ($p < 0.05$) relationship between these parameters at all NM post exposure time

points (1–28 days). Control animals were not included in the analysis because they presented with no detectable lung injury.

We subsequently calculated total lung volume from the CT data by summarizing the area under the curve of air, tissue, and fluid for each animal at each time point. Pre-exposure total lung volumes were averaged and subtracted from the animal's post exposure total lung volume, yielding a quantitative measure of the change in lung volume over time. Figure 6A shows that total lung volume increased with time in control animals, which was expected as they continued to grow and gain weight. Treatment of rats with NM resulted in a decrease in lung volume relative to control, which was evident at day 3, 7 and 28 post exposure. While anti-TNF- α antibody administration had no effect on lung volume at day 1, 3, or 7 post-NM exposure, at 28 days, lung volume was partially restored. By evaluating different radiodensities in the lung, we were able to visualize distinct changes in specific areas following NM exposure and to construct high resolution 3D images (Fig. 6B). Using our segmentation strategy, we found that NM exposure caused decreases in respiratory area, the ventilated portion of the lung that encompasses the low radiodensity portion (air) of the HU histogram, at day 1 and at day 28; this was mitigated by treatment of rats with anti-TNF- α antibody.

Discussion

Macrophages are known to play a key role in the initiation and resolution of inflammatory responses to tissue injury and infection, and in the wound healing process. However, when macrophages become overabundant or hyperactivated, they can perpetuate tissue injury and contribute to chronic diseases such as fibrosis.² Macrophages are a significant source of TNF- α , and increases in TNF- α contribute to both acute and chronic lung pathologies.^{5,6} Previous studies have shown that exposure of animals to mustard vesicants including SM and NM is associated with rapid increases in macrophage expression of TNF- α in the lung.^{3,5,36} Consistent with the role of TNF- α in the toxicity of mustard vesicants, we previously reported that treatment of rats with anti-TNF- α antibody attenuates NM-induced histopathological alterations in the lung and long-term manifestations of NM exposure including fibrosis and collagen deposition.¹² Using live-animal imaging, the present studies confirm that TNF- α is a key mediator of disease pathogenesis induced by mustards. The use of imaging techniques allowed for a greater understanding of the progression of inflammatory and fibrotic processes associated with NM exposure. These findings provide support for the idea that targeting TNF- α may be an efficacious approach for mitigating mustard-induced pulmonary toxicity.

MRI and CT have been utilized clinically to characterize lung diseases such as acute respiratory distress syndrome, cystic fibrosis, and idiopathic pulmonary fibrosis in humans noninvasively.^{37–40} The use of these techniques in animal models for mechanistic studies and to assess potential therapies is only in its early stages, and analytical approaches for data analysis are limited, but continue to be improved. The goal of the present studies was to use imaging techniques to track NM-induced lung injury in rats over time in order to quantify the disease progression. Our initial approach involved evaluating acute lung injury and the development of fibrosis by quantifying areas of edema and tissue consolidation using MRI,

with the premise that edema would correlate to areas of acute lung injury, while consolidation would be indicative of fibrosis. As the lung is largely composed of air under homeostatic conditions, MRI signal in the lung is typically easy to detect.³² Following NM exposure, opacities, indicative of lung injury were observed in MRI images throughout the 28-day time course. By examining specific areas within histologic sections prepared 28 days post-NM exposure, we were able to confirm our MRI conclusions. Using VivoQuant™ 2.0 imaging software, total lung volume and the volume of lung injury were quantified. Robust signal intensity at early time points (e.g., days 1 and 3 post-NM exposure) was attributed to edema and/or hemorrhage and inflammation. We ascribed the decrease in signal intensity at later post-NM exposure time points (e.g., days 14, 21, and 28) to tissue remodeling and consolidation, consistent with pathological changes observed in histologic sections, as these areas contain less water than edematous tissue.

Anti-TNF- α antibody treatment of rats resulted in a reduction in the percentage of lung injury as shown by MRI. This is in accord with our previous findings using classical measures of lung injury.¹² Although we were able to detect evidence of acute lung injury and fibrosis in the lung using MRI, these pathologies were difficult to precisely characterize due to the low proton density in normal lung tissue.⁴¹ Signal decay by apparent transverse relaxation time T2* of the plethora of airways and alveoli in the lung make gradient echo MRI difficult as this leads to a loss of signal.⁴² While gadolinium-based contrast agents could potentially be used to overcome this difficulty, in some diseases they do not provide increased sensitivity and may be harmful as serial administration is required.⁴³ In an attempt to increase our ability to assess lung pathology, we overlaid GRE and FSE images from the MRI scans and monitored the animals longitudinally. We found that this approach reduced interference from physiological artifacts such as respiration, blood flow, and cardiac motion^{41,44} and provided more accurate data on pathological changes such as edema and tissue consolidation in the lung.

To enhance our assessment of lung structure, we next utilized techniques in CT. This approach allowed us to evaluate different components of the lung and register alterations in tissue radiodensities. HU thresholds were selected, such that fluid-containing voxels could be identified unambiguously. Using this segmentation strategy, we were able to calculate the percentage of fluid accumulation within the lung and estimate its correspondence to the percentage of lung injury derived from MRI images. Although a correlation was observed between these measures 28 days post-NM exposure, it was relatively low ($r^2 = 0.15$), which was due in part to two animals that significantly deviated from the mean. If the animals were removed from the analysis, a stronger correlation would have been observed ($r^2 = 0.6$) providing support for our contention that our analytical approach is valid.

The calculated percentage of lung injury derived from MRI and the percentage of fluid accumulation derived from CT are independent indirect measures of lung injury. However, they are affected differentially by factors such as ventilation rate and depth of inspiration. Therefore, while the correlation is not strong between the two methodologies, the degree of the relationship across the later time points, as defined by the slope of the least squares line of best fit, is consistent and thus, appropriate to define injury. Weaker correlations at earlier time points may be attributed to difficulties in separating areas of air, tissue, and fluid in the

CT analysis due to inflammation, protein secretion, and edema that present as early as 1 day after mustard exposure and were observed in MRI images.³⁶ Although additional studies may be necessary to further refine segmentation selection criteria (e.g., air: -999 to -400 HU), statistical significance as determined by Wilcoxon-Pratt signed-rank test was observed at all time points between the two measures. Thus, animals with greater lung injury as determined by MRI also had greater fluid accumulation in the lung as defined by CT. A similar relationship between MRI and methodologies such as microCT and morphometry has been observed in lung diseases such as COPD, bleomycin-induced fibrosis, and fungal infection.^{45,46}

The segmentation strategy implemented in these studies for the CT data allowed us to distinguish between the air, tissue, and fluid components in the lung. These data were used to quantify total lung volume. Additionally, 3D reconstruction was used to assess NM-induced alterations in respiratory area of the lung. NM was found to reduce lung volume and respiratory area, responses which persisted for at least 28 days. Treatment of animals with anti-TNF- α antibody restored the NM-induced loss of lung volume and respiratory area. These observations are consistent with the ability of anti-TNF- α antibody to not only blunt lung injury, but also to improve lung function and respiratory gas exchange.

Non-invasive imaging techniques like MRI and CT are advantageous because they allow for quantification of edema and tissue consolidation, detection of changes in lung architecture, and construction of high-resolution 3D images in the same animal over time, reducing variability observed in terminal procedures with different animals. Traditional methods of analyses also fail to accurately represent dynamic changes in the lung during disease progression because of the need to euthanize many animals at multiple time points to collect longitudinal data. MRI and CT imaging overcome these limitations. Another advantage of MRI and CT is that they enhance our understanding of pathologic changes since animals are scanned prior to exposure to establish individual baseline values. Moreover, animals can be randomized between treatment groups thus reducing experimental bias.⁴⁷⁻⁴⁹

In summary, using imaging techniques and analytical methods, we were able to quantify NM-induced pathological alterations over time in live animals and confirm the ability of anti-TNF- α antibody to blunt lung toxicity. These methods can be applied to other animal models to assess lung injury and fibrosis and to enhance the prediction and potential mitigation of injury with therapeutics.

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Figure 1. Effects of NM and anti-TNF- α antibody on lung histology. Tissue sections, prepared 28 d after administration of PBS control (CTL) ($n = 6$), NM ($n = 6$), or NM followed by anti-TNF- α antibody ($n = 8$) to rats, were stained with H&E. (A) Original magnification, 4 $\times$. (B) Original magnification, 100 $\times$. Representative sections are shown.

Figure 2.

MRI acquisition and analysis. (A) T2-weighted FSE and (B) T1-weighted GRE images were collected for each animal at each time point. (C) FSE and GRE images were overlaid to determine areas of injury, edema, and/or tissue consolidation identified in FSE images that were not correlated with blood vessels or organs identified in GRE images. (D) Regions of interest (ROI) in FSE images were manually selected. The volume (mm^3) of lung injury (shown in red) was then calculated for each animal. Representative images from one animal acquired 3 d post NM exposure are shown.

Figure 3.

MRI analysis of the progression of NM-induced lung injury and the effects of anti-TNF- α antibody. (A) FSE MRI images were acquired from the same anatomical position 1, 3, 7, 14 and 28 d following CTL ($n = 6$) or NM ($n = 6$) exposure. Lung volume is indicated by the area within the blue outline and lung injury by the area within the red outlines. Representative images are shown. (B) Total lung volume (mm³) and volume of lung injury (mm³) were quantified 1, 3, 7, 14, 21 and 28 d following exposure of rats to NM ($n = 6$) and NM + anti-TNF- α antibody ($n = 8$). Data are presented as percentage of injured lung area detected in MRI images relative to total lung volume. Data are mean $\pm$ SE.

Figure 4. CT acquisition and analysis. (A) CT scans were acquired from each animal/treatment group/ time point. Lung tissue was selected and remaining non-lung tissue excluded; lung was segmented by radiodensity into areas of (B) air (red; –999 to –400 HU), (C) tissue (green; –399.9 to –250 HU), (D) fluid (cyan; –249.9 to –70 HU), and (E) 3D rendering was generated using segmented lung. Images and 3D rendering are representative of data acquired from 6 rats 28 d post NM exposure.

Figure 5.

Comparison of lung injury in MRI and CT images. Upper panels. Representative images of MRI derived lung injury (shown in red) and CT derived fluid accumulation (shown in cyan) 28 d following NM administration to rats. Lower panel. Slope of the least squares line of best fit and the correlation coefficient between areas identified as fluid by CT and lung injury by MRI. Dashed lines, 5–95% confidence interval for slope = –1.2–6.17; 5–95% confidence interval for intercept = –3.22 to 32.15 ($n = 14$).

Figure 6.

Anti-TNF- α antibody blunts NM-induced decreases in lung volume and respiratory area. (A) Total lung volume at 1, 3, 7, and 28 d post exposure was calculated from the lung CT analysis by summarizing areas under the curve of air, tissue, and fluid radiodensities. Total lung volume was subtracted from mean pre-exposure total lung volume to determine the change in total lung volume. Data are mean $\pm$ SE ($n = 6-8$ rats/treatment group/time point). (B) 3D rendering of rat lung respiratory area used to quantify changes corresponding to air in the lung at days 1 and 28 after exposure of rats to CTL ($n = 6$), NM ($n = 6$), or NM + anti-TNF- α antibody ($n = 8$). Areas of lung that are absent reflect hemorrhage, edema and/or consolidated tissue. One representative 3D rendering is shown.

Table 1.

Scan settings used to generate GRE and FSE images for MRI analysis

	T1-Weighted gradient echo	T2-Weighted fast spin echo
Number of slices	20	20
Slice thickness	1.5 mm	1.5 mm
Acquisition matrix size	256 × 256	256 × 250
Field of view (FOV)	100 mm	100 mm
Repetition time (TR)	15 ms	3786 ms
Echo time (ET)	3.79 ms	73 ms
Acquisition time	8 min	20 min
Flip angle	30°	180°
Number of excitations	10	2, 4 averages
Spatial resolution	0.39 mm	0.39 mm
Acquisition bandwidth	40 kHz	40 kHz
Acquisition plane & phase encoded direction	Coronal	Coronal