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Data Article

Comprehensive dataset to assess morphological changes subsequent to bleomycin exposure

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ABSTRACT

Intratracheal bleomycin causes pulmonary injury, inflammation and fibrosis. The characteristic patchy nature of the injury makes analysis challenging. Histological assessment of lung injury is a useful tool to evaluate damage, however quantification is not standardized. We propose a multifactorial approach to assess morphological changes subsequent to intratracheal bleomycin mediated lung injury. Lungs were inflation fixed with paraformaldehyde, sectioned and stained with hematoxylin and eosin. Whole slide images were scanned and ten 400x images were randomly chosen throughout the tissue for further analysis. Using ImageJ software, alveolar wall width was measured, nuclei were counted and airspace was quantified. Morphological changes were identified in mice instilled with bleomycin. This combination offers a robust measure of lung morphology especially in a heterogenous injury.

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Specifications Table

Subject	Immunology
Specific subject area	Histological assessment of pulmonary injury
Type of data	Image Graph Figure
How data were acquired	Hematoxylin and eosin stained lung sections were scanned on Olympus VS120 microscope. Ten 400x fields were randomly selected from scanned whole images on Olyvia software.
Data format	Raw
Parameters for data collection	Lungs were harvested from mice 7- and 21-days post instillation of PBS or bleomycin. Instillation treatment groups were subdivided into either saline or anti-TNFα antibody. Each group has 5-6 mice.
Description of data collection	Ten high magnification (400x) fields were randomly chosen throughout the tissue. Using Image J software, alveolar walls were measured, nuclei counted and airspace quantified for each image. Alveolar walls were identified in each high magnification field and their width measured with the line tool. Nuclei were manually counted in each high magnification field. Finally, the image was converted to binary and the background (airspace) quantified. Each measurement is included in the Mendeley Data repository as separate sheets in the Excel worksheet.
Data source location	Rutgers University Piscataway, NJ USA
Data accessibility	Repository name: Mendeley Data repository Data identification number: http://dx.doi.org/10.17632/9djkffg5k5.1 Direct URL to data: http://dx.doi.org/10.17632/9djkffg5k5.1 Instructions for accessing these data: Data is accessible on data.mendeley.com and http://dx.doi.org/10.17632/9djkffg5k5.1
Related research article	Venosa, A., Gow, J.G., Taylor, S., Golden, T.N., Murray, A., Abramova, E., Malaviya, R., Laskin, D.L., Gow, A.J., 2021. Myeloid cell dynamics in bleomycin-induced pulmonary injury in mice; effects of anti-TNFalpha antibody. Toxicol Appl Pharmacol **417**, 115470. http://dx.doi.org/10.1016/j.taap.2021.115470

Value of the Data

- Acute lung injury can result in a patchy phenotype that is challenging to rigorously analyze. Using a robust multi-factorial approach allows for improved assessment of therapeutic interventions. By increasing the sensitivity of measurement, one can more accurately assess overall injury. Also, the inclusion of several measures has the potential to identify the involvement of particular pathological pathways.
- This approach to assess lung morphology is potentially useful in models of pulmonary injury, fibrosis and inflammation. While it is used here to assess injury subsequent to intratracheal bleomycin, it evaluates commonly disrupted structures thus having broad applicability.
- This data set also includes a robust measure of normal C57BL/6 lung structure. The variability seen in control animals is informative for powering future studies in which one wants to compare a model of injury or therapeutic intervention.

1. Data Description

Fig. 1. Morphological assessment of lungs following intratracheal instillation of PBS or bleomycin. Whole lung images allow for global assessment and identification of high-power fields (A). High power field images at 400x magnification (B) were used to measure alveolar wall width (C), number of nuclei (D) and percent of area that is airspace (E). Raw data and images are uploaded to Mendeley Data Repository accessed via http://dx.doi.org/10.17632/9djkffg5k5.1.

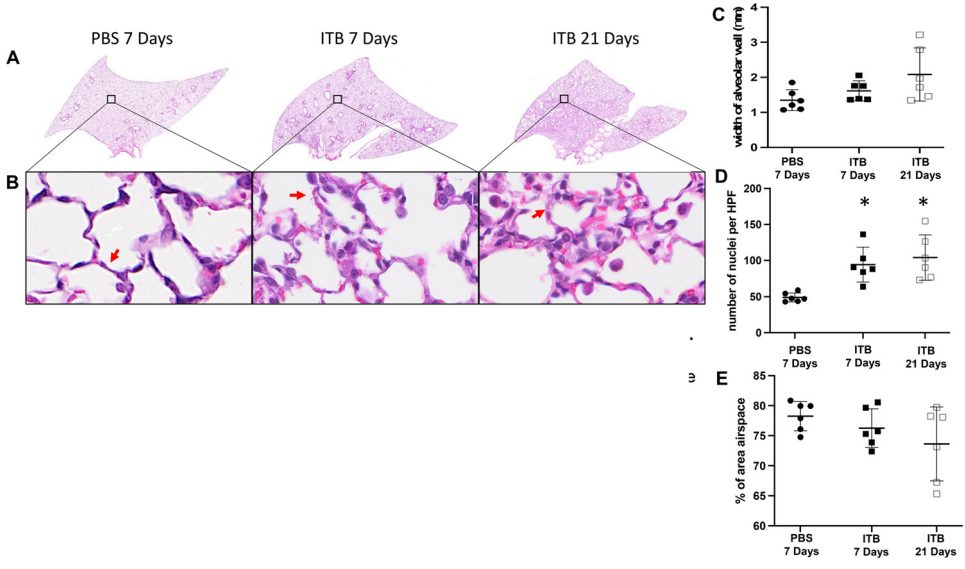

Fig. 1. Lungs were histologically assessed for morphological changes. Ten 400x fields were randomly chosen throughout the tissue (A) and field of view adjusted to avoid large airways. The following measurements were made using Image J software (B): Alveolar walls were identified and the width of the wall measured (red arrow) (C). Nuclei were manually counted (D). Images were converted to binary and the percent of white (airspace) was calculated (E). Values are reported as mean ±SD. One-way ANOVA tested PBS and bleomycin (ITB) instillation at 7 and 21 days. Upon significant ANOVA, t-test was used to compare ITB to PBS treatment (* $p < 0.05$).

Fig. 2. Histological assessment following bleomycin with or without TNF**α** treatment quantifies morphological changes to the tissue architecture. Images were captured and assessed as described in Fig. 1. Data is also available in Mendeley Data Repository[6].

2. Experimental Design, Materials and Methods

Twenty-nine C57BL/6 male mice were intratracheally instilled with either PBS (control) or bleomycin (injury; ITB) [1]. The two cohorts received either saline or anti-TNFα antibody (7.5mg/kg; tail vein) starting on experiment initiation and repeated every fifth day. There are 5-6 mice in each treatment group. On day 7-and 21- after intratracheal instillation, lungs were lavaged and left lungs inflation fixed with paraformaldehyde. Lungs were then dehydrated and paraffin embedded for sectioning. Sections (5 micron in thickness) were stained with hematoxylin and eosin. Histology slides were scanned using Olympus VS120 microscope at 400x magnification. Slides were visualized on Olympus Olyvia software and ten high power field (400x) images were randomly chosen throughout the tissue. Field of view was adjusted to avoid large airways.

Images were assessed in Image J to measure changes in lung morphology in all study groups. Morphological assessment was based on those commonly reported in the literature [2–5]. Alve-

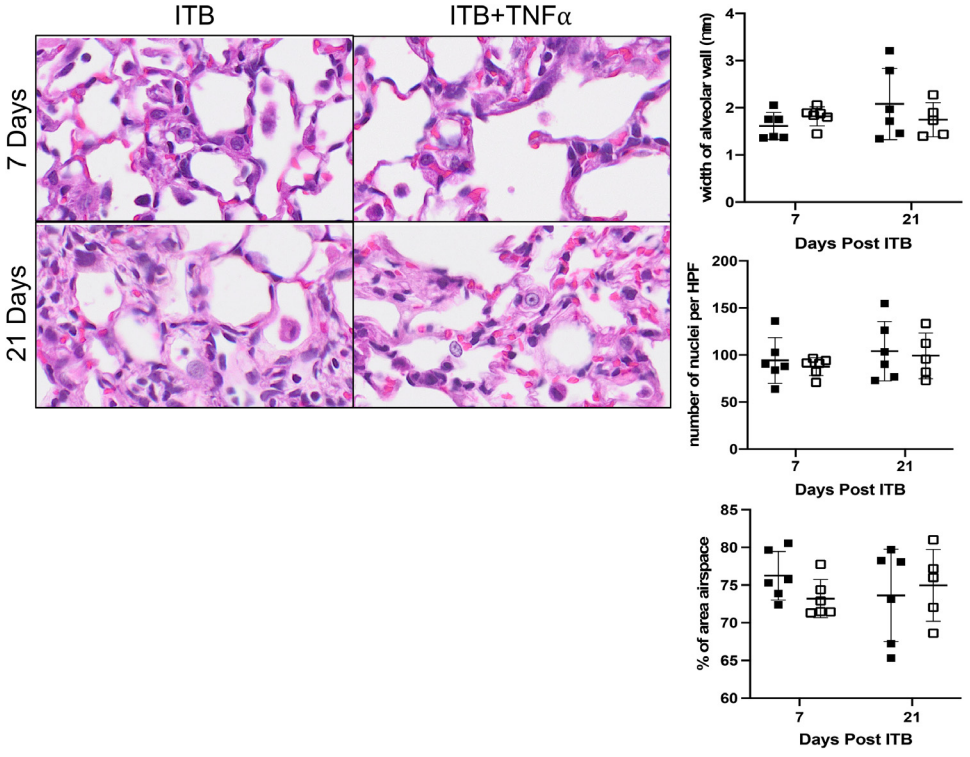

Fig. 2. Lungs were histologically assessed for morphological changes. Representative pictures demonstrate increased cellularity and airway thickening in lungs post intratracheal bleomycin instillation (ITB) (solid square) regardless of TNFα treatment (hollow square) at 7 and 21 days. Values are reported as mean ± SD and tested by two-way ANOVA

olar walls were identified and width measured using Image J scale and line tool. Nuclei were also counted manually per field. To quantify the percent airspace, the image type was converted to 8-bit, threshold set, and then the image converted to binary. The background (airspace) was quantified by creating a selection and measurement made. The total field measurement was used as the denominator.

Ethics Statement

Animal studies were completed in accordance with Rutgers Institutional Animal Care and Use Committee approval (protocol 06-025) and follow NIH guidelines for care and use of animals.

CRediT Author Statement

Thea Golden: Methodology, Formal analysis, Writing – review & editing; **Alexa Murray:** Methodology; **Alessandro Venosa:** Formal analysis, Writing – review & editing; **Andrew J. Gow:** Methodology, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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