

Supplementary Materials: Diacetyl Vapor Inhalation Induces Mixed, Granulocytic Lung Inflammation with Increased CD4⁺CD25⁺ T Cells in the Rat

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Table S1. Flow Cytometry Antibody Panel

Scheme 1. Flow Cytometry Markers.	Catalog #	Source	Fluorochrome	Dilution
CD3	201414	Biologend	APC	1:150
CD8	25-0084-82	Invitrogen	PE-Cyanine 7	1:150
CD4	201518	Biologend	APC-Cyanine 7	1:100
CD25	202103	Biologend	Fitc	1:100
FoxP3	IC8970P	R&D	PE	1:100
UltraComp Beads	01-2222-42	Invitrogen		

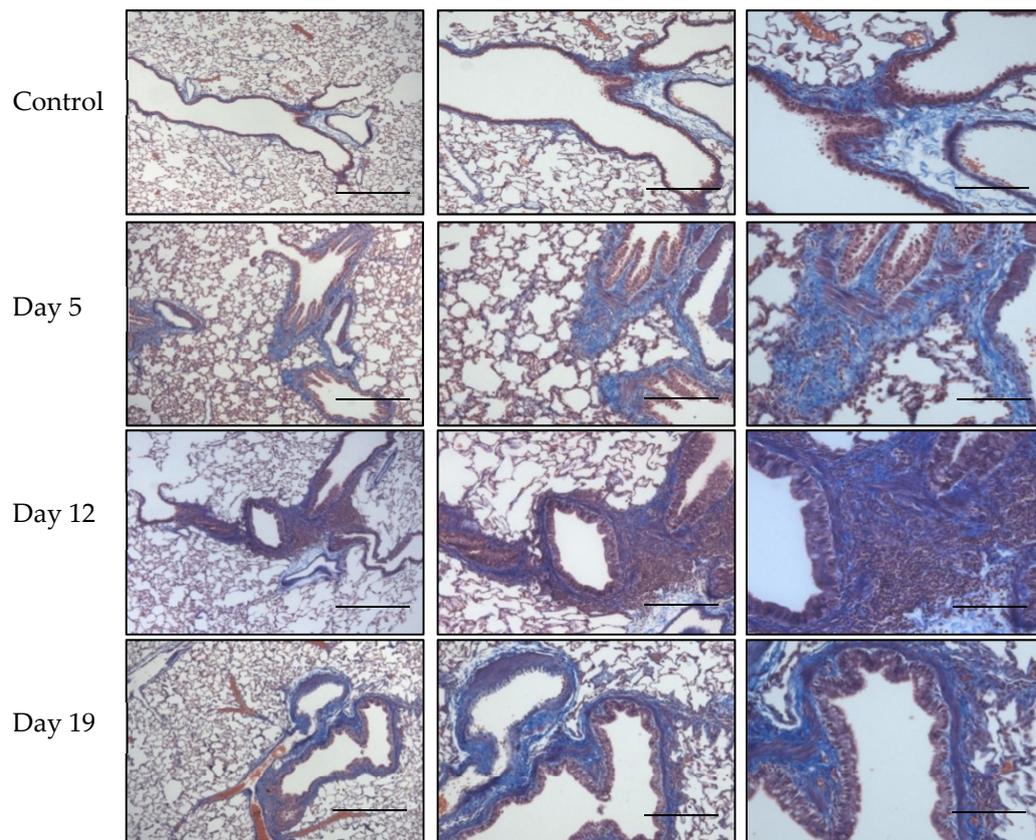


Figure S1. Representative rat lung sections stained for Masson's Trichrome demonstrating collagen I deposition (blue) (bar: 250 μm (left column), 75 μm (middle column), and 50 μm (right column)). (a) Room air (Control)-exposed rat airways, (b) DA Day 5 (immediately post-exposure) rat airways (c) DA Day 12 (1 week following DA exposures) rat airways, and (d) Day 19 (2 weeks post-exposure) rat airways. Organization of collagen I is noted in DA-exposed rats as early as Day 5 and persists to Day 19.

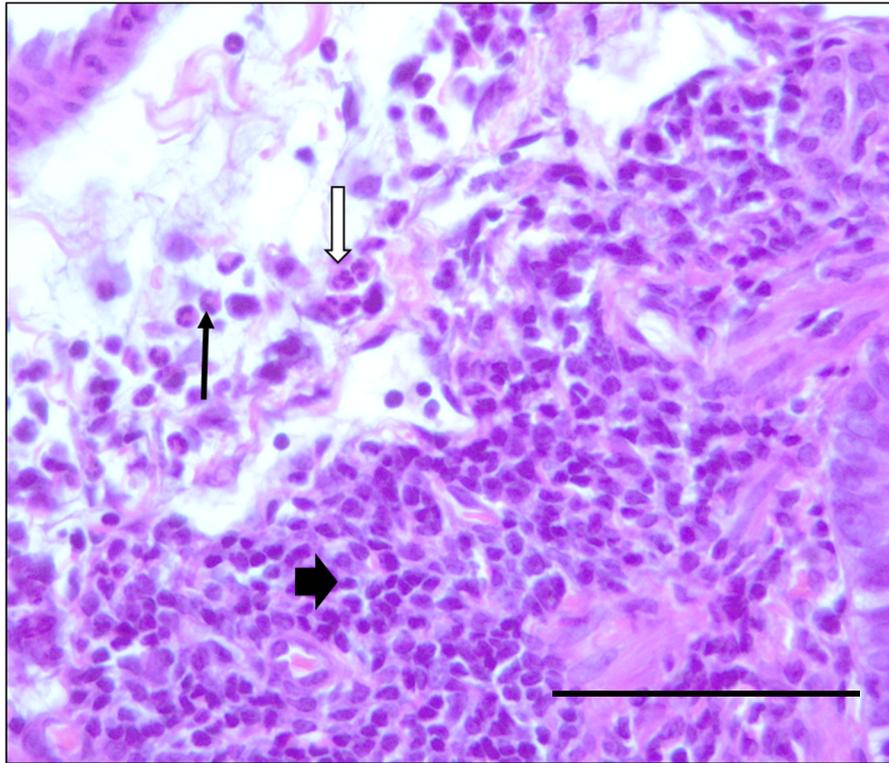


Figure S2. Higher magnification image of hematoxylin & eosin (H&E) stained rat lung section at Day 12 post-diacetyl exposure. Mixed inflammatory infiltrate adjacent to DA affected airway with neutrophils (white arrow), eosinophils (black arrow), and clustered lymphocytes (arrow head) (bar: 25 μ m).

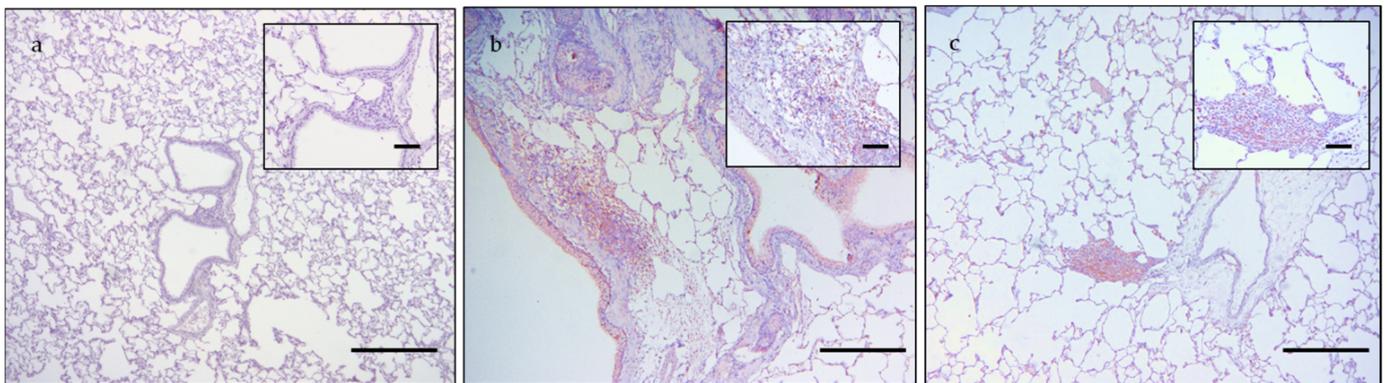


Figure S3. Rat lung sections stained for CD3, a T cell marker, and counterstained for hematoxylin in room air controls (a), DA-exposed Day 12 (b), and DA-exposed Day 19 (c) (bar: 250 μ m). Inserts highlight the airway-centric, lymphoid aggregates with prominent CD3+ T cell staining within lymphoid aggregates (bar: 25 μ m).