Interpreting pulmonary alveolar macrophage increases in rodent inhalation toxicity studies

Risk assessment of pharmaceuticals

Society of Toxicologic Pathologists
Alveolar Macrophage Working Group

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Introduction

- Alveolar macrophages respond to changes in the local micro-environment
  - Adaptations that maintain normal function vs. processes with the potential to altered function and lead to adverse sequelae

- Need for generally accepted criteria to distinguish non-adverse versus adverse findings
  - Overly conservative approach to identify the NOAEL with regard to alveolar macrophage increases may unduly impact dose escalation in the clinic
Histopathology Terminology

• Terminology should
  – Distinguish between alveolar macrophage increases without other lung findings and those complicated by inflammation or tissue changes
  – Allow use of data summary tables to distinguish between exposure levels associated with adaptive alveolar macrophage increases and those associated with potentially adverse changes

• “No threshold” approach recommended
  • Record increased alveolar macrophages, alveolar epithelial hyperplasia, and inflammation in controls
Recommended Terminology

• Increased Alveolar Macrophages
  – Stand-alone diagnosis; doesn’t include other changes

• Other changes should be entered separately from Increased Alveolar Macrophages. Examples:
  – Inflammation
  – Debris
  – Alveolar proteinosis
  – Type II pneumocyte hyperplasia
  – Bronchiolar epithelial cell hyperplasia/hypertrophy
  – Fibrosis

• Inflammatory change consisting of a variety of inflammatory cell types including macrophages would be recorded using the single term “Inflammation”
Increased Alveolar Macrophages
Increased Alveolar Macrophages with Bronchoalveolar Hyperplasia
Alveolar Inflammation
Increased Alveolar Macrophages as a Non-Specific Effect of Poorly Soluble Material

- Inhaled “nontoxic” dusts are removed from alveolar airspaces by macrophages
  - Translocation to lymph nodes or removal via the mucociliary escalator
  - Clusters of macrophages occasionally observed in control animals
  - Dust accumulation in autopsy specimens with normal pulmonary architecture
Control Rat
High Dose Group Rat
Uncomplicated Macrophage Increase
Increased Alveolar Macrophages as a Non-Specific Effect of Poorly Soluble Drugs

• Large amounts of particulate material reach the alveolar spaces at the high exposure levels in toxicity studies of inhaled particulate pharmaceuticals
  – Historically, pharmaceuticals were relatively soluble
    • not associated with increases in alveolar macrophages
  – Newer drug candidates are poorly soluble (PS)
    • Maximize lung efficacy and minimize systemic exposure
    • May accumulate and induce a macrophage response
    • Non-specific response to particulate material independent of chemically or pharmacologically mediated effects of the drug
  – Typically, vehicles are soluble and not associated with a macrophage response
Interpreting Non-specific Macrophage Responses to PS Drugs – Use of Insoluble Particle Data

- Published rat inhalation studies using inert, insoluble particles: titanium dioxide ($\text{TiO}_2$), carbonyl iron (CI), carbon black (CB)
  - Frequently conducted to study lung “overload” but lower doses provide relevant data to understand nonspecific responses to PS pharmaceuticals
  - Up to 2 years of exposure to respirable particles
    - Often included BALF analyses, proliferation indices, morphometry in addition to histopathology

- Analyses of these studies show that the development and outcome of alveolar macrophage responses are well documented and understood
Non-specific Macrophage Responses – TiO$_2$ and Cl

- 4-week inhalation exposure
- 5 mg/m$^3$ → minimal, diffuse increase in alveolar macrophages
  - No BALF analyte changes
  - Reversed within one week after exposure
- 50 and 250 mg/m$^3$ → dense aggregates of macrophages (alveolar ducts/adjacent alveoli), local epithelial cell hyperplasia/hypertrophy, and neutrophils
  - Not completely reversed 6 months after exposure
- Non-adverse vs. adverse exposure levels are distinguishable; 5 mg/m$^3$ NOAEL
Non-specific Macrophage Responses – CB

(Elder et al, 2005, Tox Sci, 88)

- 13-week inhalation exposure; rat, mouse, hamster
- 1 mg/m³ → particles within alveolar macrophages
  - No other morphological changes; no BALF changes
- 7 mg/m³ → macrophage accumulation (alveolar ducts/adjacent alveoli), local epithelial cell hyperplasia and minimal inflammation
  - Epithelial changes and inflammation reversed 3 months after exposure
- 50 mg/m³ → macrophage accumulation and degeneration, local epithelial cell hyperplasia, local inflammation, cell debris, alveolar proteinosis
  - Changes not reversed after 11 months and fibrosis developed
- Body weights not affected at any dose; no CB-related morbidity/mortality; 1 mg/m³ NOAEL
Non-specific Macrophage Responses – CB

(Driscoll et al, 1996, Tox Appl Pharmacol, 136)

• 13-week inhalation exposure
• 1.1 mg/m³ → accumulation of alveolar macrophages
  – No other morphological changes; no BALF changes
• 7.1 mg/m³ → macrophage accumulation, local epithelial cell hyperplasia, neutrophils in alveolar spaces, and mild interstitial fibrosis (trichrome stain)
  – Particle burden not reduced and changes not reversed after 8 months
• 52.8 mg/m³ → similar but more severe changes than 7.1 mg/m³
  – Particle burden not reduced and changes not reversed after 8 months; fibrosis increased during the recovery period
• 1.1 mg/m³ NOAEL
Non-specific Macrophage Responses – TiO$_2$

(Lee et al, 1986, *Environ Res*, 41)

- 2-year inhalation exposure
- 10 mg/m$^3$ → clusters of alveolar macrophages, local epithelial cell hyperplasia, increased macrophages in local lymph nodes
  - No changes in: body weight, adverse signs, or morbidity/mortality vs. control
  - Dose sufficient to induce slight epithelial hyperplasia in association with alveolar macrophage increases over the lifetime of the rats did not compromise health or cause adverse sequelae
- 50 and 250 mg/m$^3$ → lung overload, debris, proteinosis, cholesterol granulomas, bronchioloalveolar hyperplasia
Lessons From These Studies

• Removal of insoluble material by macrophages is slow (~100 day T1/2 in rat lung)
  – At higher doses, essentially all insoluble inhaled material was still present and exerting its effect in the lung

• Findings at the lower exposures to inert, insoluble material were typical of findings at the highest doses in inhalation tox studies of PS pharmaceuticals in rats
  – However, inert particle represent “worst case” because even PS pharmaceuticals are slightly soluble such that dissolution is the predominant mode of particle removal
  – Macrophage stimulus would not be as persistent; reversal not as long

• Reasonable to conclude that changes in macrophage distribution/size/number centered on alveolar ducts with minimal or no changes in the adjacent epithelium or inflammation represent the same non-specific, adaptive response to particles and would have the same benign course, even if present for lifetime

• Inclusion of additional BALF, morphometric, or macrophage functional assays corroborated but did not change NOAEL based on histopathology
PS Inhaled Pharmaceutical Candidates

(Jones and Neef, 2012, *Xenobiotica*, 42)

- Reviewed data from 4 PS candidates and estimated lung particulate burden
- Lung burdens greater than 1 mg/g lung were associated with adverse changes consistent with those described (previous slides) for inert, insoluble particles at the higher doses
- Lung burdens ~ 0.5 mg/g (0.1 – 1.0) represented transition point between adaptive, non-adverse and adverse changes
- Similarity to published data on insoluble particles supports the conclusion that the pathogenesis of the changes due to the PS candidates may be related to low solubility and high particle burdens
Marketed Drugs

• Public-domain FDA data
• Increased alveolar macrophages in studies supporting registration of:
  – Salmeterol, fluticasone/salmeterol, mometasone, formoterol, memetasone/formoterol, budesonide, tobramycin, zanamivir
  – Each of these products has been used in humans many years
  – All but zanamivir powder (Relenza) and Tobi Nebulizer are “insoluble” or “practically insoluble in water”
    • Macrophage increase at much higher doses with zanamivir
    • Tobramycin associated with phospholipidosis
  – Provides evidence that the increased alveolar macrophages (except tobramycin) is a non-specific effect related to phagocytosis of undissolved particles (and/or surfactant in the case of corticosteroids) in the lung
Clinical Pathology: Current Measurements in Standard Inhalation Studies

- Marketed compounds that elicited increased alveolar macrophages did not alter clinical pathology parameters
  - No indication of inflammation or pulmonary injury
  - Correlation between histopathology and clinical pathology
Clinical Pathology: Additional Parameters

- Bronchoalveolar lavage (BAL) fluid analysis
  - Studies (TiO₂, Cl, CB) have shown an inflammatory cell response at exposures where inflammation was observed histopathologically
  - BAL analytes indicative of inflammation not present at lower exposures where uncomplicated increases in macrophages were observed histopathologically

- No specific, proven BAL biomarkers that identify early changes in alveolar macrophages prior to morphologic changes or contribute to an adverse versus non-adverse designation
  - Markers that corroborate changes complicated by inflammation
    - Serum C-reactive protein and fibrinogen
    - BAL C-reactive protein, interleukins, interferons, total protein and electrophoreses, cell differentials and mRNA analysis
Lung Functional Parameters Support Uncomplicated ↑Macrophages as Non-Adverse

• Marketed inhaled drugs with preclinical alveolar macrophage response
  – PFTs only for mometasone/formoterol
    • Slight ↓ minute and tidal volume attributed to decreased body weight

• Diesel exhaust (DE; complex mixture)
  – 7 or 0.35 mg/m³, 7 h/d, 5 d/wk for 12 or 30 m
    • Increased alveolar macrophage size and number
    • Particle (soot) accumulation
    • No change in pulmonary function parameters
Lung Functional Parameters (2)

- Diesel exhaust at higher exposures
  - 3 to 7 mg/m³, 7 h/d, 5 d/wk for 24+ m
    - Morphologic inflammation and parenchymal changes
    - Lung overload demonstrated
    - Restrictive pulmonary function changes
      - ↓ volume/compliance, ↓ gas exchange, non-uniform gas distribution

- Elastase-induced emphysema and DE
  - 3.5 mg/m³, 7 h/d, 5 d/wk for 24 m
  - No changes in respiratory function parameters vs corresponding air controls after 6, 12, 18, or 24 months exposure
  - No functional evidence for an effect of increased alveolar macrophages in this model of a susceptible population
Weight of the Evidence Indicates…

• Uncomplicated alveolar macrophage increases should be considered non-adverse based on:
  – origin in a physiological process
  – small magnitude of the change
  – presence of similar changes in occasional control rats
  – lack of clinical signs
  – lack of inflammation or tissue destruction
  – lack of progression
  – reversibility
Points to Consider in Determining the NOAEL when Increased Alveolar Macrophages are Observed

- Increases in alveolar macrophage number and/or size should not be interpreted as adverse in the absence of other, adverse lung changes.

- Findings similar to those observed in studies conducted with approved products at a similar exposure multiple should not be interpreted as adverse.
Points to Consider in Determining the NOAEL when Increased Alveolar Macrophages are Observed

• Resolution of increased alveolar macrophages during recovery supports the interpretation that increased alveolar macrophages did not initiate a pathway leading to adverse findings.
Control Rat:
Uncomplicated Macrophage Increase
Test Article-Treated Rat: Uncomplicated Macrophage Increase
Test Article-Treated Rat: Inflammation and Alveolar Epithelial Hyperplasia