

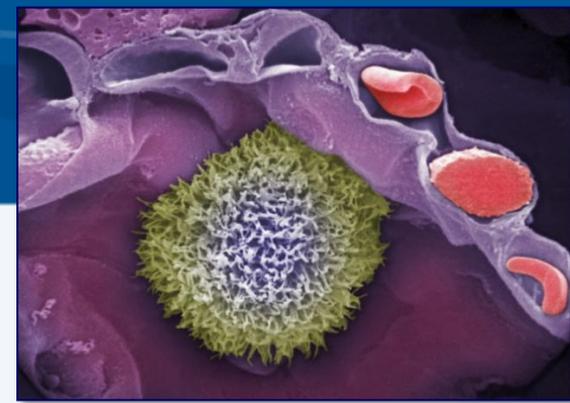
2011 HESI Emerging Issues

Alveolar macrophage changes in response to inhaled drugs:
Factors distinguishing adaptive from adverse effects.

HESI Annual Meeting, June 2011

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Overview



- Alveolar macrophages are a **normal feature of the lung**
 - Responsible for phagocytosis and immune surveillance
- Drugs administered by inhalation to animals in toxicity studies can induce **varying degrees of alveolar macrophage response**
 - Simple increase in number of macrophages → complex reaction involving also other (inflammatory and epithelial) cell types
- There is **varying opinion** between and among industry and Regulatory Authorities about which of these responses are adaptive (to foreign material) and which are adverse (involving eg tissue damage)
- Consequences include over-cautious dose escalation, reduced clinical doses with potential **delay or cessation of therapies of medical benefit**

Why Administer Pharmaceuticals by Inhalation?

Lung can be...

- ...intended target of therapy:
 - eg asthma, COPD, idiopathic pulmonary fibrosis, cystic fibrosis, bronchiolitis obliterans, acute lung injury
 - Maximize delivery to target / minimise systemic absorption* (therefore toxicity / secondary pharmacological effects)
- ...route of administration for systemic treatment / effect:
 - eg inhaled insulin for diabetes, anaesthetics
 - Maximise / control systemic absorption

(* Eg by decreasing solubility or maximising first-pass metabolism)

Range of Pharmacologies / Targets

- Beta-agonists – M, L
- Muscarinic antagonists – M, L
- Corticosteroids – M, L
- Novel targets eg kinases, cytokine pathways – E, L
- Dual pharmacophores eg muscarinic antagonist / beta agonist (MABA) – L

E = Early Development / Discovery; L = late-stage development; M = marketed

Diverse attributes

- Small molecules
- Peptides
- Monoclonal antibodies
- Domain antibodies
- Oligonucleotides / siRNAs

Administered as:

- Solution
- Suspension
- Dried powder

The lung sees the drug as “foreign material” which has...

- Physico-chemical properties
 - Particle
 - Liquid
 - Size
 - Shape
 - Solubility (under physiological conditions)
 - Lipophilicity
 - pH
 - Reactivity (of parent, breakdown products & counter-ion)
 -
- Pharmacological properties
 - Intended
 - Off-target

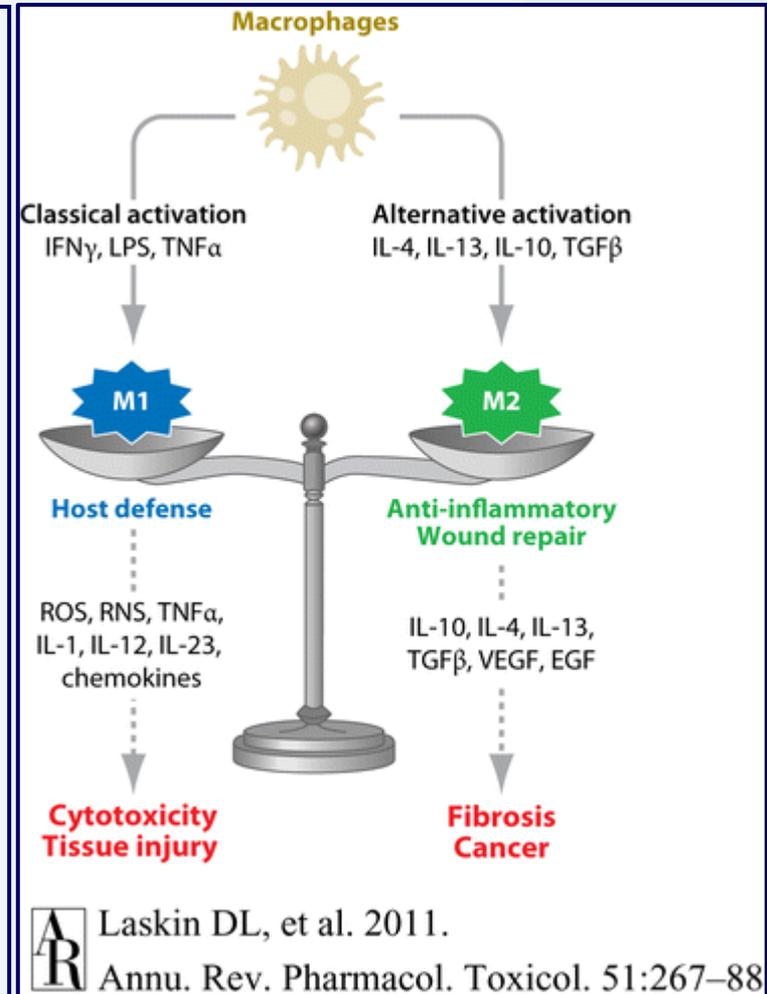
Consequences

The physico-chemical-pharmacological properties of the compound will affect:

- Distribution, absorption, metabolism, elimination of compound itself – Pharmacokinetics
- Lung function – airway diameter, mucus secretion, inflammatory cell trafficking – Pharmacodynamics
- Lung integrity - Toxicity

Response of the Lung

- The single most common cellular component of the lung response is the Alveolar Macrophage
- Alveolar macrophage has multiple functions / phenotypes:
 - Phagocytosis – ingestion of particulate materials – clearance via muco-ciliary escalator (or via tissue lymphatics)
 - Immune surveillance – infectious / antigenic agents
 - Orchestrates immune response – M1 and M2 immunophenotypes
- But what is significance of alveolar macrophage in toxicology studies?



There are differing perspectives on this issue

Macrophage accumulations in control animals

Characteristic morphological features;

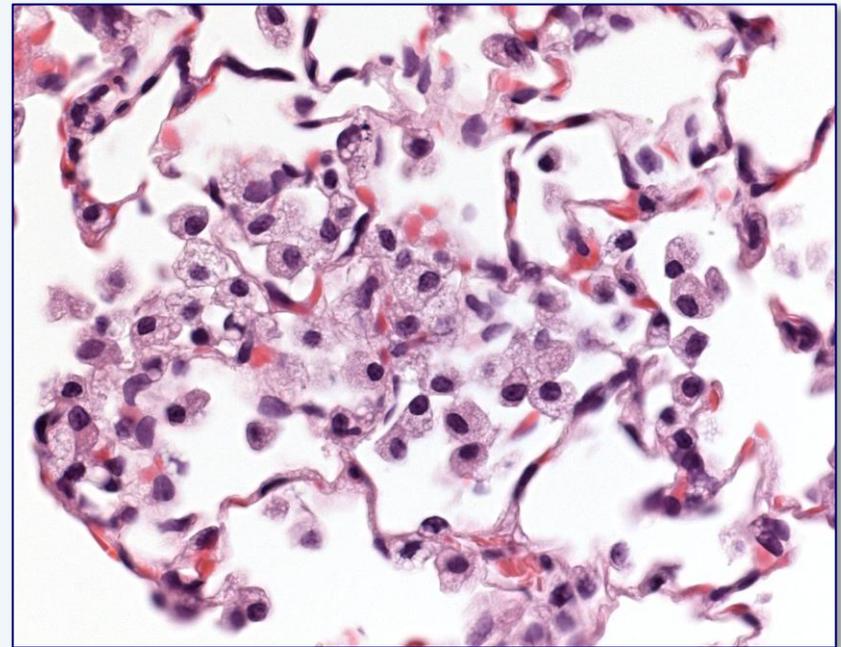
Accumulations

- Small and few
- Alveolar lumens near terminal airways

Macrophages

- Foamy cytoplasm
- May form aggregates
- No degeneration or necrosis
- Very occasional apoptosis

No lung injury or inflammation



Macrophage accumulation in a control animal

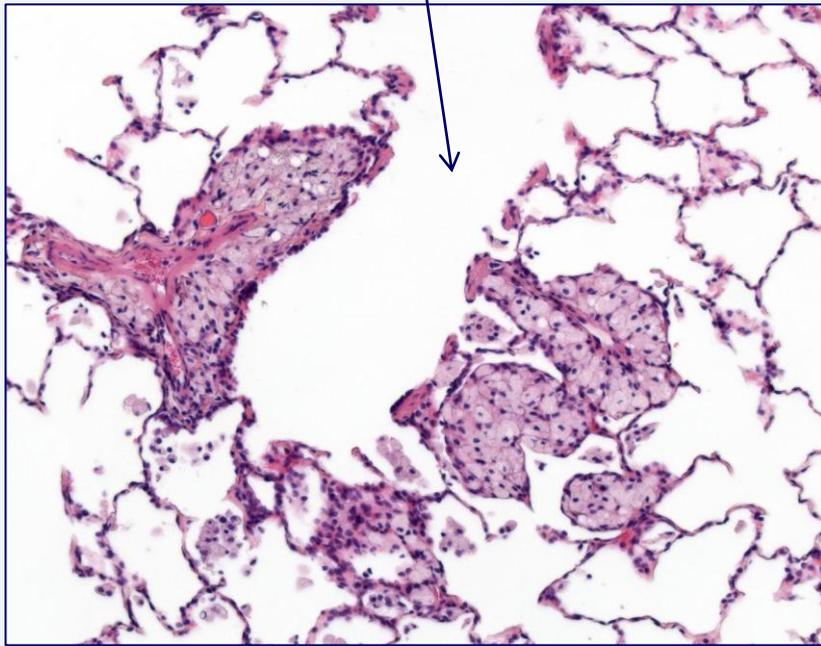
Treated animals: an increased incidence

- Common observation
- Usually dose-related
- Control incidence generally low
- Treated incidence range from low to high
- Morphological features of the accumulations are essentially similar to control animals
- *Typical example of an increased incidence;*

	Males				Females			
Dose group	Control	Low	Inter	High	Control	Low	Inter	High
No. animals	10	10	10	10	10	10	10	10
Macrophage accumulations								
Grade 1	2	3	7	9	1	2	5	10

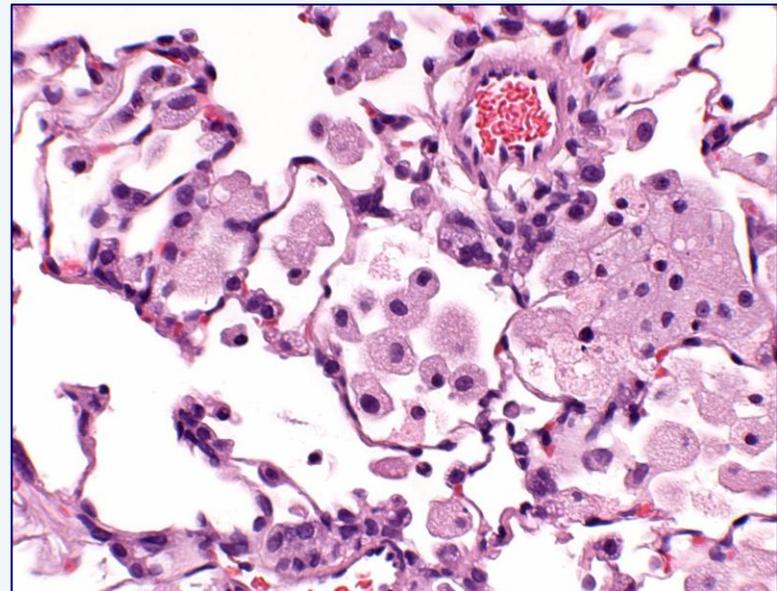
Treated animals: an increased extent

Respiratory bronchiole



- Accumulations are larger and/or more numerous
- *Greater numbers* of macrophages
- Common observation
- Range is wide
- In most cases;
 - No macroscopic observations
 - Lung weights not increased

- Usually;
 - Macrophages do not degenerate or show necrosis
 - No lung injury or inflammation
- Even around fairly large accumulations

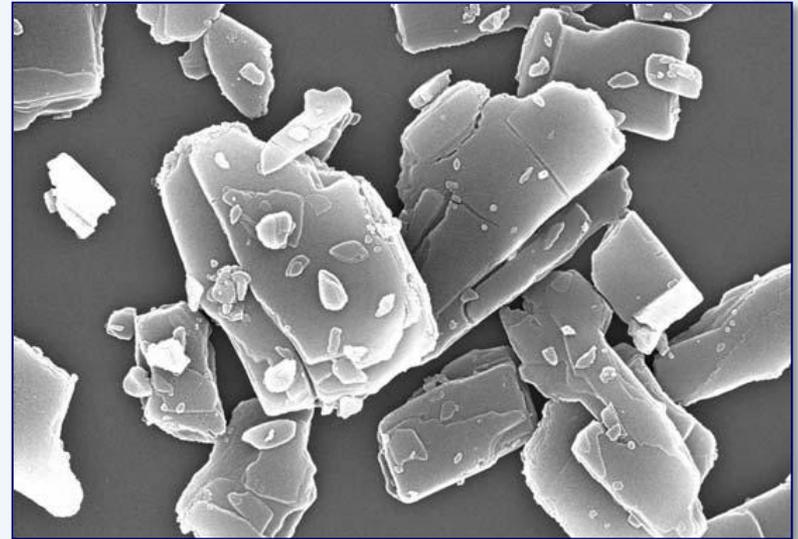


Evidence in support of particle-mediated response

1. Much more common in inhalation toxicology
2. Accumulations can occur following;
 - Diverse pharmacological mechanisms
 - Excipients
 - Inert environmental and mineral particulates

Particles are the common denominator

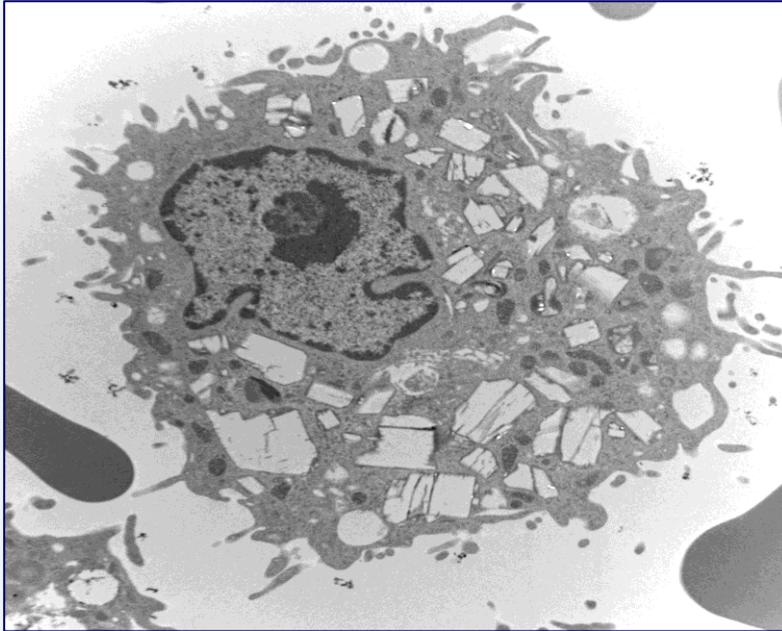
3. Varied shapes but their typical target size range is 1-5 microns. A proportion will deposit in terminal airways and alveoli where accumulations occur
4. Dissolution rates vary from minutes to hours...



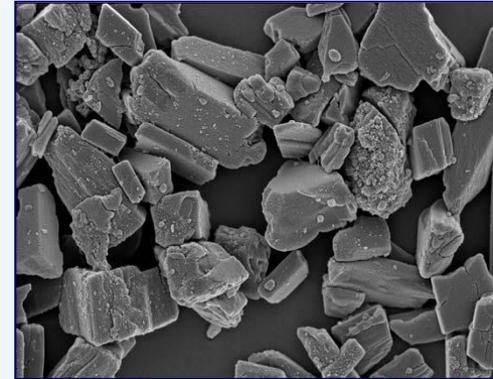
A pharmaceutical particulate (SEM)

Evidence in support of particle-mediated response

5. .. Which is ample time for alveolar macrophages to avidly phagocytose drug particles *in vivo*



*Alveolar macrophage, laden with phagocytosed drug particles. BAL-derived, 4 hours after a single inhaled dose. **No evidence of injury** (TEM)*



A pharmaceutical particulate (SEM)

6. Accumulations more likely when large doses of relatively poorly soluble drug particles are administered for long periods = high particle burdens

However....

-there is a concern that even these responses could be adverse....
-leading to a lowering of No Observed Adverse Effect Levels (NOAELs).....

What Impact Does a Low NOAEL Have?

- Affects starting dose in human trials...
 - Prolongs dose escalation phase of early clinical trials
- Affects maximum allowable dose in human trials....
 - May prevent drug reaching efficacious levels
 - Avoidable drug attrition

Proposal – what do alveolar macrophages mean – adversity vs adaptation?

1. Identify a work group – 10 leaders in (alveolar) macrophage biology / pathology
 - Teleconferences to structure symposium
 - Pre-competitive information sharing?
 - Retrospective analysis of marketed drugs?
2. Conduct a symposium – Broader, larger symposium group
 - 2-day meeting
 - Open debate between Industry, Academia and Government / Regulators
3. Publish proceedings – Consensus statement
 - Peer-reviewed journal

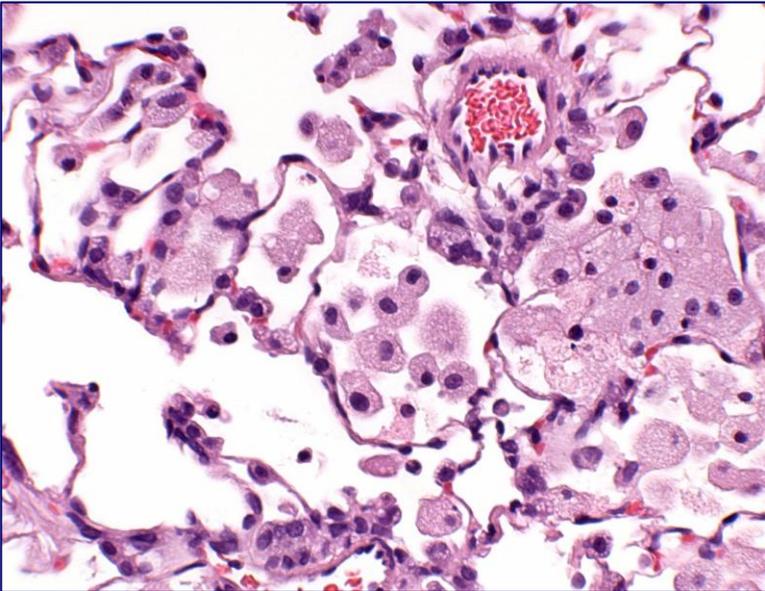
NB Not proposing any new experimental work through HESI. But work is ongoing and we may propose to co-ordinate this better.

Questions to be Addressed

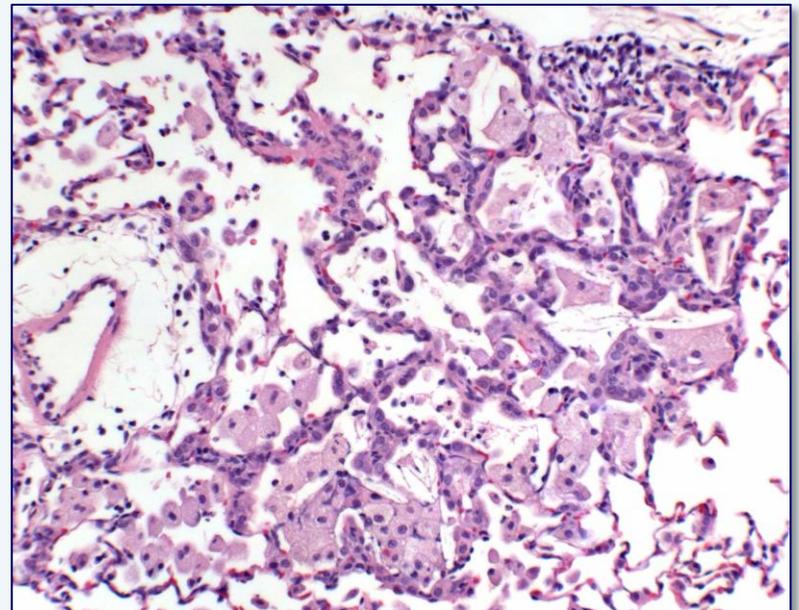
- Do alveolar macrophage responses exist [to inhaled pharmaceuticals] which are purely physiological / adaptive responses to the presence of foreign material?
 - Input required from: experts on (alveolar) macrophage physiology and responses to inhaled particulates / chemicals
- Experimentally, macrophages (incl alveolar) can be induced to activate into different functional / immunological phenotypes; how do these correlate with what we see in response to pharmaceutical agents?
 - Do increased numbers of morphologically unremarkable macrophages have an M1 or M2 phenotype of something different?
 - Can they be considered “activated” in the classical sense?
- What currently available endpoints (*in vivo*, *ex vivo*, *in vitro*) could be used to address these questions in Regulatory toxicology studies?
- What further endpoints could realistically become available to address this question?
 - What is the likelihood that they would be accepted by Regulatory Agencies?

In essence....

...which factors convert this type of response....



...into one that looks like this....



All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals

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