# Pulmonary Disease, Animal Modeling, & Vaccine Development


Tori Baxter, DVM, PhD


Introduction to Pathology of Disease

NC A&T Biol 342, NCCU Special Course

October 7, 2021


THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

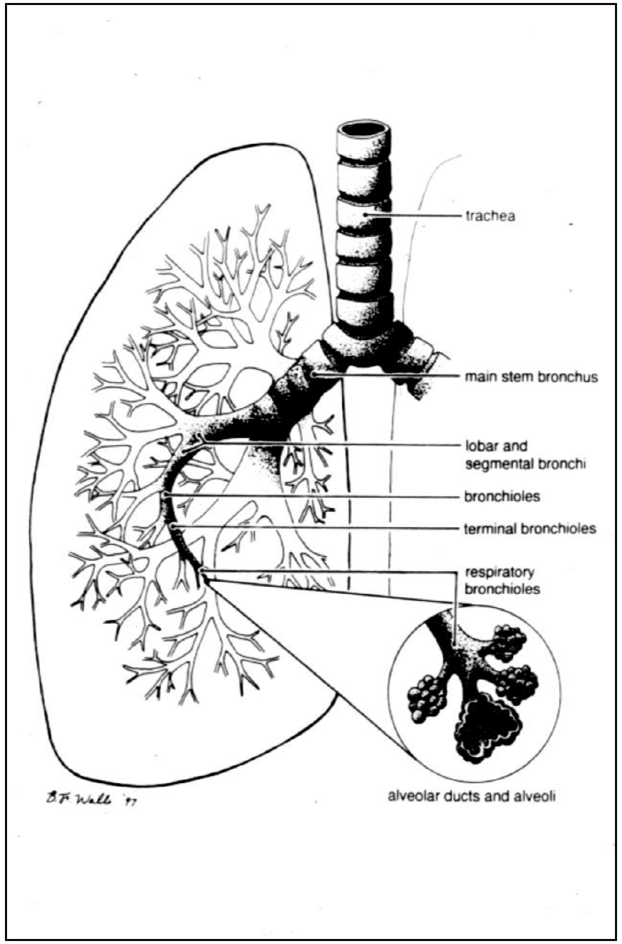
# What Are We Going to Cover Today?

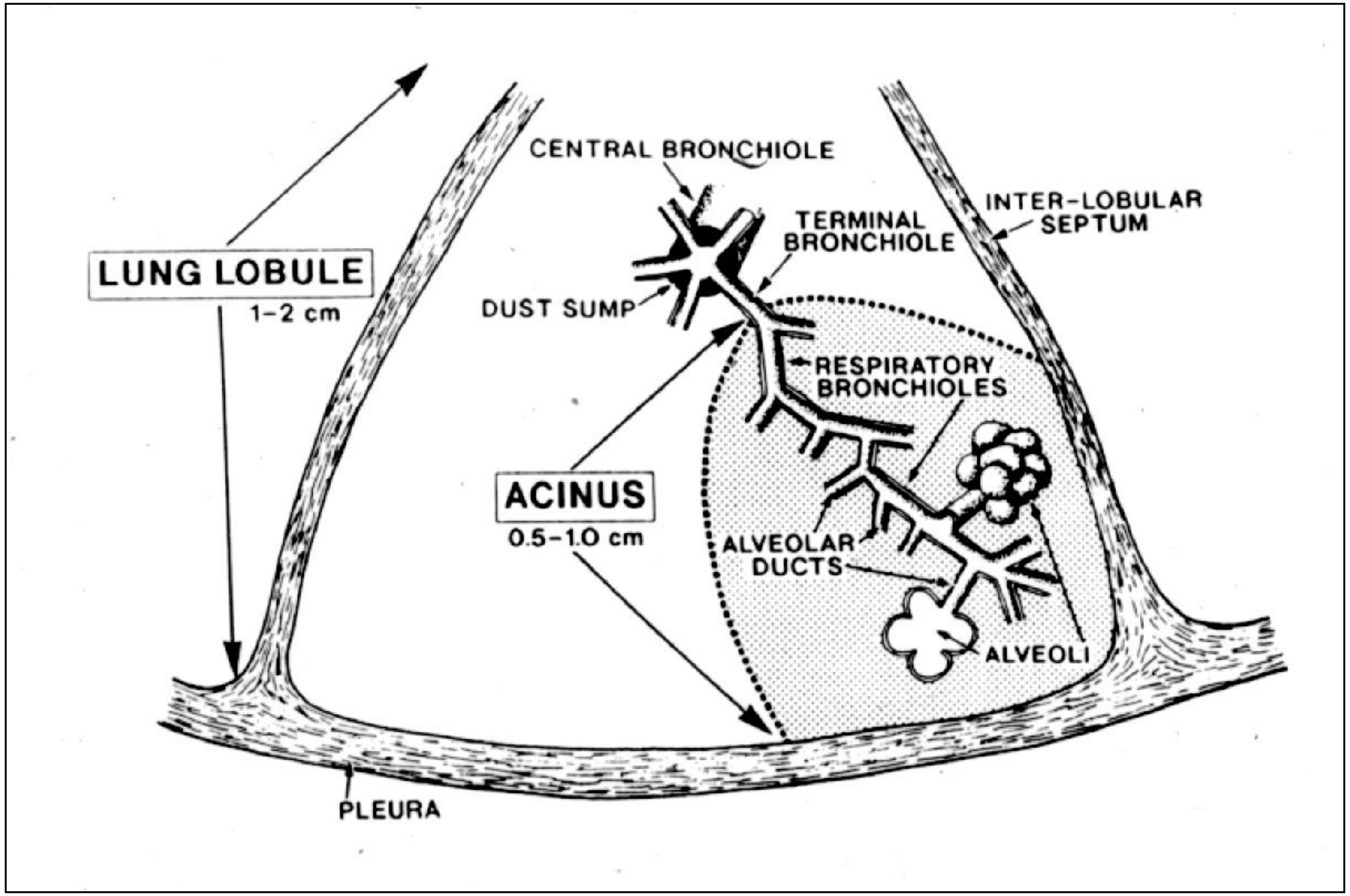
- Normal lung anatomy and histology
- Evaluating lung function
- Pathological changes to the lung
- Using animal models to study respiratory disease
- Vaccine development

# What Are We Going to Cover Today?

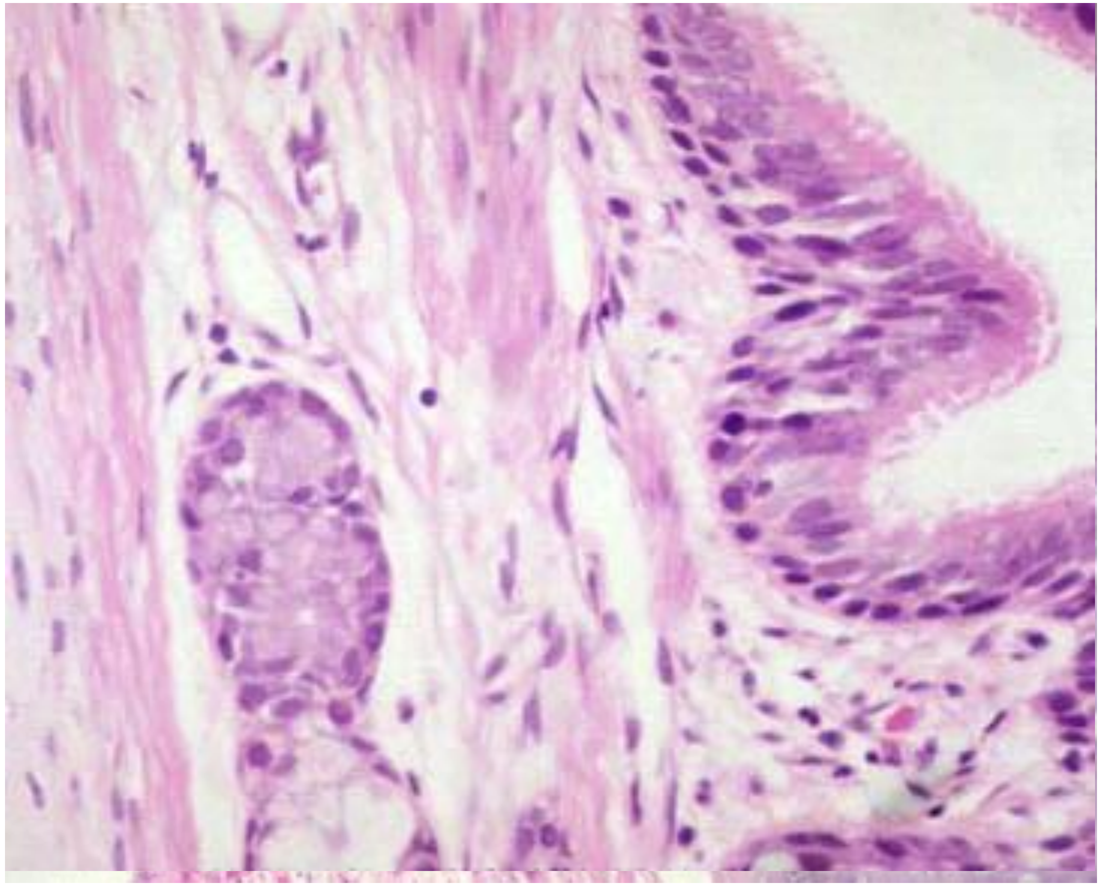
- **Normal lung anatomy and histology**
- Evaluating lung function
- Pathological changes to the lung
- Using animal models to study respiratory disease
- Vaccine development

# Normal Lung Anatomy

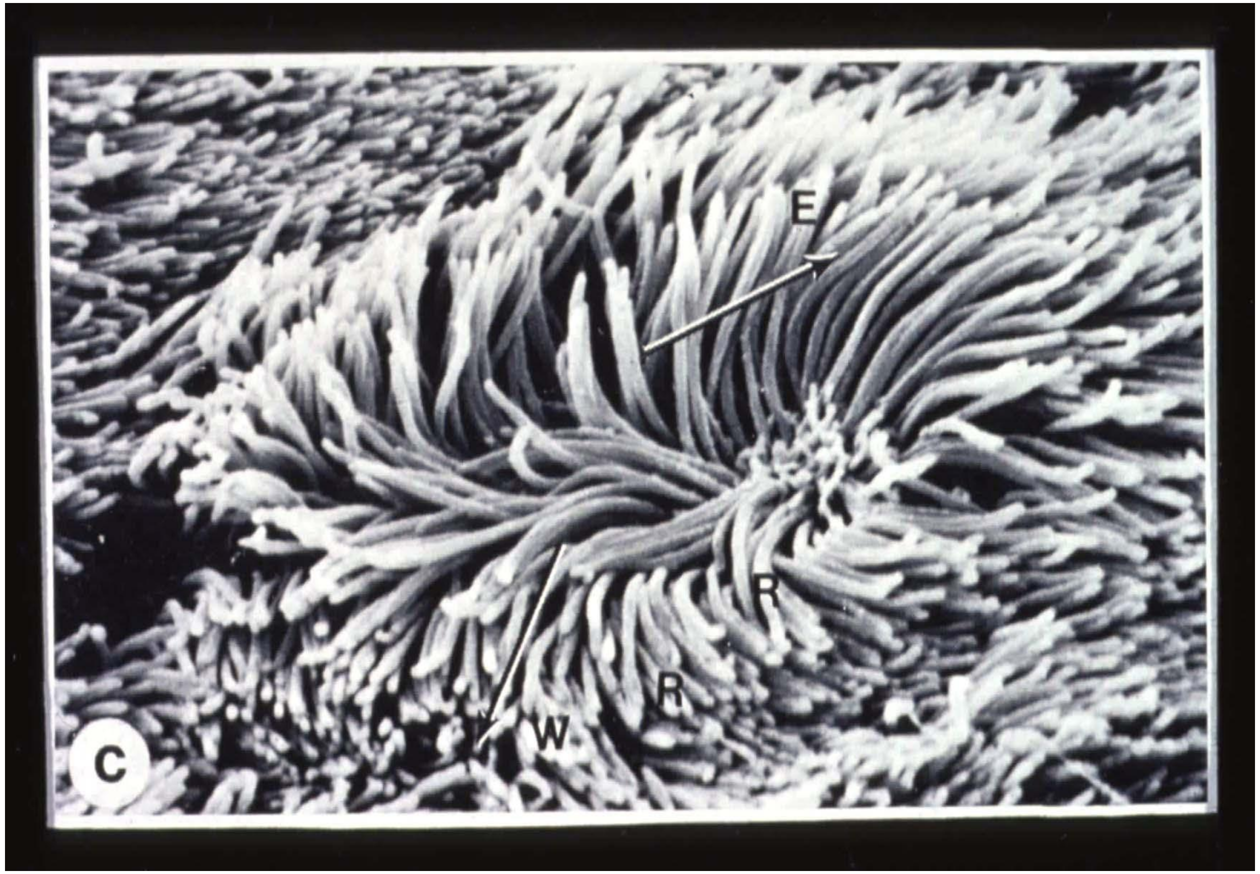


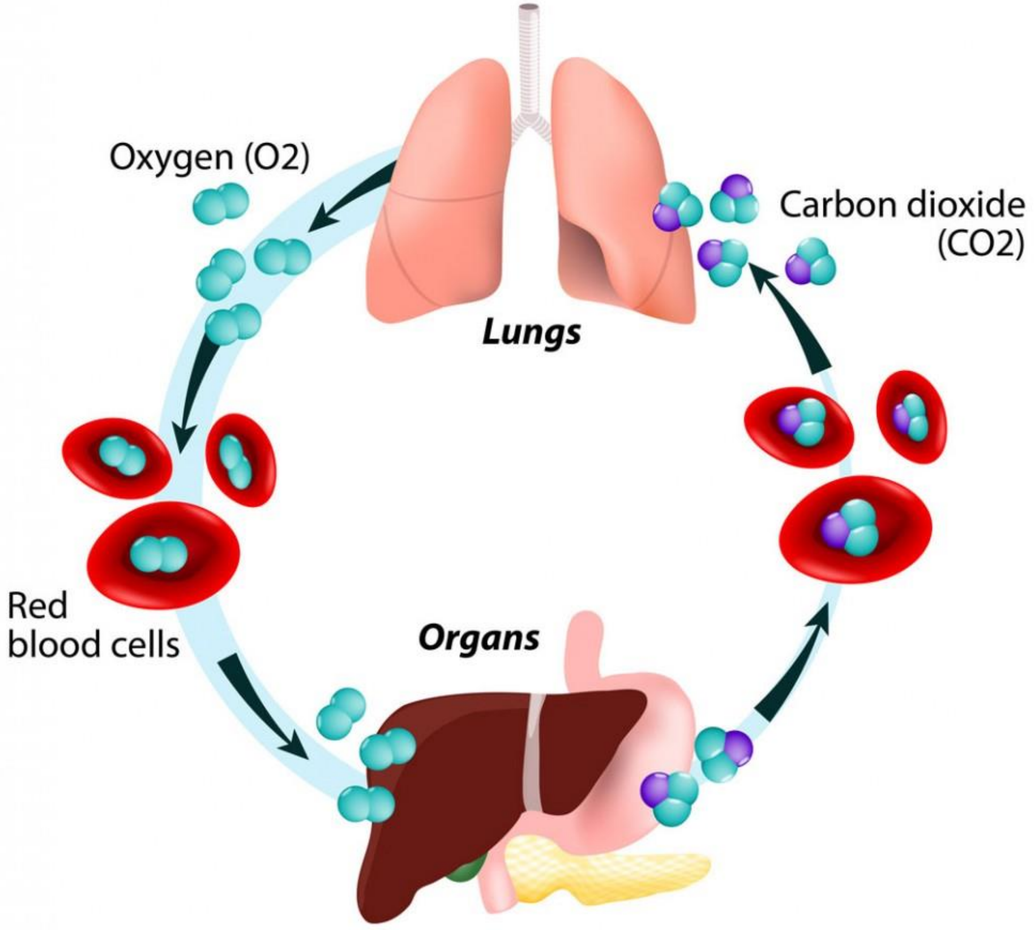
# Bronchus



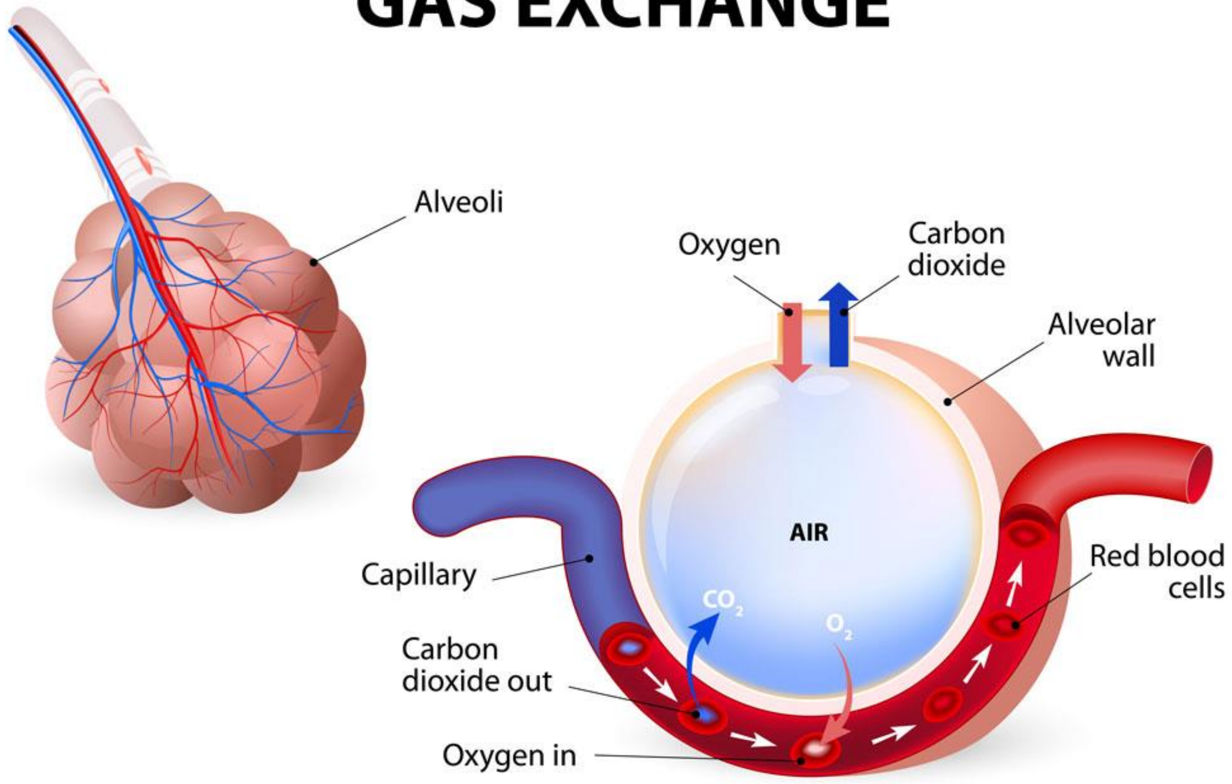
meddean.luc.edu



# GAS EXCHANGE IN HUMANS

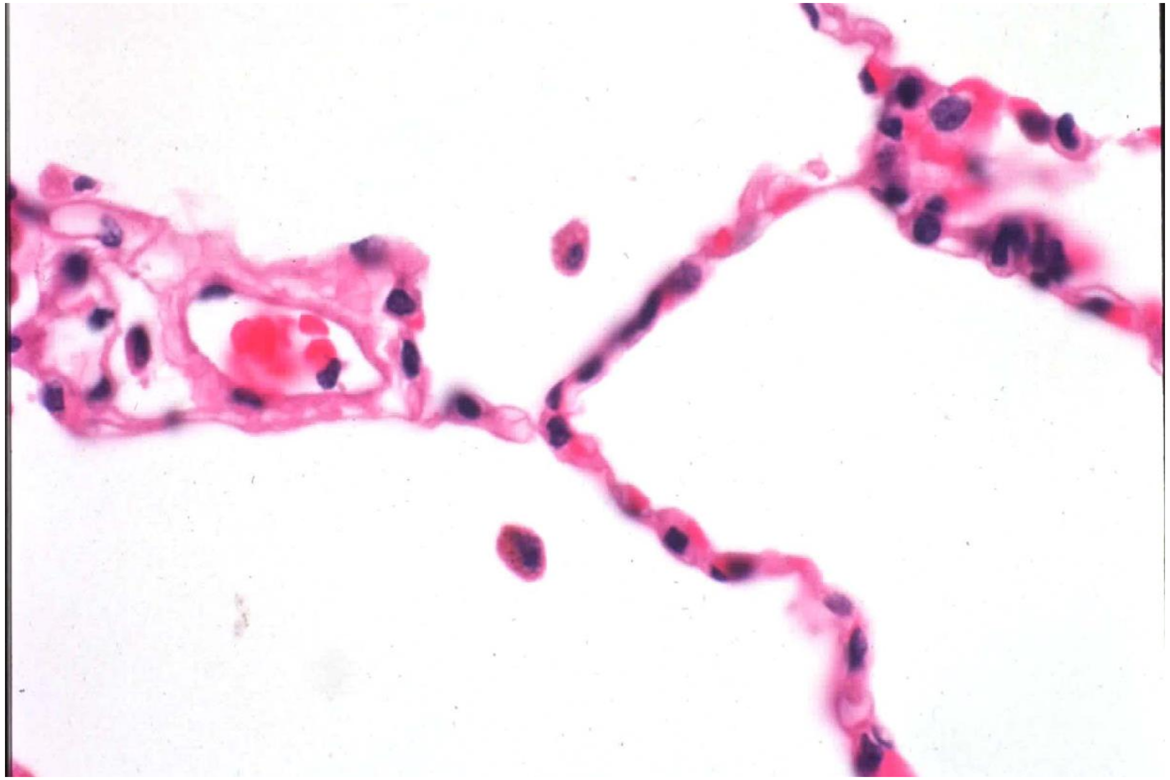



# ALVEOLUS GAS EXCHANGE

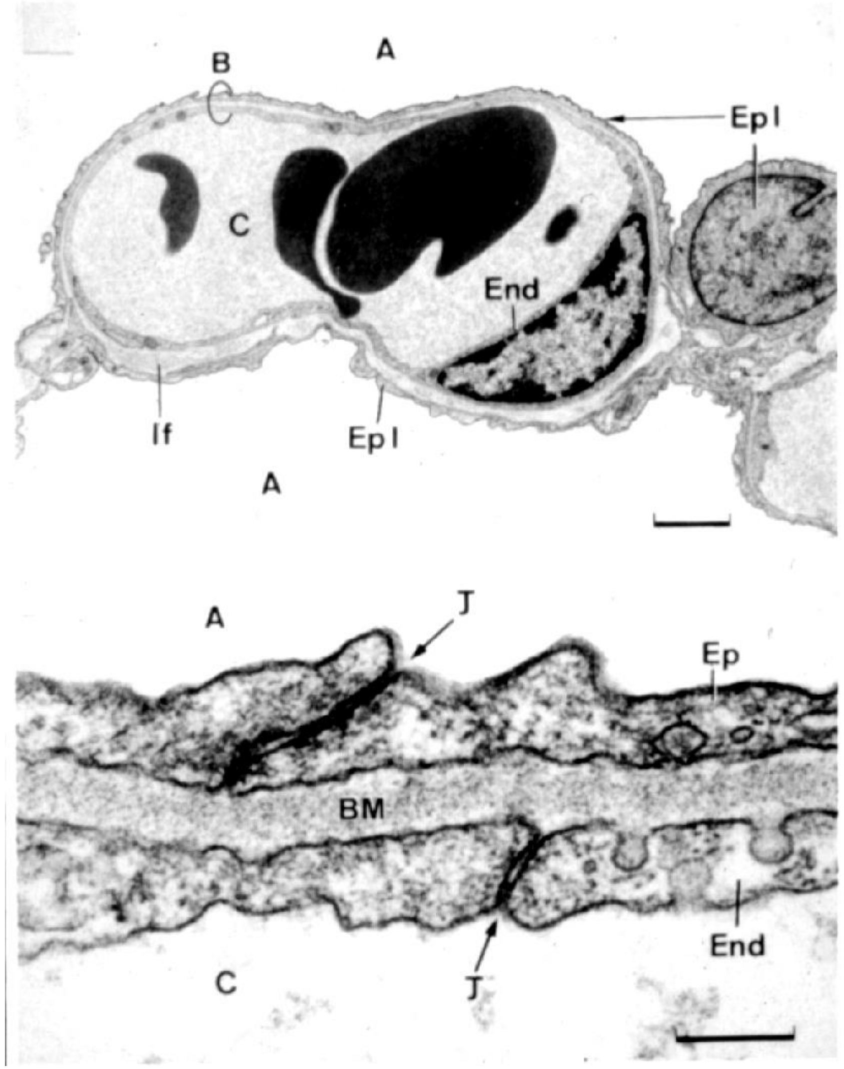



www.pedilung.com

# Alveoli



meddean.luc.edu



# What Are We Going to Cover Today?

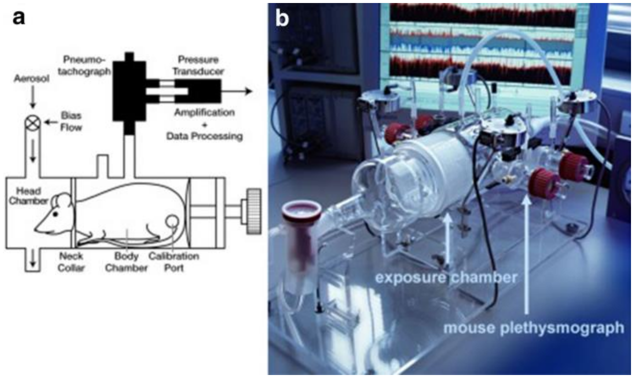
- Normal lung anatomy and histology
- **Evaluating lung function**
- Pathological changes to the lung
- Using animal models to study respiratory disease
- Vaccine development

# Pulmonary Function Tests

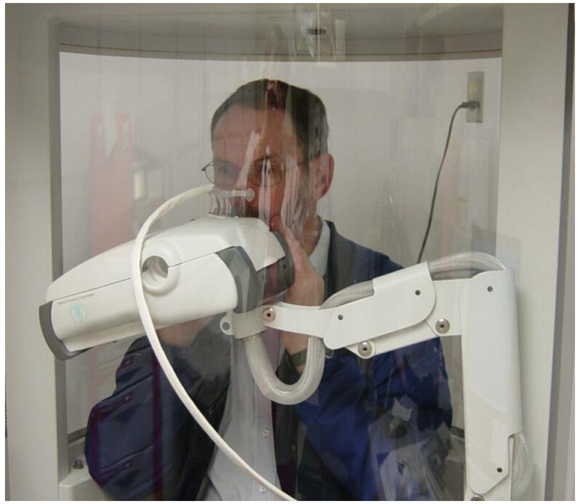
- **Spirometry** – measures the rate of air flow and estimates lung size
- **Plethysmography** – measures lung volume changes
- **Lung Diffusion Capacity** – assesses how well oxygen gets into blood
- **Pulse Oximetry** – estimates oxygen levels in blood
- **Arterial Blood Gas** – directly measures levels of gases in blood
- **Fractional Exhaled Nitric Oxide** – measures how much nitric oxide is in exhaled air

# Modeling Pulmonary Function Tests

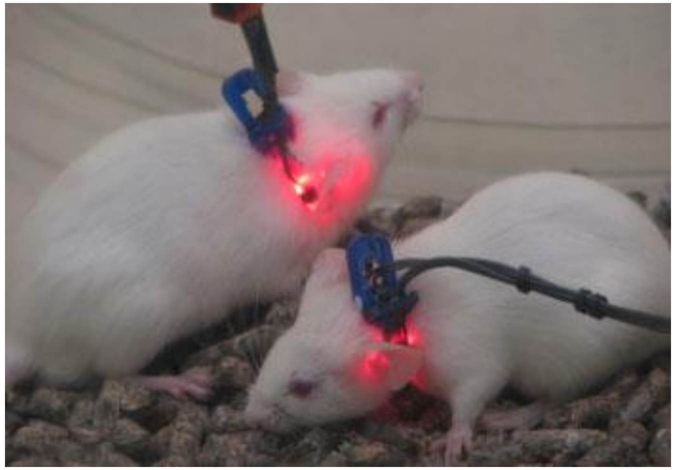
## Spirometry




## Plethysmography



## Pulse Oximetry

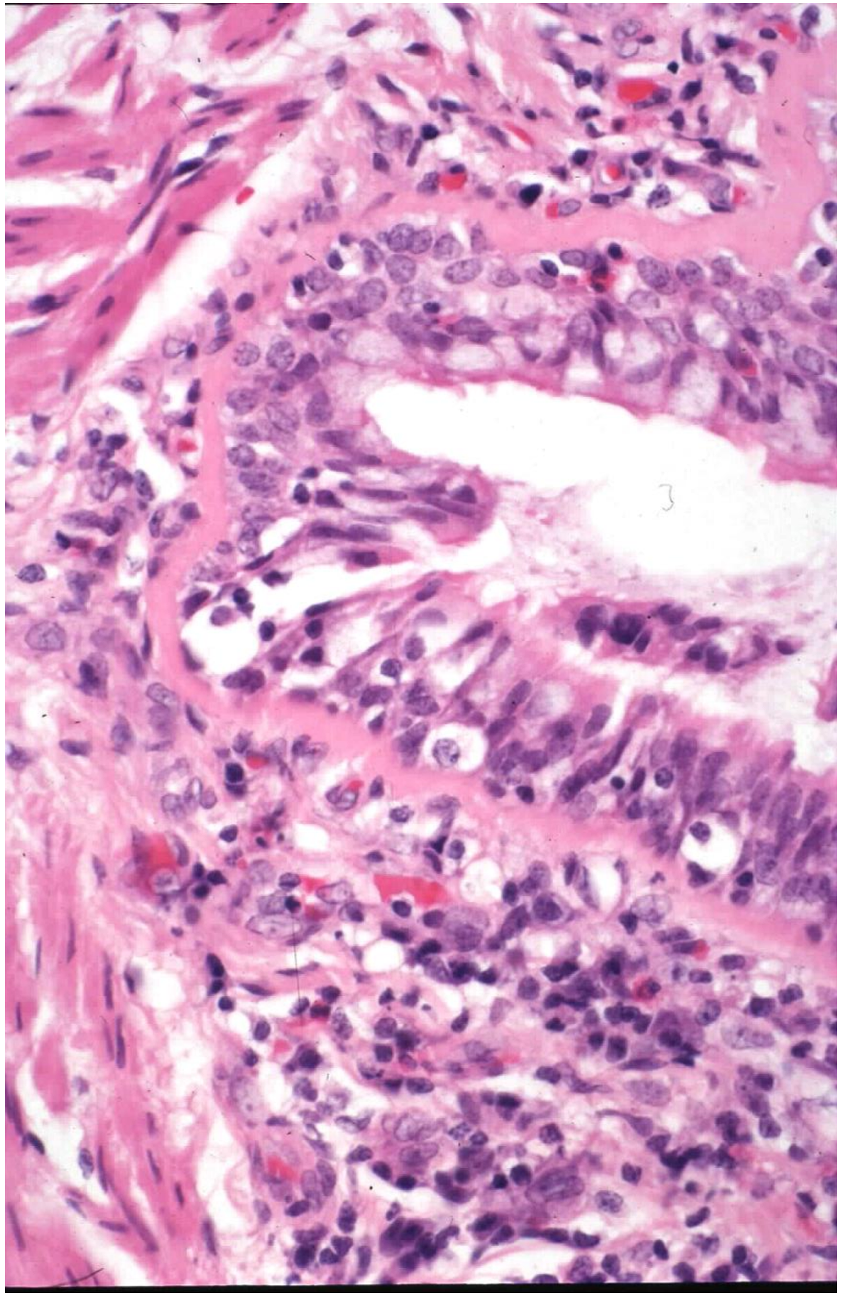



# What Are We Going to Cover Today?

- Normal lung anatomy and histology
- Evaluating lung function
- **Pathological changes to the lung**
- Using animal models to study respiratory disease
- Vaccine development

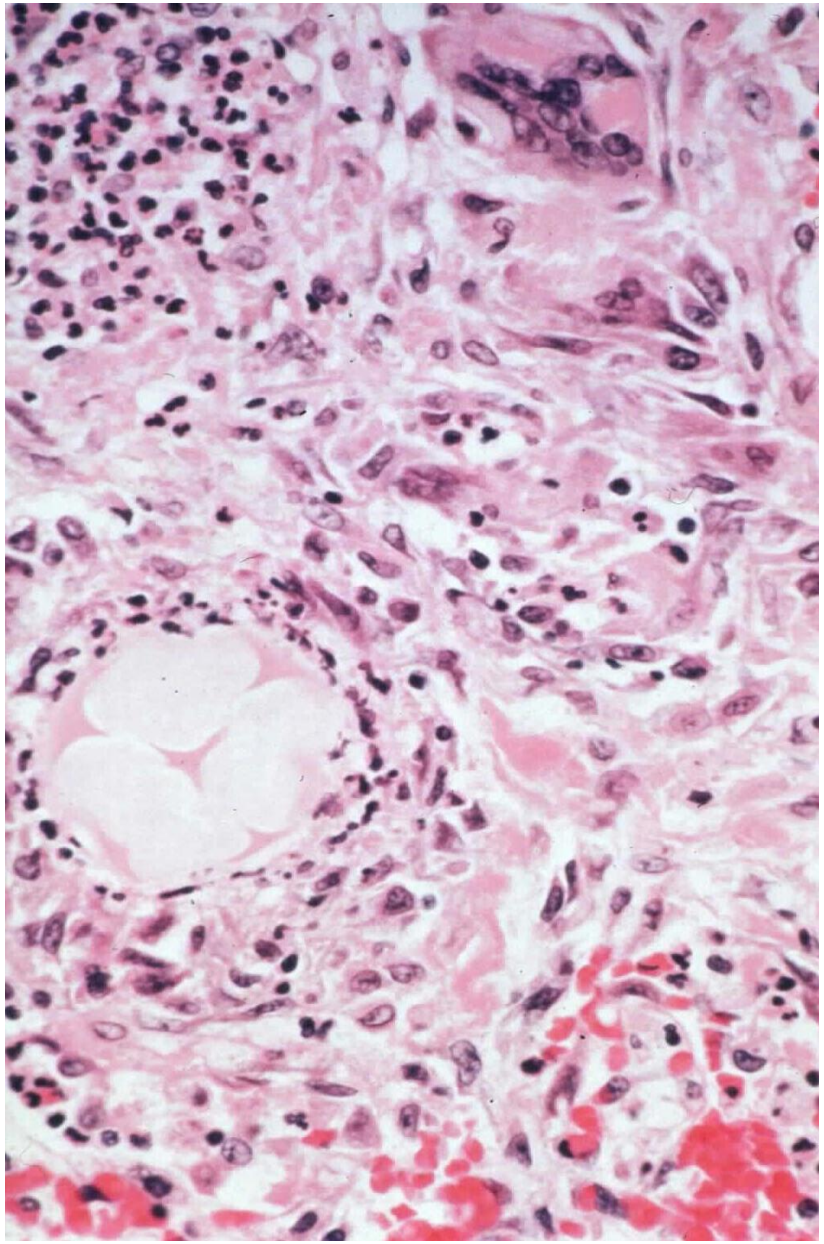
# Asthma

- Clinical:
  - Airway hyper-responsiveness
  - Triggers: antigens, exercise, drugs, infections, stress
  - Acute, usually reversible diffuse bronchial narrowing
  - Wheezing, dyspnea
- Pathology:
  - Edema, smooth muscle thickening, basement membrane thickening, mucous cell hyperplasia, increased submucosal eosinophils, thickened intralumenal mucus



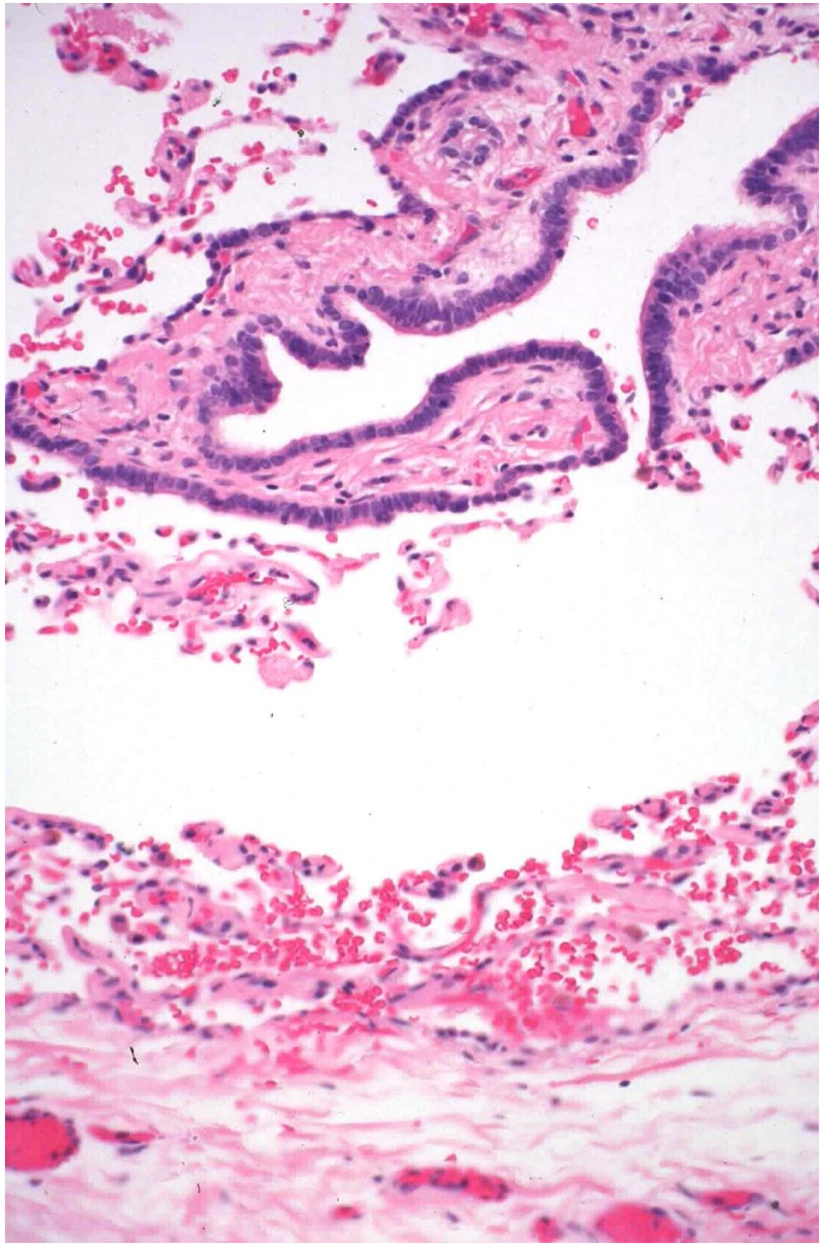
# Aspiration

- Clinical:
  - Children - foreign bodies
  - Adults - gastric acid, food, foreign bodies
    - Lipids in nasal drops
- Pathology:
  - Foreign material → foreign body giant cell reaction with exogenous material



# Emphysema

- Clinical:
  - Increased elastase activity
    - Cigarette smoking
    - Inherited $\alpha$1-antitrypsin deficiency
- Pathology:
  - Dilation of distal airspaces with septal destruction

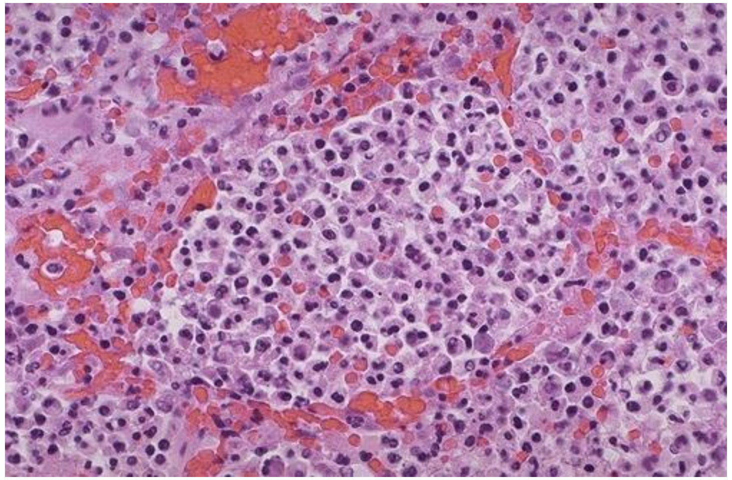


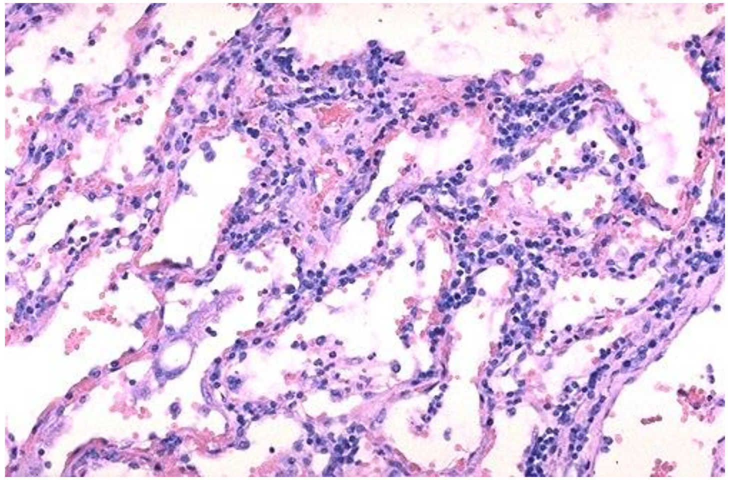
# Lung Infections

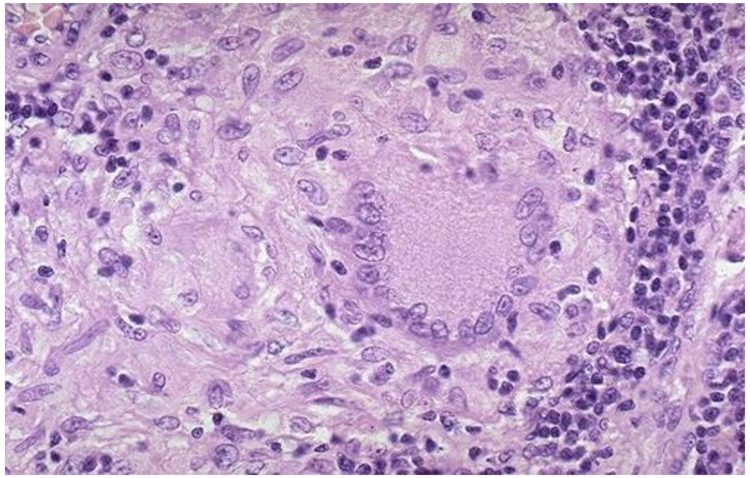
- Types of inflammatory response
- Bacterial pneumonia
- Viral
- Mycobacteria
- Fungal

# Types of Inflammatory Responses

- Neutrophils
  - Usually bacterial
  - Usually in alveoli
- Lymphocytes
  - Usually viral
  - Usually in interstitium / septae
- Granulomatous inflammation
  - Usually mycobacterial or fungal

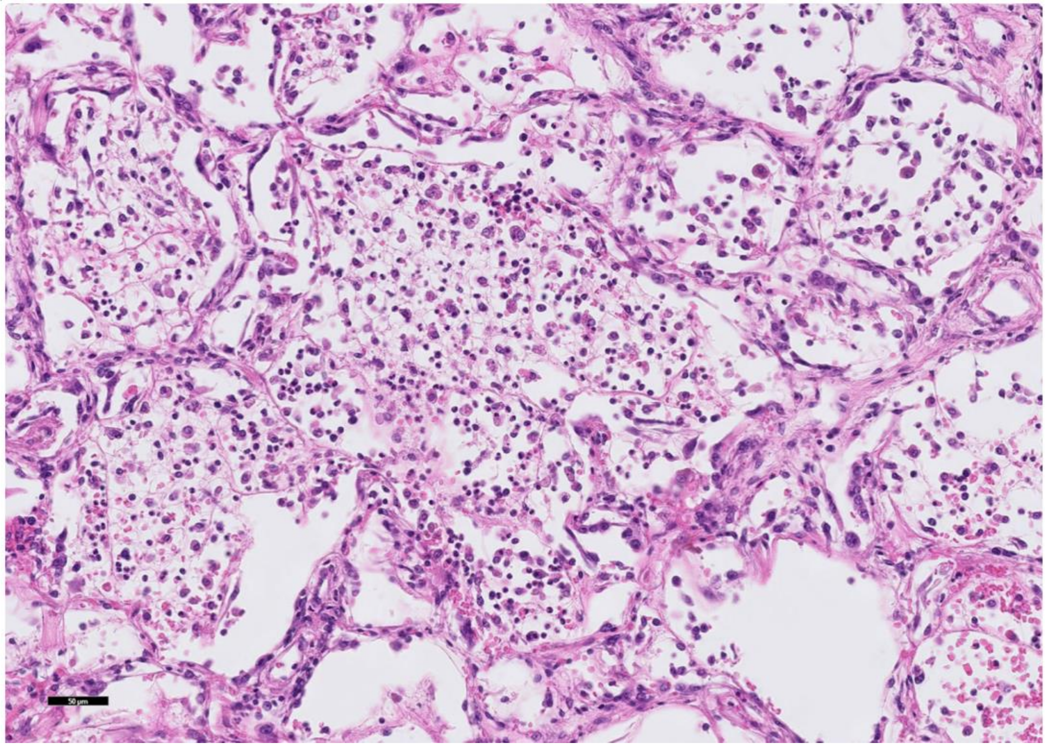






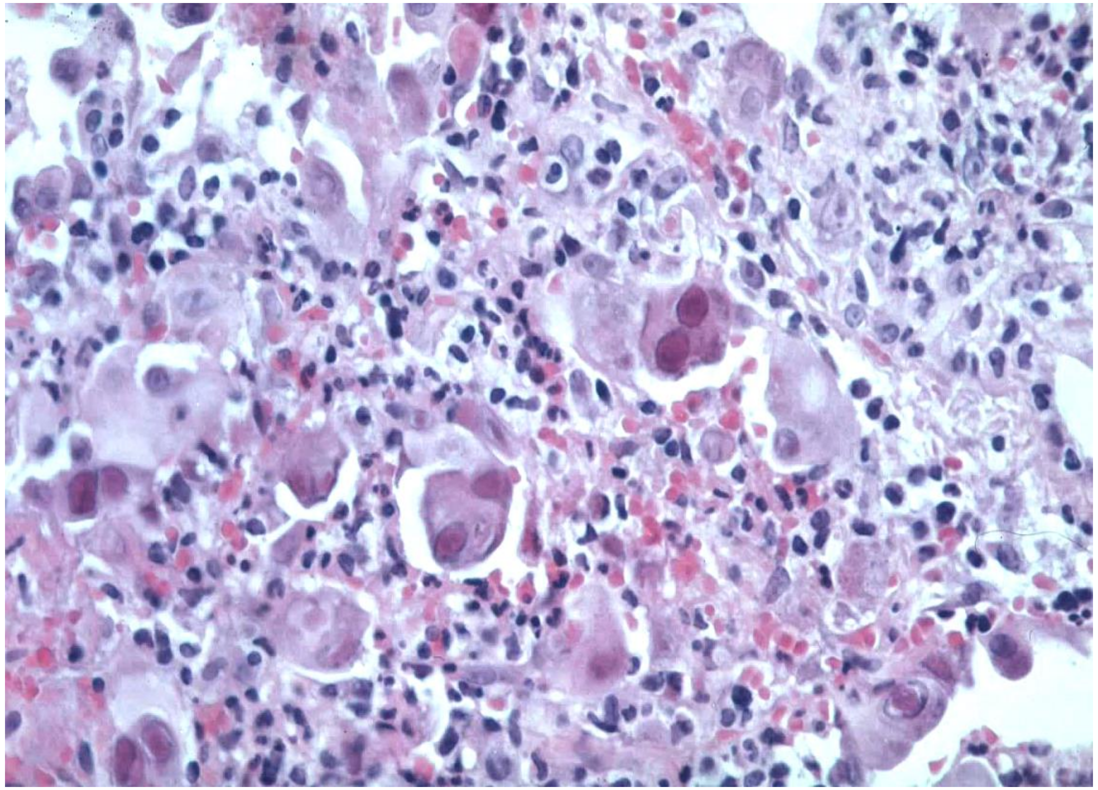
# Bacterial Pneumonia

- Most bacteria are normal inhabitants of the nasopharynx or oropharynx
- Reach alveoli by
  - Aspiration (most)
  - Inhalation
  - Hematogenous seeding
  - Direct spread from adjacent site (rare)
- Pneumococcal pneumonia
  - *Streptococcus pneumoniae*
  - Pulmonary edema, bacterial proliferation, neutrophils



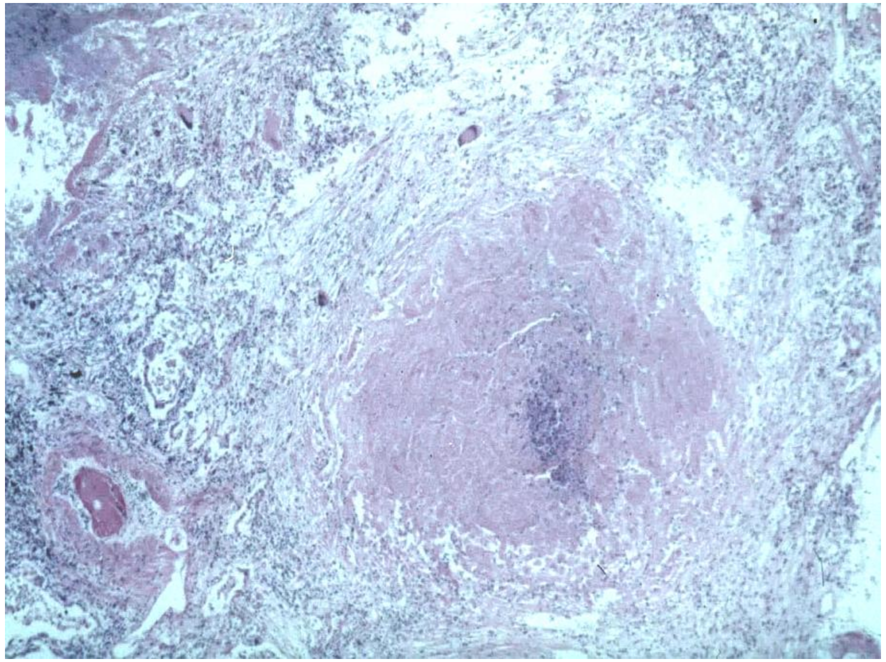
# Viral Pneumonia

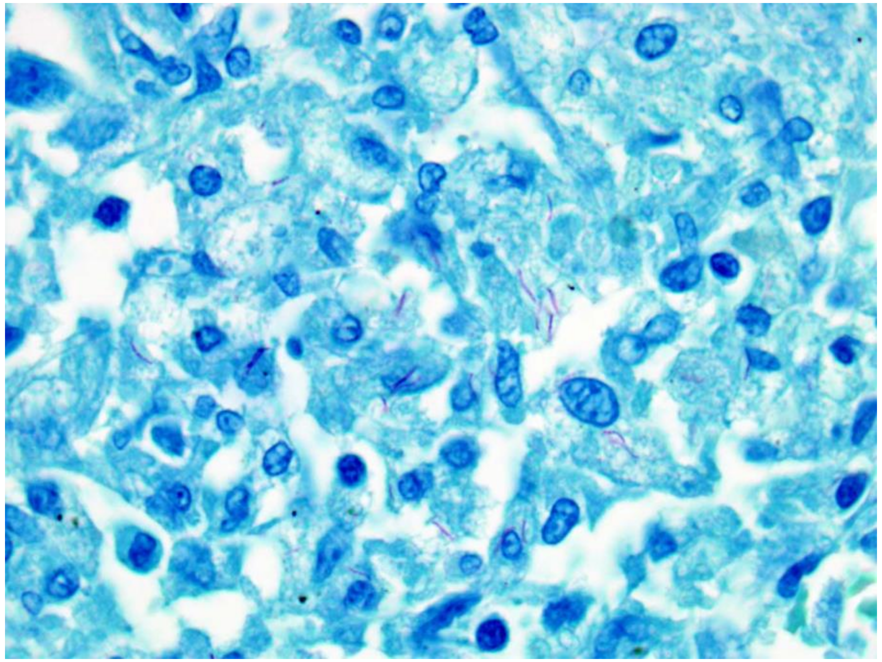
- Immune compromised
  - HIV, organ transplants
- Infants may get CMV
- Lymphocytes in the interstitium
- Distinct cytologic inclusions for some viral infections



# Tuberculosis

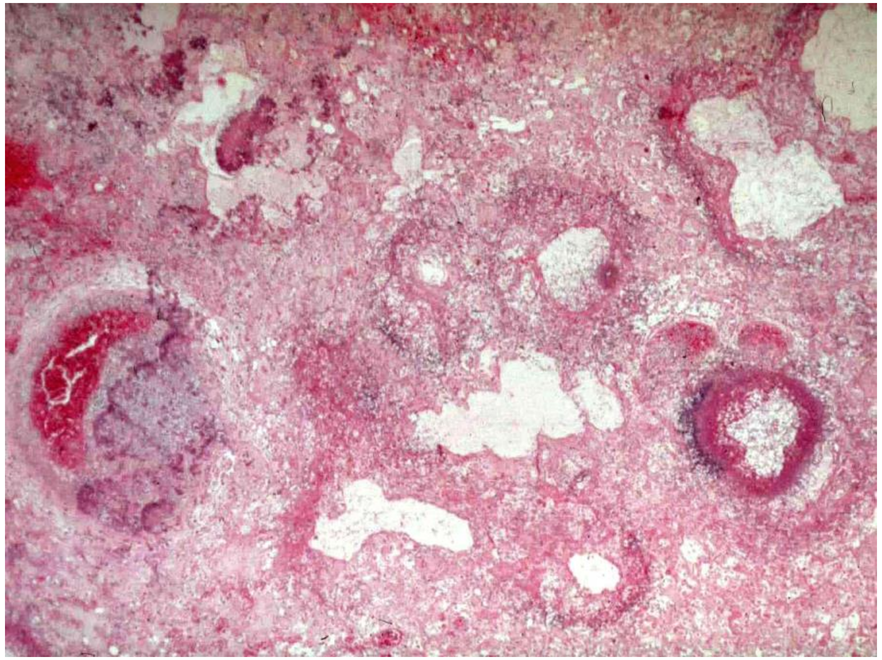
- *Mycobacterium tuberculosis*
- Infection by inhalation of aerosolized droplets containing organisms
- Caseous granulomatous inflammation results
- 90% of primary exposures asymptomatic

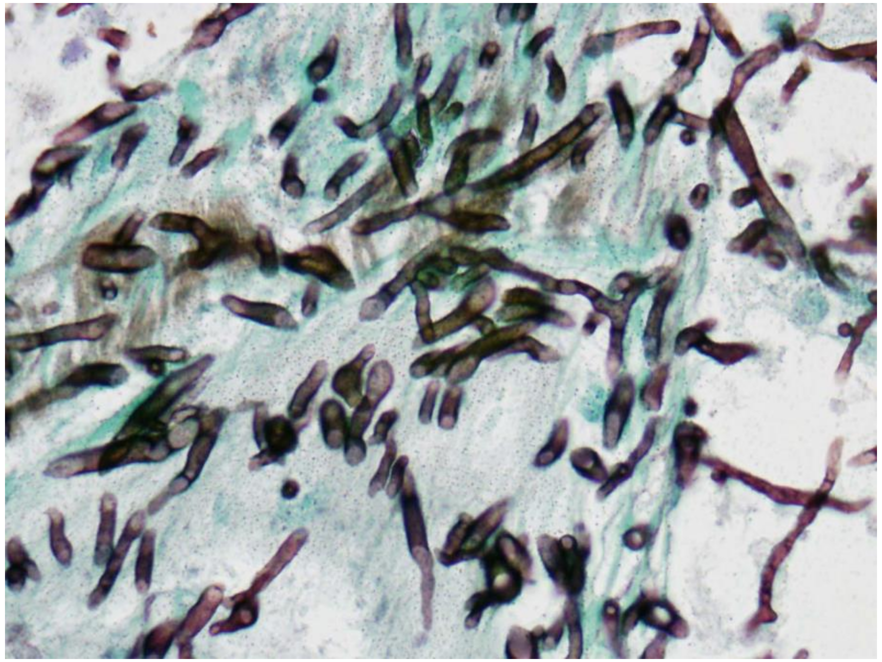




# Fungal Pneumonia

- Fungi are ubiquitous in soil and air
- Most exposure fails to produce infection
  - Body temperature arrests growth
  - Phagocytosis by neutrophils and macrophages
  - Risks: chemo, steroids, T cell deficiencies
- Usually granulomatous inflammation
- Silver stains demonstrate organisms



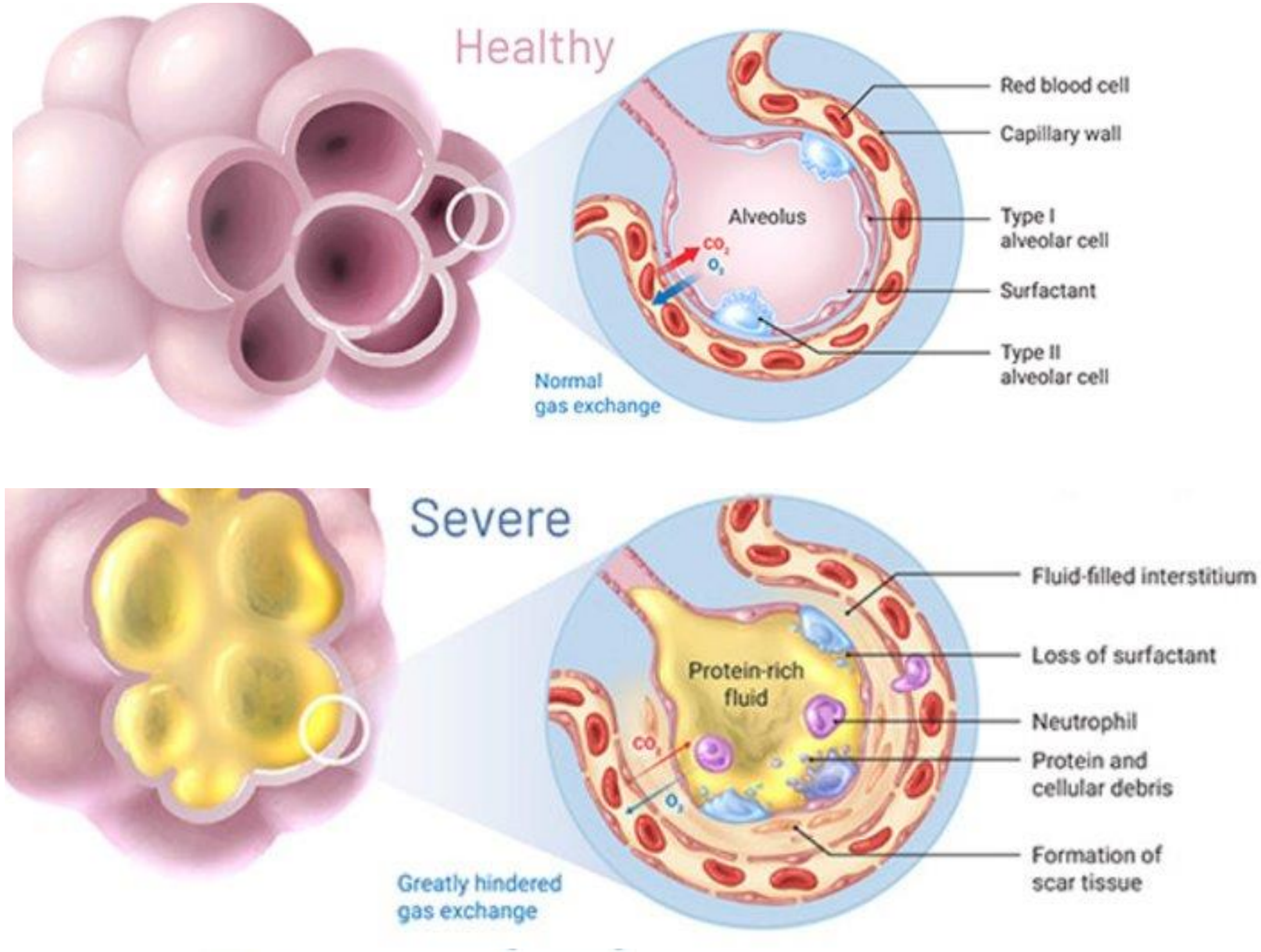


# What Are We Going to Cover Today?

- Normal lung anatomy and histology
- Evaluating lung function
- Pathological changes to the lung
- **Using animal models to study respiratory disease**
- Vaccine development

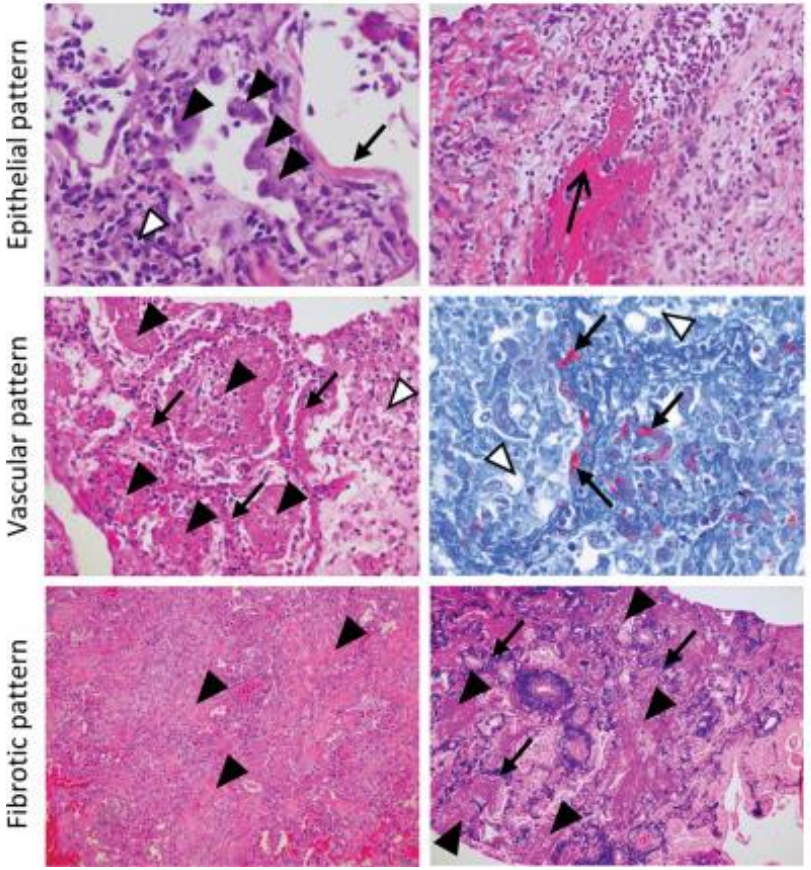
# COVID-19 in Humans

- **Flu-like illness:** fever, chills, fatigue, cough, congestion, shortness of breath, sore throat, body aches, headache, diarrhea
- **Loss of taste or smell**
- **Severe disease manifestations:** pneumonia, respiratory failure, sepsis, multi-organ failure, cardiomyopathy, acute kidney injury, stroke

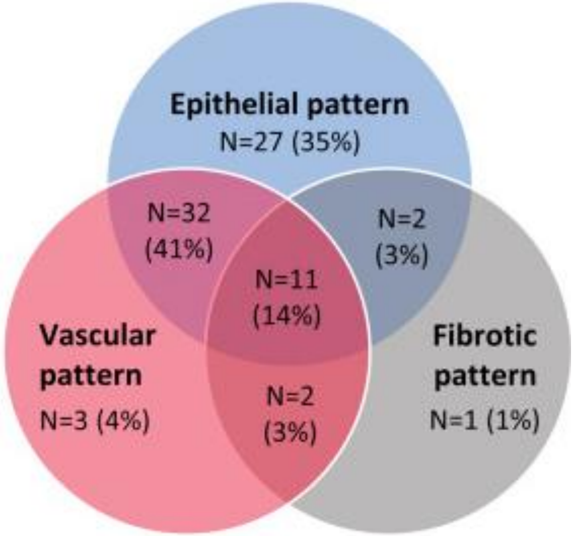


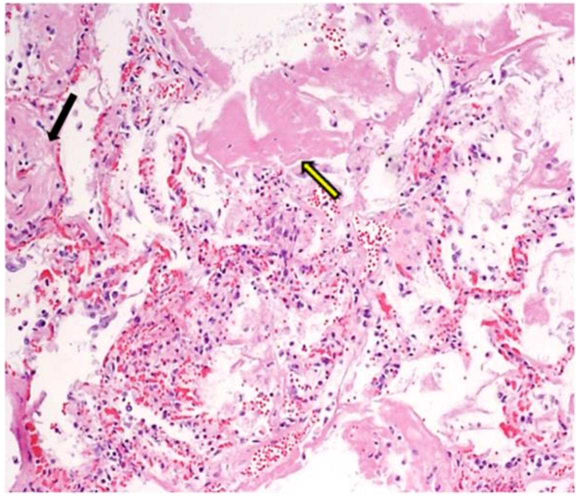

Avesta Rastan (www.azuravesta.com)

# Lung Pathology in COVID-19 Patients




Polak et al, *Mod Pathol*, 2020

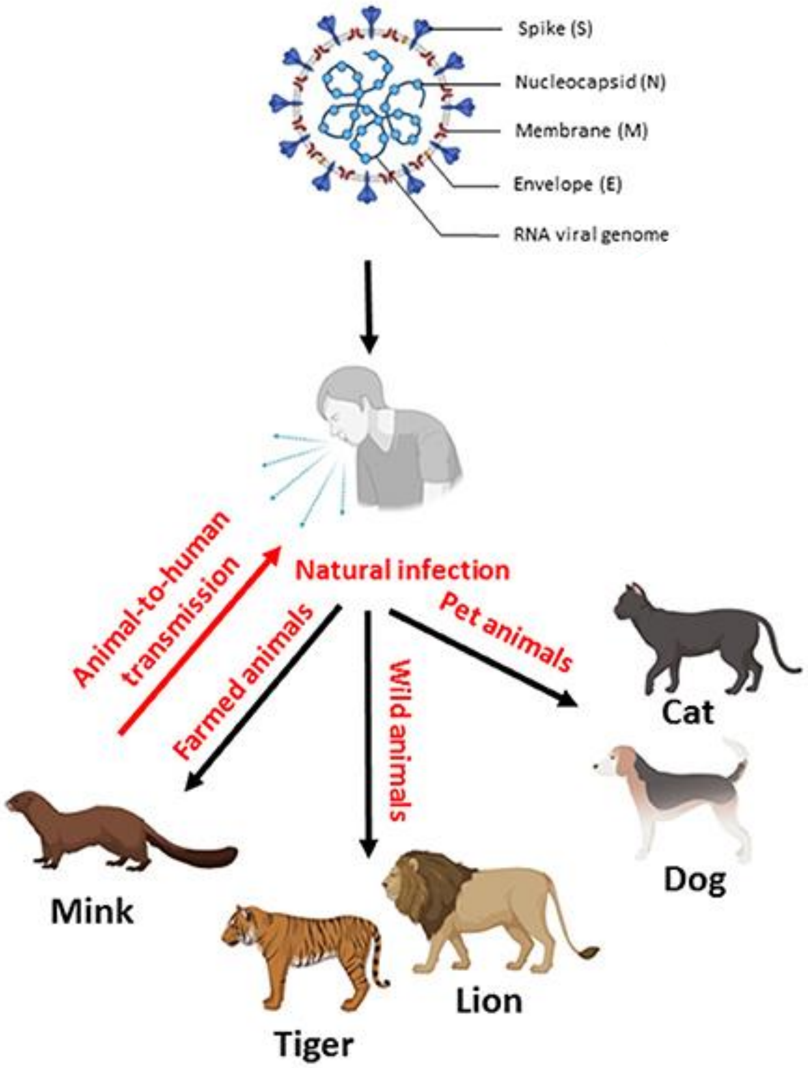


Scendoni et al, *Diag Pathol*, 2020

- Atypia and detachment of type II pneumocytes
- Hyaline membranes
- Interstitial inflammation
- Epithelial denudation
- Hyaline thrombi
- Fibrinous pneumonia
- Edema
- Intra-alveolar fibroelastosis

# SARS-CoV-2 Host Range



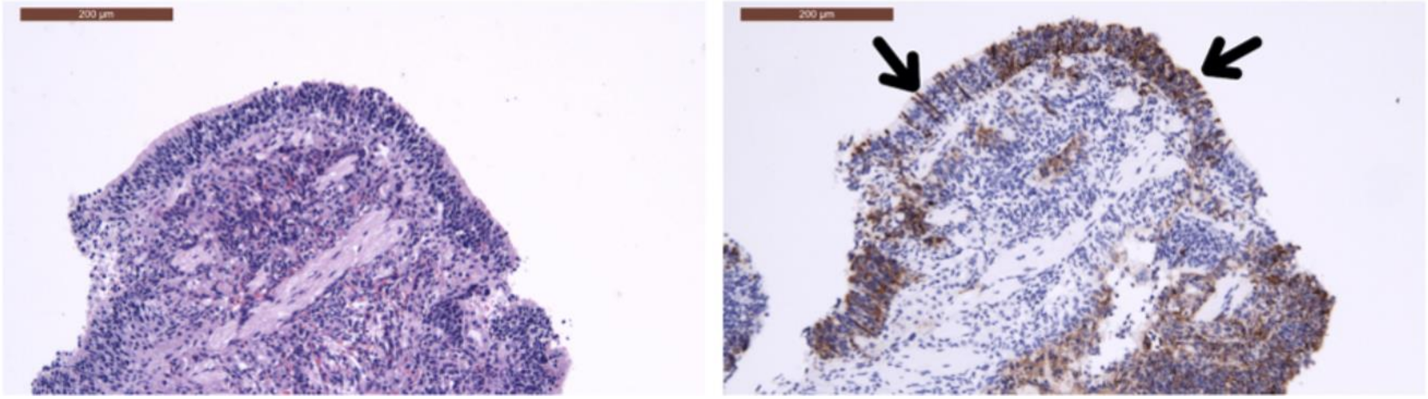

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Mahdy, *Front Vet Sci*, 2020

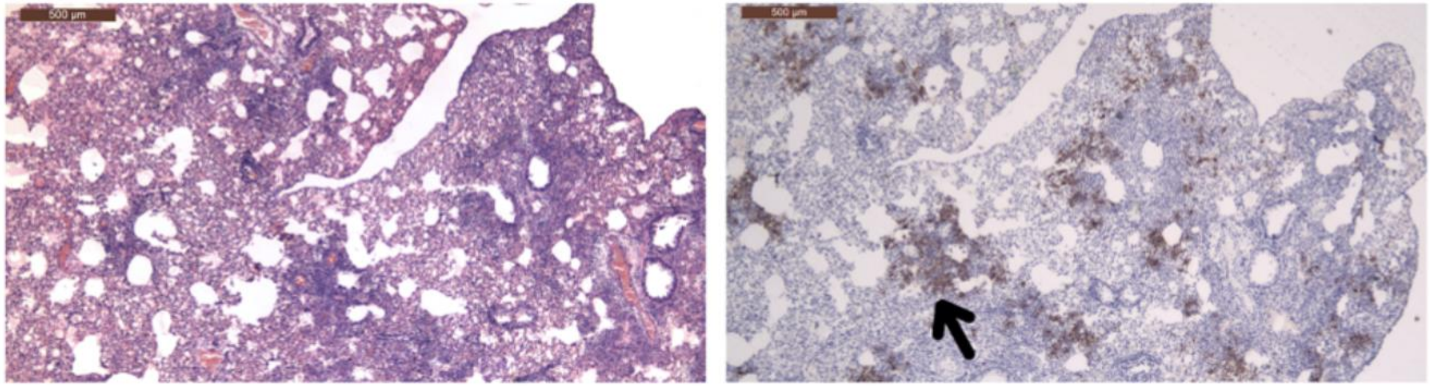
# Hamsters as an Animal Model for SARS-CoV-2 Infection

- **Clinical Signs:**
  - Fever
  - Dyspnea
  - Weight loss
- **Tissue Tropism:**
  - Upper and lower airways
  - Nasal and bronchiolar epithelium
- **Potential Applications:**
  - Pathogenesis
  - Transmission dynamics
  - Vaccines and therapeutics

**Nasal Epithelium – 2 DPI**



**Lung Parenchyma – 5 DPI**



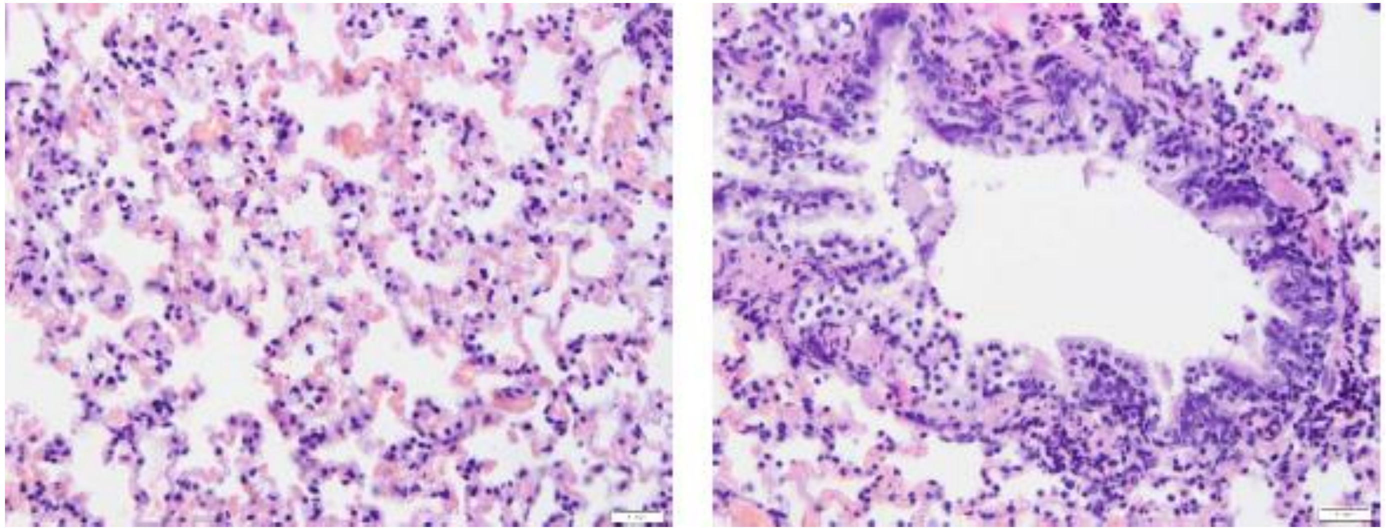
Sia et al, *Nature,* 2020

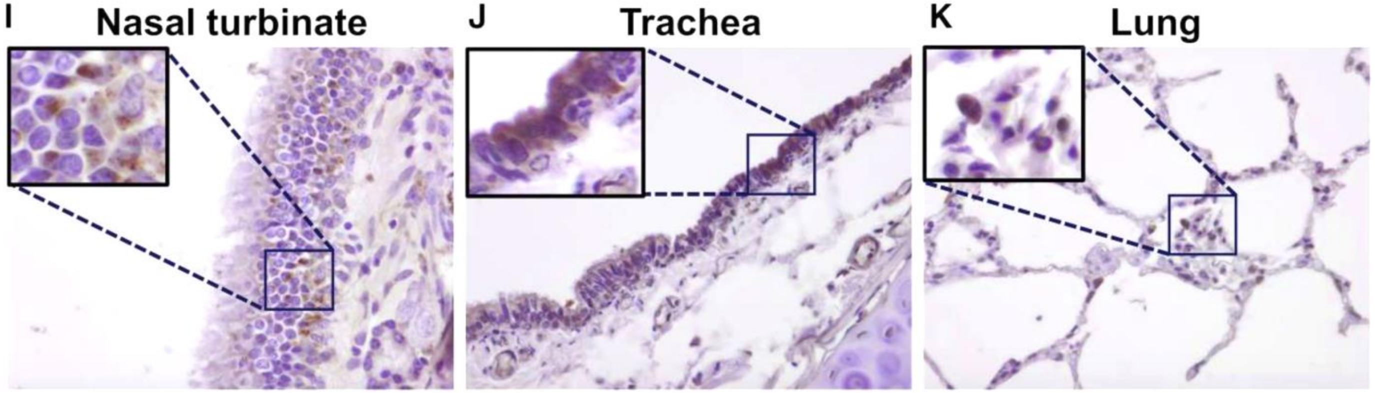
THE UNIVERSITY
*of* NORTH CAROLINA
*at* CHAPEL HILL

# Ferrets as an Animal Model for SARS-CoV-2 Infection

- **Clinical Signs:**
  - Fever
  - Appetite loss
- **Tissue Tropism:**
  - Upper respiratory tract
  - GI tract
- **Potential Applications:**
  - Vaccines and anti-virals
  - Transmission dynamics

4 DPI

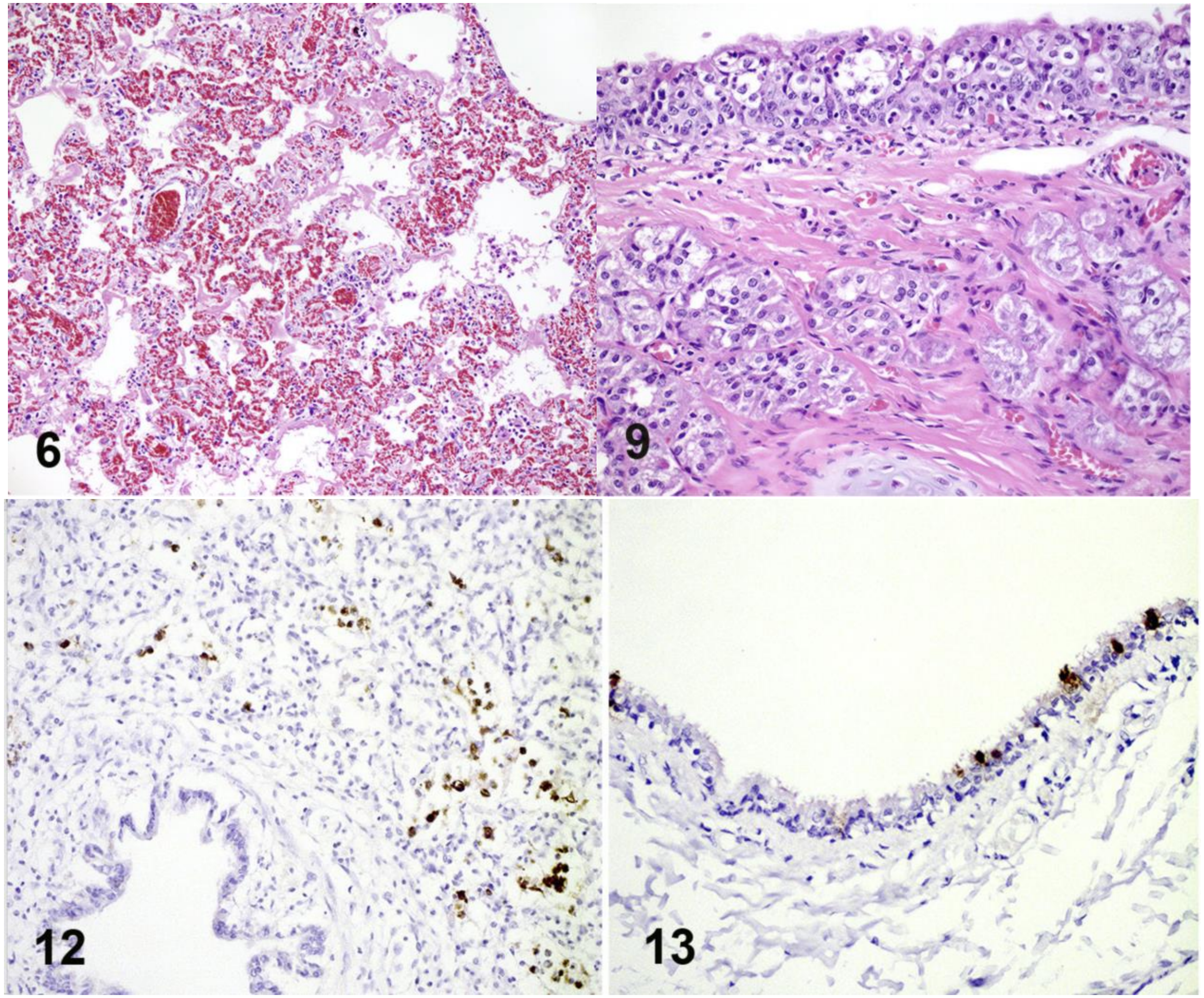





THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Kim et al, *Cell Host Microbe*, 2020

# SARS-CoV-2 Infection of Mink

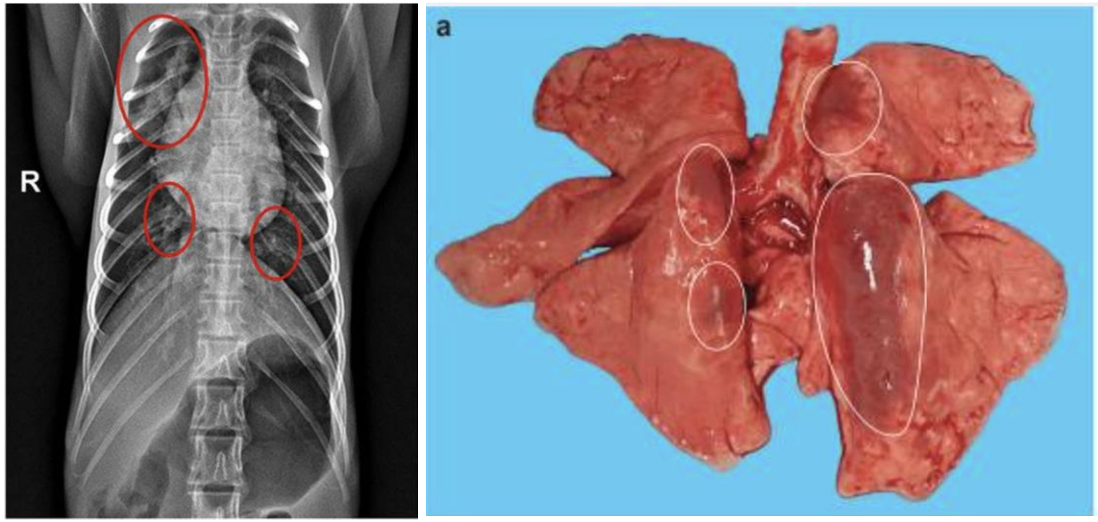


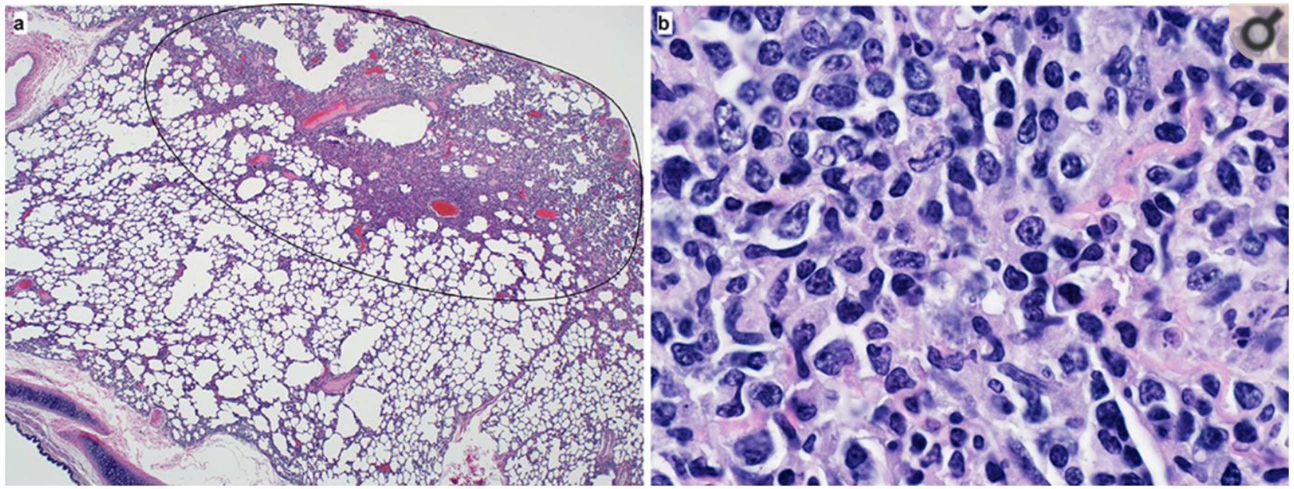

Molenaar et al, *Vet Pathol*, 2020

# Macaques as an Animal Model for SARS-CoV-2 Infection

- **Clinical Signs:**
  - Changes to respiratory pattern
  - Fever
  - Weight loss
- **Tissue Tropism:**
  - Lung
  - Lymphoid Tissue
  - GI Tract
- **Potential Applications:**
  - Pathogenesis
  - Vaccines and therapeutics
  - Chronic effects

3 DPI



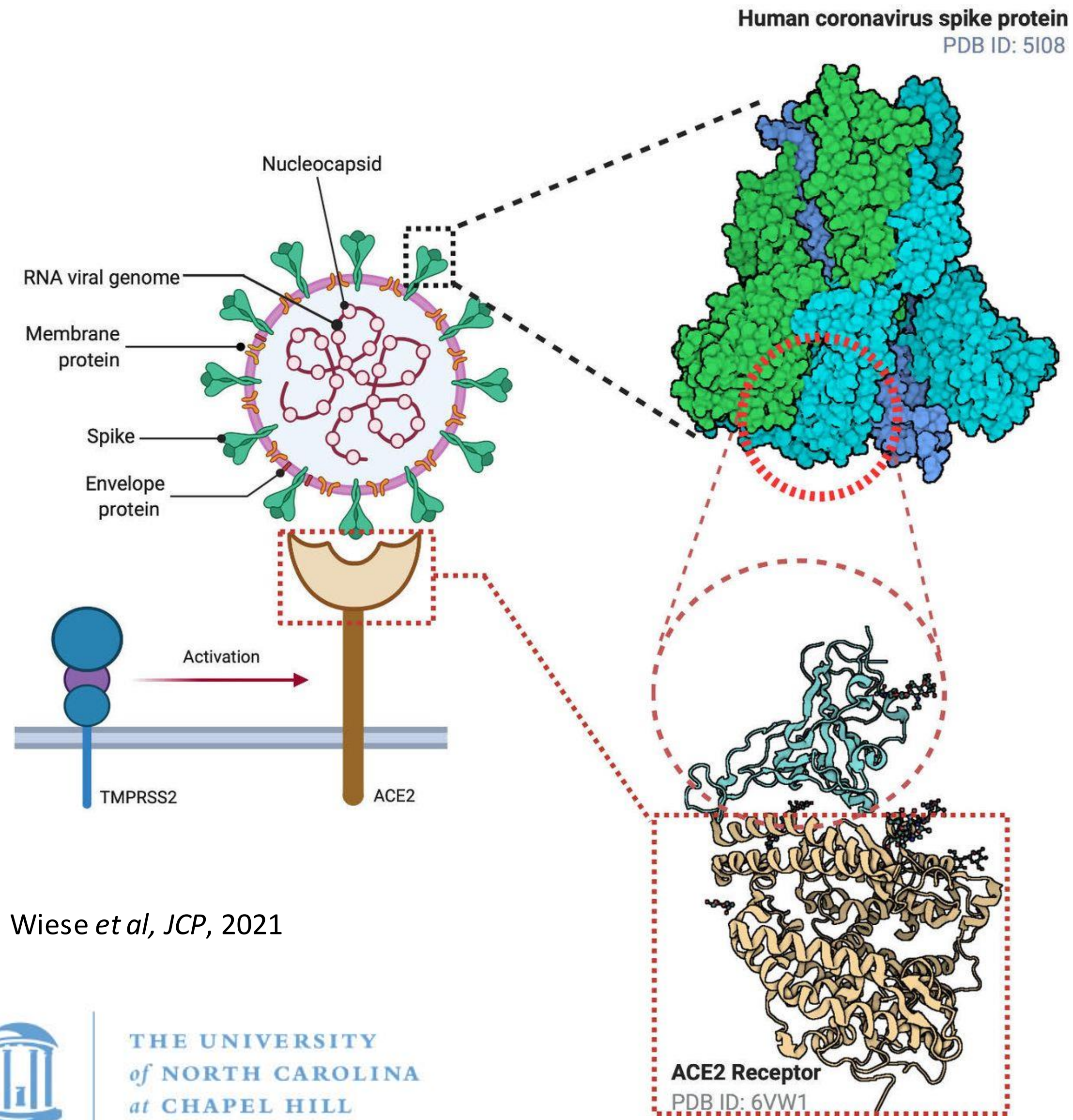




Munster et al, *Nature*, 2020

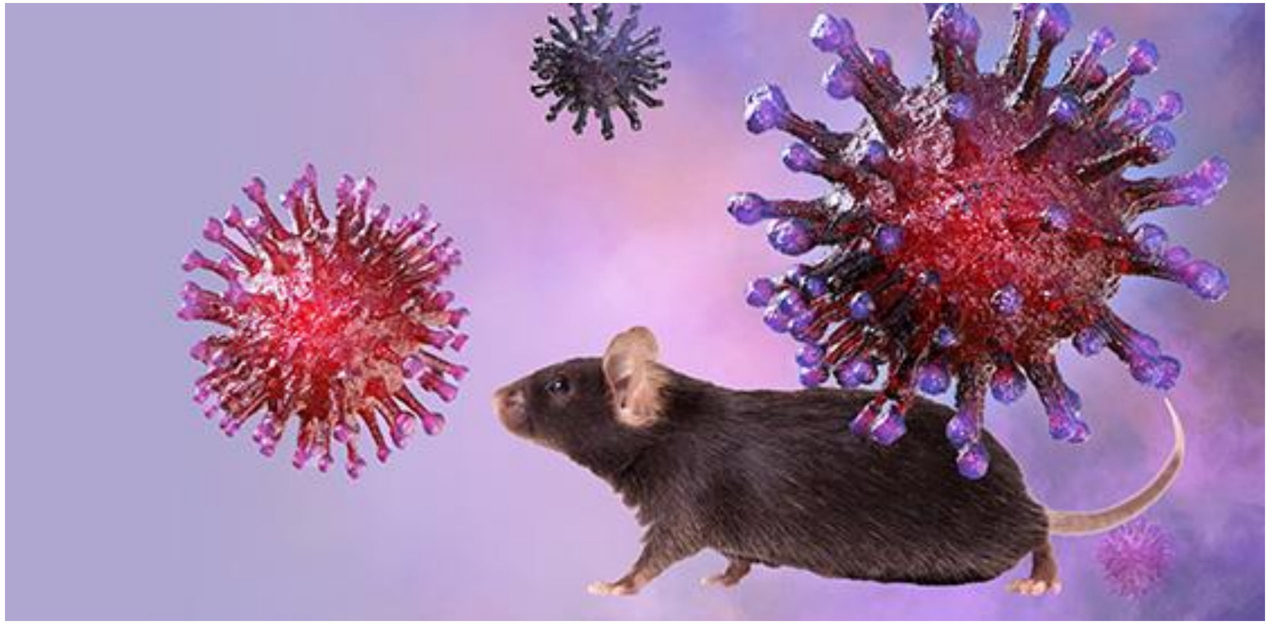
# Advantages to Using Mice

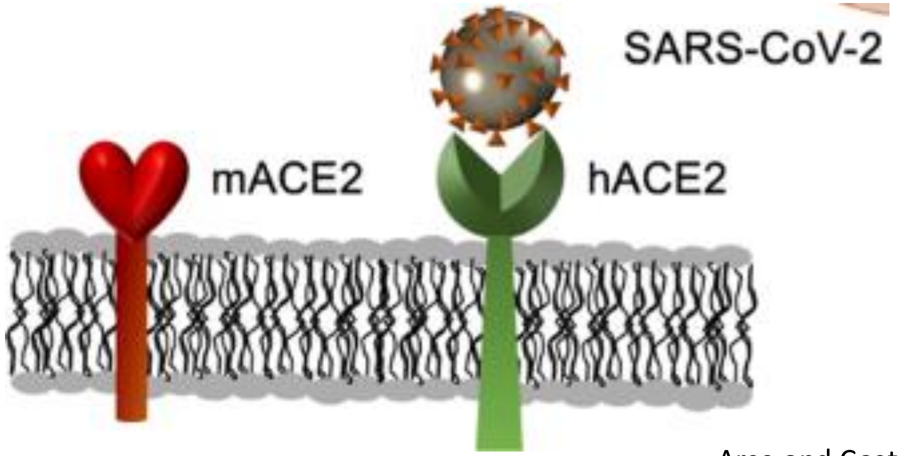
- Similar anatomy, physiology, and genetics to humans
- Relatively cost effective
- Genetically modifiable
- Small and easy to house
- Short gestation time and lifespan
- Quick maturation
- Good array of reagents for research

# Barriers to Using Mice to Study COVID-19




Wiese *et al*, *JCP*, 2021





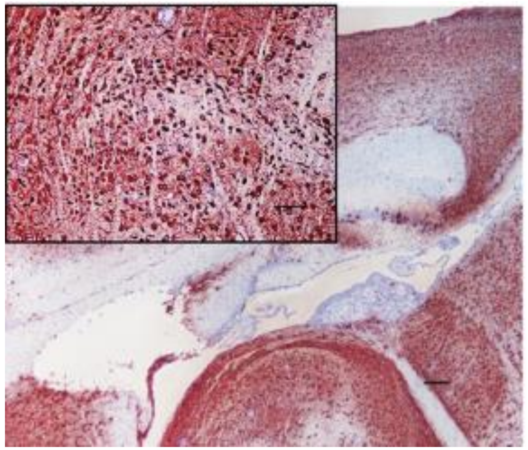

Arce and Costoya, *C&MI*, 2021

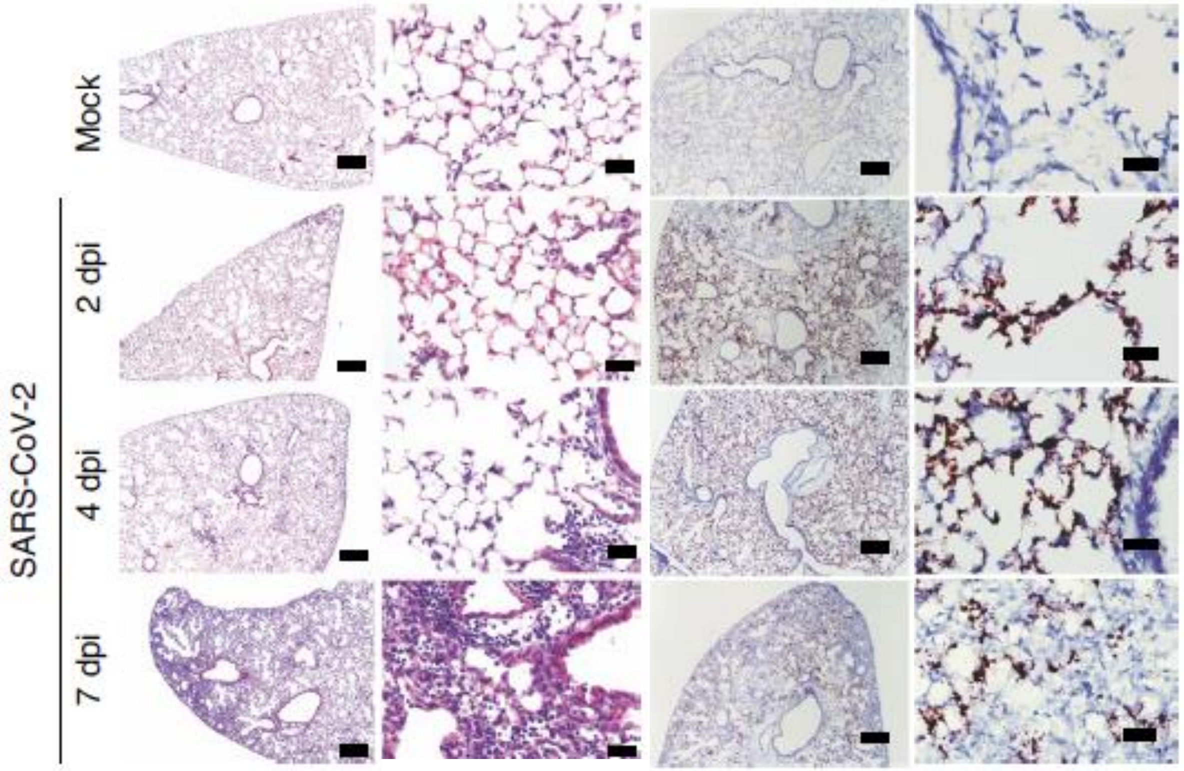
# Mouse Model Approaches

- Transgenic Mice
- Viral Vector-Mediated Sensitization
- Humanized Mice
- Mouse-Adapted SARS-CoV-2 Strains

# K18-hACE2 Mice

- Transgenic mice with human ACE2 constitutively expressed in epithelial cells
- Can use human clinical SARS-CoV-2 isolates
- **Clinical Signs:**
  - Weight loss
  - Hunched posture
  - Mortality
- **Tissue Tropism:**
  - Lung
  - Brain
  - Systemic replication
- **Potential Applications:**
  - Pathogenesis
  - Vaccines and therapeutics

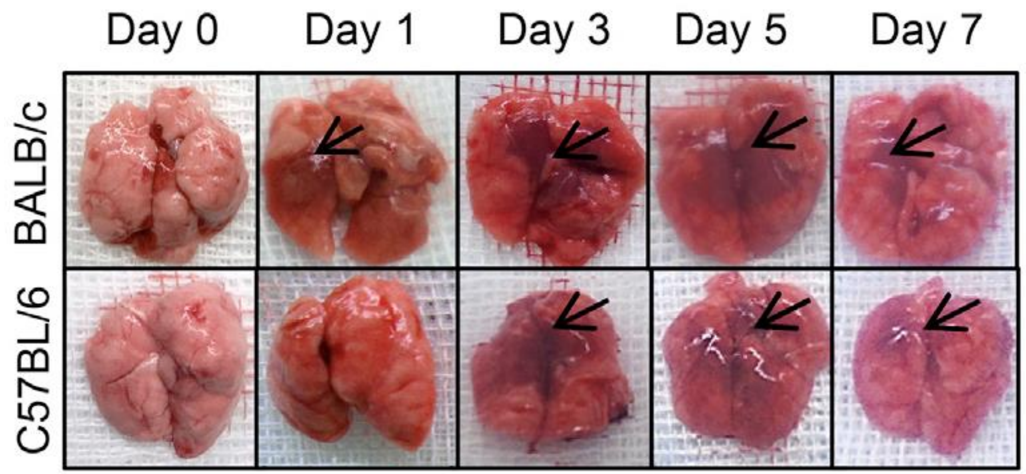


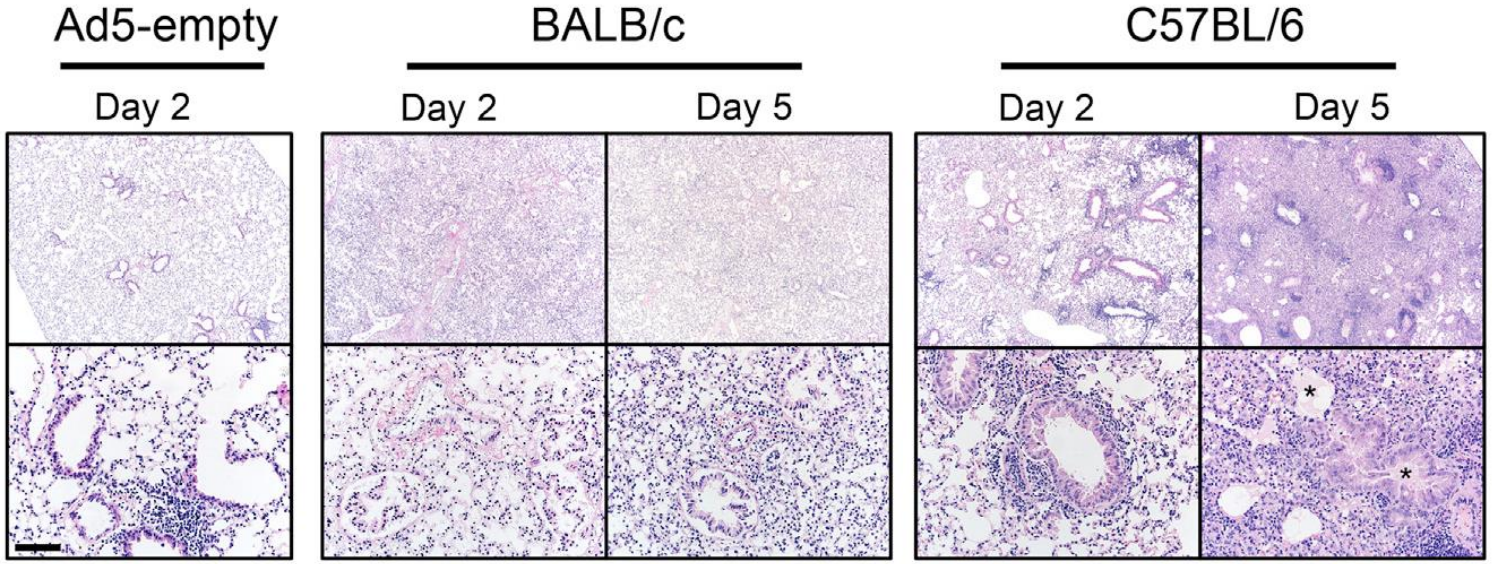



Winkler, et al, *Nature Immnol*, 2020

# Ad5-hACE2 Mice

- Exogenous delivery of human ACE2 with a replication-deficient adenovirus under control of a CMV promoter
- Can use human clinical SARS-CoV-2 isolates
- **Clinical Signs:**
  - Weight loss
  - Hunched posture
- **Tissue Tropism:**
  - Lung
- **Potential Applications:**
  - Pathogenesis
  - Vaccines and therapeutics

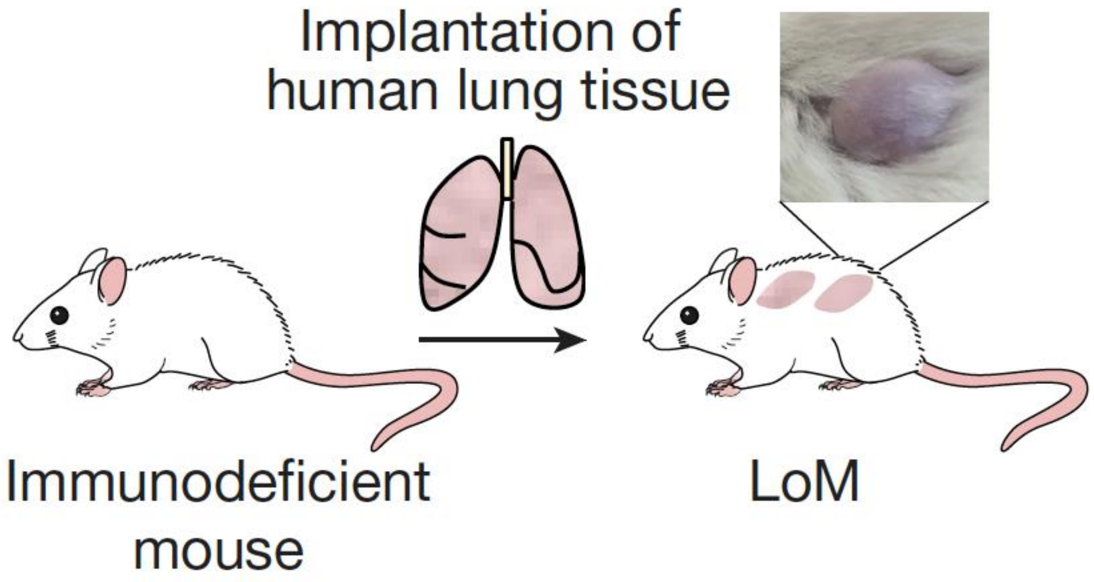


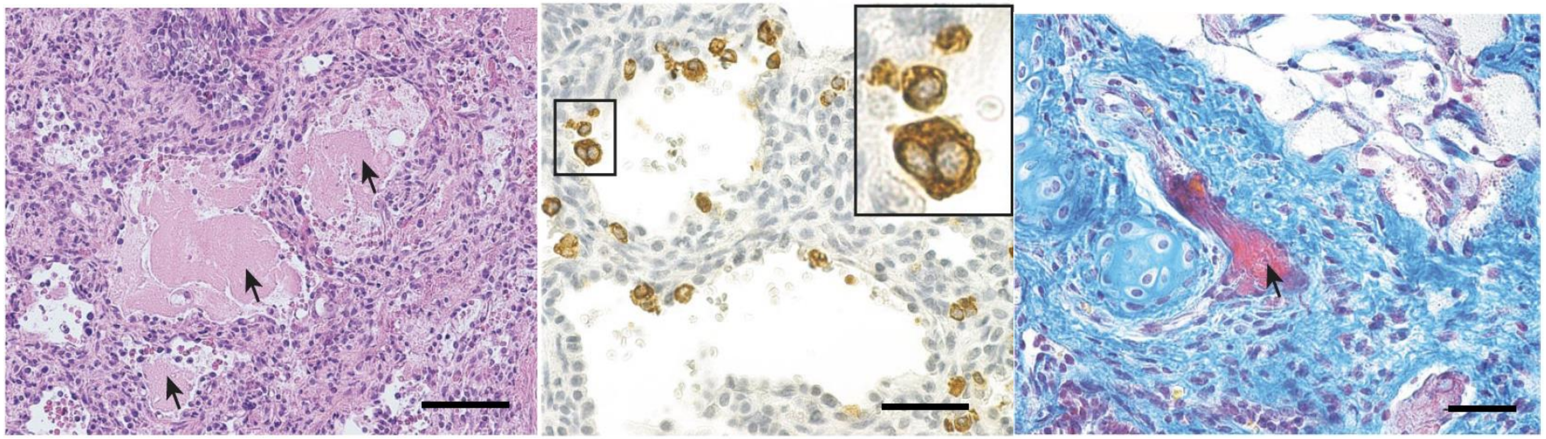




Sun, et al, *Cell*, 2020

# Humanized Mice

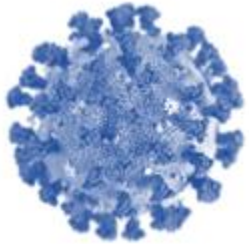
- Subcutaneous implantation of human lung tissue into the backs of immunodeficient mice
- Can use human clinical SARS-CoV-2 isolates
- **Clinical Signs:**
  - None?
- **Tissue Tropism:**
  - Human Lung Implant
- **Potential Applications:**
  - Viral Replication Kinetics
  - Lung Pathology
  - Human Immune Response
  - Prophylactics and Therapeutics



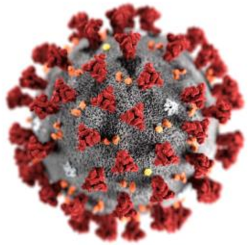



Wahl and Gralinski, et al, *Nature*, 2021

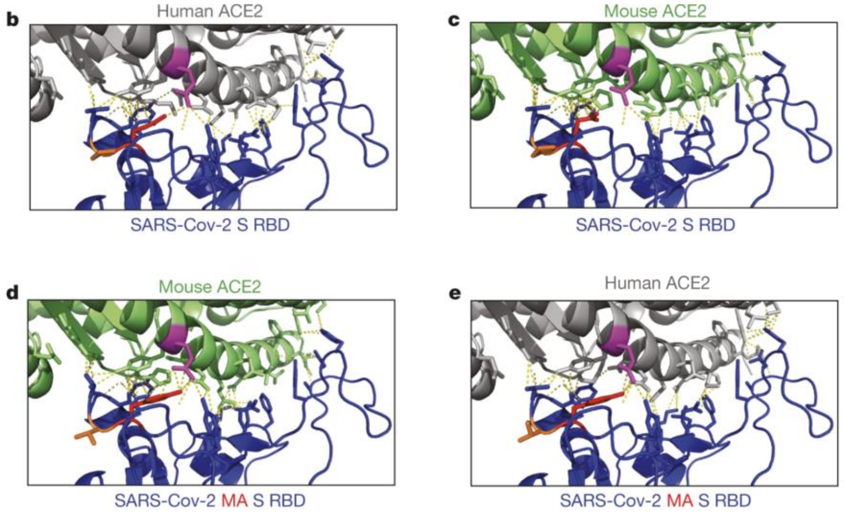
# Mouse Adaptation of SARS-CoV-2

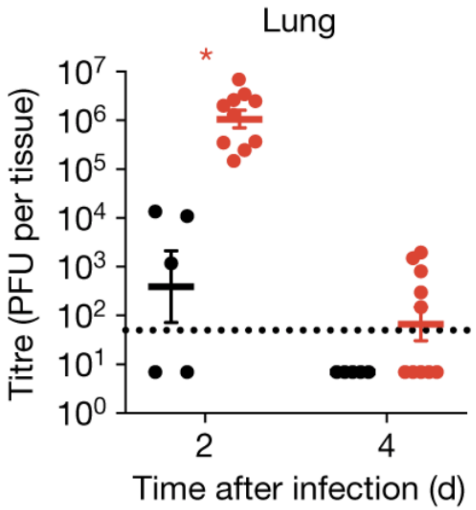


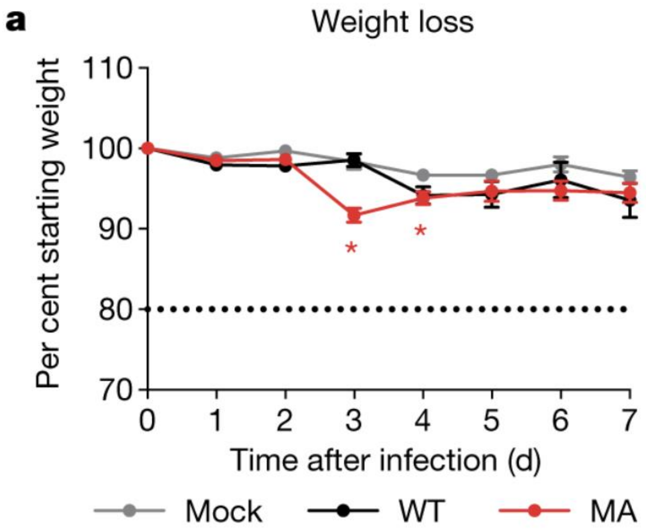
**Original SARS-CoV-2**



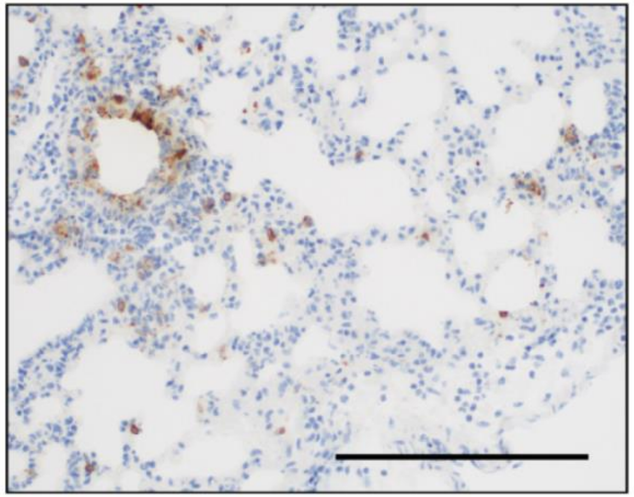
**Q498Y/P499T SARS-CoV-2-MA**



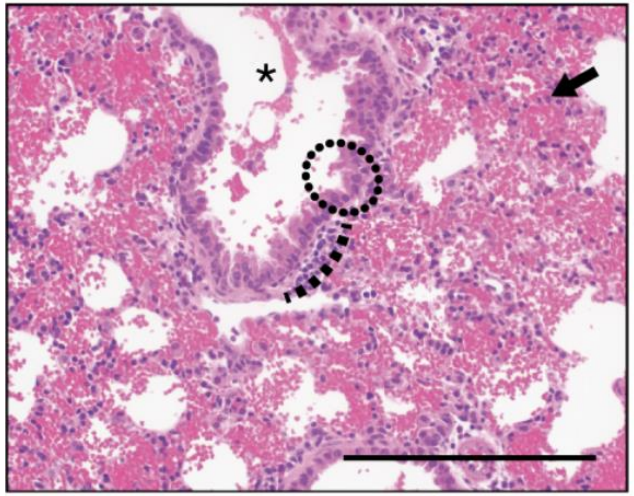



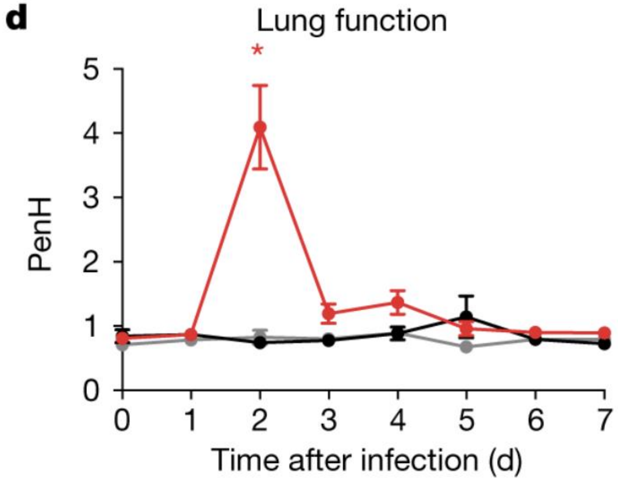




**2 DPI**



**4 DPI**

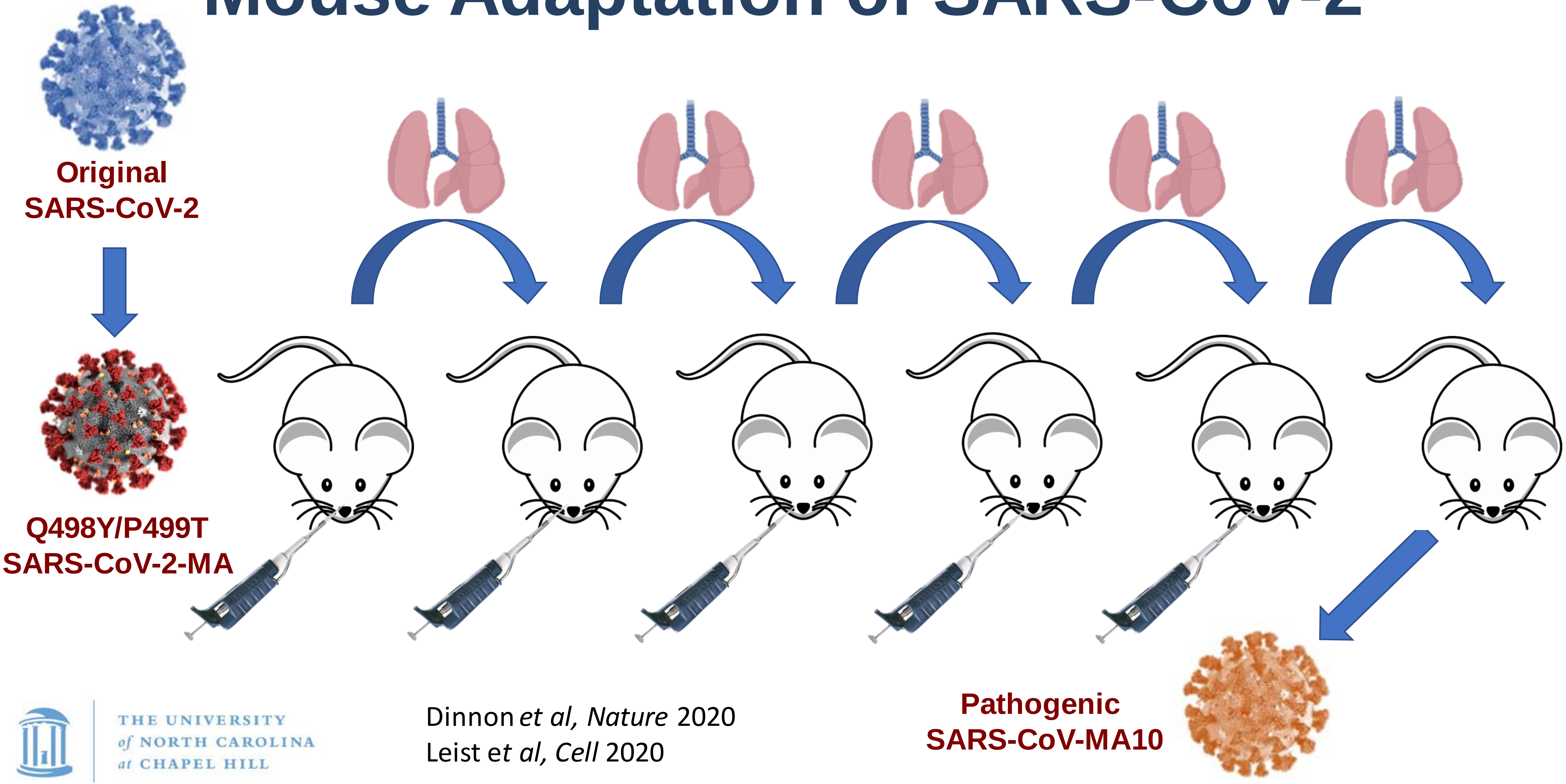





THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Dinnon *et al, Nature* 2020

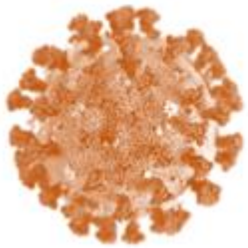
# Mouse Adaptation of SARS-CoV-2




Dinnon *et al*, *Nature* 2020
Leist *et al*, *Cell* 2020

THE UNIVERSITY
*of* NORTH CAROLINA
*at* CHAPEL HILL

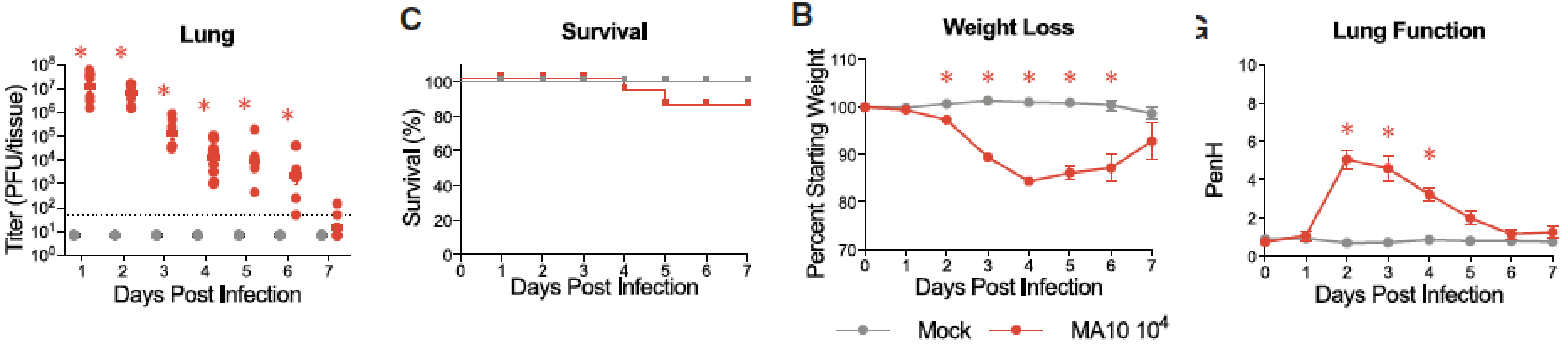
# Mouse Adaptation of SARS-CoV-2

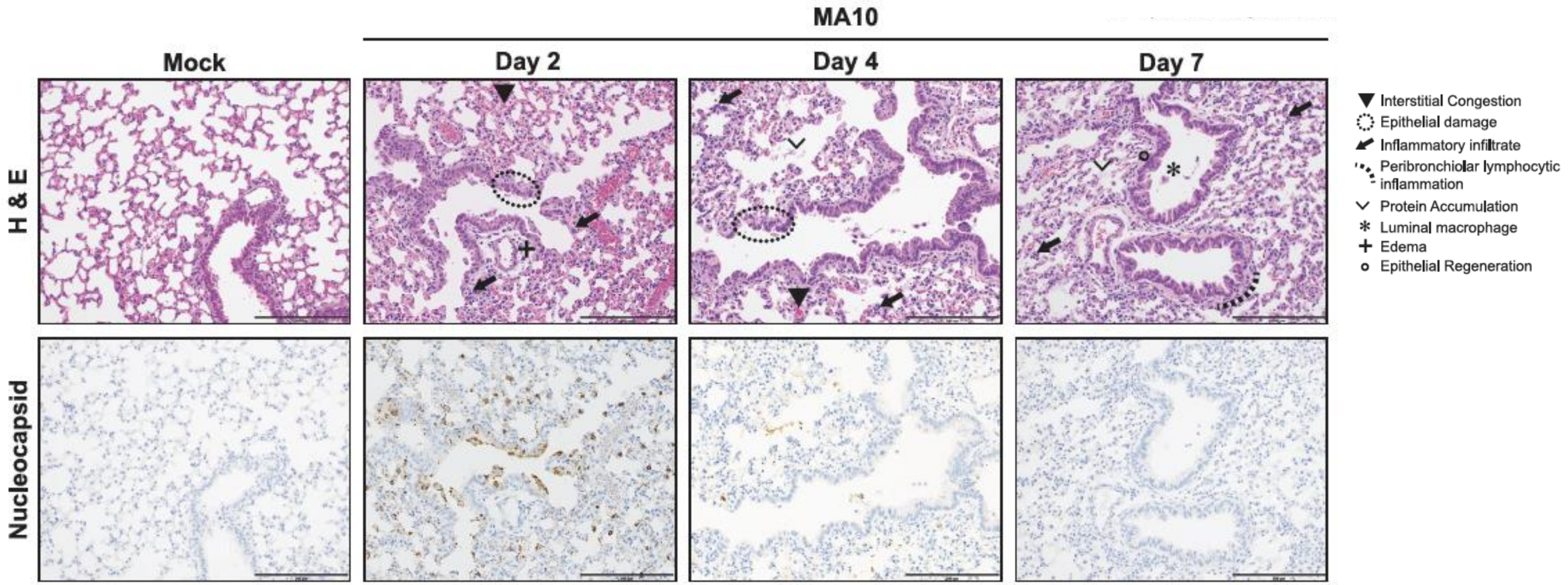


**Pathogenic SARS-CoV-MA10**

| Mutation | Gene | Coding Change |
|---|---|---|
| C9438U | nsp4 | T285I |
| A11847G | nsp7 | K2R |
| A12159G | nsp8 | E23G |
| C23039A | Spike | Q493K |
| U27221C | ORF6 | F7S |

Leist *et al*, *Cell* 2020



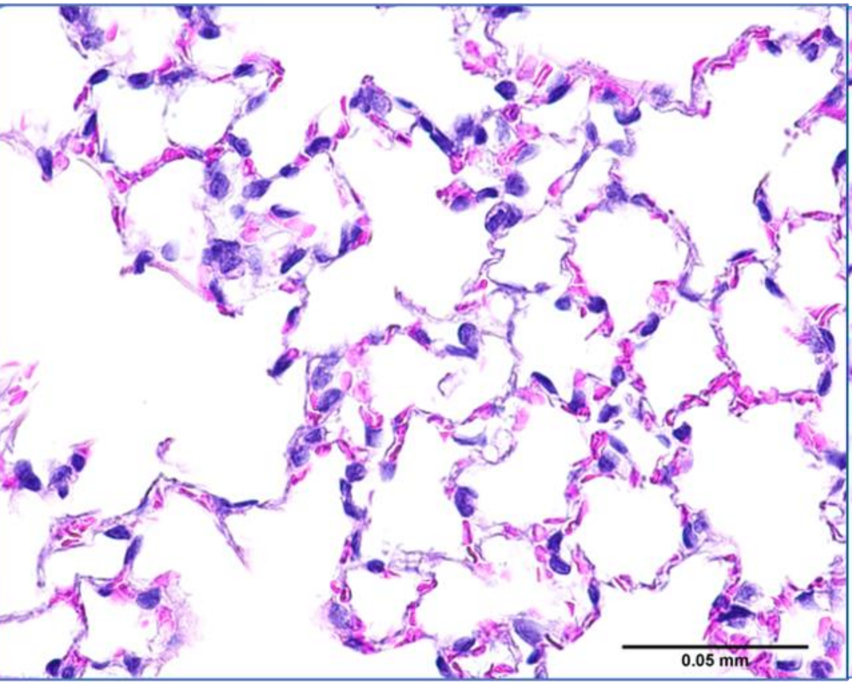

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

# Lung Pathology Scoring

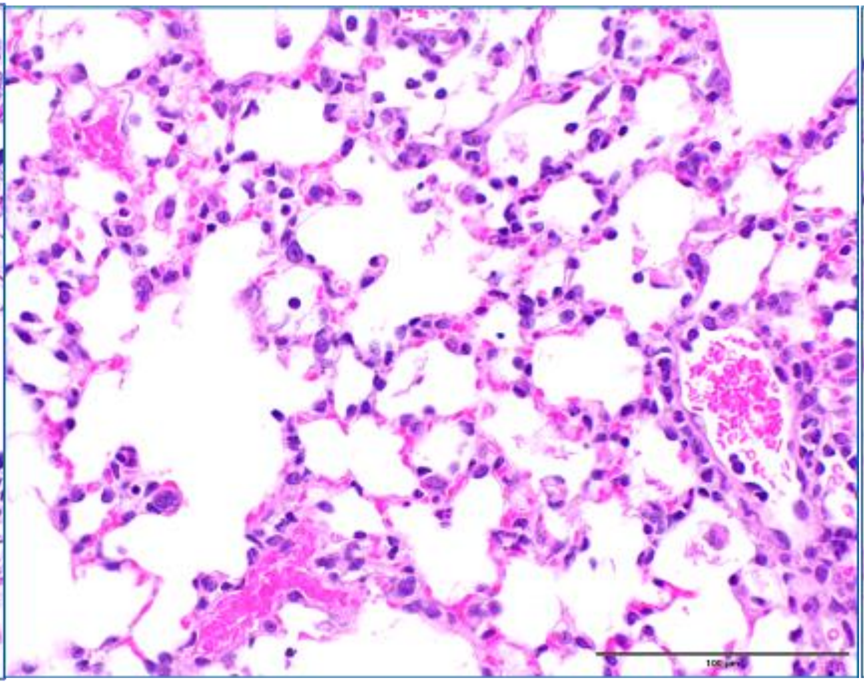
| ATS Acute Lung Injury Score (2011 Matute-Bello et al.) | | | | | |
|---|---|---|---|---|---|
| | Neutrophils in the alveolar space | Neutrophils in the interstitial space | Hyaline membranes | Proteinaceous debris filling the airspaces | Alveolar septal thickening relative to mock |
| **0** | None | None | None | None | < 2X |
| **1** | 1-5 | 1-5 | 1 | 1 | 2X - 4X |
| **2** | >5 | >5 | >1 | >1 | >4X |

| M.E. Schmidt DAD Score (Schmidt et al. 2018) | |
|---|---|
| **1** | Absence of cellular sloughing and necrosis |
| **2** | Uncommon solitary cell sloughing and necrosis (1-2 spots) |
| **3** | Multifocal cellular (3+) sloughing with uncommon septal wall hyalinization |
| **4** | Multifocal cellular sloughing (>75% field) with common and/or prominent hyaline membranes |

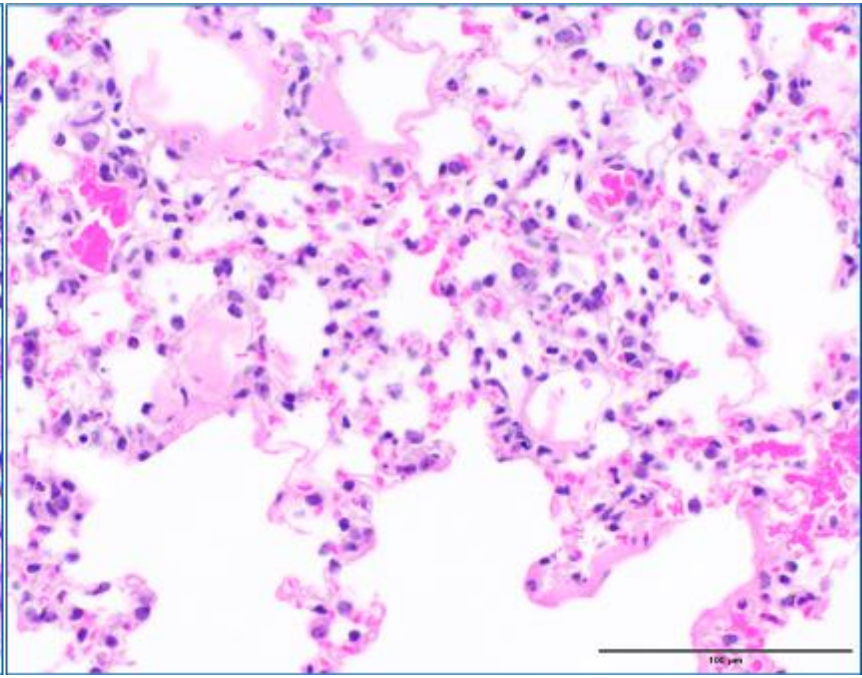
## Mock-Infected




## Moderate Lung Injury

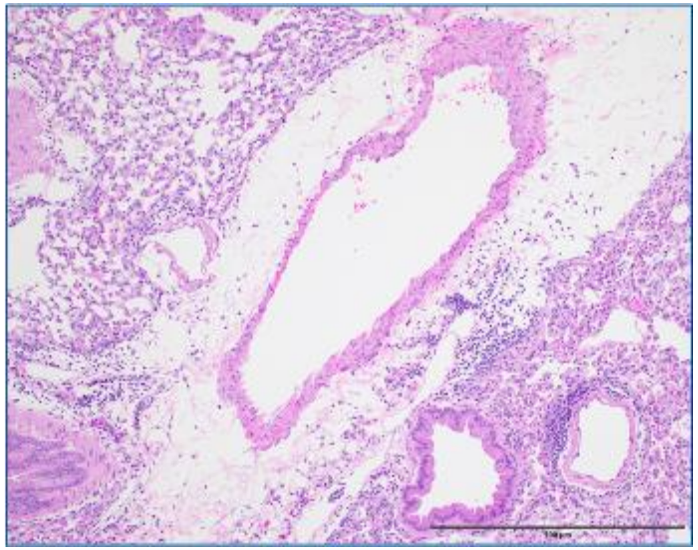


## Severe Lung Injury

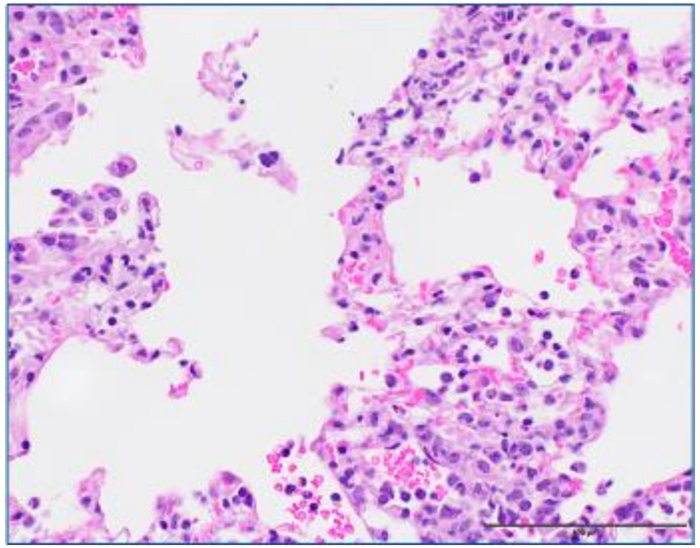


# Other Pathological Changes

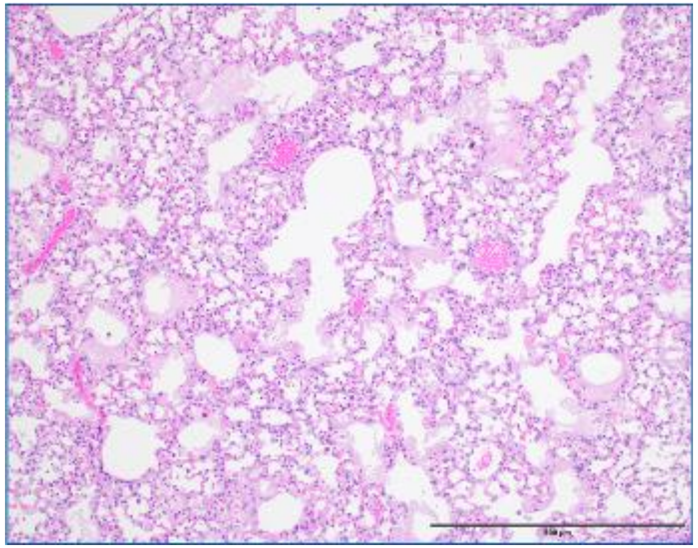
**Perivascular edema**



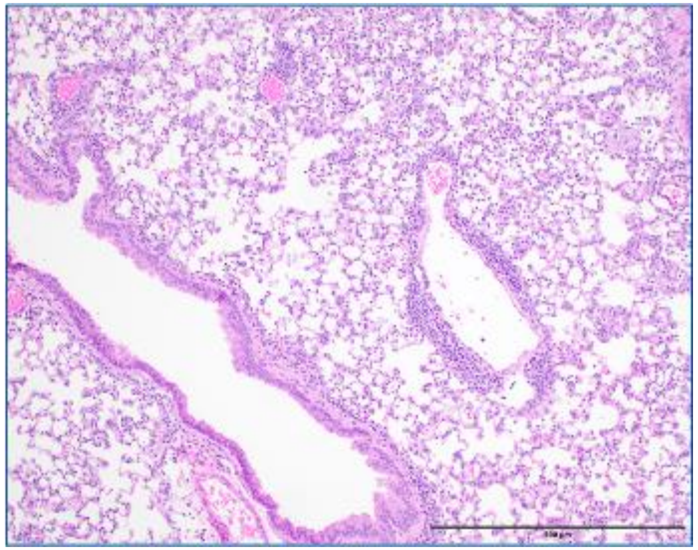
**Loss of interstitial architecture**



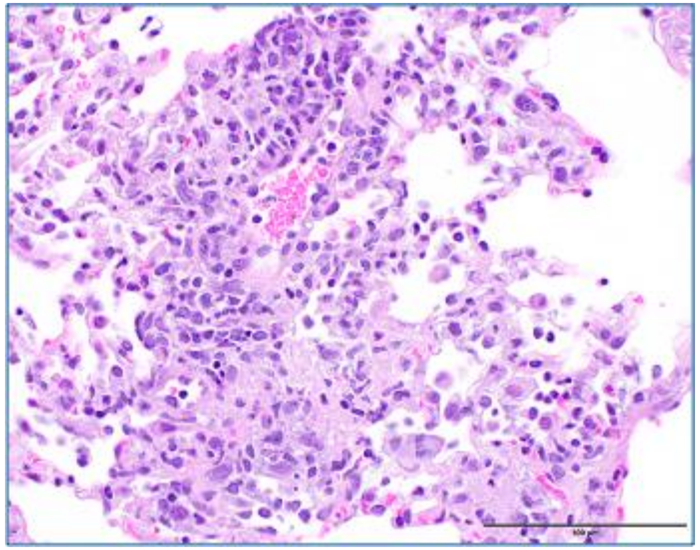
**Extensive hyaline membranes**



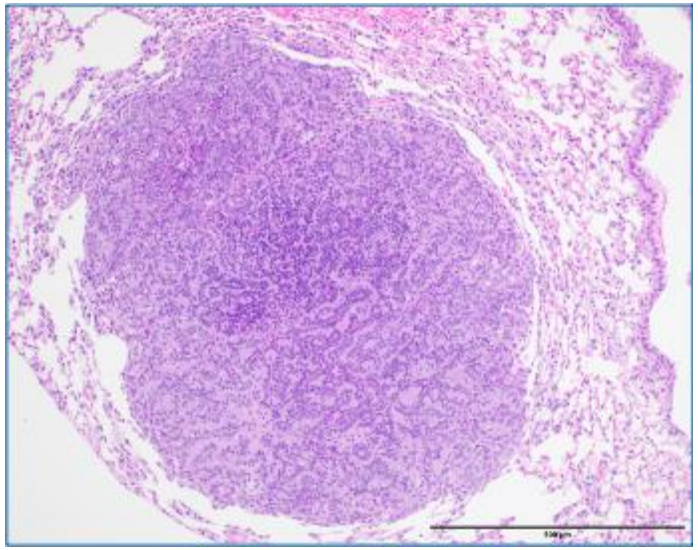
**Perivascular cuffs**



**Early regenerative changes**



**Pulmonary adenoma**

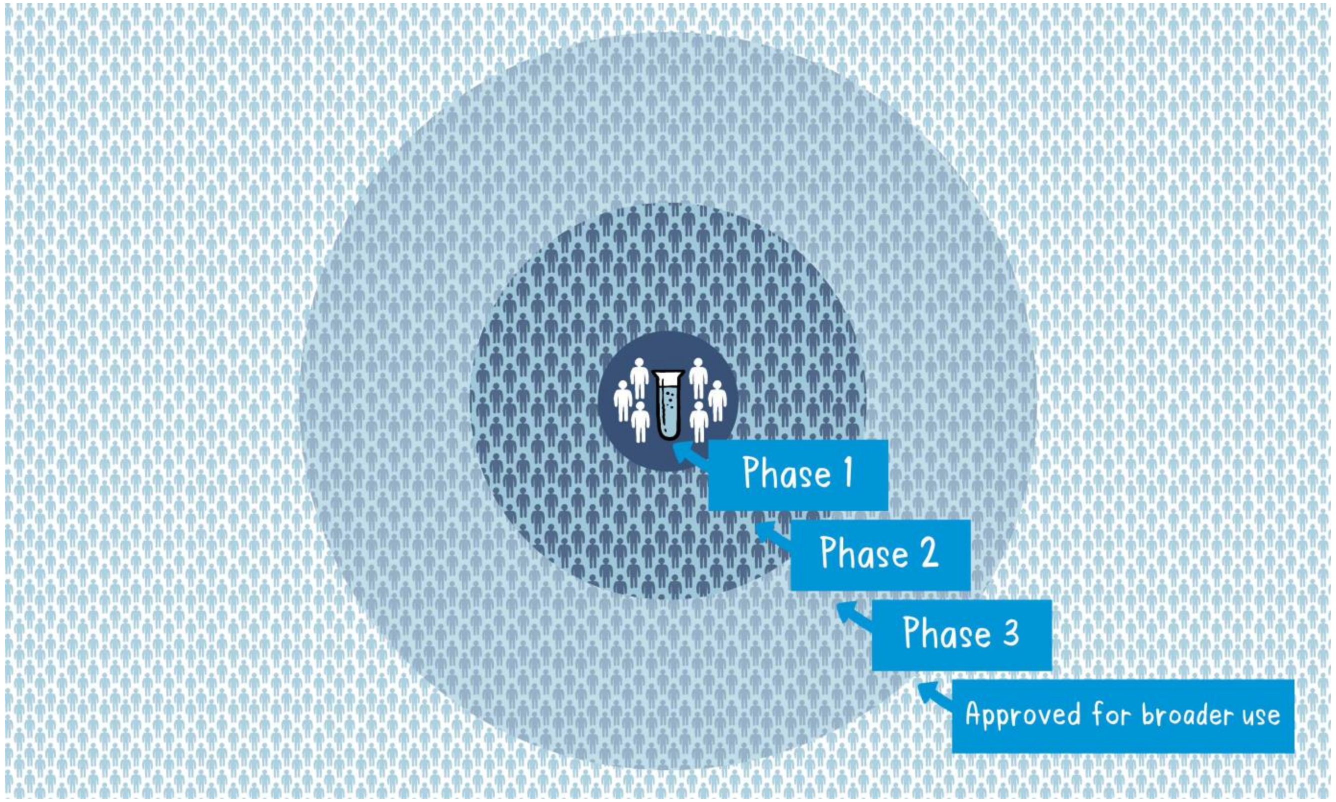


# What Are We Going to Cover Today?

- Normal lung anatomy and histology
- Evaluating lung function
- Pathological changes to the lung
- Using animal models to study respiratory disease
- **Vaccine development**

# Vaccine Development Process

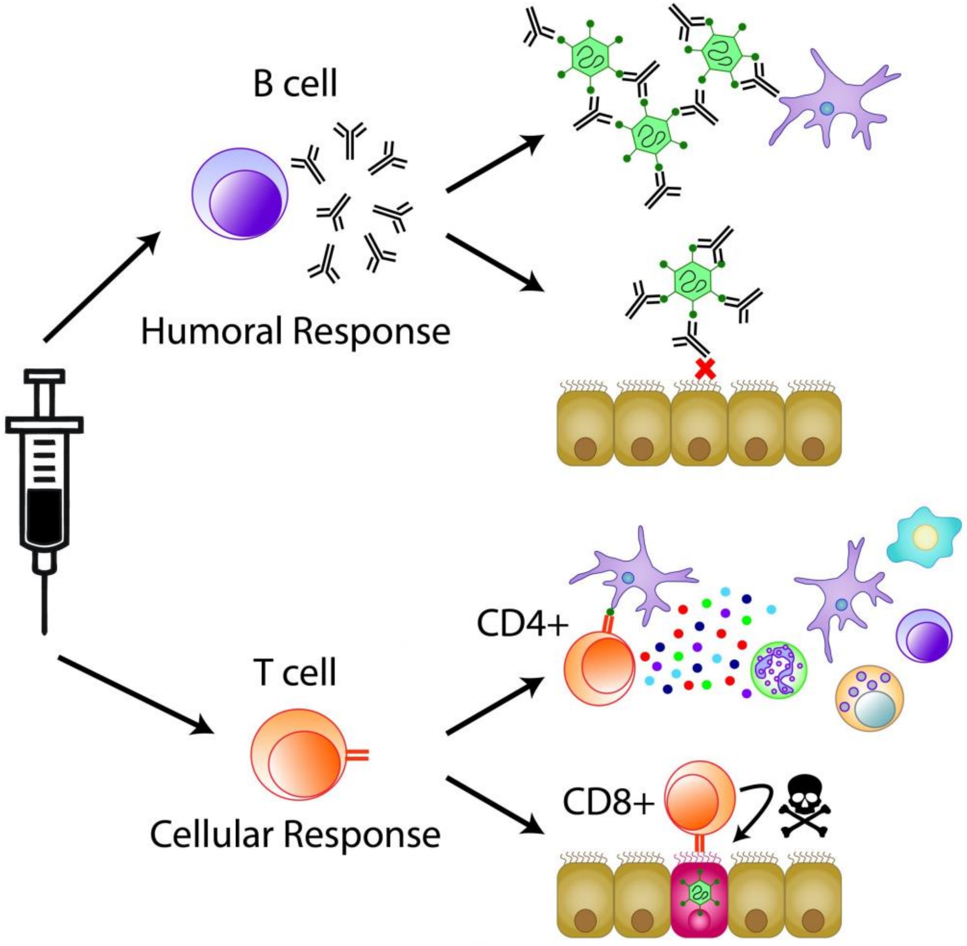
- Preclinical Studies
- Clinical Trials
  - Phase I
    - Safety, immunogenicity, dosage
    - Young, healthy adult volunteers
  - Phase II
    - Target population
    - Placebo group
  - Phase III
    - Efficacy studies
    - Large diverse populations
    - Placebo group




WHO.int

# Immune Responses to Vaccines

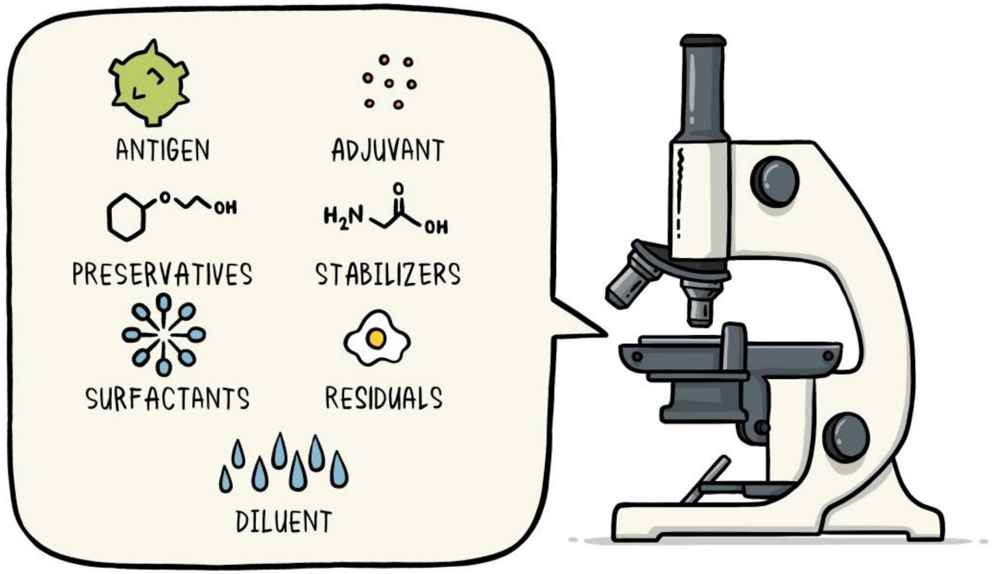
- Humeral Response
  - Antibody
- Cellular Reponses
  - CD4+ T cells
  - CD8+ T cells
- Ideally a vaccine elicits both a strong humeral response and a strong cellular response

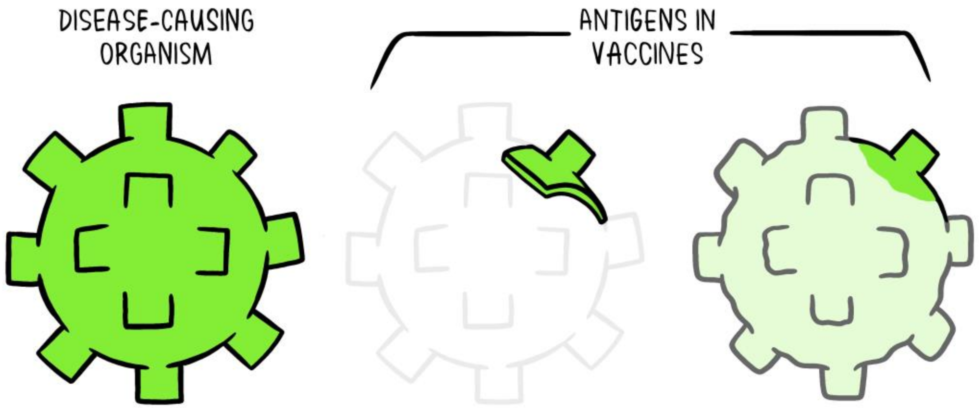



Crl.com

# Vaccine Components

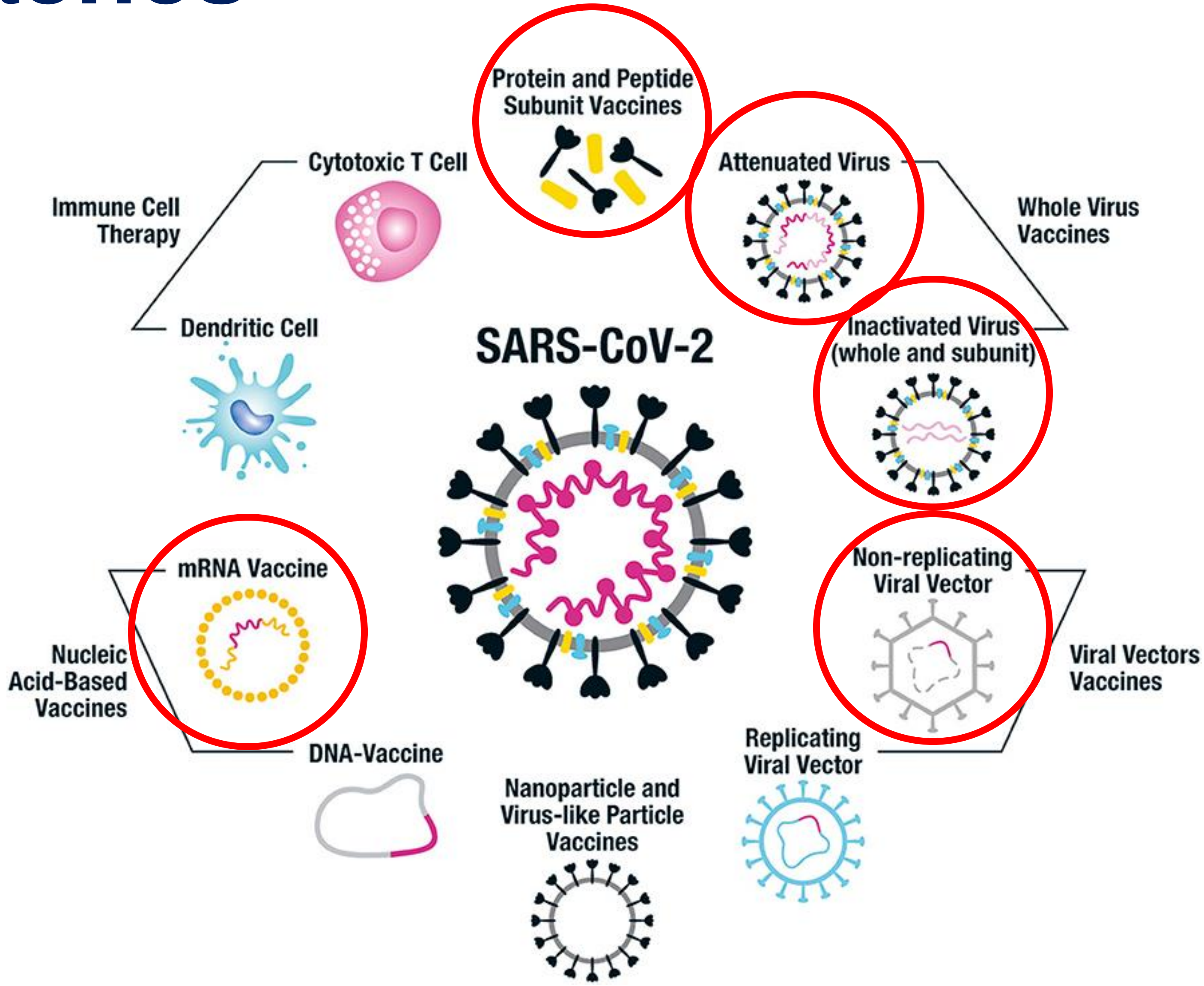
- Antigen
- Preservatives
- Stabilizers
- Surfactants
- Residuals
- Diluent
- Adjuvant



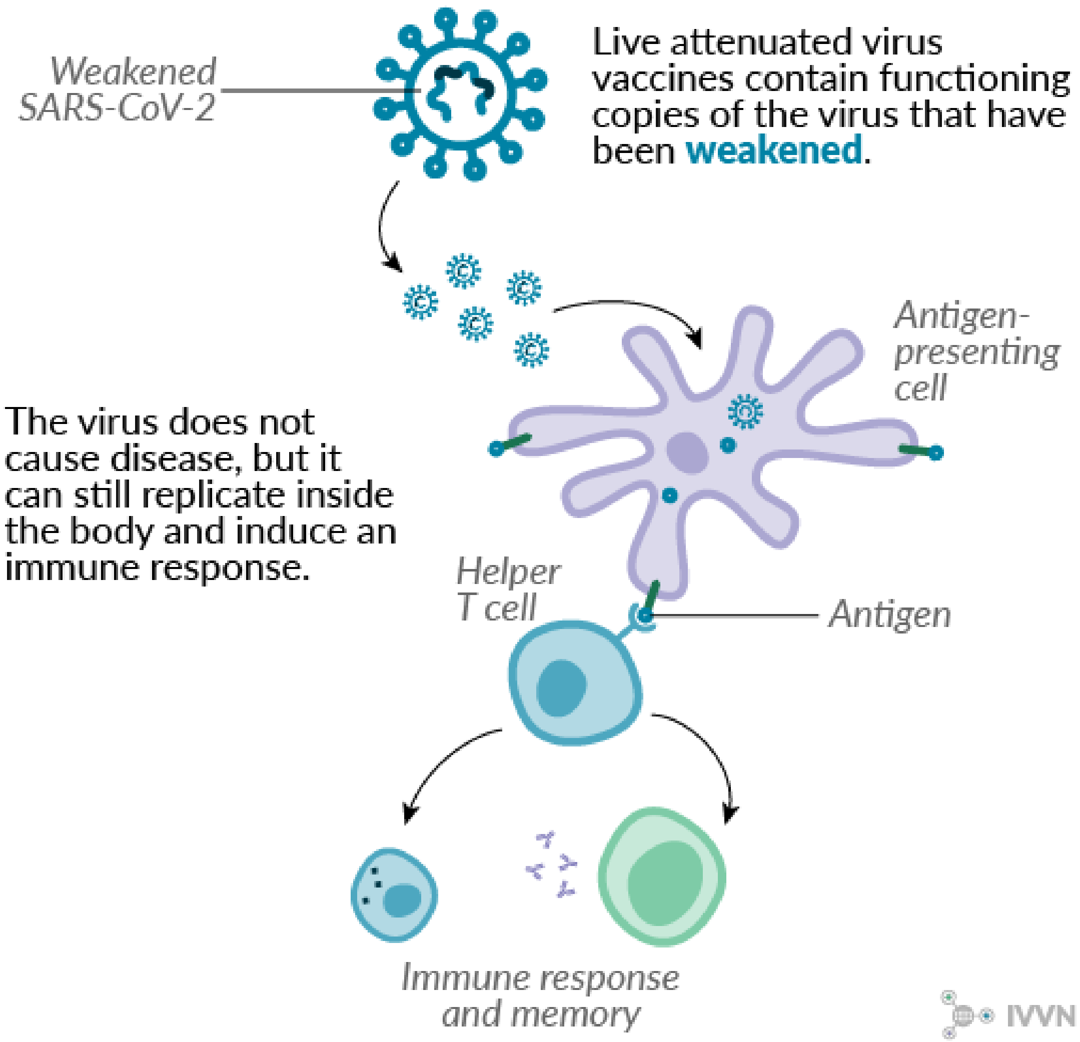
# Vaccine Approaches

- Live attenuated
- Inactivated
- Viral vectored
- Protein subunit
- mRNA



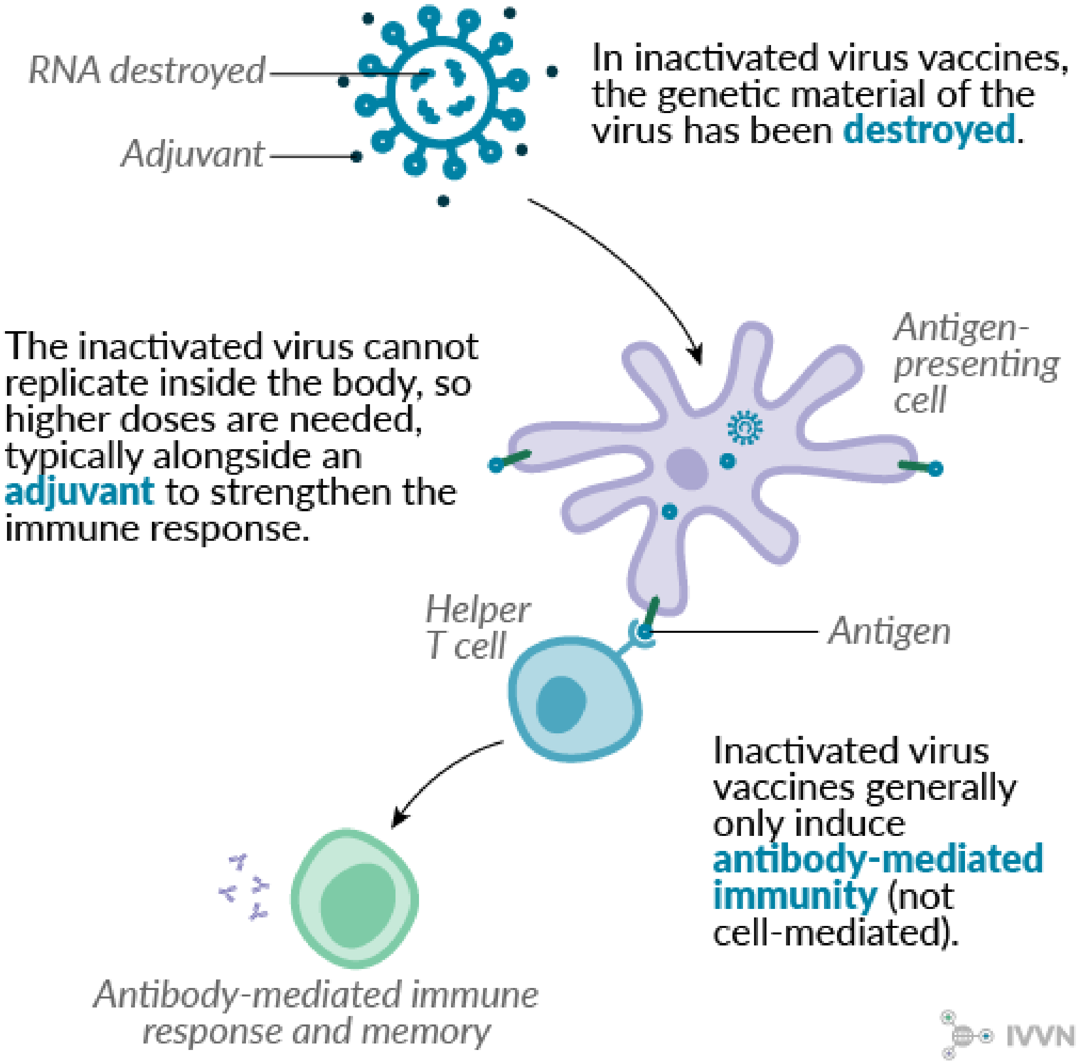
# Live Attenuated Vaccines

- Weakened version of the actual virus
- Present all target antigens
- Tend to elicit strong immune responses
- Examples
  - MMR
  - Chickenpox
  - **COVID:** Codagenix, Indian Immunologicals



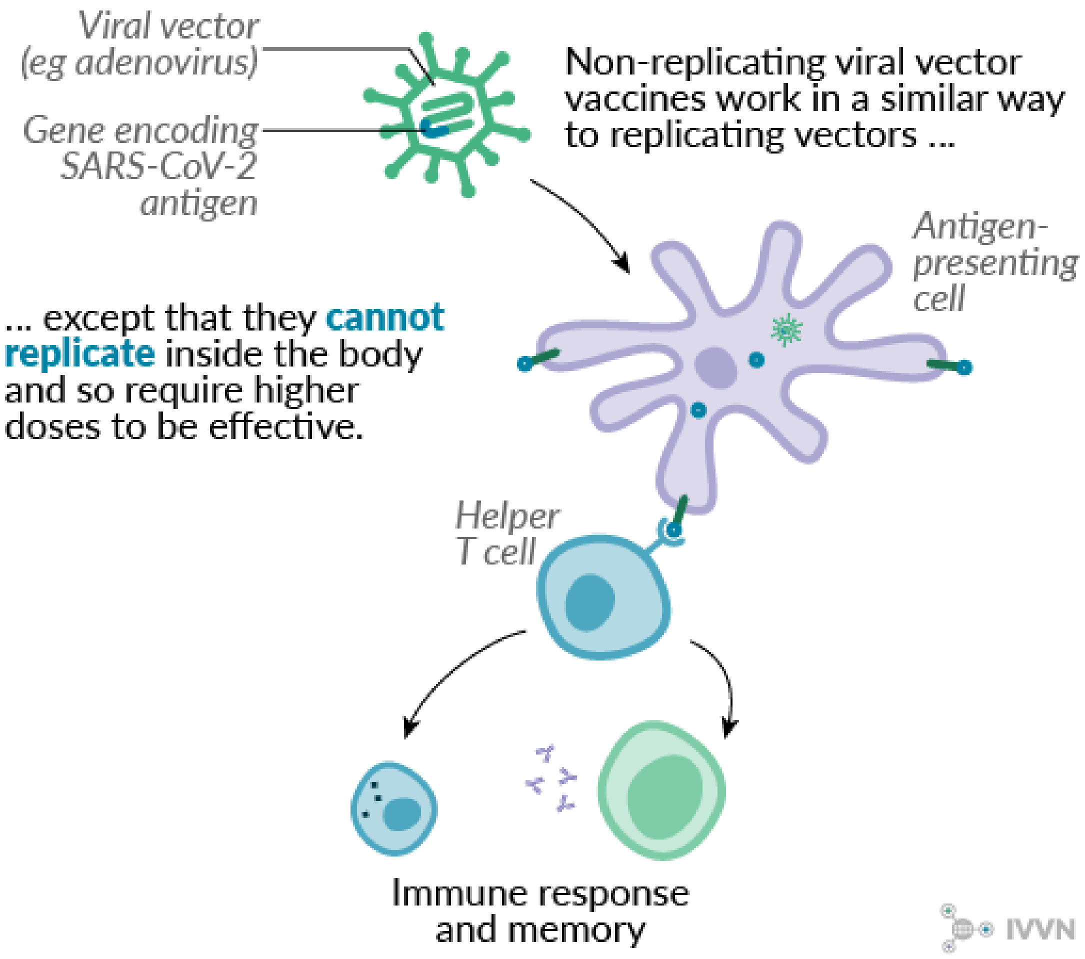
# Inactivated Vaccines

- Uses the whole virus after it has been killed with heat or chemicals
- Contain all antigens but not necessarily in native form
- Tend to primarily elicit antibody responses
- Often include adjuvants
- Examples
  - Polio
  - Rabies virus
  - **COVID:** Sinovac, Sinopharm



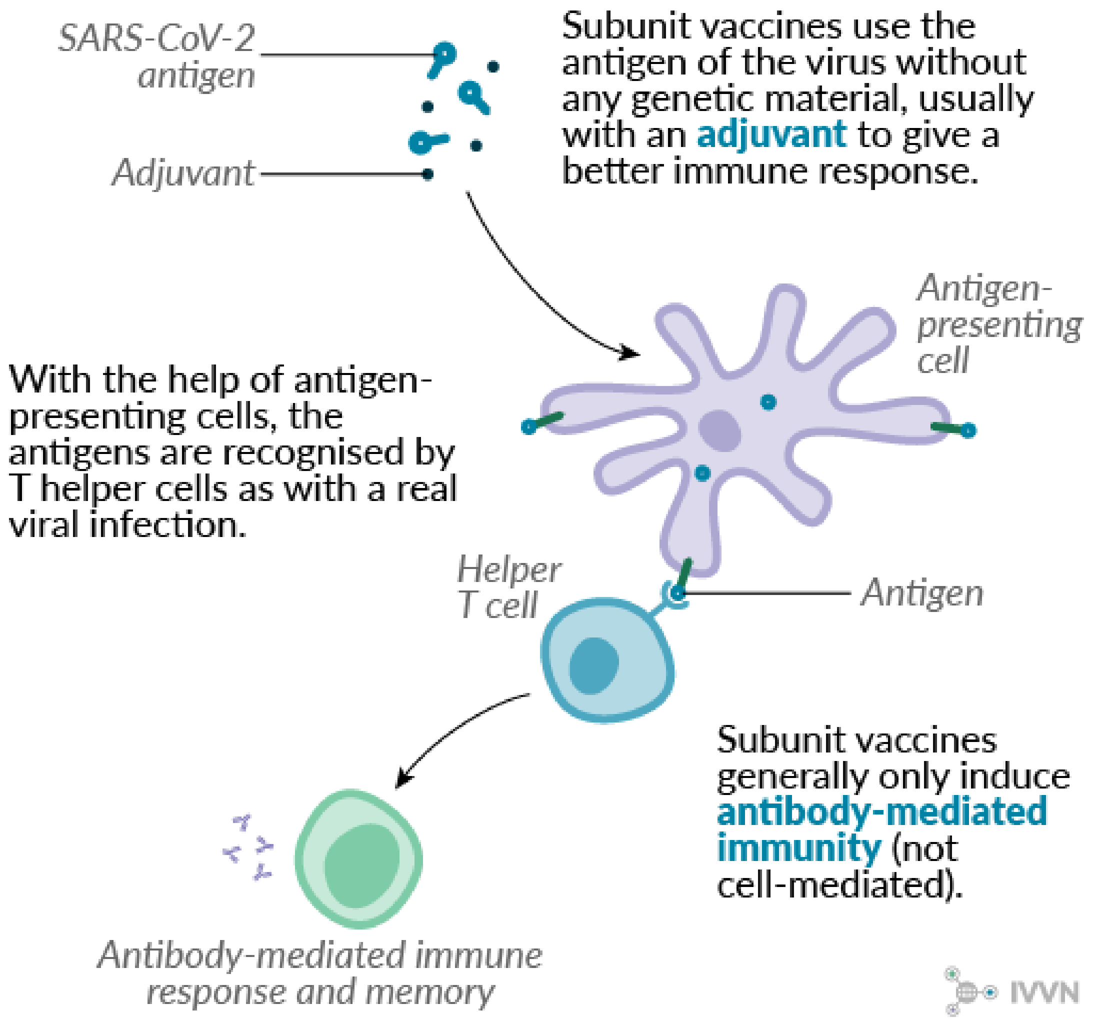
# Viral Vectored Vaccines

- Uses the backbone of a virus to deliver viral genes
- Present specific antigens
- Tend to elicit strong immune responses
- Examples
    - Ebolavirus
    - Canine Distemper Virus
    - West Nile Virus (horses)
    - **COVID:** AstraZeneca, Johnson & Johnson



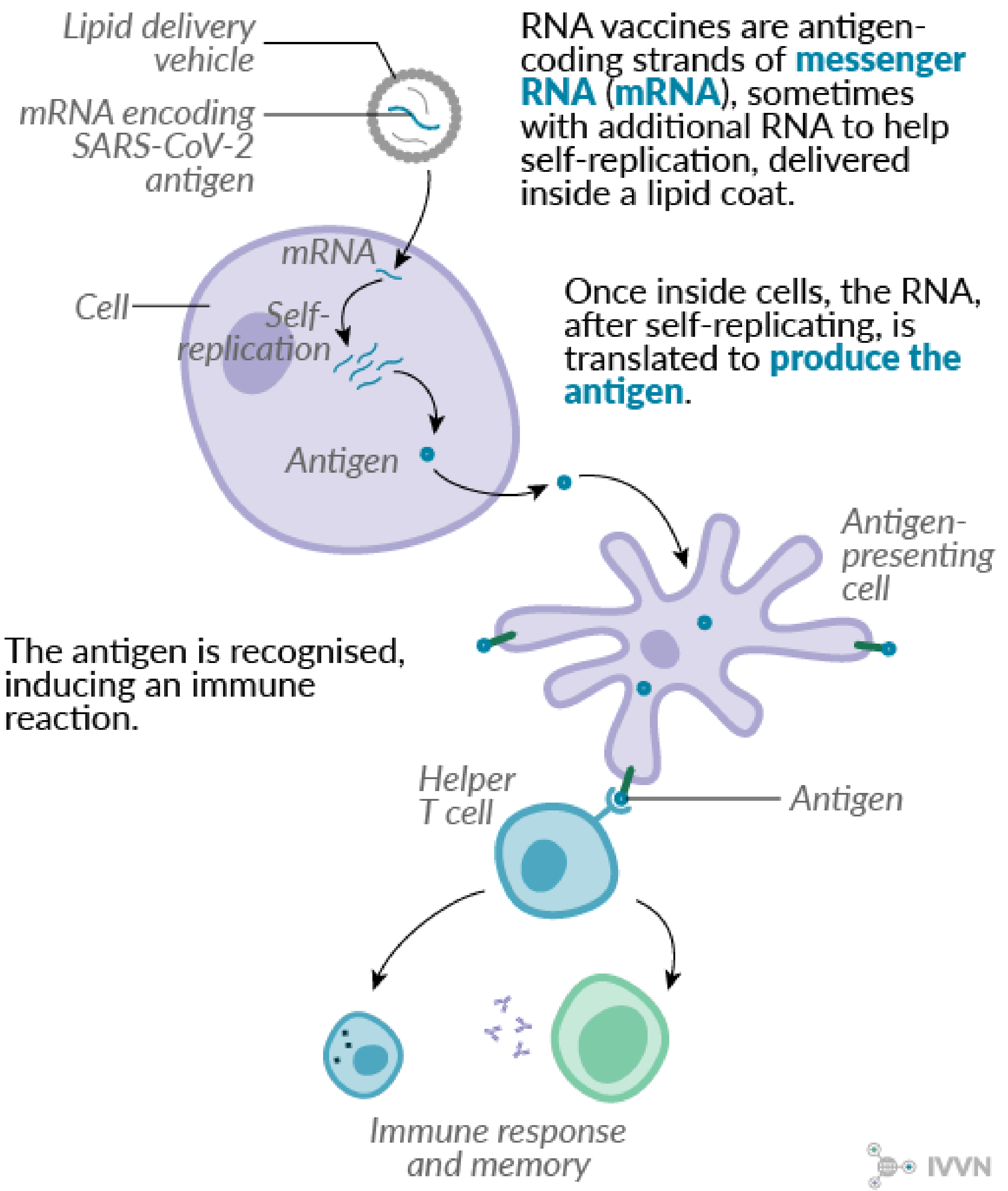
# Protein Subunit Vaccines

- Uses a piece of a virus's protein
- Focuses on a small, important portion of the virus
- Tend to primarily elicit antibody responses
- Often include adjuvants
- Examples
  - Pertussis
  - Hepatitis B
  - HPV
  - **COVID:** Novavax



# mRNA Vaccines

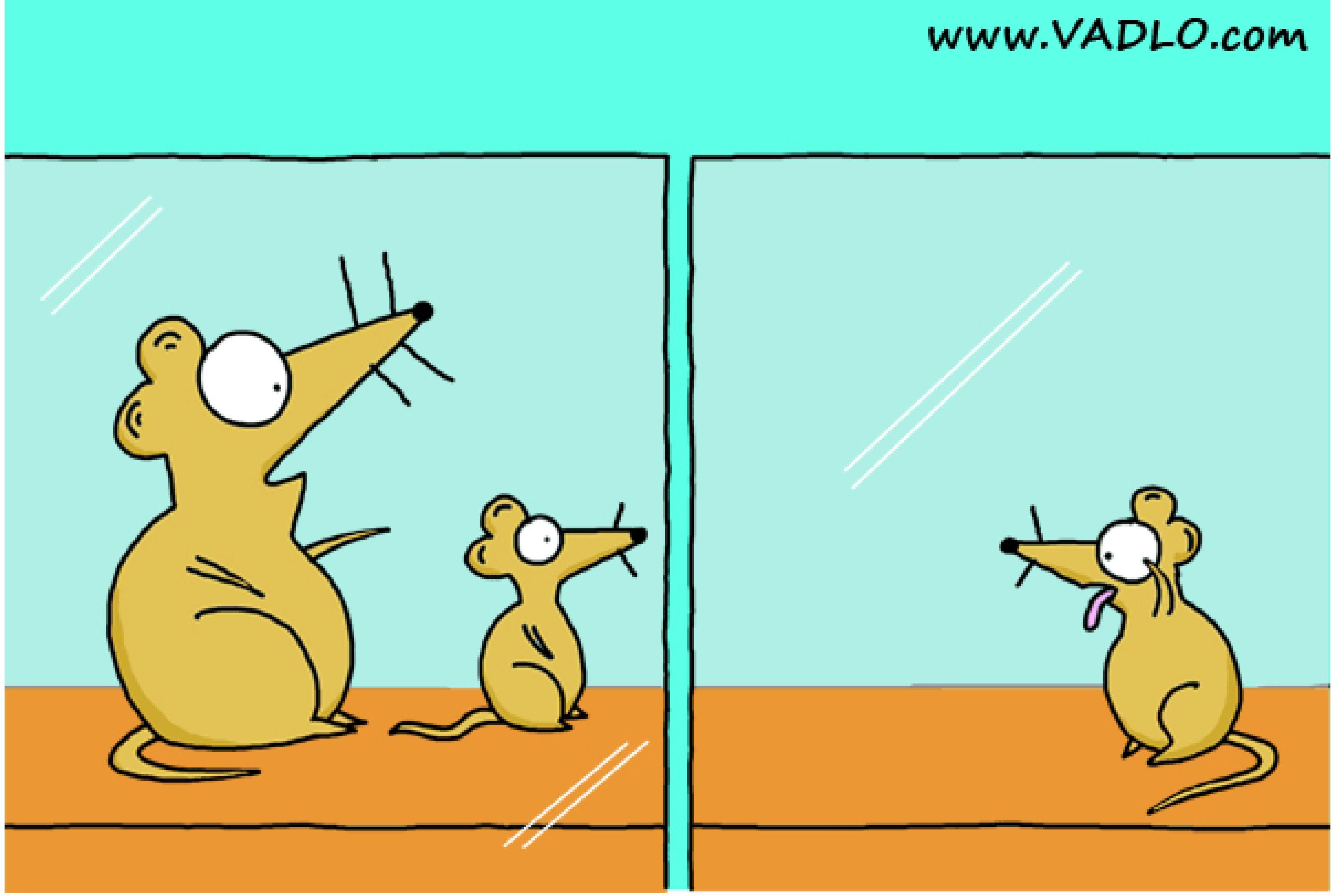
- Contains the coding portion of a virus protein
- Present specific antigens
- Tend to elicit strong immune responses (we think)
- Examples
  - **COVID:** Pfizer BioNTech, Moderna



# What Are We Going to Cover Today?

- Normal lung anatomy and histology
- Evaluating lung function
- Pathological changes to the lung
- Using animal models to study respiratory disease
- Vaccine development

# Any Questions?




"Don't play with him, he is **Wild Type**."