# *In vitro* and *in vivo* models used in pathogenicity research

- *in vivo*
- *in vitro*
  - tissue culture
  - explants
- worms and flies
- humans

# Learning outcomes

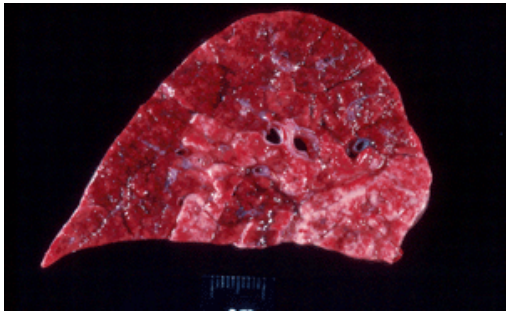
Be able to give examples and describe the advantages and disadvantages of the use of the following in infectious disease research:

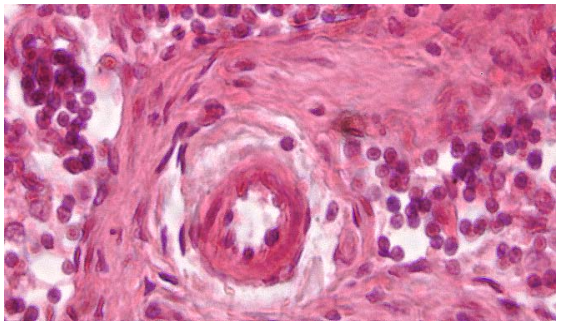
- animals
- in vitro and explant surrogates
- alternative hosts
- humans

# Animal models

- Relevance and appropriateness
- NOT convenience and accessibility

- Mimics pattern found during natural infection
  - pathology
  - location
  - severity





- Infection and disease at high prevalence
  > 50 % → smaller groups

- Natural route
  - aerosol, intranasal (in)
  - subcutaneous
  - intraperitoneal (ip)

BUT - depends on what you want to measure
e.g. intranasal *vs* intraperitoneal

# Choice of animal species

- Inbred laboratory rodents
  - most widely used
  - wide range of reagents available
  - transfer immune cells between animals
  - knockout mice
  - introduce transgenes

# But…

- Atypical responses to specific infections
- Limited host range
- Inbreeding - restricted MHC
- In general not good for IC pathogens

# Outbred laboratory animals

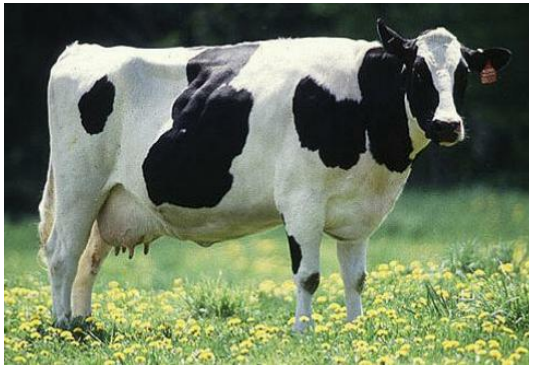
- Primates, guinea pigs, rabbits, ferrets, armadillo, chickens

- Reagents increasingly available

- Same pathogenicity as humans?

# Large animals

- Pigs, sheep, goats and cows
- Natural host → natural responses
- Reagents increasing available
- Genetic polymorphisms known (breeds)
- Costs high especially if category 3/4



# Dose

- Minimum necessary to establish infection in target host (vaccines/pathogenesis)
- Suprainfective doses (therapeutics OK?)
- Dose and route linked

  - e.g. *H. influenzae* i.p *vs* i.n.

# Indicators for infectious outcome

- Recovery → indicates infection
- Time points → indication of severity
- Pathology → patterns of disease

- Clinical symptoms
  - Weight loss, ruffled fur, behavioural

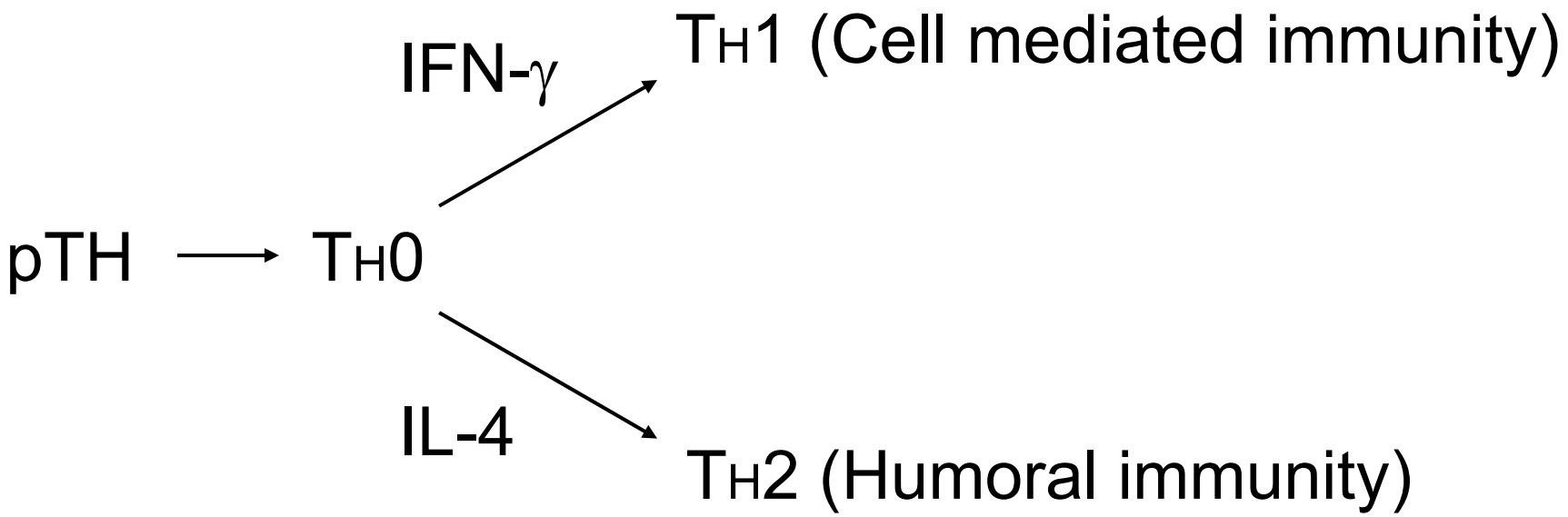
- Death - increasingly unacceptable

Endpoints should allow evaluation of presence and severity of infection or disease

e.g. vaccine efficacy

- does the vaccine protect against infection or disease or both?

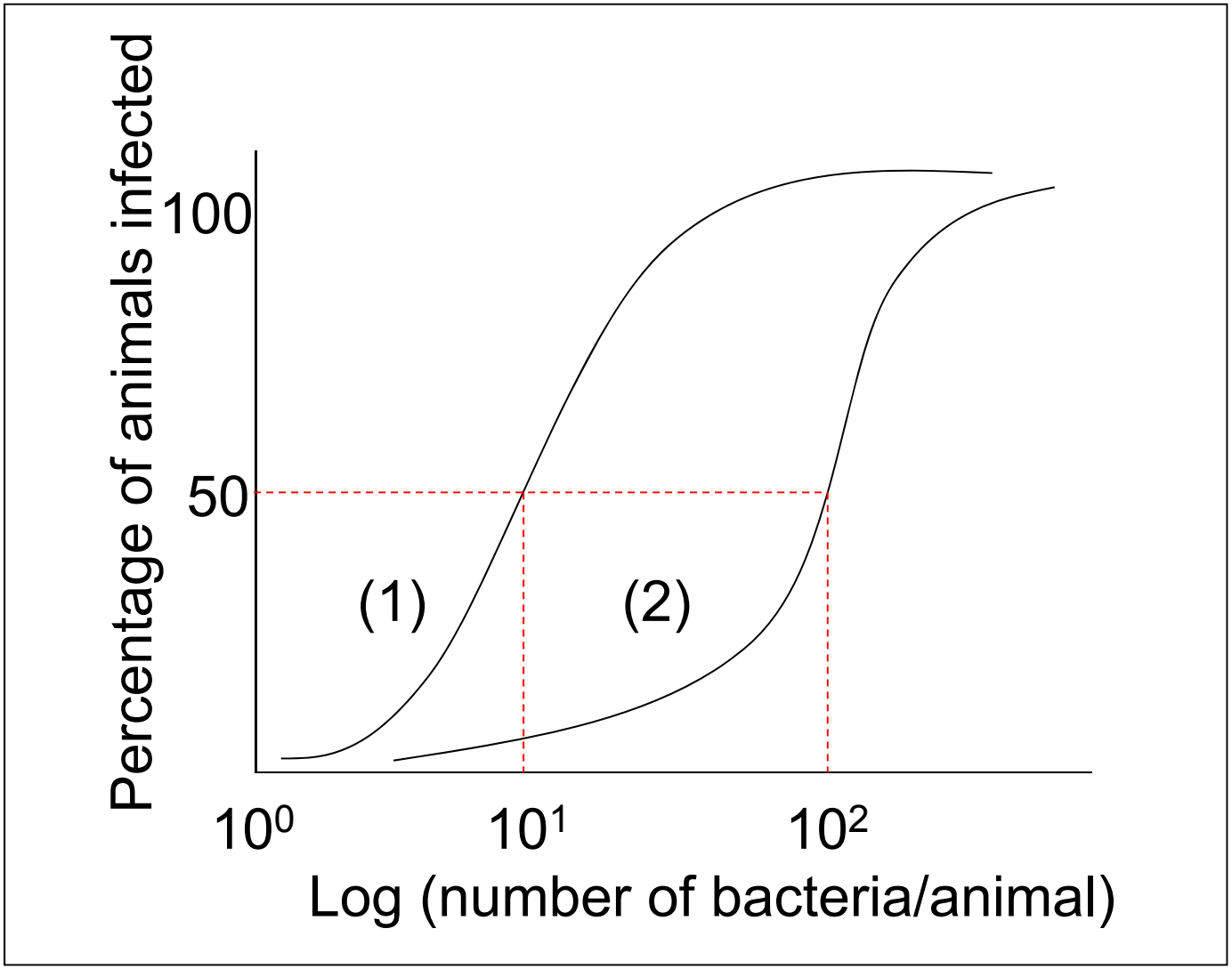
# Immune markers in infection and protection



# $ID_{50}$ and $LD_{50}$

| | |
|---|---|
| $ID_{50}$ | The number of bacteria necessary to infect 50% of the animals exposed to the bacterium |
| $LD_{50}$ | The number of bacteria necessary to kill 50% of the animals |

# $ID_{50}$ values for bacteria of different infectivity



- Potentially misleading e.g.
  - human $ID_{50}$ cholera is 10,000 cfu
    shigella dysentery 10-200 cfu
  - cholera can be fatal; dysentery rarely fatal

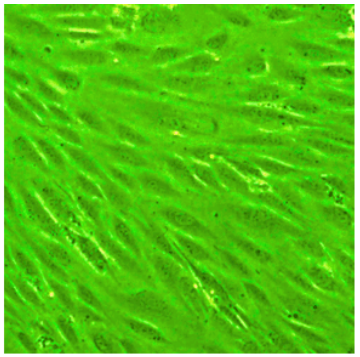
- LPS - too many bacteria (non-physiological)
  die of shock

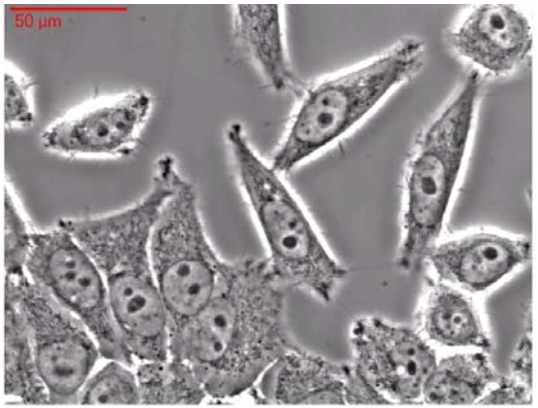
- Best applied to relative infectivity or lethality of closely related strains

# *In vitro* models

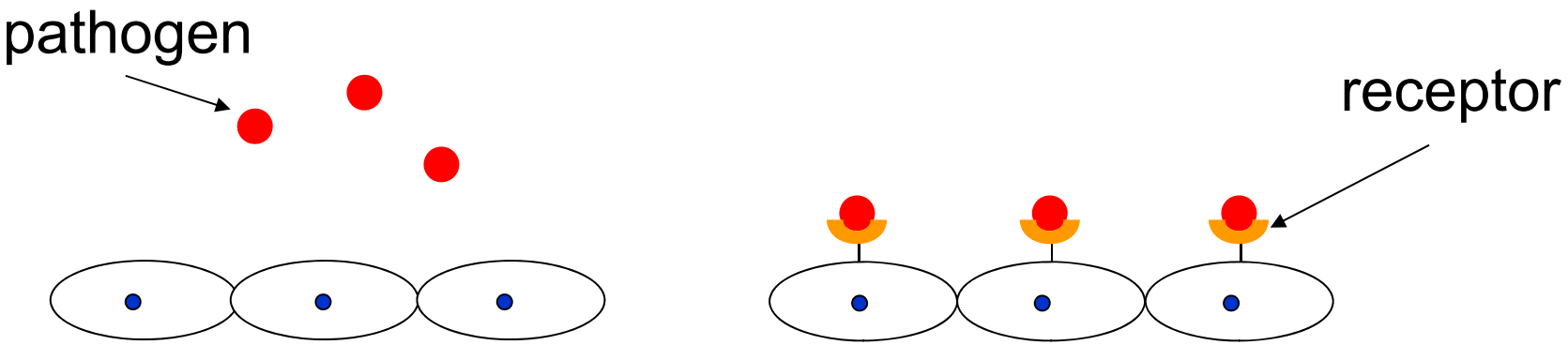
# Cultured cell lines - the mainstay of pathogenicity research

- Epithelial (bronchial, lung, gut etc)
- Endothelial (HUVECs, BBB)
- Macrophages (J779)
- Cancer derived (Hela)
- Immortalised (SV40)





# Transfectants




Cell line

Cell line expressing
host pathogen receptor
(transfectant)

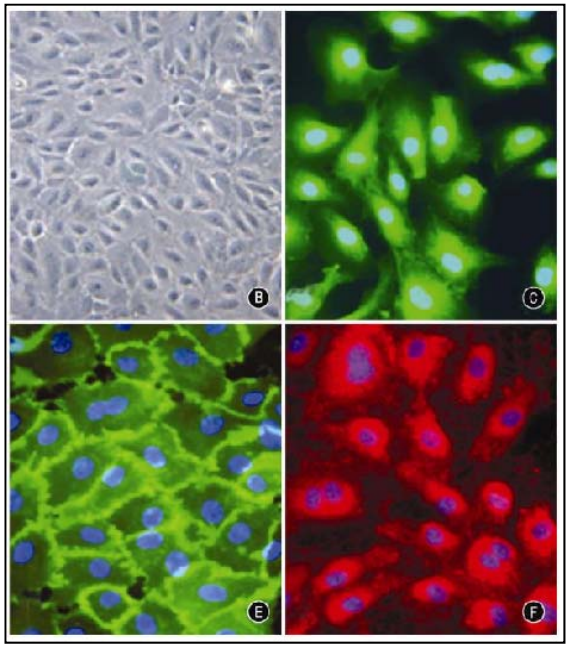
But is it a primary or secondary effect?

# Problems

- Passage
  - phenotypic changes from *in vivo*
  - surface molecule expression changes with time
- Typically use bovine serum, FCS, serum-free
- Speed of growth - slow BBB vs fast fibroblasts
- Static not dynamic (mimic this e.g. ECs and flow)
- Primary passage - genetic variability humans
  - polymorphisms!

# Important to have criteria for cells

- Morphology (cobblestone)
- Surface expression or uptake (acetylated-LDL)
- Specific markers (CD31, vWF)
- Mycoplasma free



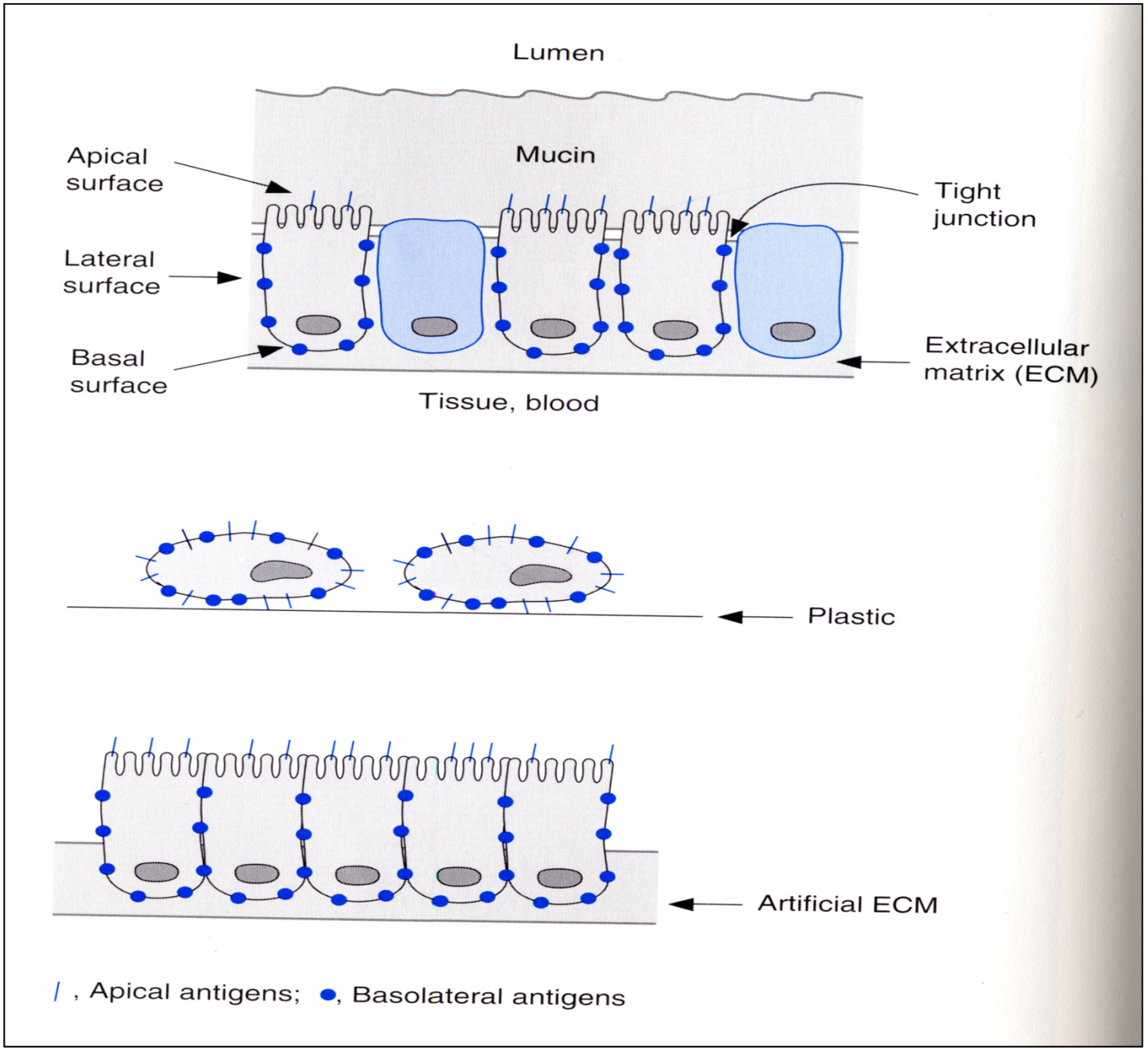
- Seed stocks from culture collections (e.g. ATCC)
- But not infallible e.g. contamination of many cell lines with HeLa!

# Make phenotype *in vivo* like by:

- Adding (or deleting) components
  - e.g. add endothelial cell growth factor
  - astrocyte conditioned media (BBB)

- Growth on different surfaces
  - collagen, gelatin, fibronectin etc….

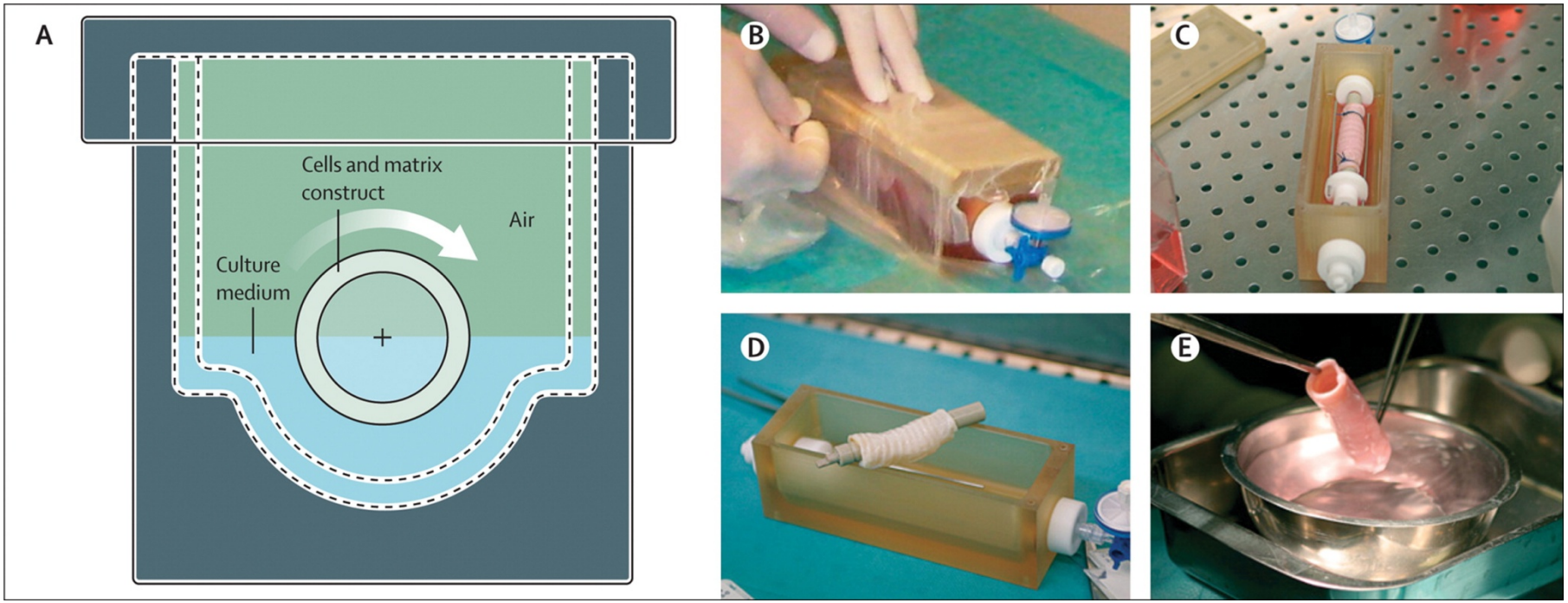
- Polarisation


Lumen
Mucin
Apical surface
Lateral surface
Basal surface
Tight junction
Extracellular matrix (ECM)
Tissue, blood
Plastic
Artificial ECM
/ , Apical antigens; ●, Basolateral antigens

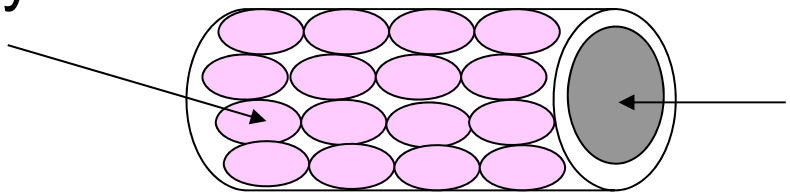
- Macchiarini P et al.
  Lancet 372 (2008) 2023 – 2030.

- post-tuberculous chronic tracheitis

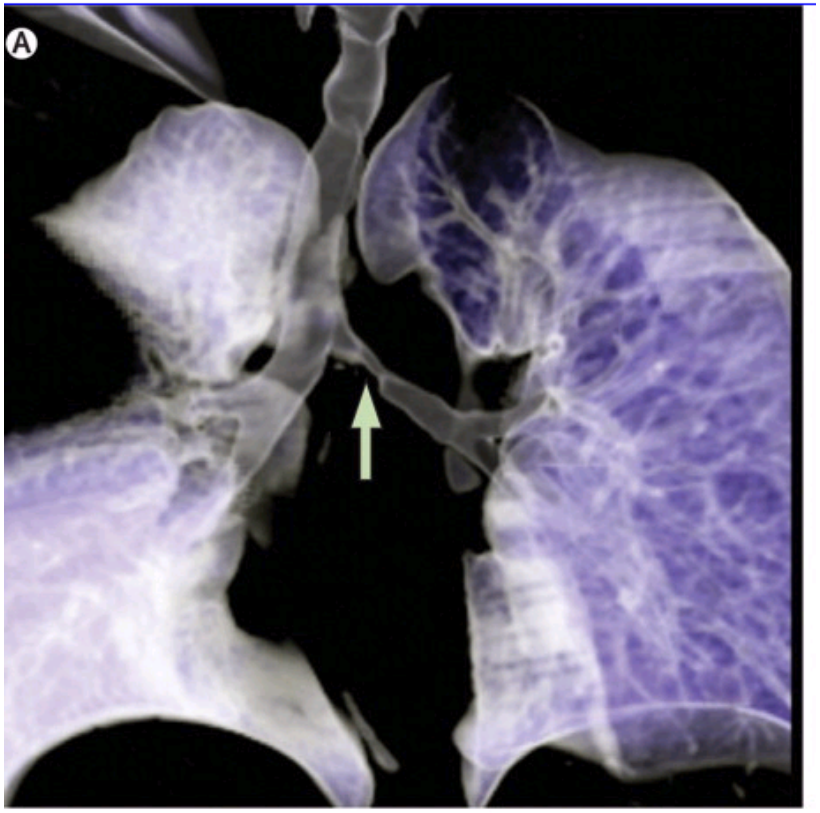
- trachea from a donor = scaffold




chondrocytes



bronchial epithelial cells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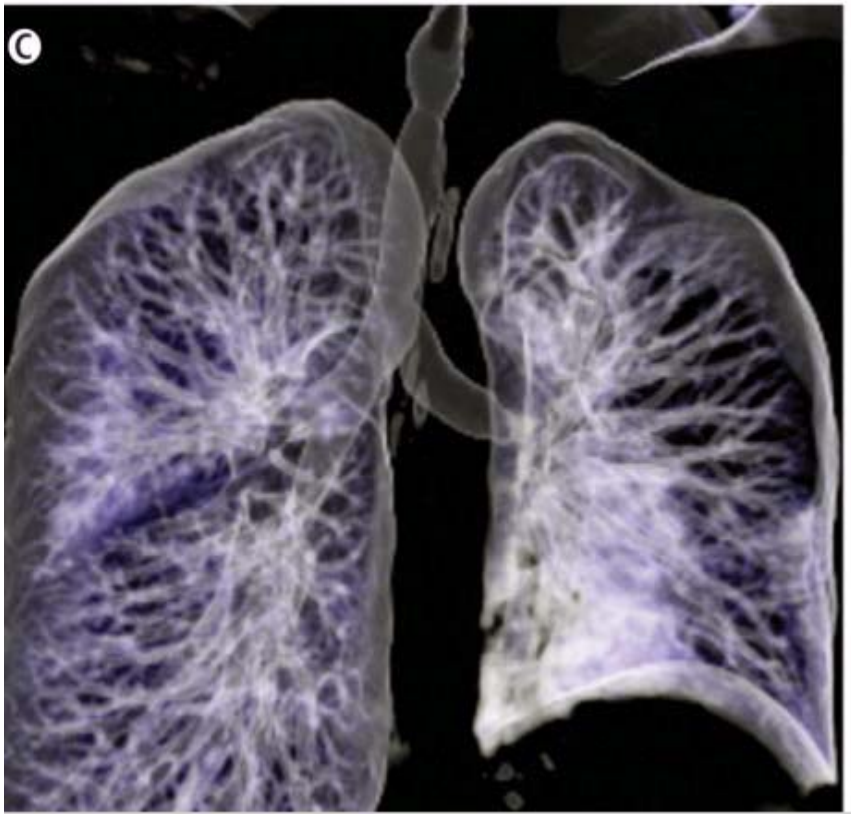
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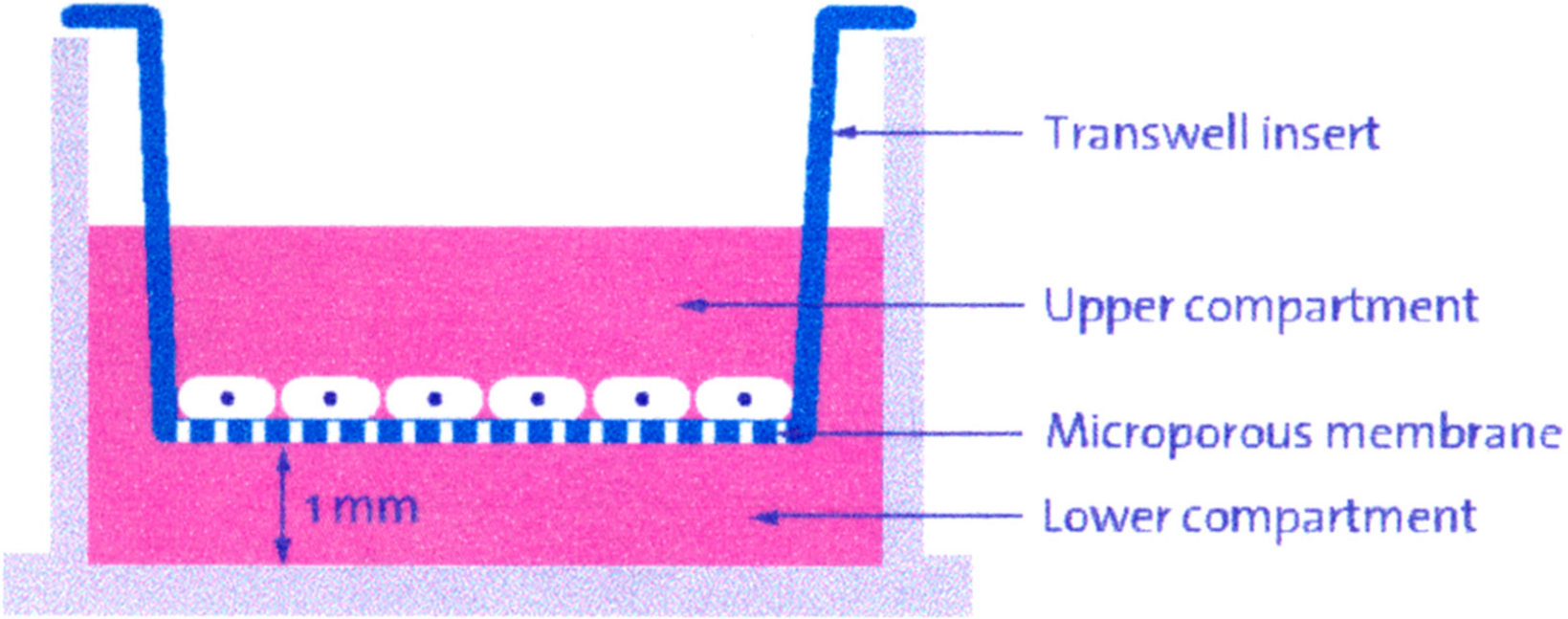
# Transwells




**Transwell Permeable Support**

Normal human bronchial epithelial cells (inc stem cells)

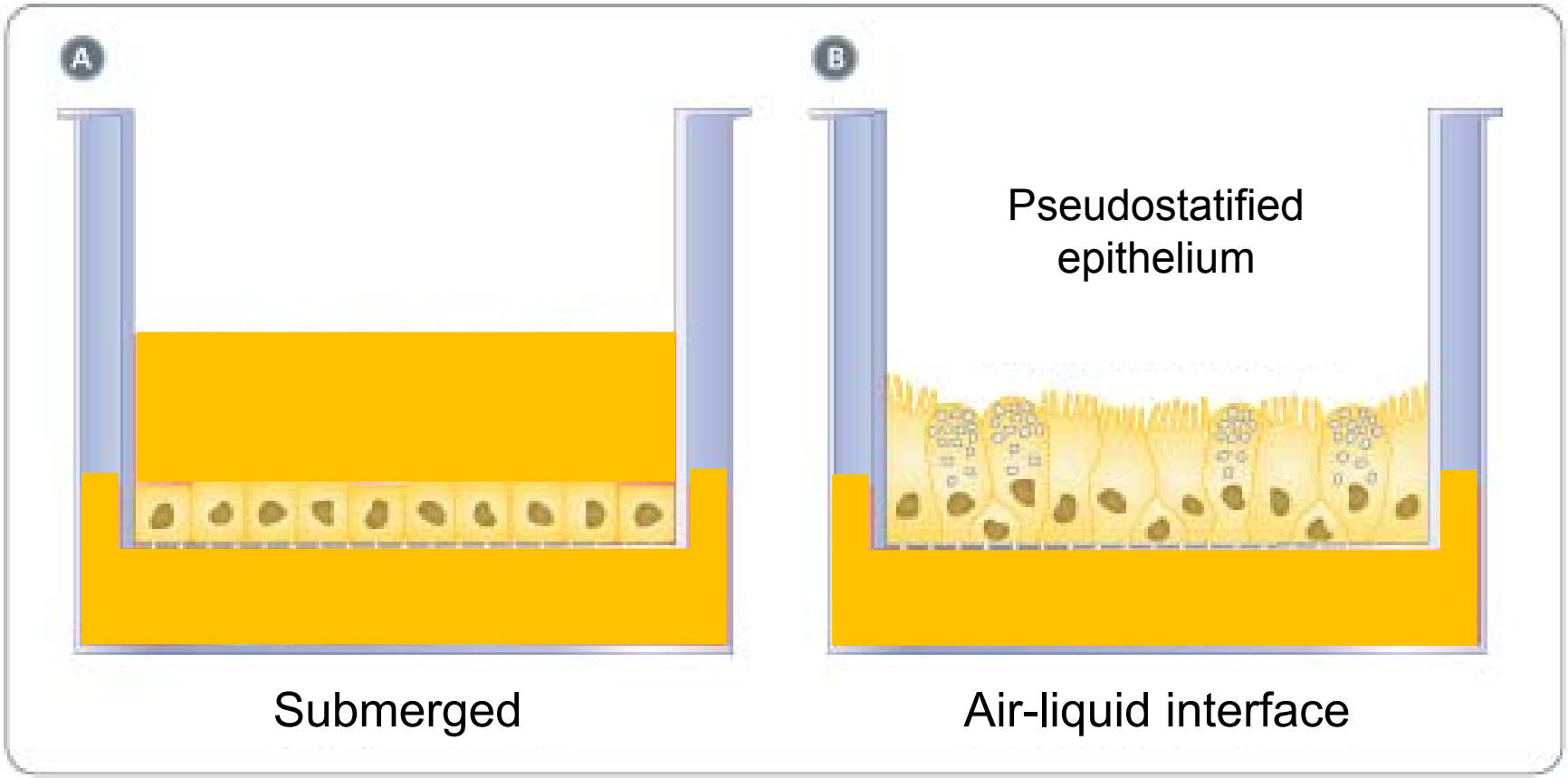
↓

Differentiate

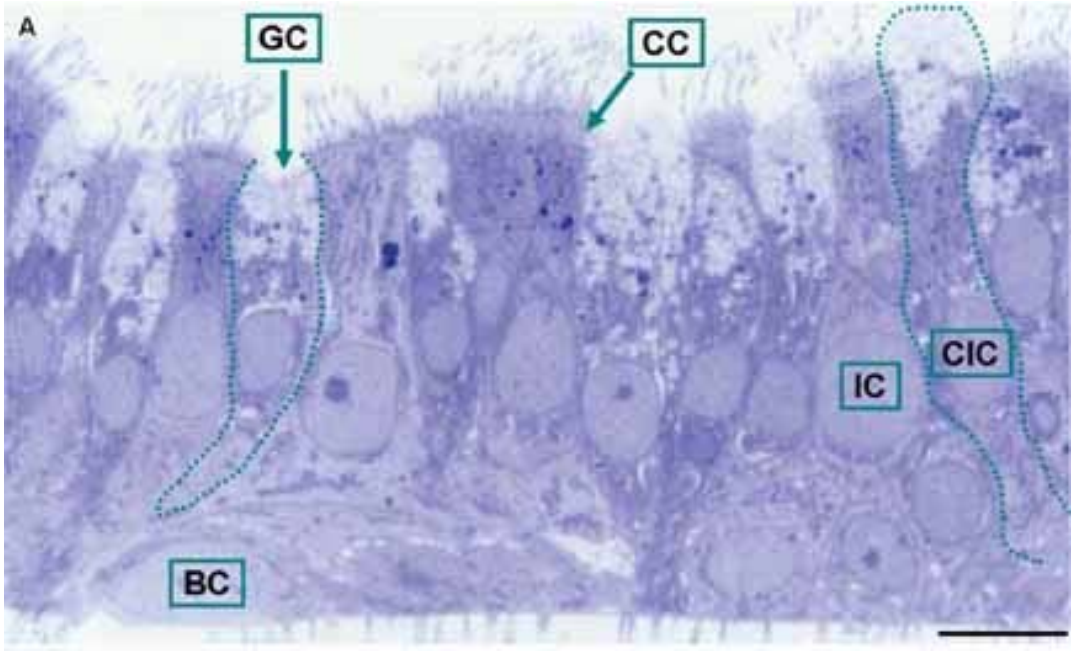
↓

Cell line e.g. A549 respiratory epithelium

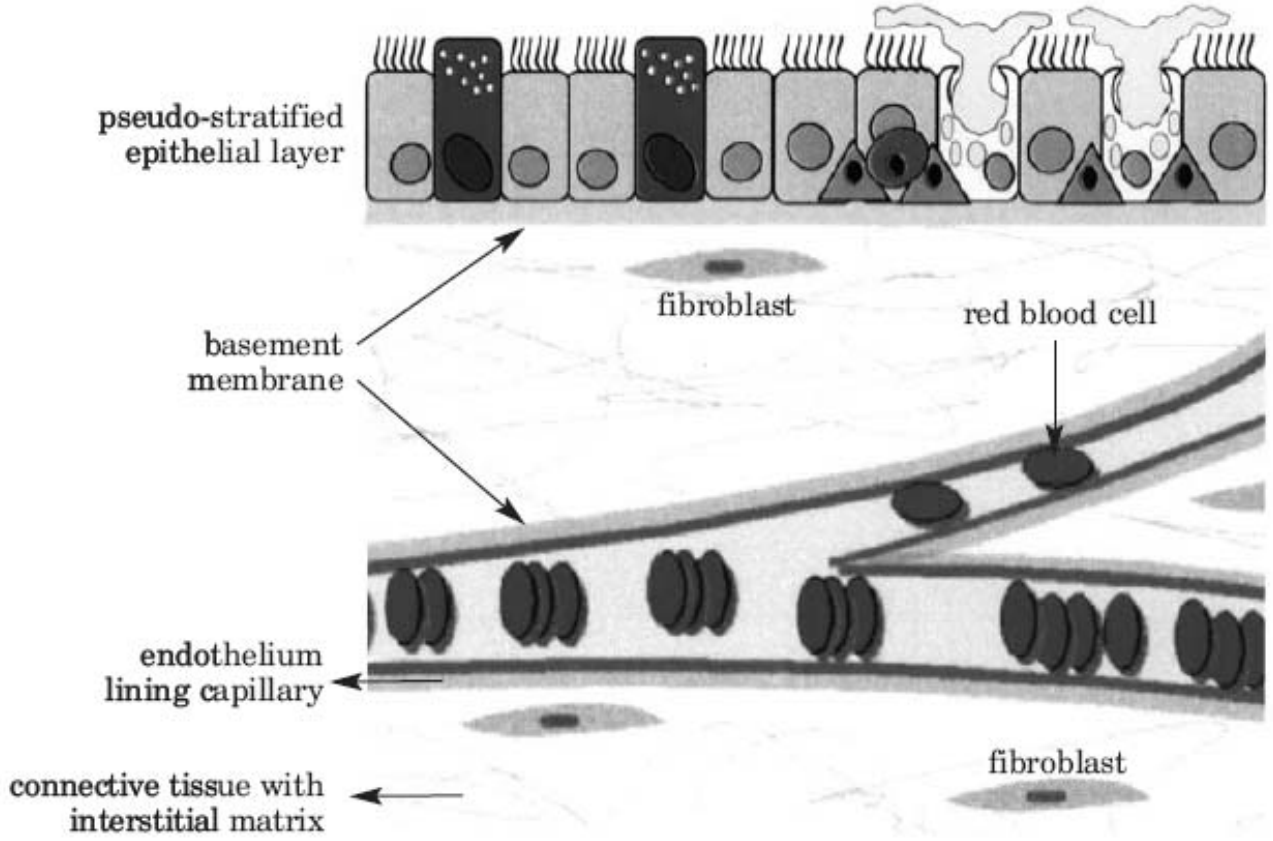
↓



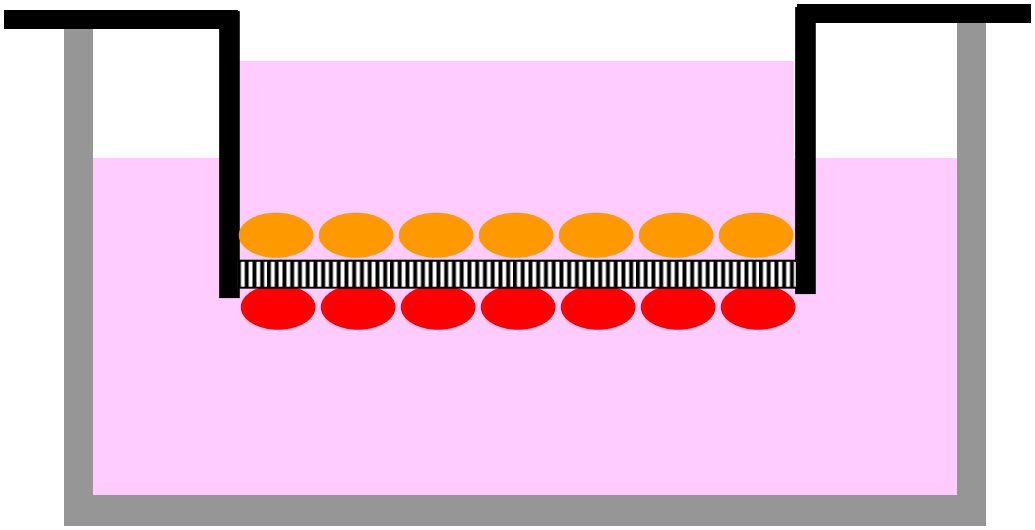
# Tissue-specific stem cell differentiation in an in vitro airway model



# 3D environment of lung epithelial cells




*The pseudo-stratified epithelial layer in airway tissues is characterised by an apical surface, exposed to an air–liquid interface, and the baso-lateral surface, which is embedded in a complex three-dimensional matrix.*



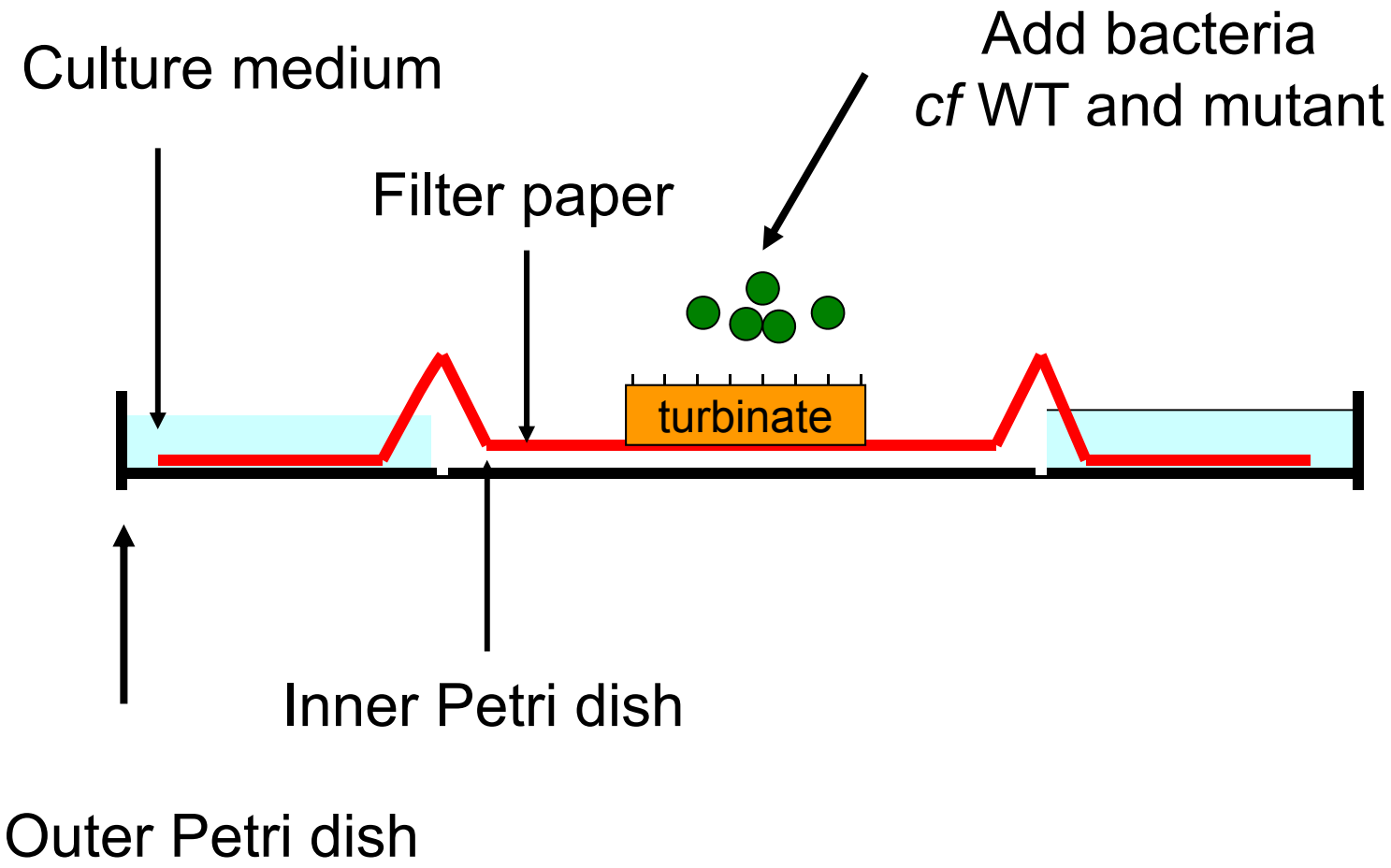
Epithelial cells

Endothelial cells

# Explants (*ex vivo* models)

- Explant material (e.g. biopsy material, nasal turbinates etc. - got mucus!)
- Organ cultures (human rare material)
- Limited use  (but could immortalise)

# Air model - nasal turbinate



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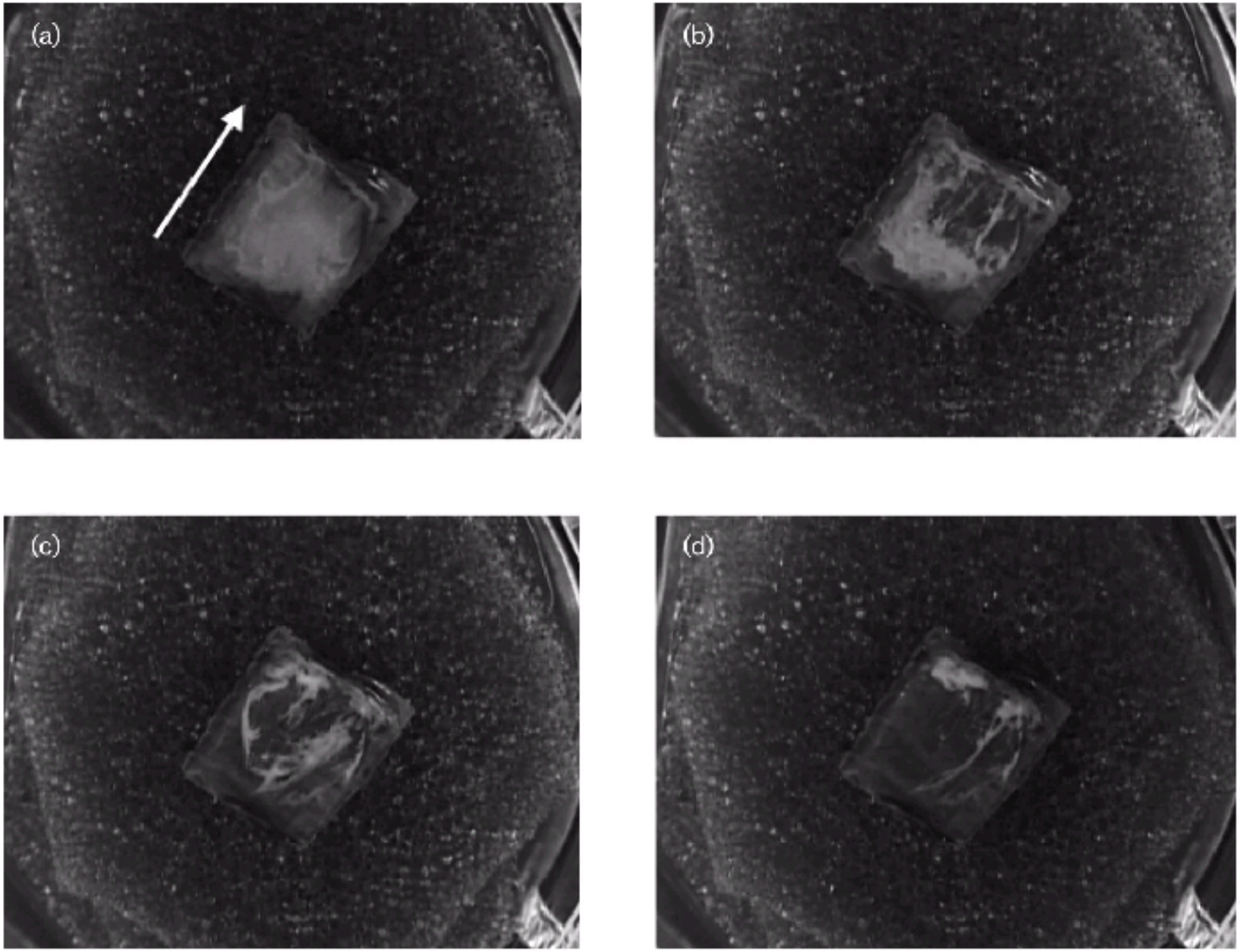
Anderton T et al. (2004) Microbiology **150**:2843 - 2855


(a)
(b)
(c)
(d)

# More accessible material

- serum *vs* whole blood *vs* individual cells
- isolate individual cells by
  - FACS
  - immunomagnetic separation
- but can activate cells alone!

# Choice of bacterial strain and species

- Common isolate (but care e.g. BCG)
- Genome sequence available
- Manipulate genetically
- Fresh isolate (animal passage) or lab “pet”
- Culture collections, seed stocks
- Natural isolates e.g. *Mycobacterium marinum*

# How do you grow pathogens?

- *In vivo* - pathogens are Fe-restricted
- *S. typhimurium* gut - anaerobic

  spleen/blood - aerobic
- Mimic by growth in serum, milk etc
- Static vs dynamic, pH changes *in vitro* e.g. CSF

# Up and coming models…

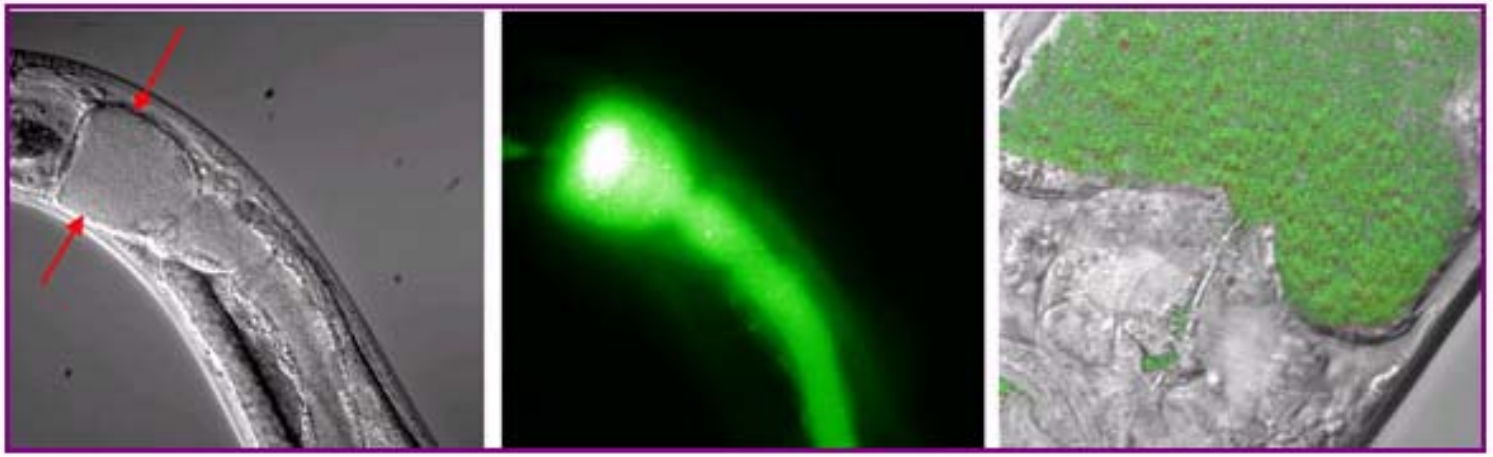
# *C. elegans*

- Easy to grow, fast
- Genetically well defined
- Genome sequences known
  - arrays (transcriptome)
  - mutants available
  - high throughput systems
  - iRNA technology

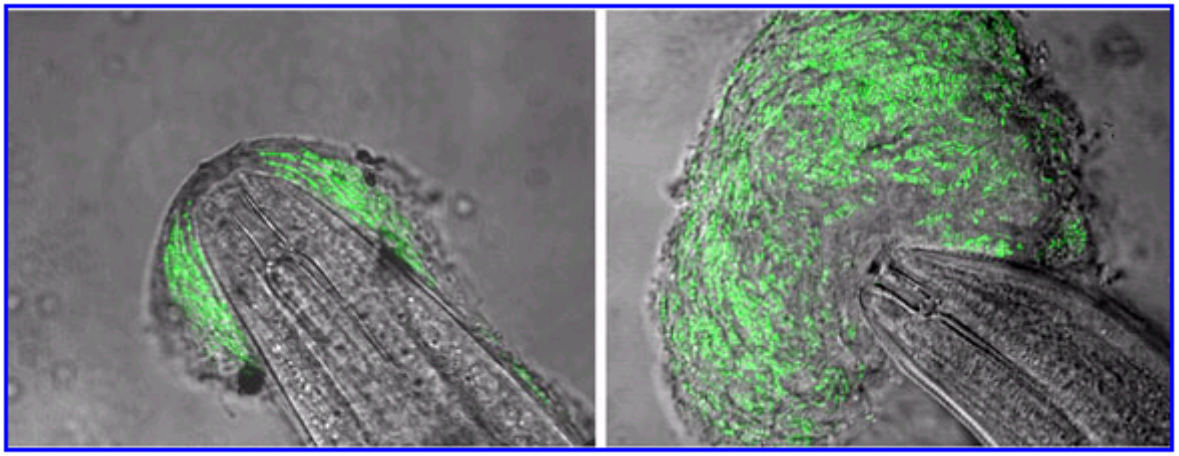
Allows KOs in host vs KOs in pathogen

- *Salmonella typhimurium*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Enterococcus feacalis*
- *Yersinia pseudotuberculosis*

Not: *N. meningitidis*!

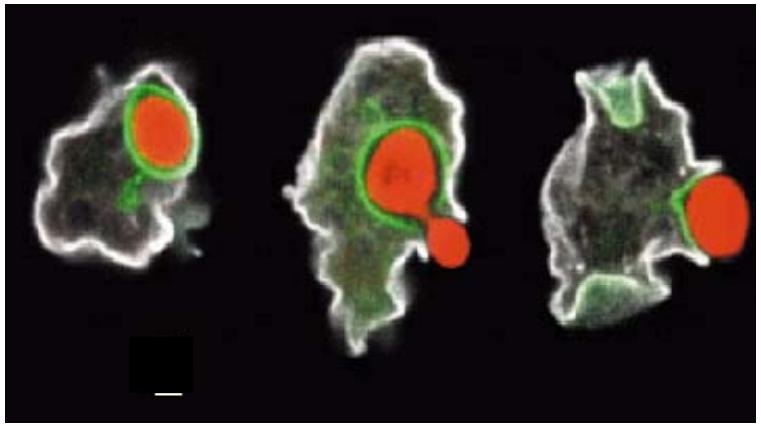


*S. aureus* expressing GFP



*Y. pseudotuberculosis* expressing GFP

Amoeba



Wax moth

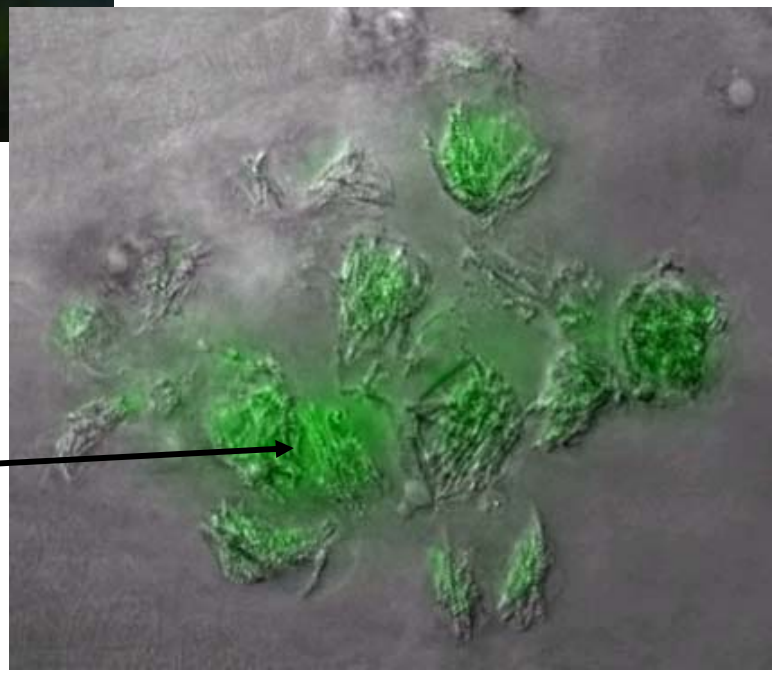
Drosophila

Dictyostelium

Zebra fish

*M. marinum* (expressing GFP)

infection results in granuloma formation



# Adult “volunteers”

- Live vaccines, infection “models”…
- May need facilities (e.g. St George’s)
  - shedding, persistence
- Logistics, statistics
- Follow up
- Ethics …


23. 1. 2003


23. 1. 2003


23. 1. 2003

# St George's facilities

- Single bedded barrier isolation rooms (X2)
- Total control air handling (-ve pressure)
- En-suite shower and WC which can also be isolated to prevent release
- Suitable for live attenuated respiratory bacterial or viral vaccines, gene therapy agents, or other GMOs

# Messages

- What question are you asking
- Relevance of model to answer question
- Reproducibility, statistics
- The pathogen matters as well