

The macrophage as a pharmacological target in airway disease

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Airway Disease
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Macrophages in the Lung

- **Host Defence**

Maintain sterility of the airways

- **Clearance:**

Inhaled particles

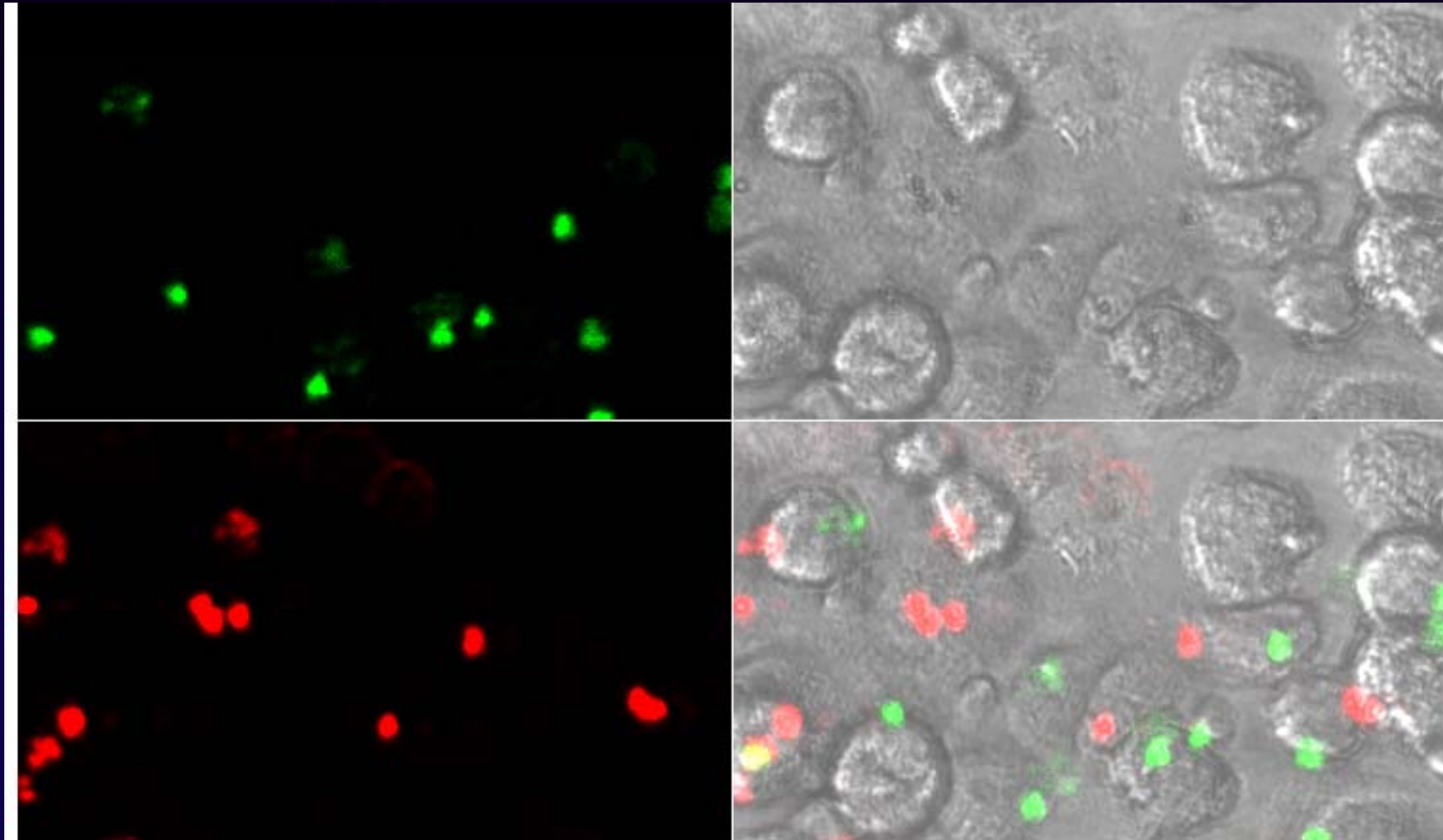
Bacterial pathogens

Apoptotic cells

Phagocytosis

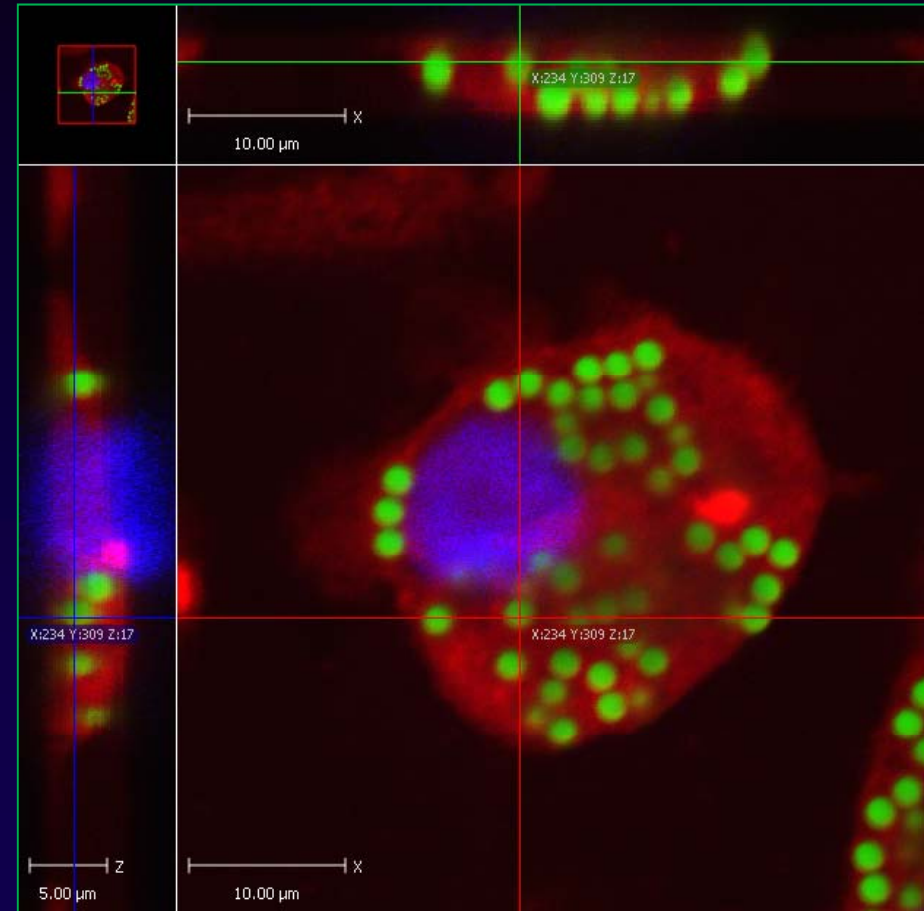
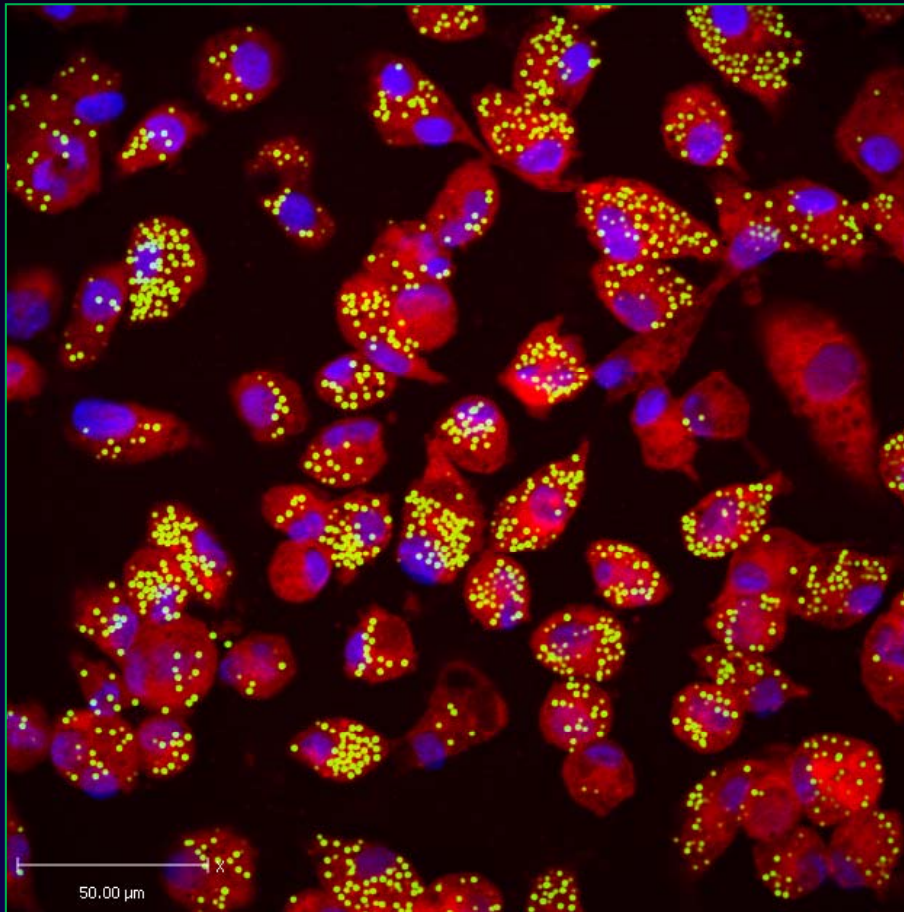
- Efferocytosis

Macrophage Phagocytosis



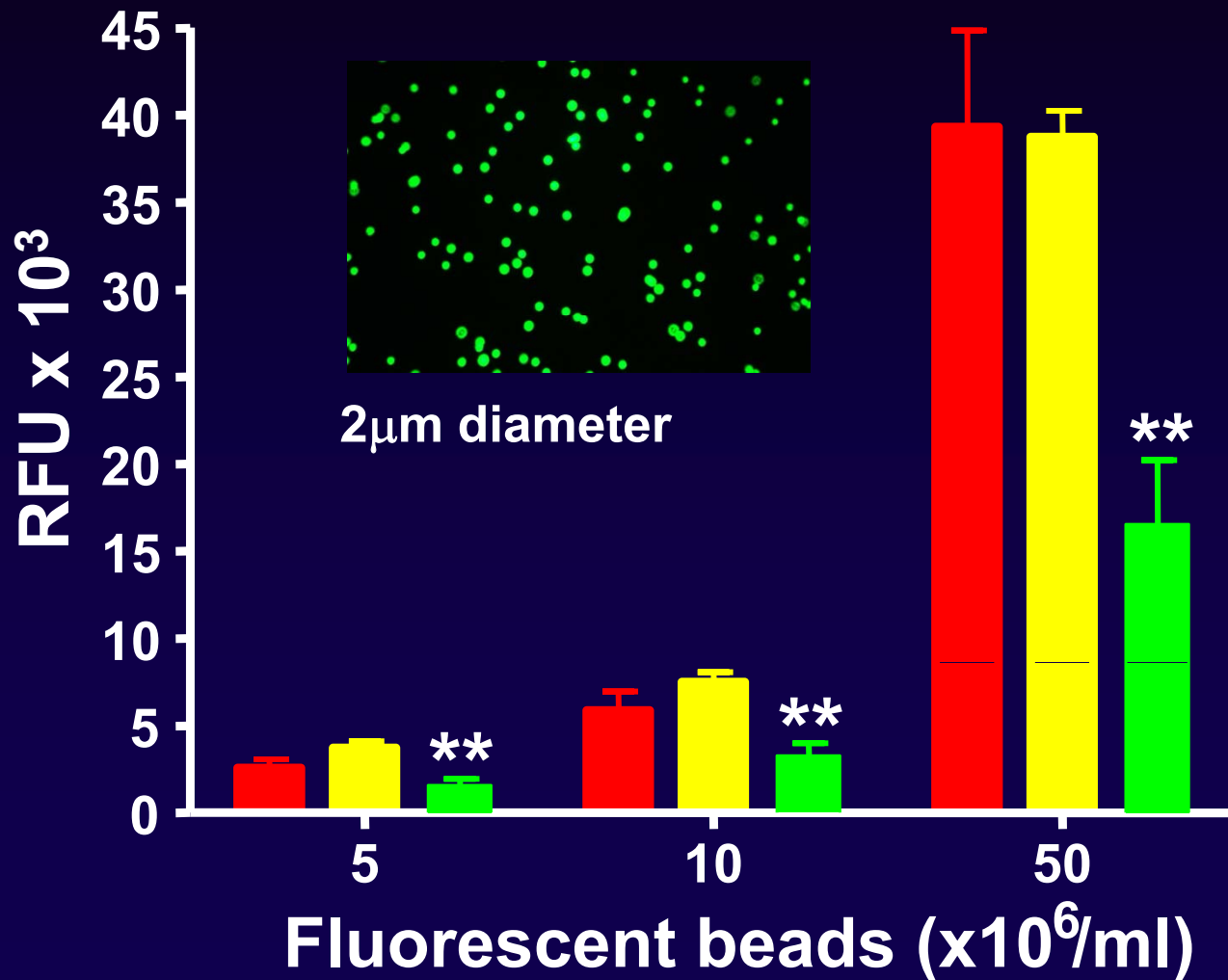
Macrophage Phagocytosis

Confocal imaging of phagocytosis in macrophages and dendritic cells



Phagocytosis of Beads

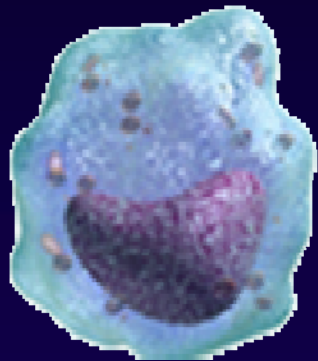
Alveolar Macrophages, MDM,
Tissue Macrophages



Different Types of Pulmonary Macrophages

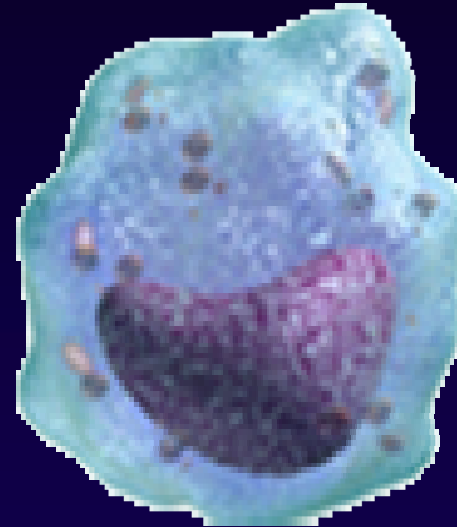
Interstitial M ϕ

8 μm



Alveolar M ϕ

16 μm



Macrophage Polarization

M1

M2

Membrane receptors



IFN_γ/LPS

IL-10

Cytokines



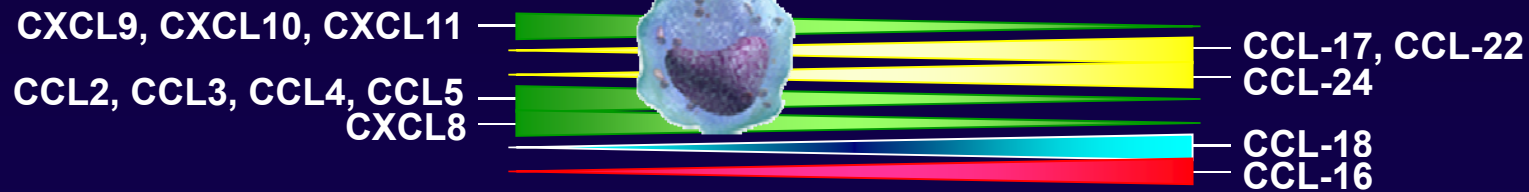
IL-4/IL-13

Cytokine receptors



IL-4/IL-13, IL-10

Chemokines



Chemokine receptors



Effector molecules



*Mantovani et al, (2002)
Trends in Immunol.
23: 549-555*

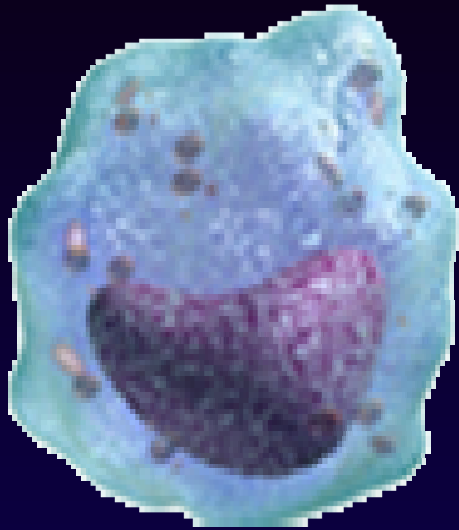
Changes in Lung Inflammatory Cells

Inflammatory cells	Asthma	COPD
CD45 ⁺	2	2
CD3 ⁺	2	4
CD4 ⁺	3	3
CD8 ⁺	2	8
Macrophages	0	9
Neutrophils	-2	2
Eosinophils	93	4

Fold change in number of cells vs healthy controls

Jeffery, P.K (2000) Chest 117: 251S-260S

Macrophages and Asthma



**Lack of AHR
suppression**

Remodelling

↑
**MMP-9
TGF- β**

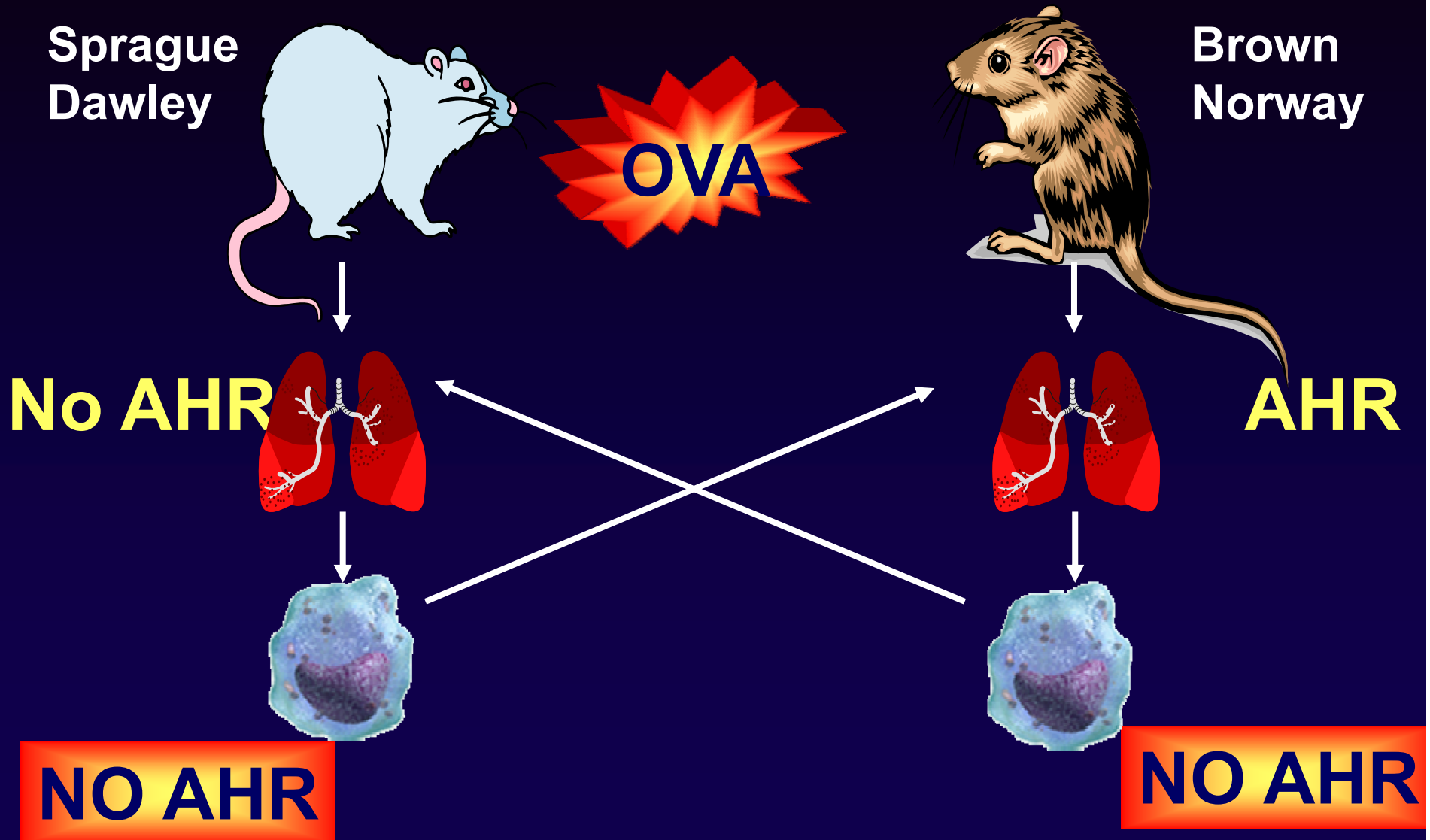
T-cell responses

↑
CD4+ IL-5

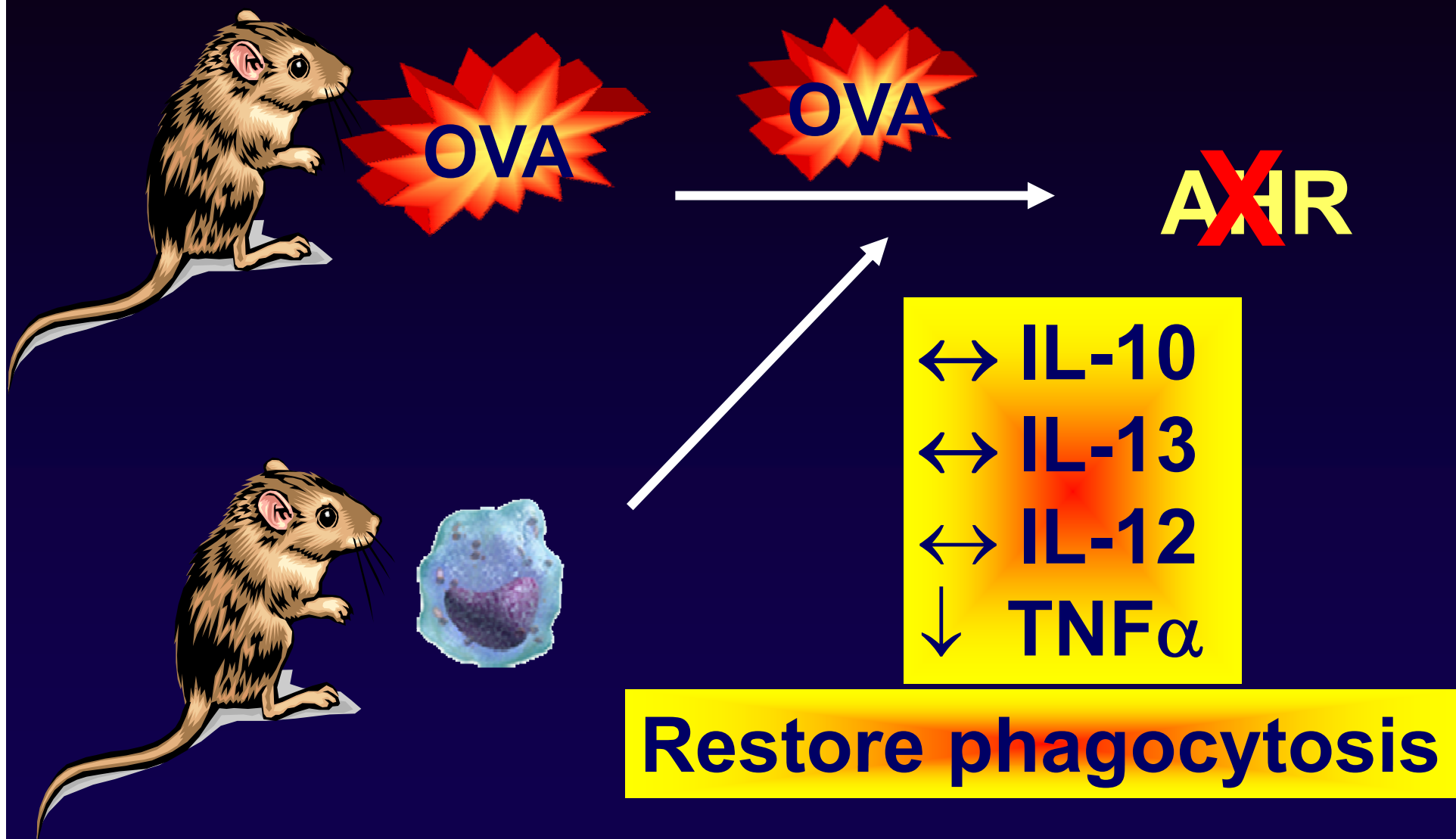
Mediators

↑
**CO
IL-12, IL-18**

Macrophage Suppression of AHR



How can Macrophages Suppress AHR?



Summary: Macrophages in Asthma

- **Modulate the inflammatory response**
 - ↓ IL-10, IFN λ , ↑ IL-4
- **Remodelling**
- **Regulate AHR**

Chronic Obstructive Pulmonary Disease

- Chronic bronchitis

mucus

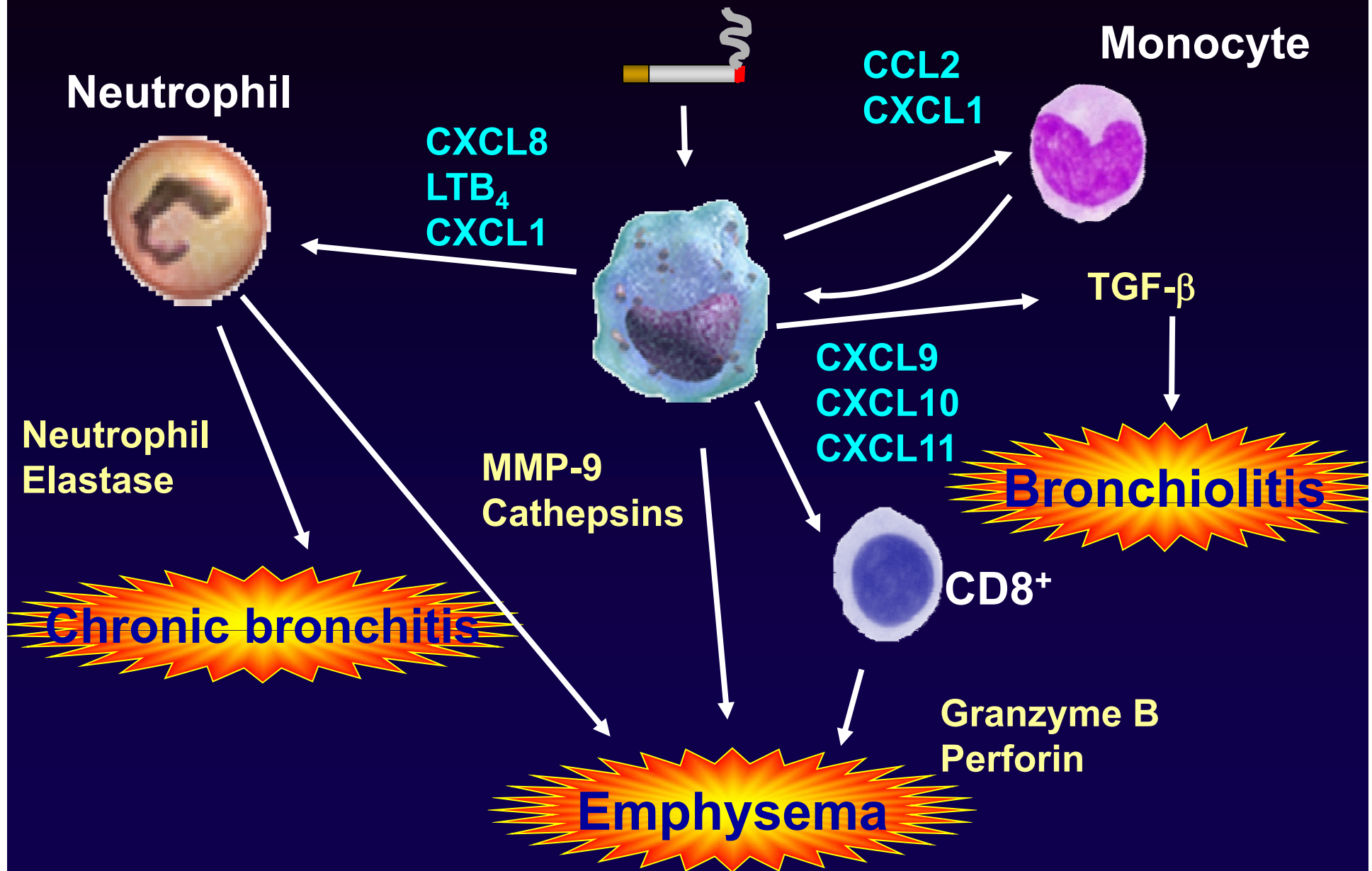
Chronic
Inflammation

'small'
'collapse'

- Emphysema



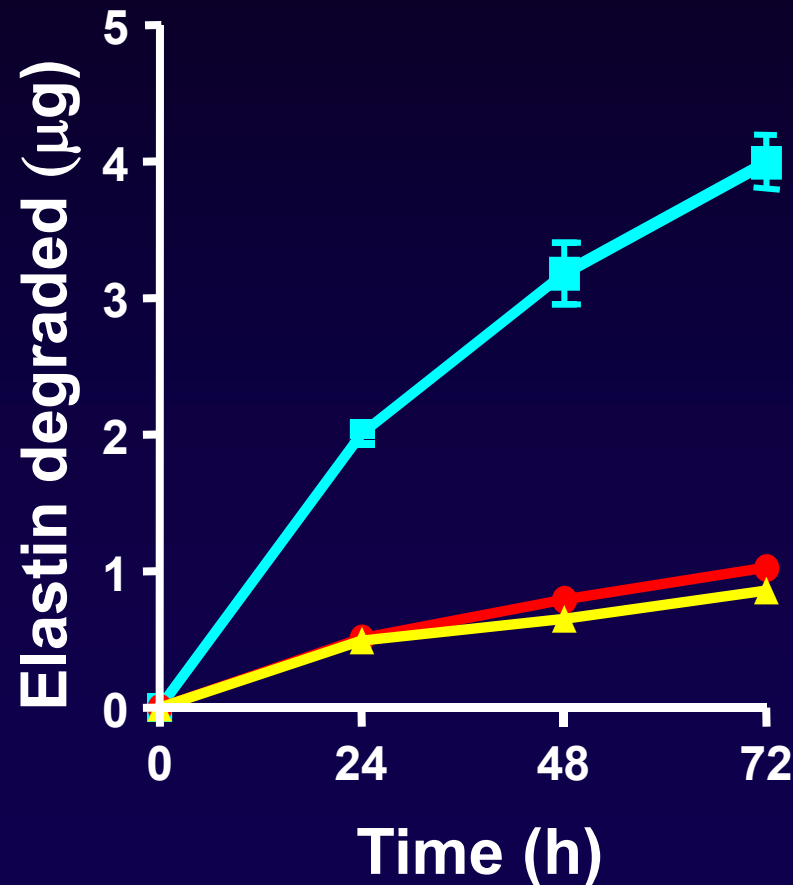
Macrophages in COPD



Macrophages and COPD

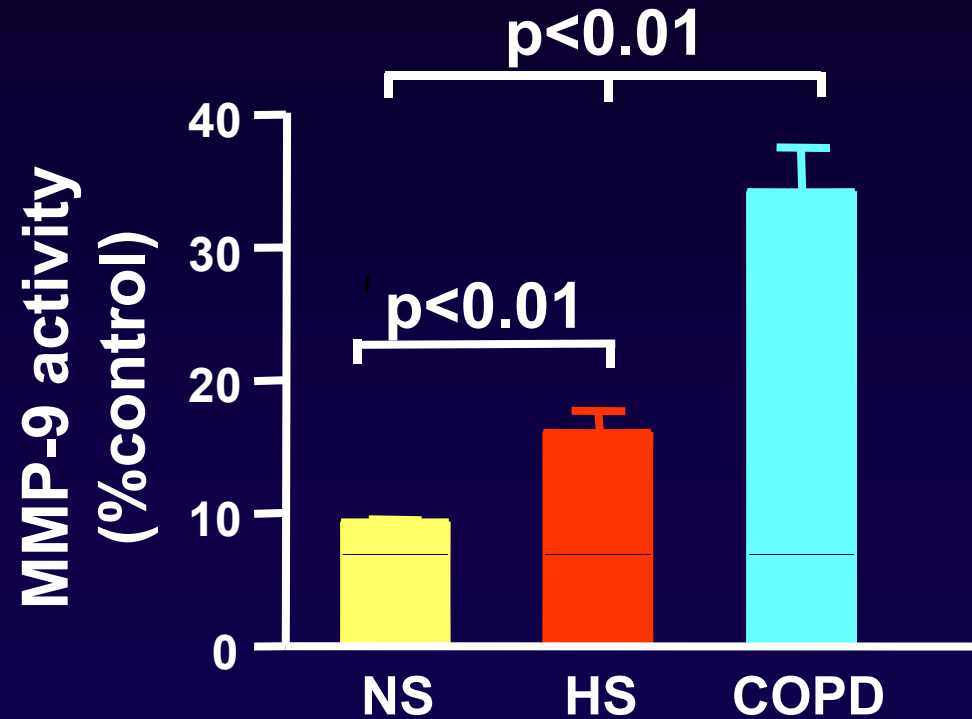
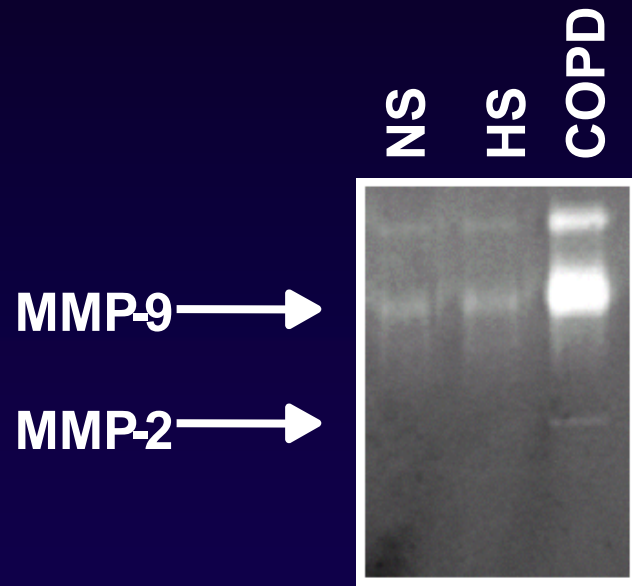
- ↑ Macrophages in BAL and sputum
- ↑ Macrophages at sites of proteolytic damage
- ↑ Macrophages produce increased proteases and chemokines
 - **MMP-9, IL-8**

Release of Elastolytic Activity by Alveolar Macrophages (normal, smokers, COPD subjects)

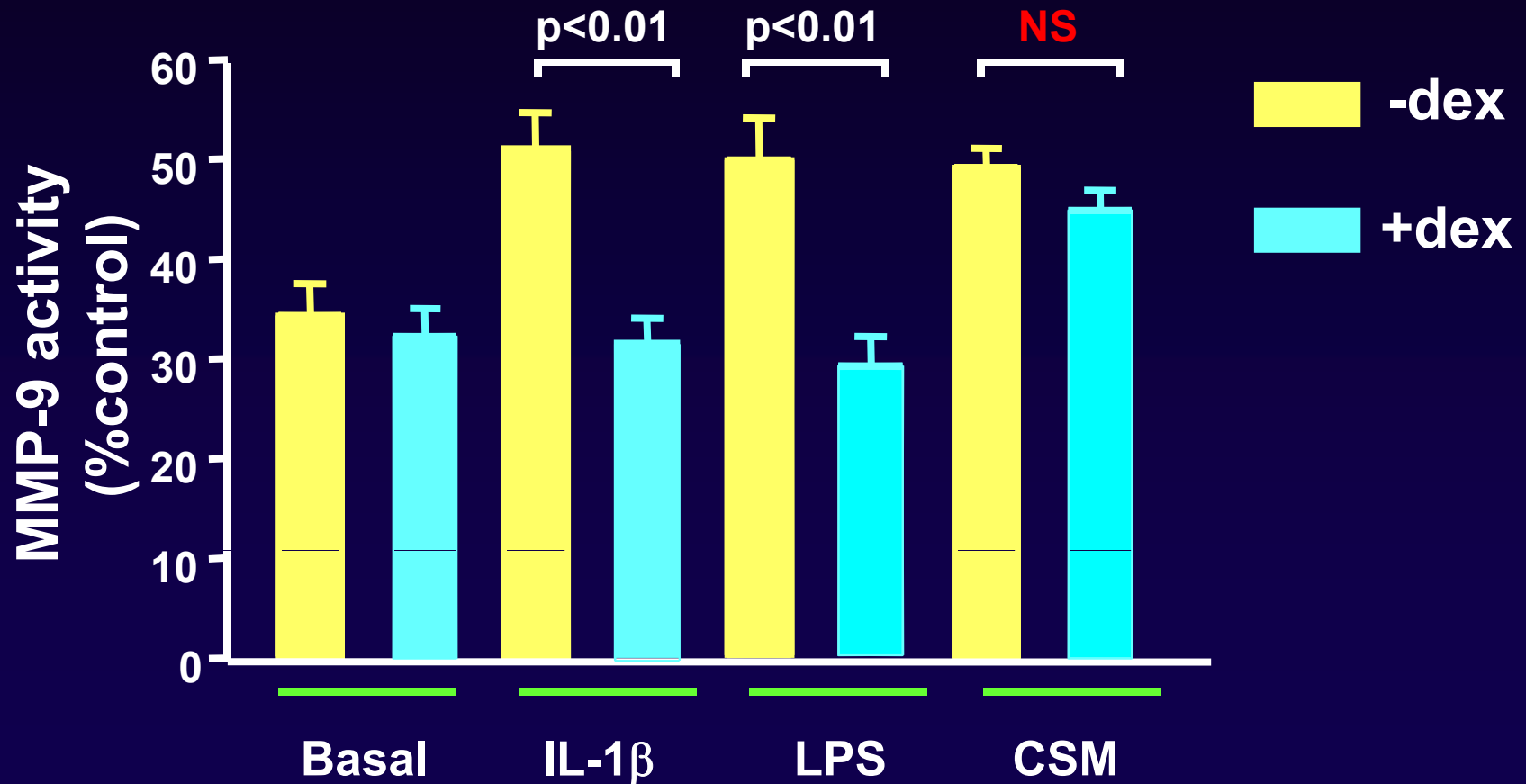


Russell et al., (2002) AJP Lung Cell Mol. Physiol. 283:L867-873

Release of MMP Activity by Alveolar Macrophages



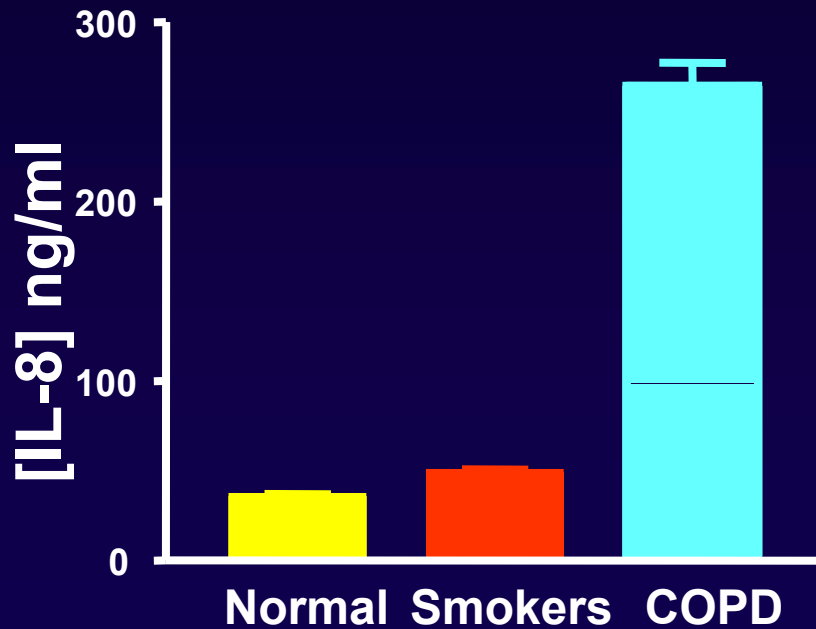
Effect of Dexamethasone on Release of MMP Activity by Macrophages from COPD Patients



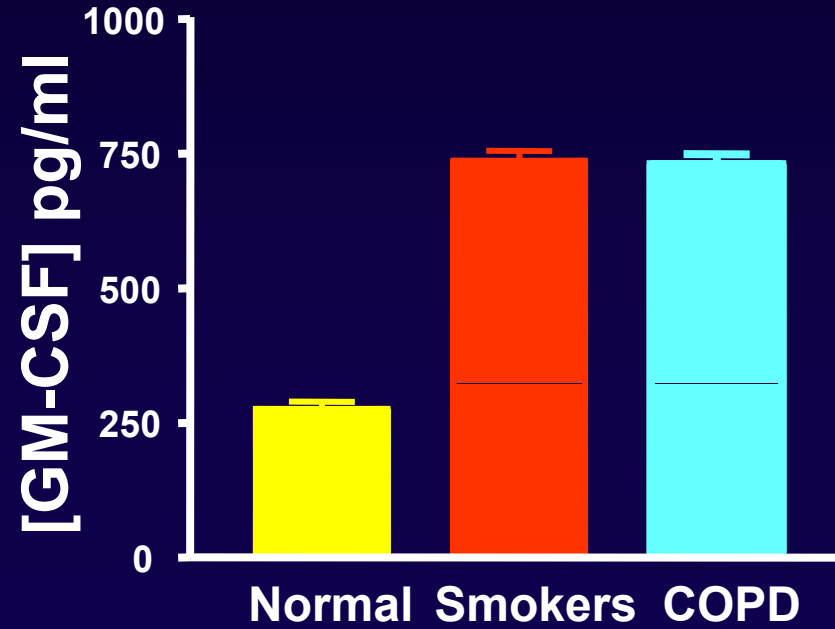
Russell et al., (2002) AJRCMB 26:602-9

Cytokine Release by Alveolar Macrophages

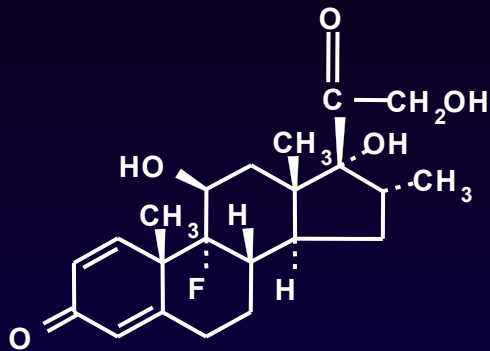
IL-8



GM-CSF

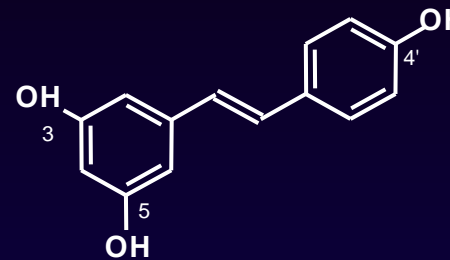


Anti-inflammatory agents



Dexamethasone

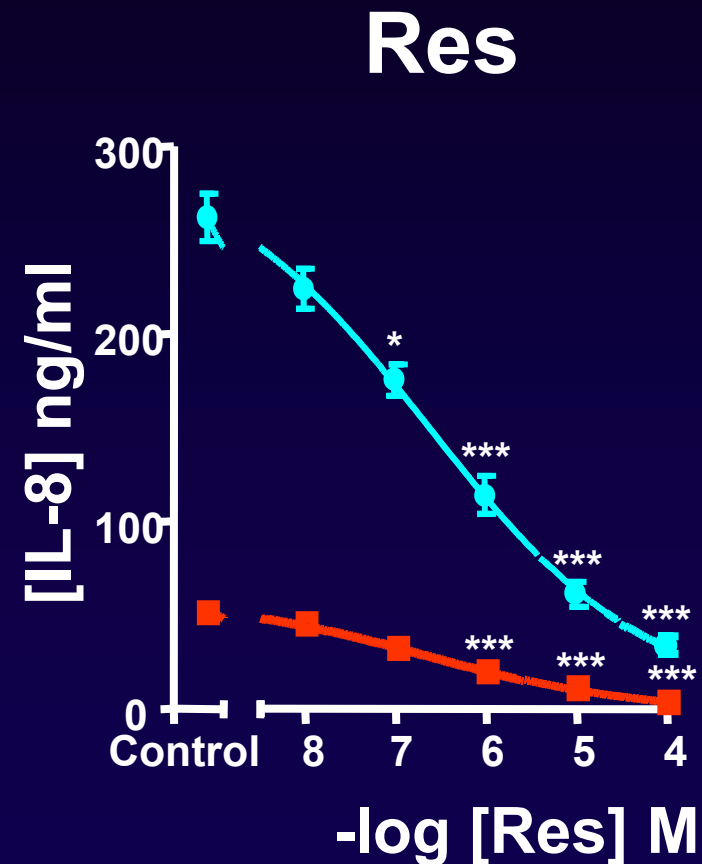
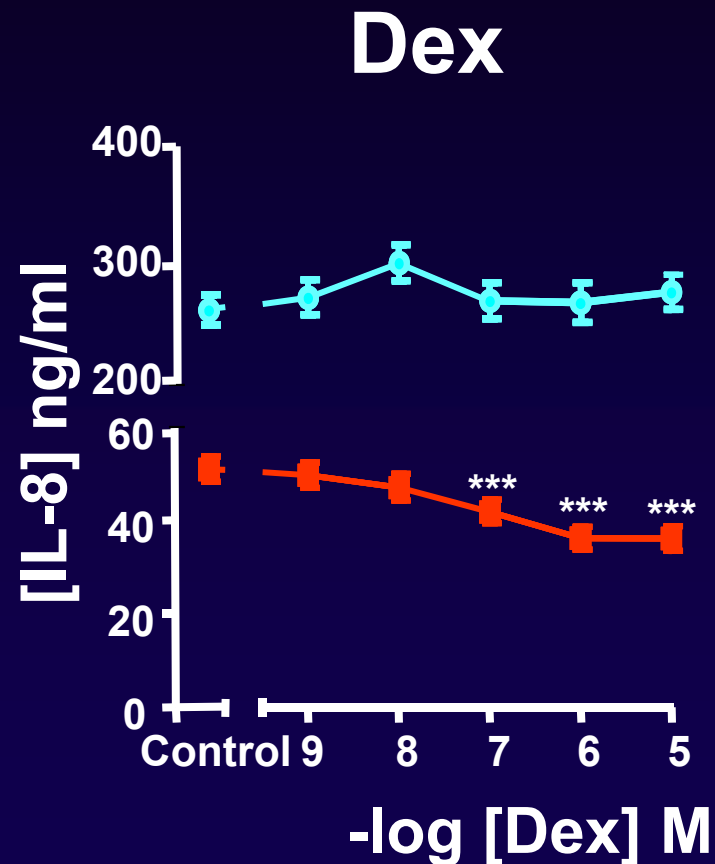
- **Glucocorticosteroid**
- **Anti-inflammatory**



Resveratrol

- **Found in the skin of peanuts, red fruit (plums and grapes) and red wine**
- **Anti-oxidant and anti-proliferative properties**

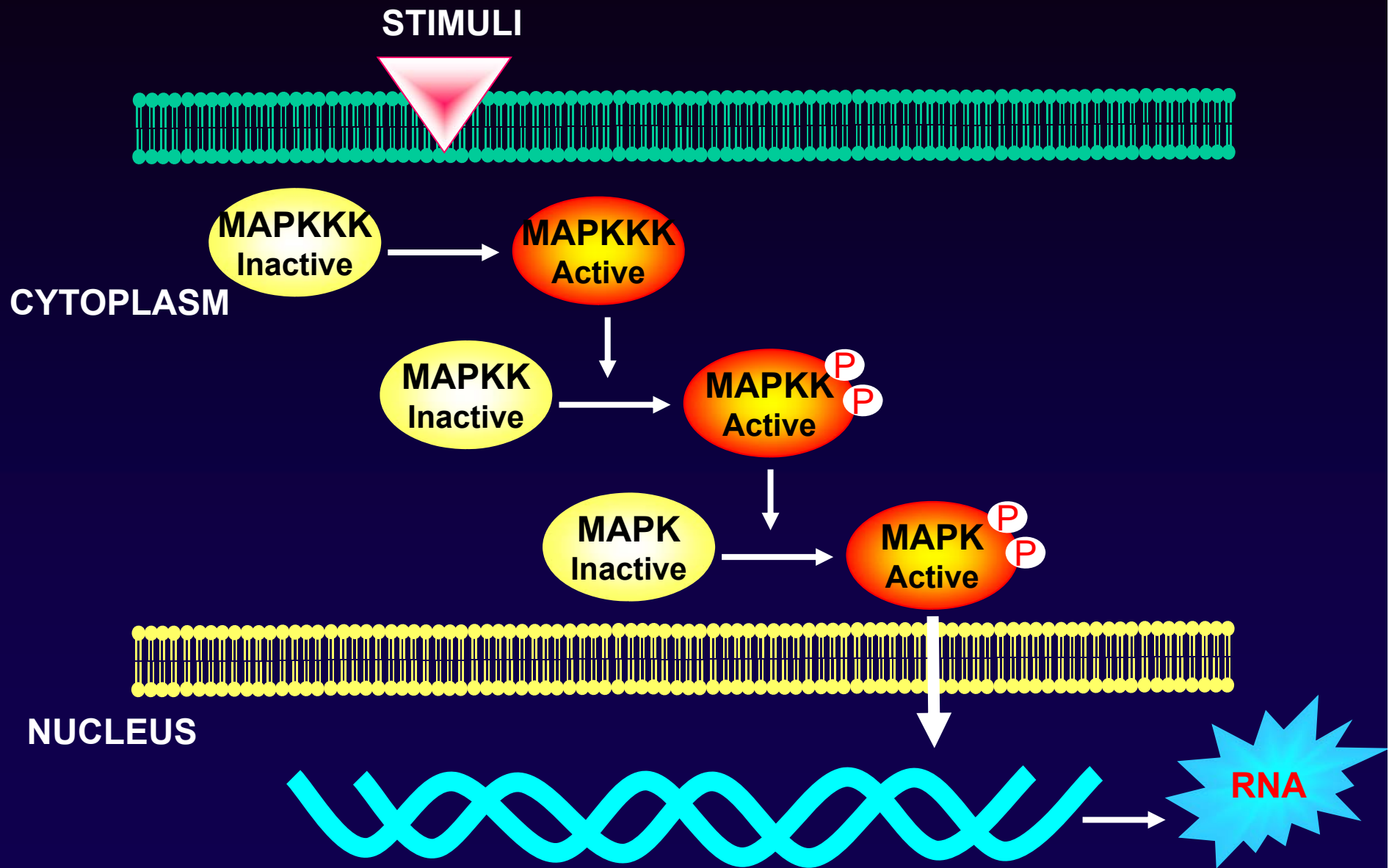
Effect of Dexamethasone and Resveratrol on IL-8 release by Alveolar Macrophages (smokers, COPD)



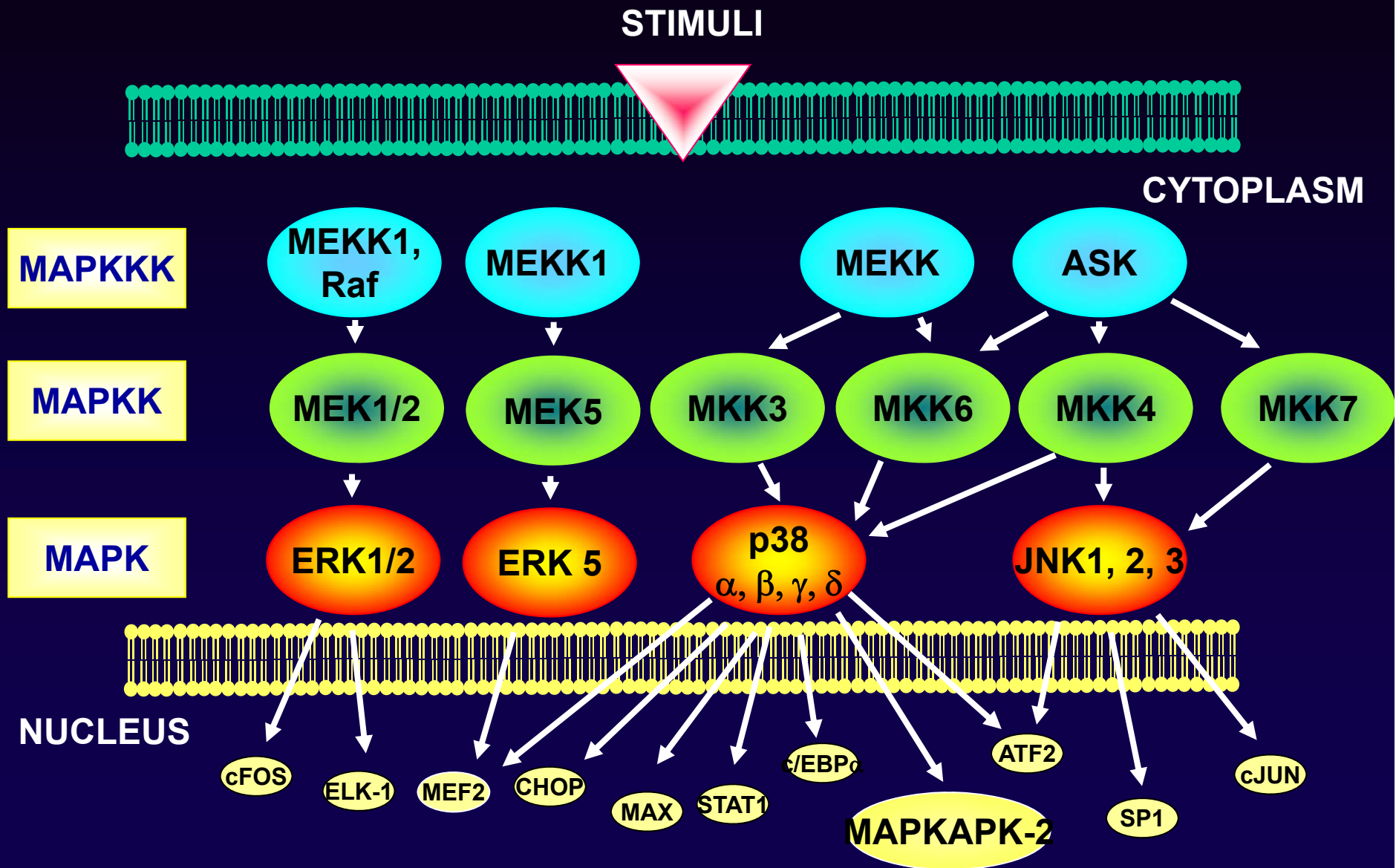
Culpitt et al., (2003) AJRCCM. 167:24-31

Culpitt et al., (2003) Thorax 58:942-946

MAP Kinase Pathways

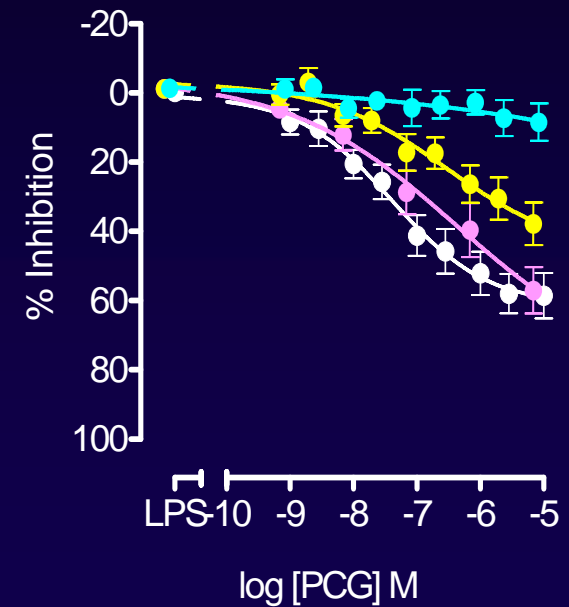
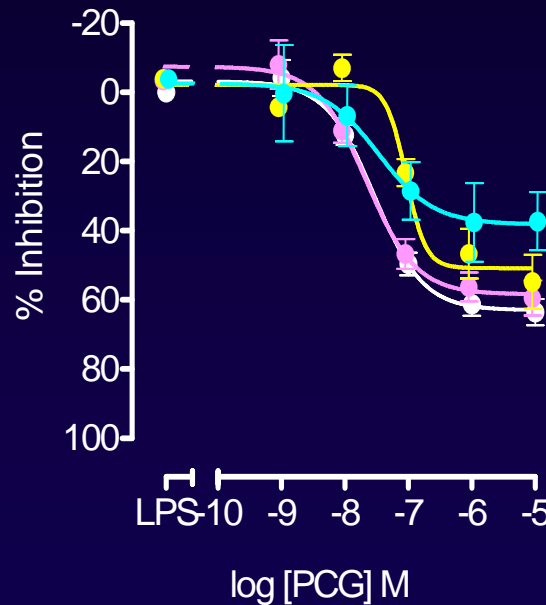
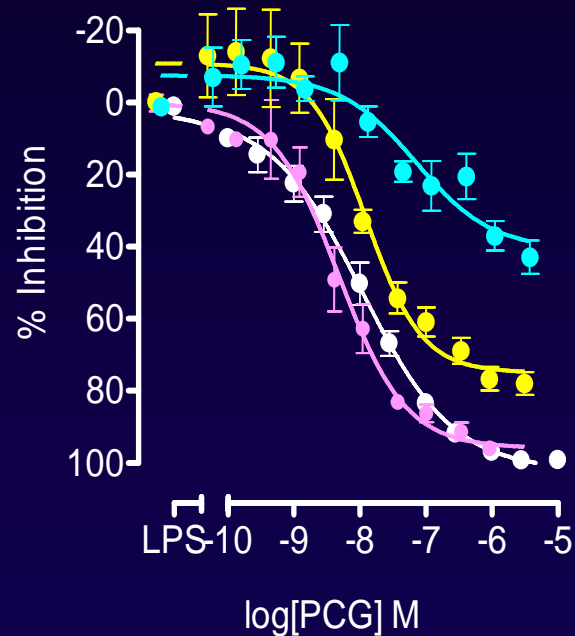


MAP Kinase Pathways



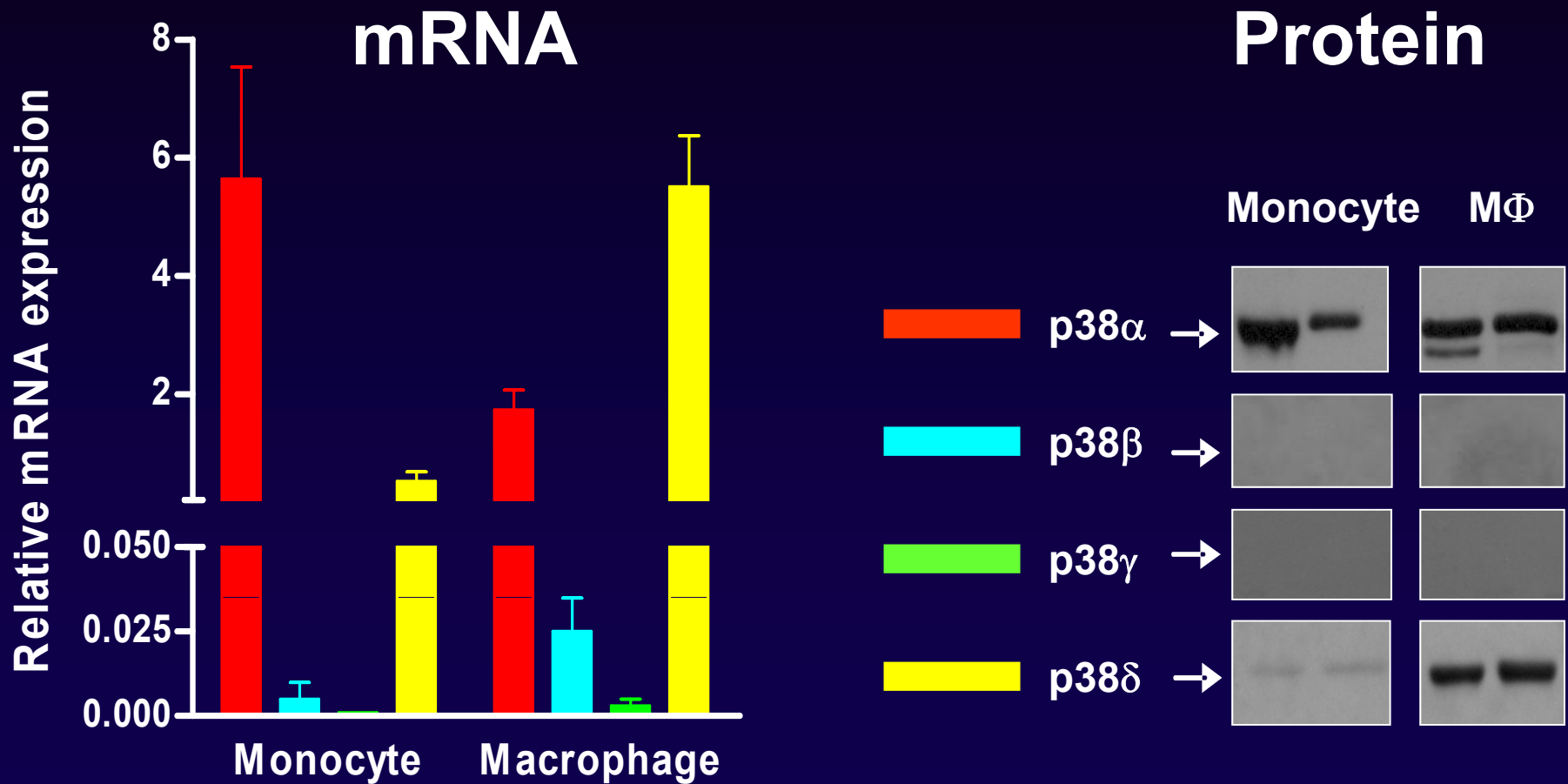
Effects of a PCG on Cytokine Release

MONOCYTES > **Derived Macrophages** > **MACROPHAGES**

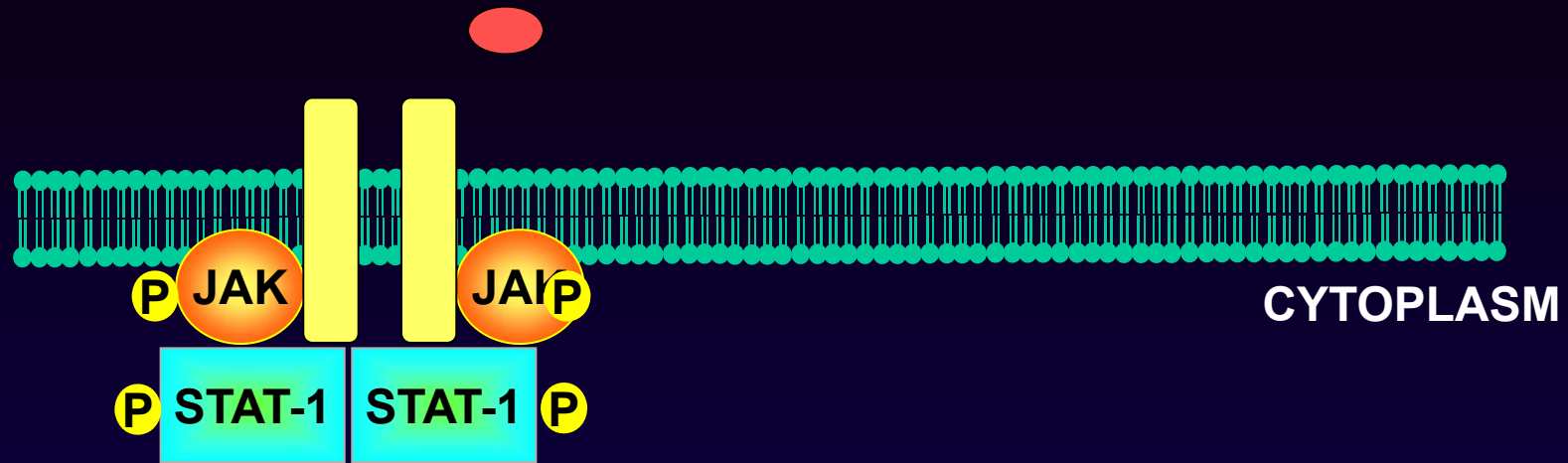


TNF-α = GM-CSF > IL-6 > IL-8

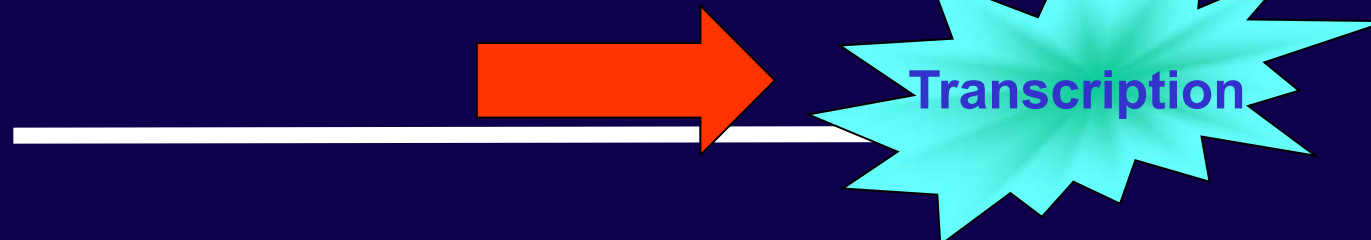
Differential Expression of p38 MAP Kinase Isoforms in Monocytes and Macrophages



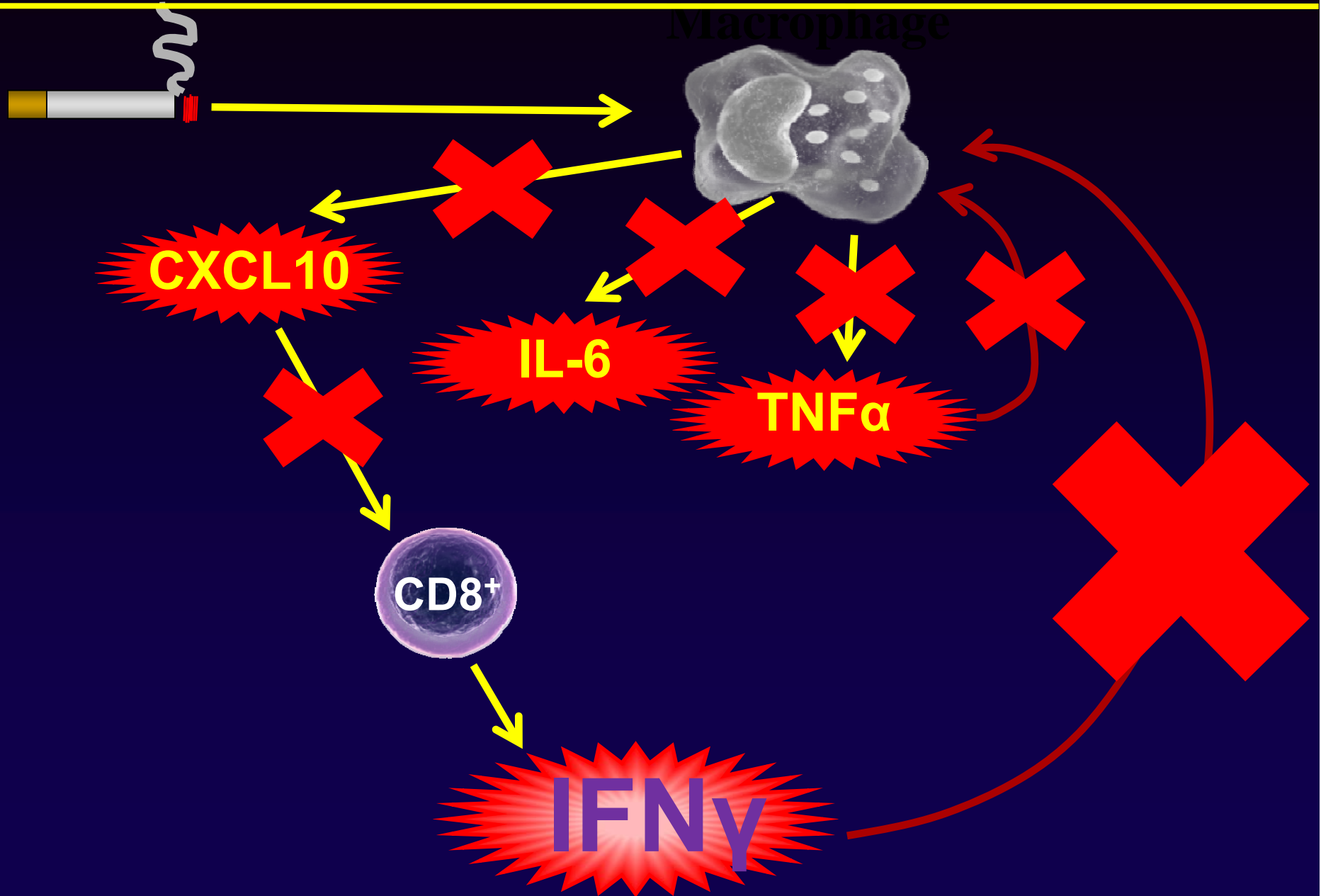
IFN γ Signalling



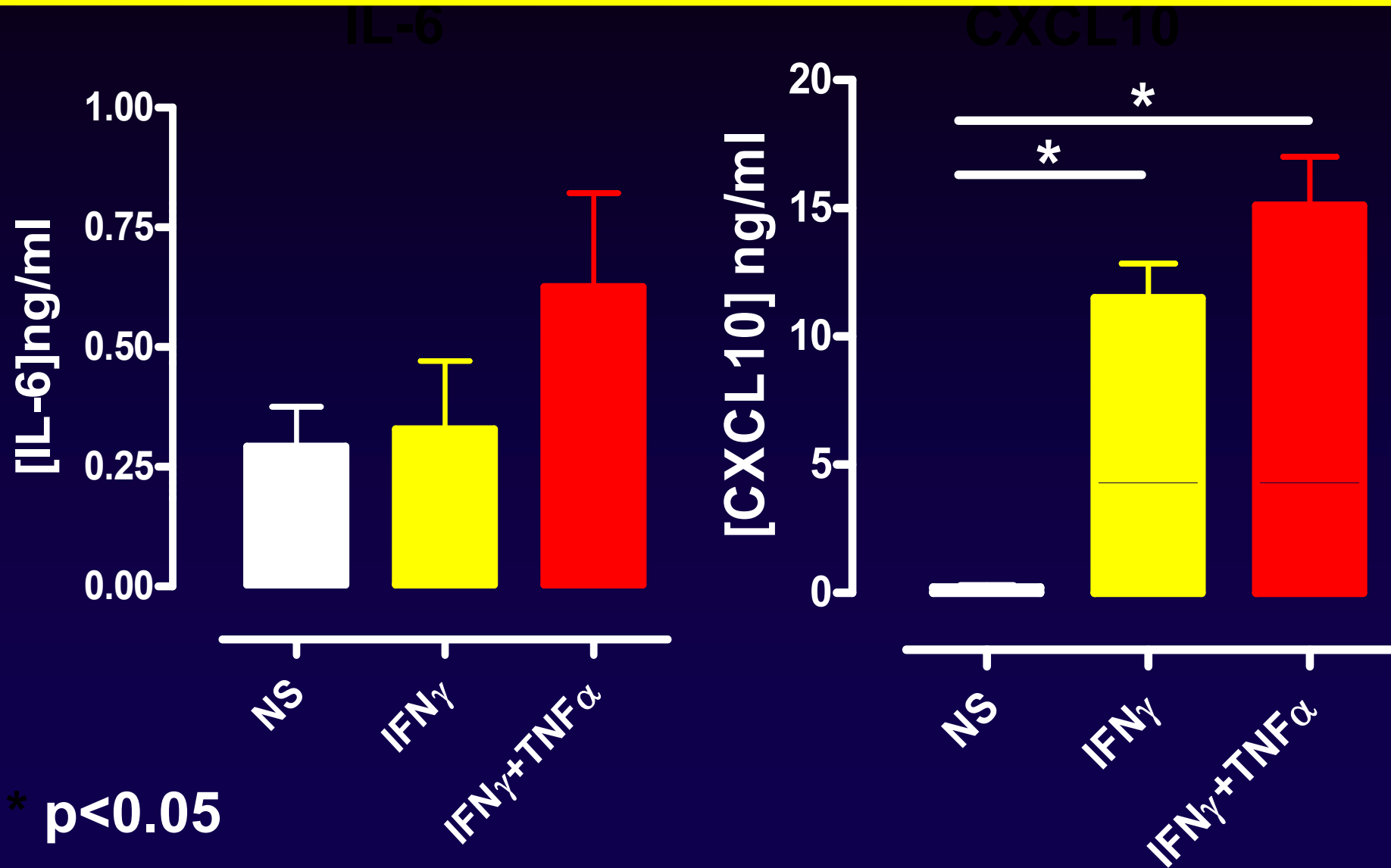
NUCLEUS



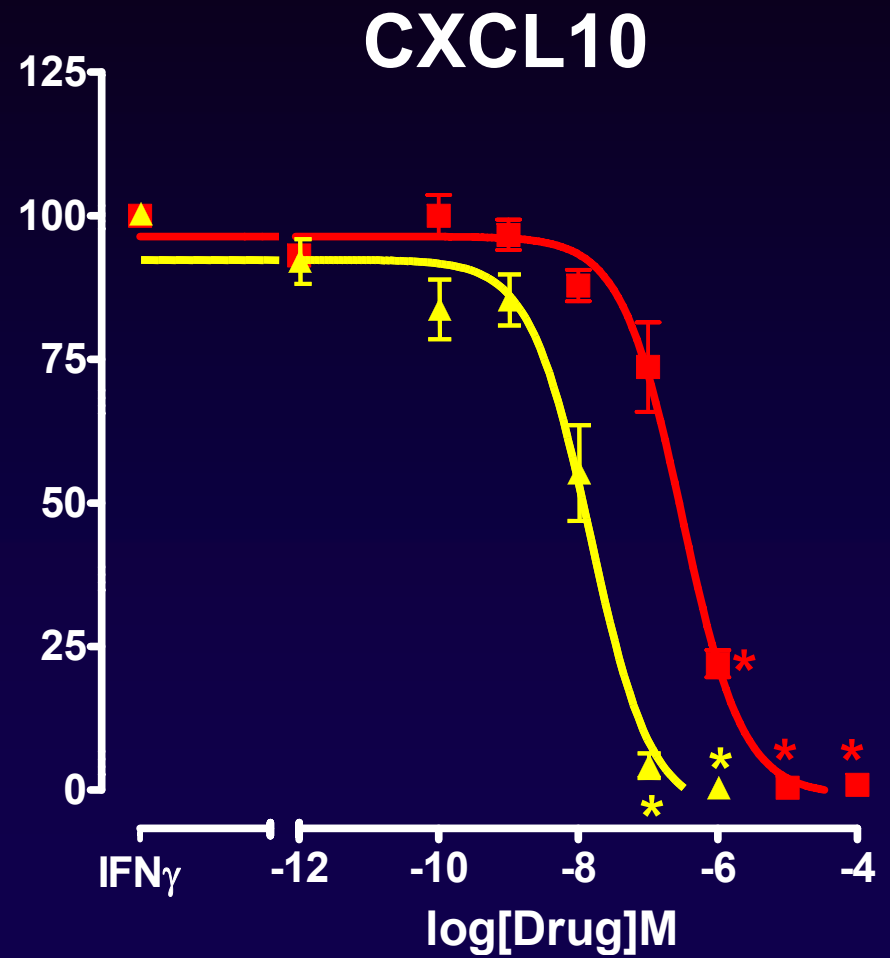
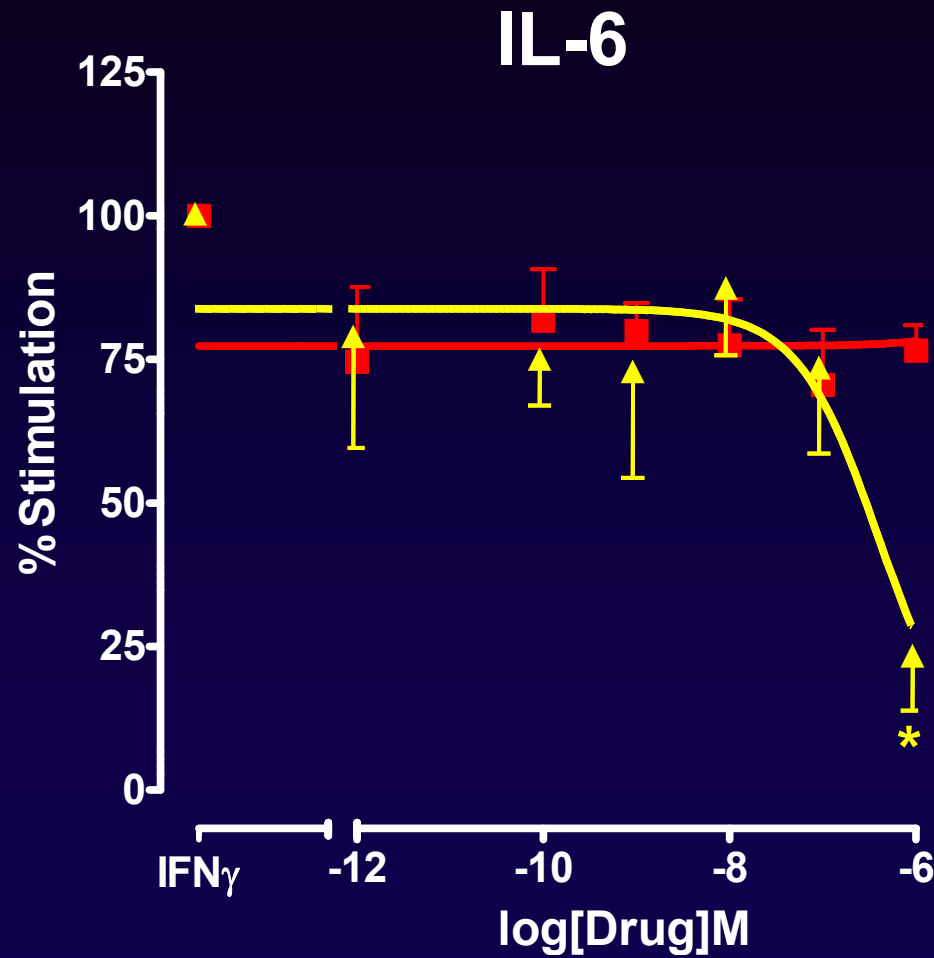
Inflammation in COPD



Effect of IFN γ \pm TNF α on Macrophage Stimulation of IL-6 and CXCL10 (n=4-5)



Effect of JAK/STAT inhibitors (PF95, PF13) on IFN γ stimulated cytokine release (n=4-5)

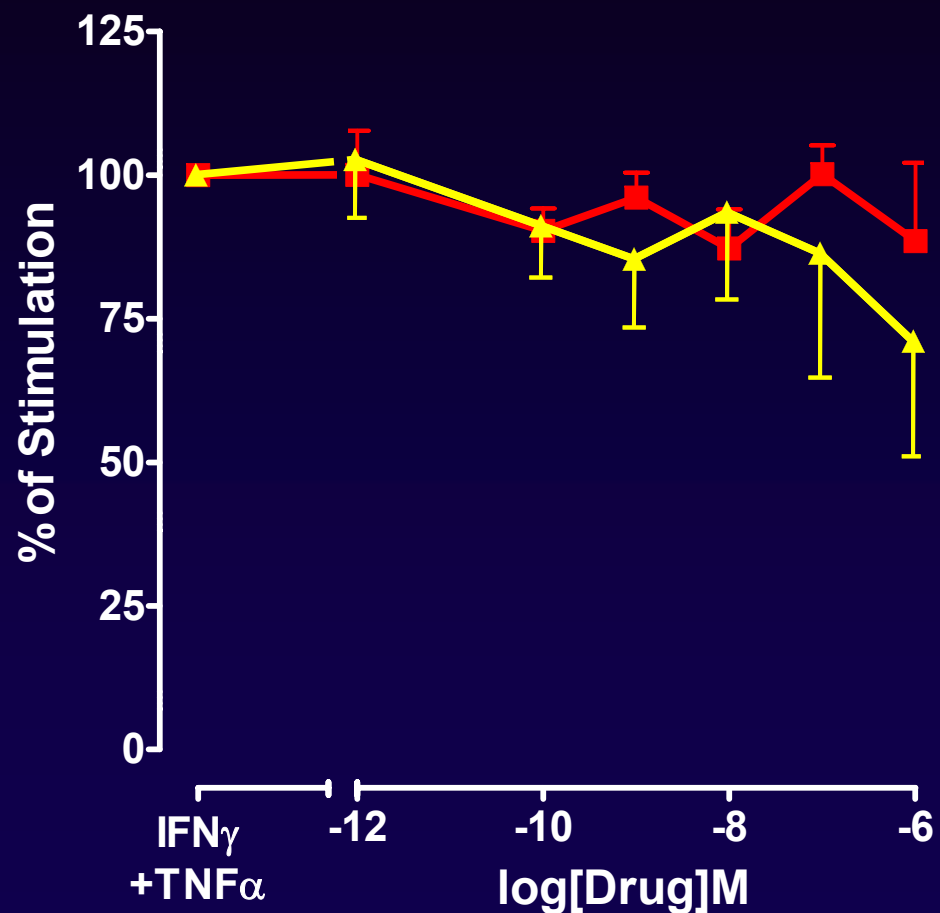


* $p < 0.05$ EC_{50} PF13: $0.5 \pm 0.3 \mu M$

EC_{50} PF95: $0.3 \pm 0.1 \mu M$
 EC_{50} PF13: $14.6 \pm 4.2 nM$

Effect of JAK/STAT inhibitors (PF95, PF13) on IFN γ +TNF α stimulated cytokine release (n=4-5)

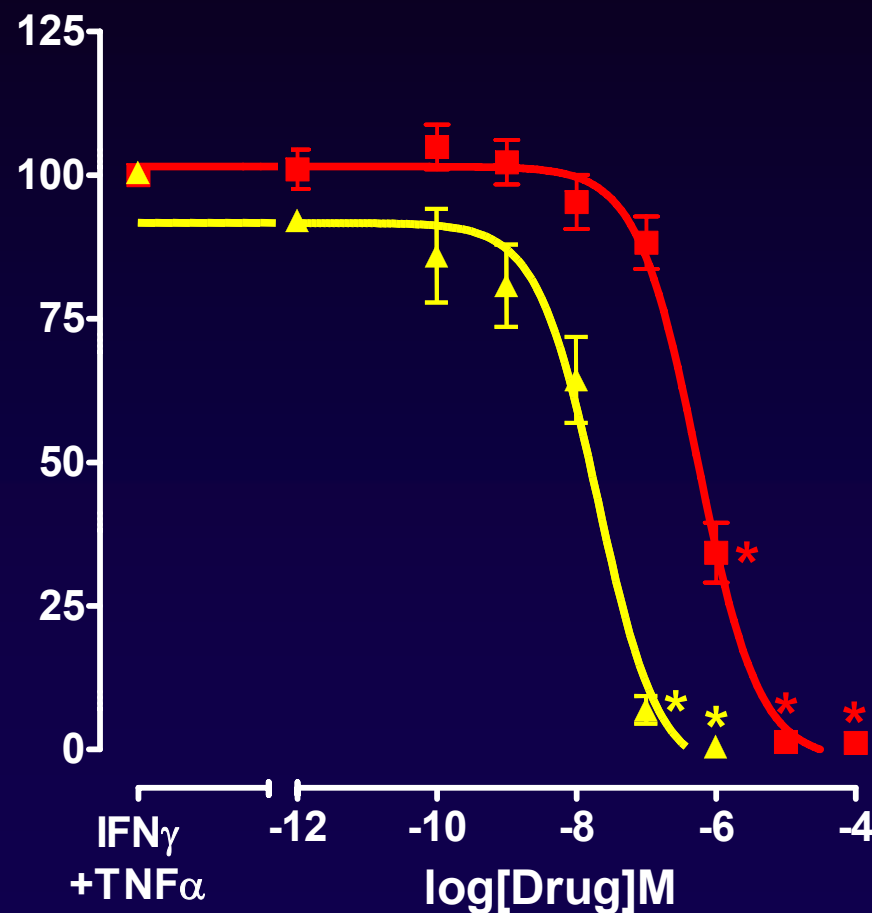
IL-6



EC₅₀ PF13: 2.3 ± 0.9 μM

* p < 0.05

CXCL10



EC₅₀ PF95: 0.6 ± 0.1 μM

EC₅₀ PF13: 18.9 ± 3.7 nM

Summary

Inhibition of signal transduction pathways could provide anti-inflammatory therapies

However, macrophage differentiation state may alter these responses

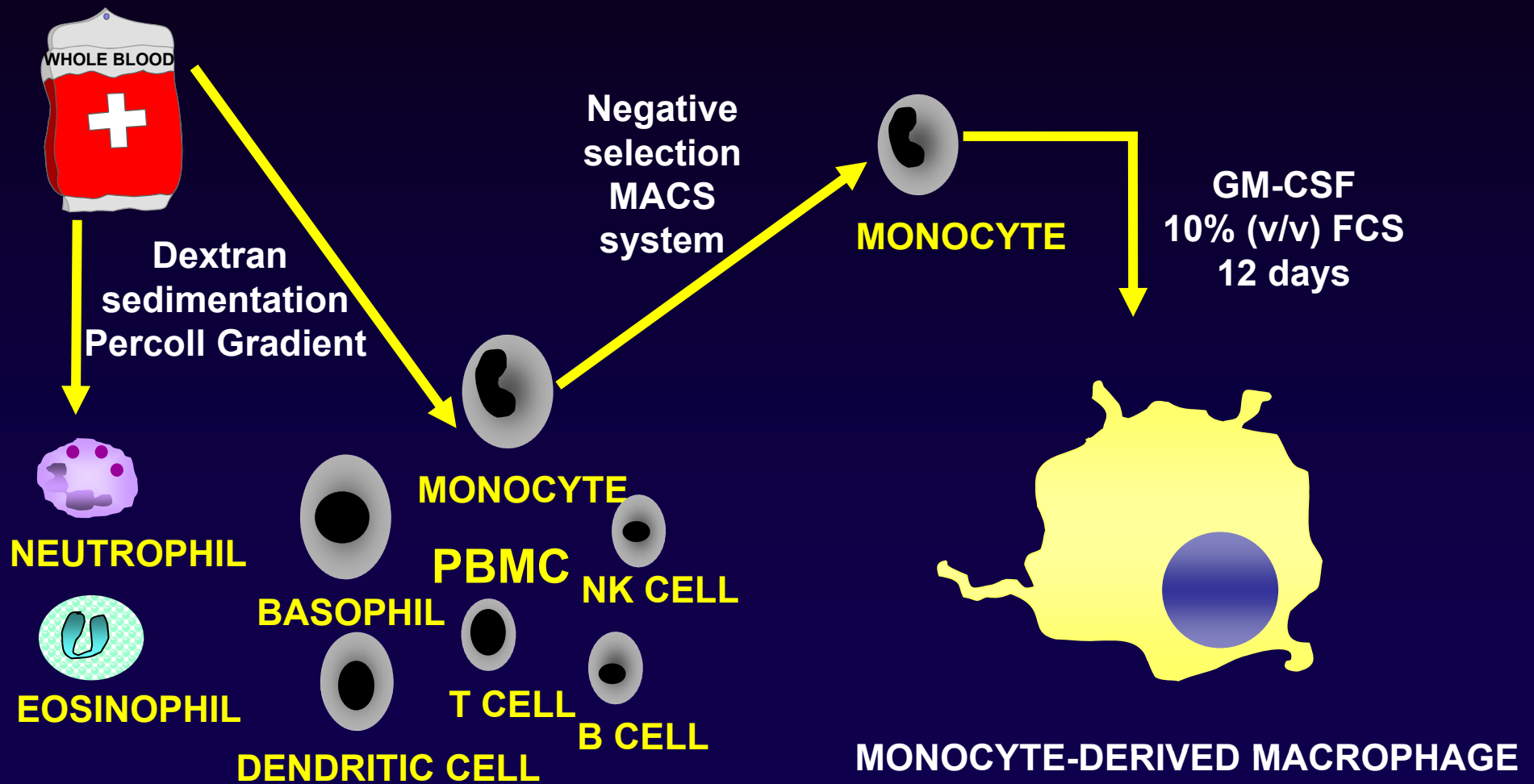
Macrophage Phagocytosis in COPD

- Alveolar macrophages phagocytose inhaled particles and pathogens and maintain sterility of the airways
- Despite increased numbers of macrophages in COPD the lower airways are colonised
- This suggests defective pathogen clearance by macrophages in COPD

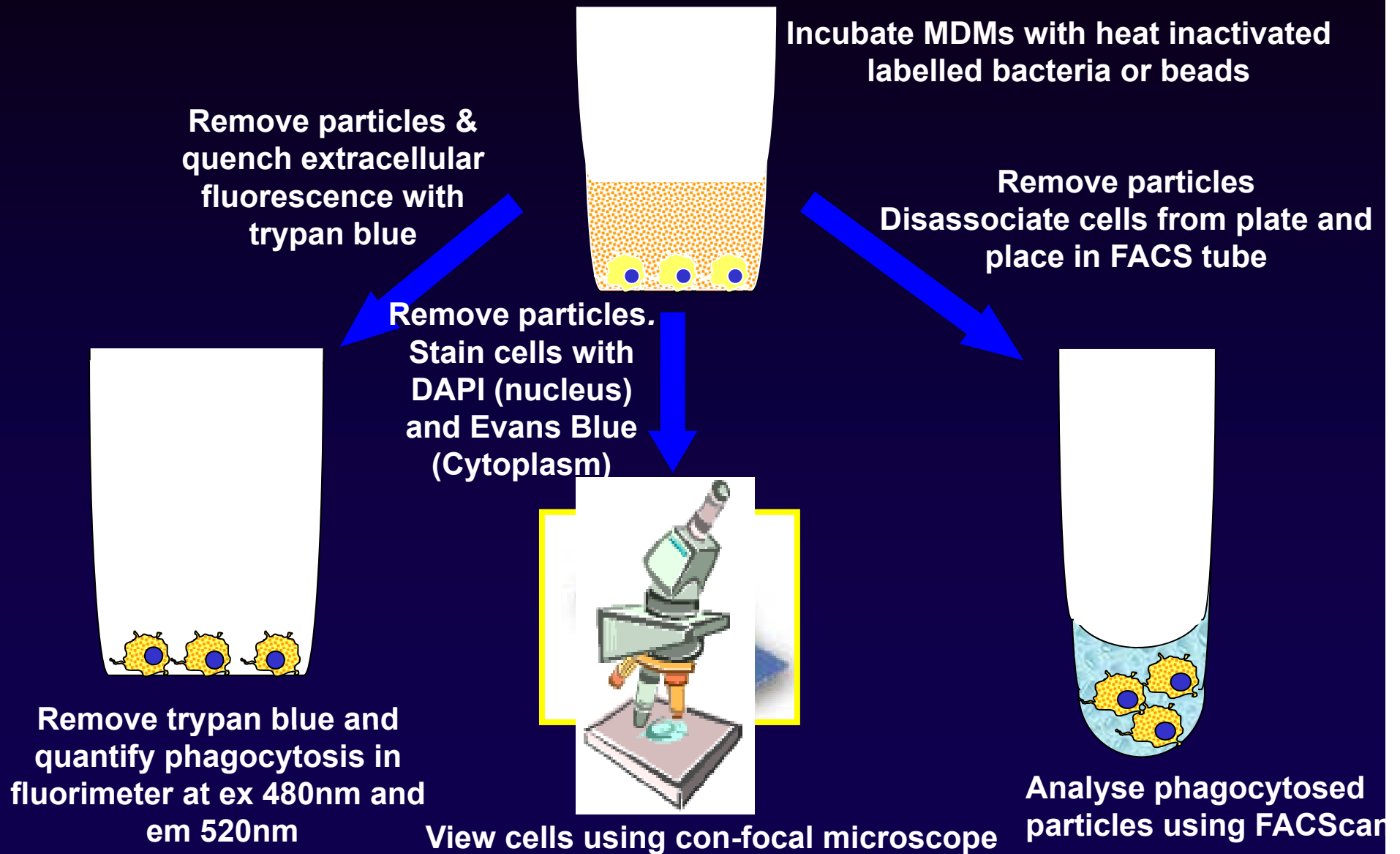
COPD and Exacerbations

- COPD exacerbations are the most common cause of hospital admissions in the UK
- Increased numbers of exacerbations are associated with decline in lung function
- Approx 40% of exacerbations are caused by bacterial infections
 - *H. influenzae*
 - *S. pneumoniae*

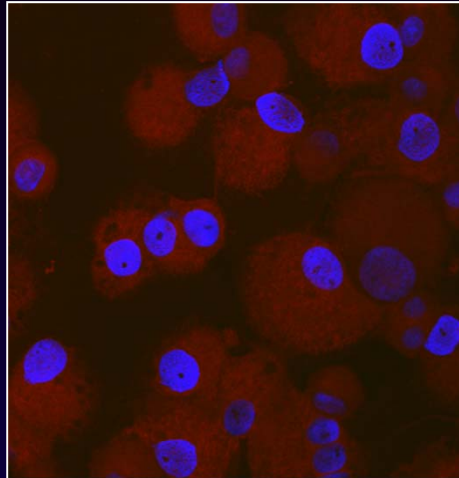
Leukocyte Isolation and Differentiation of MDM



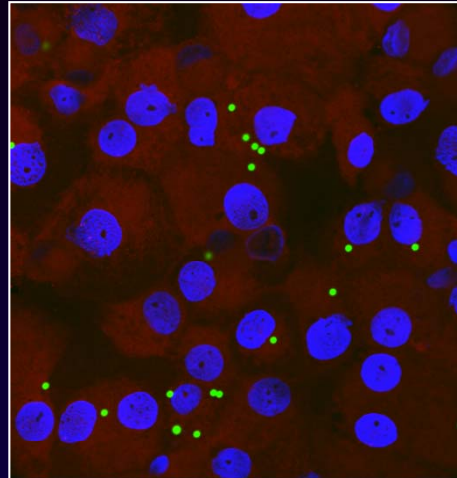
Measurement of Phagocytosis



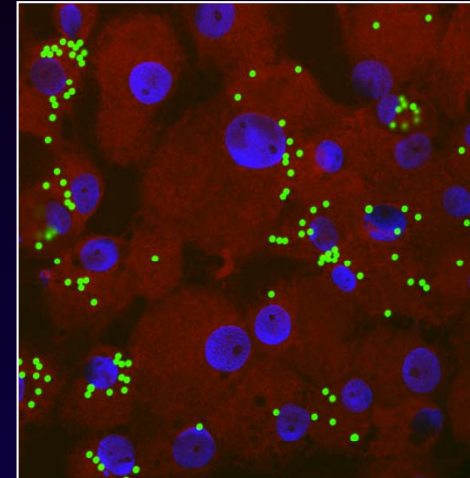
MDM phagocytosis of Beads



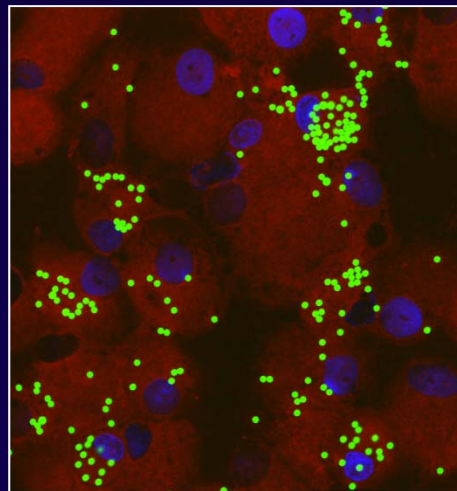
No beads



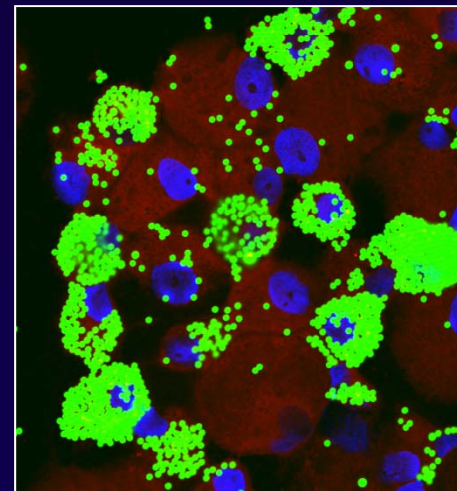
1×10^6 beads/ml



5×10^6 beads/ml



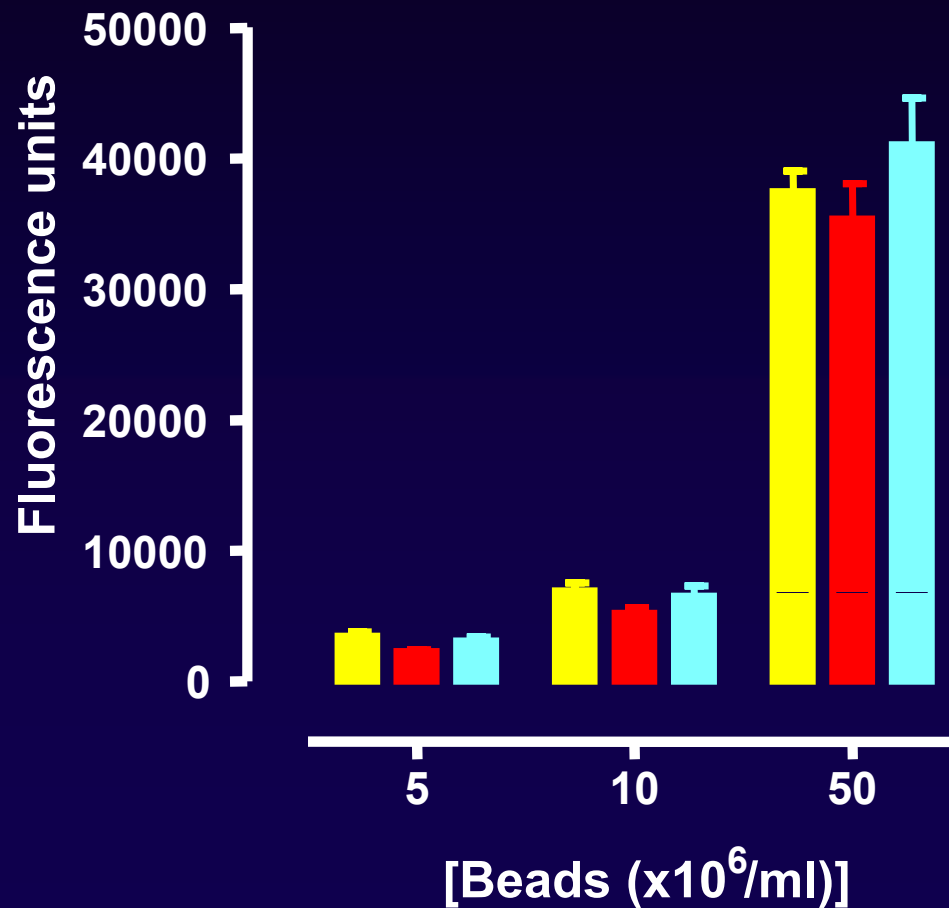
10×10^6 beads/ml



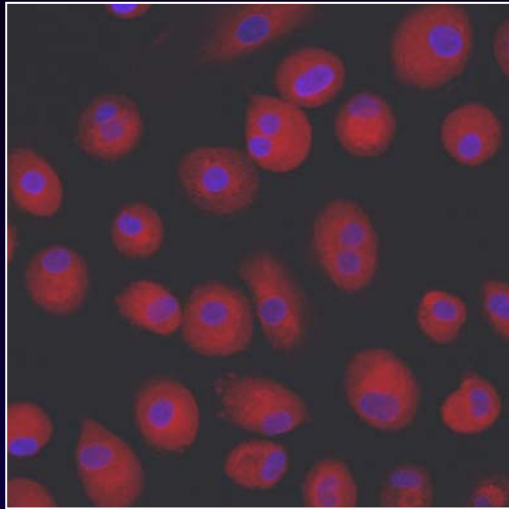
50×10^6 beads/ml

MDM phagocytosis of beads

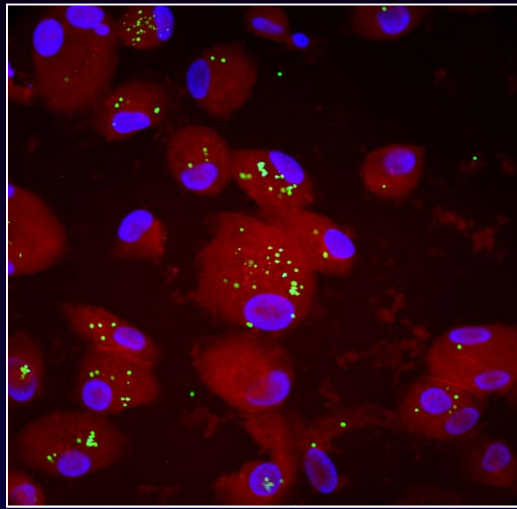
Non-smokers (n=9), smokers (n=13) and COPD subjects (n=11)



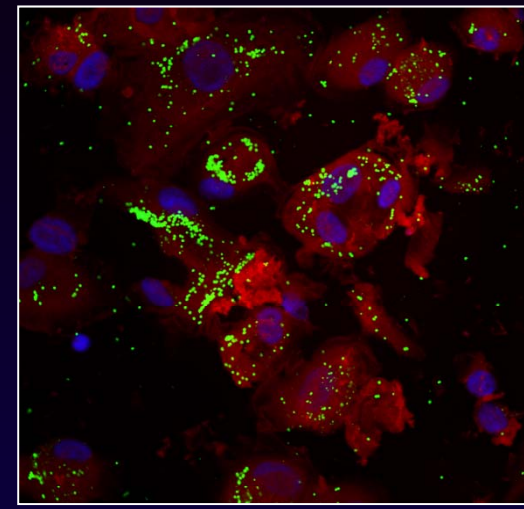
Phagocytosis of *E.coli*



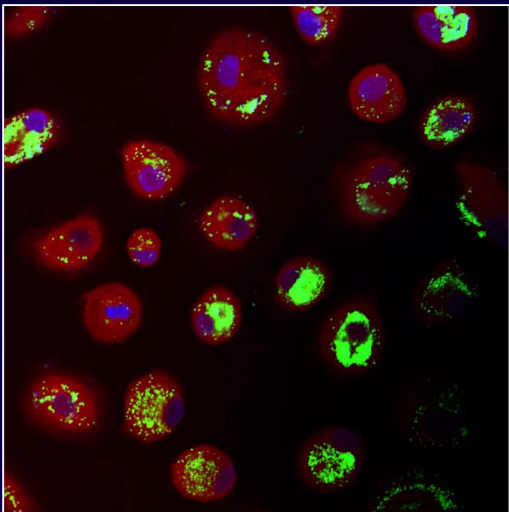
No *E.coli*



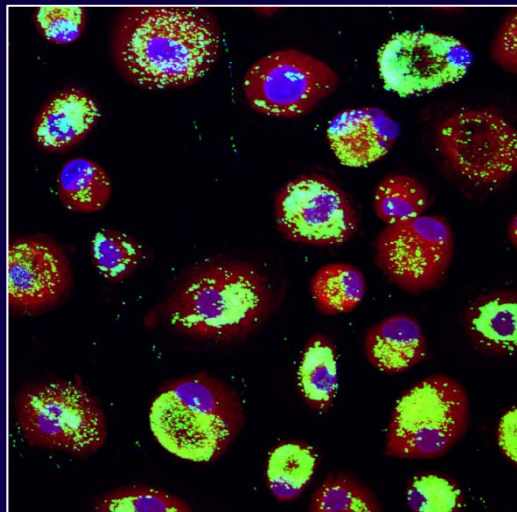
1 µg/ml *E.coli*



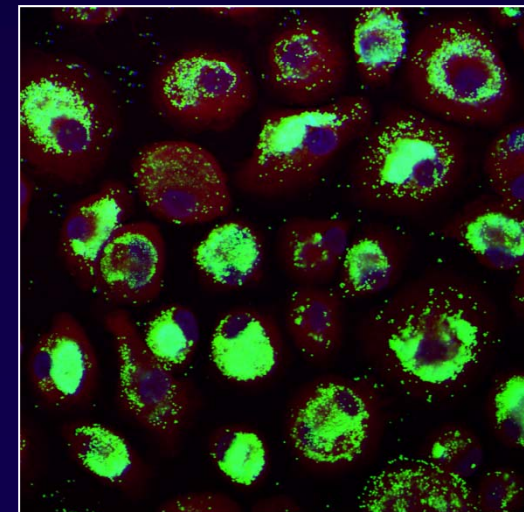
2 µg/ml *E.coli*



25 µg/ml *E.coli*



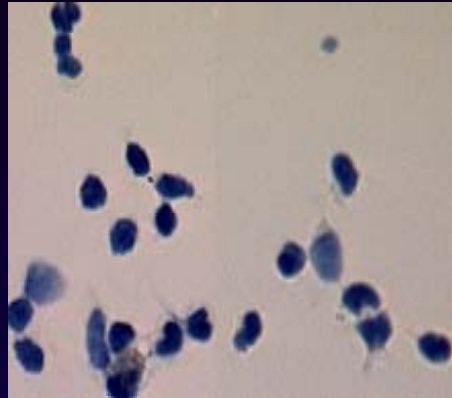
100 µg/ml *E.coli*



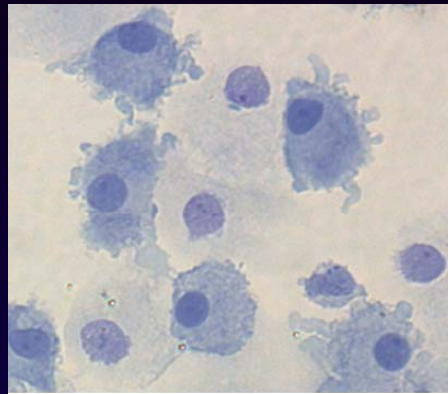
400 µg/ml *E.coli*

Does Disease Affect Phagocytosis?

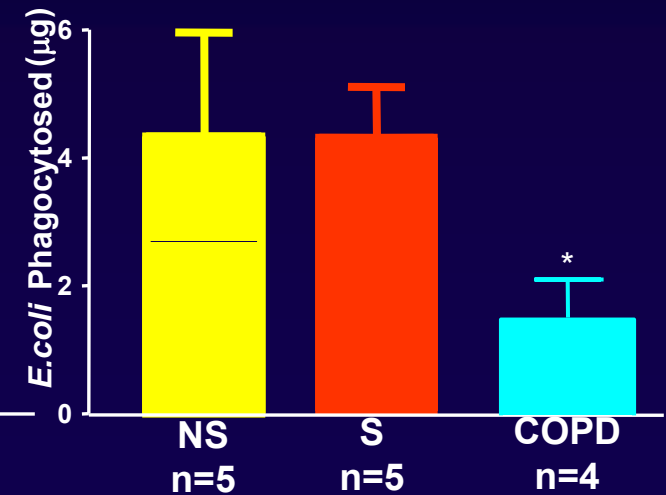
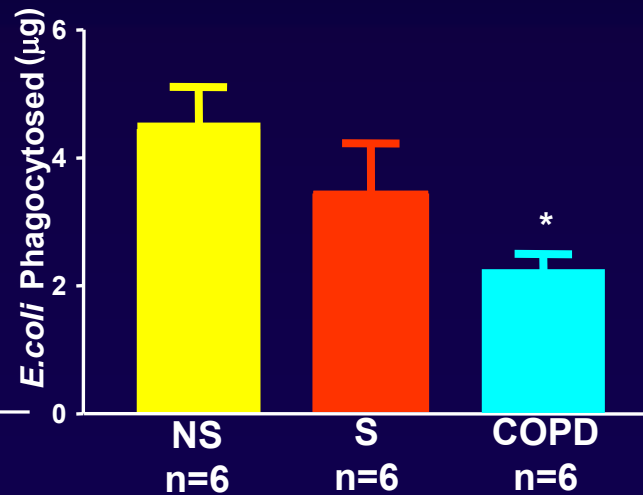
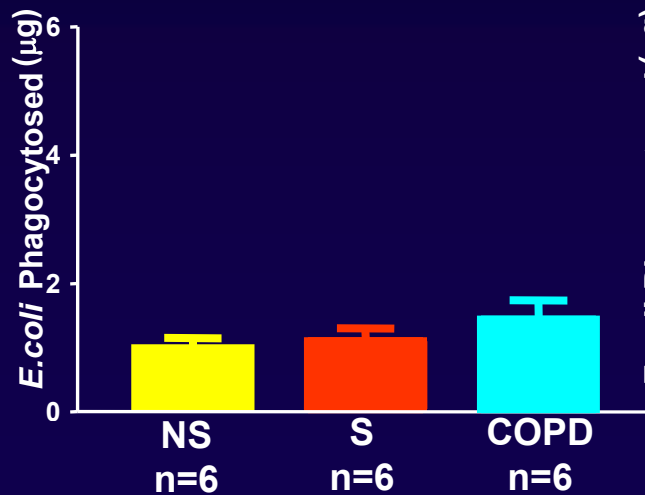
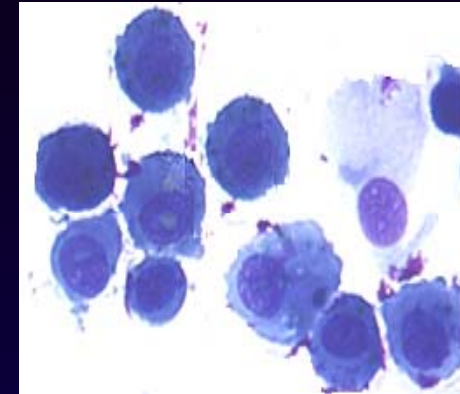
Monocytes



MDM

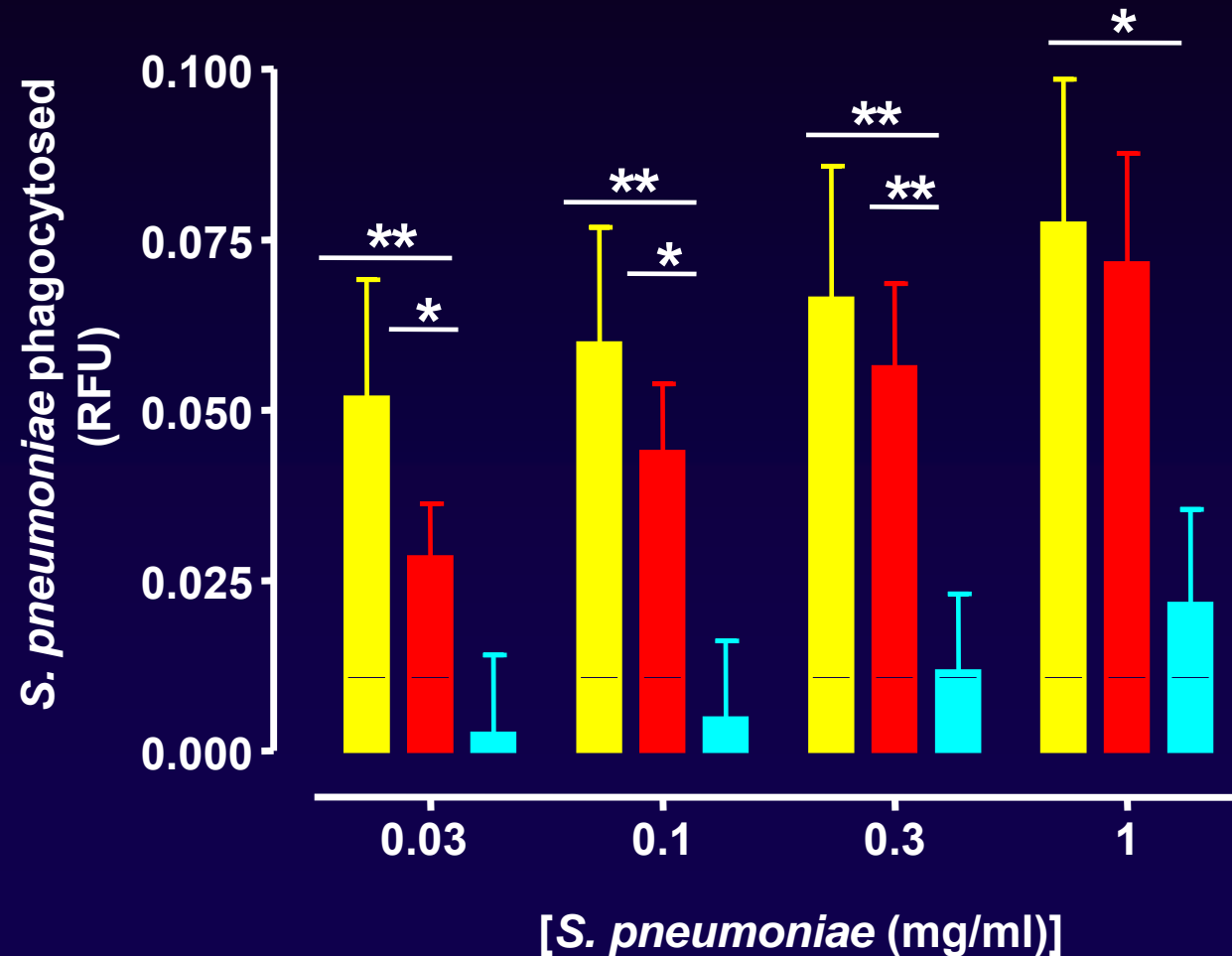


Alveolar Macrophages



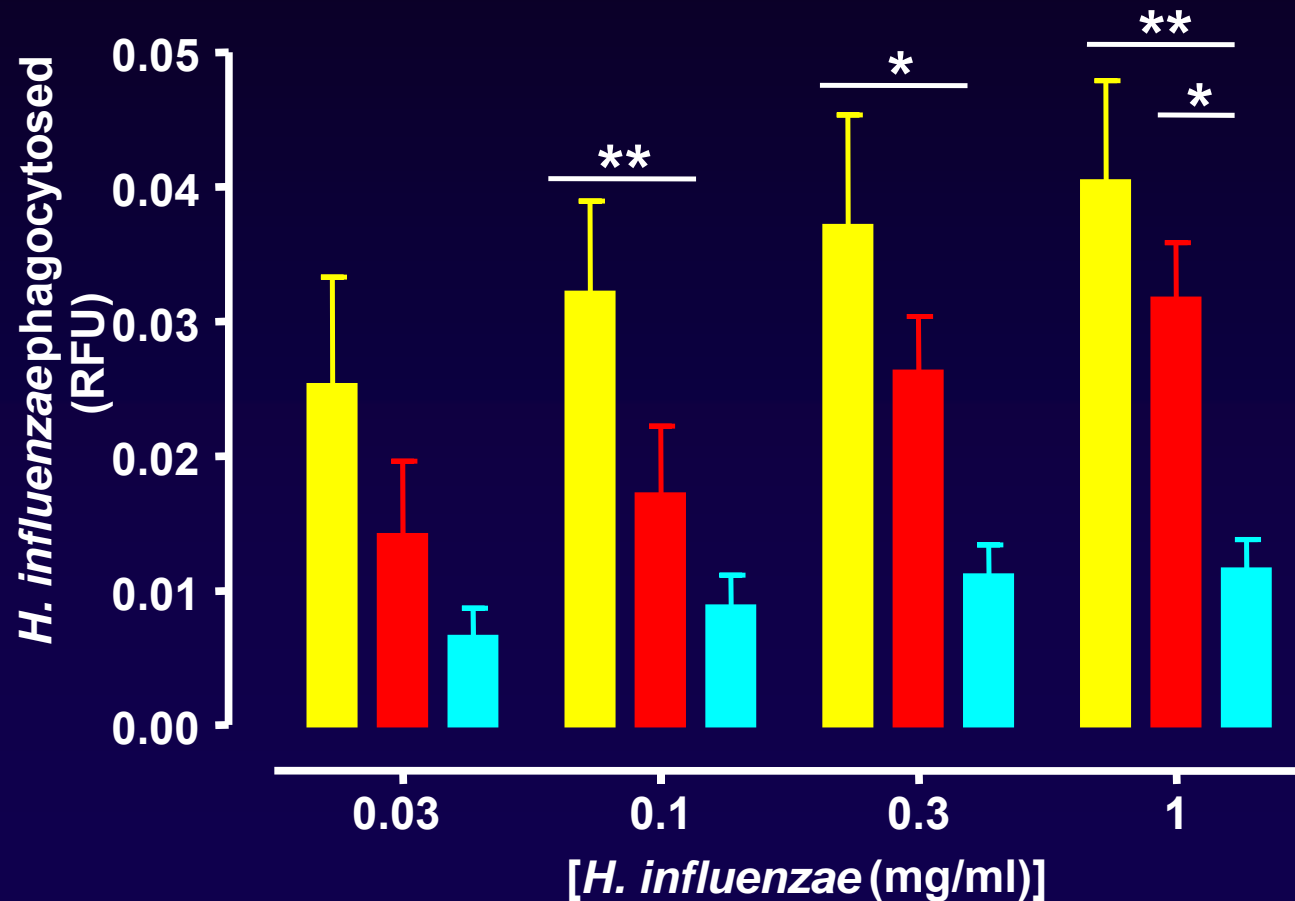
MDM phagocytosis of *S. pneumoniae*

Non-smokers (n=16), smokers (n=11) and COPD subjects (n=12)



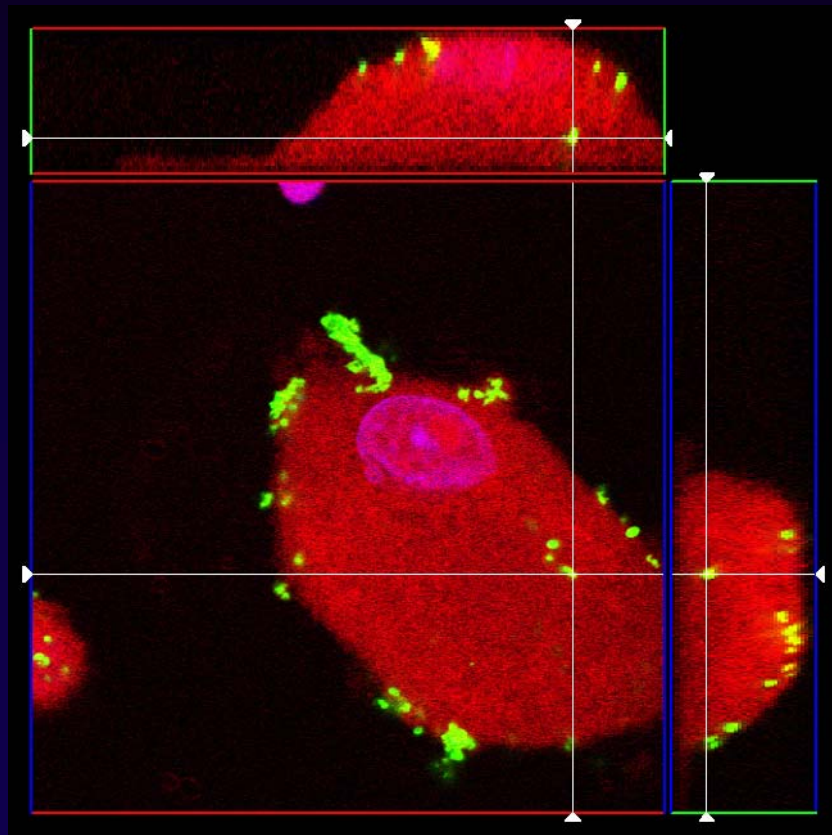
MDM phagocytosis of *H. influenzae*

Non-smokers (n=15), smokers (n=11) and COPD subjects (n=16)

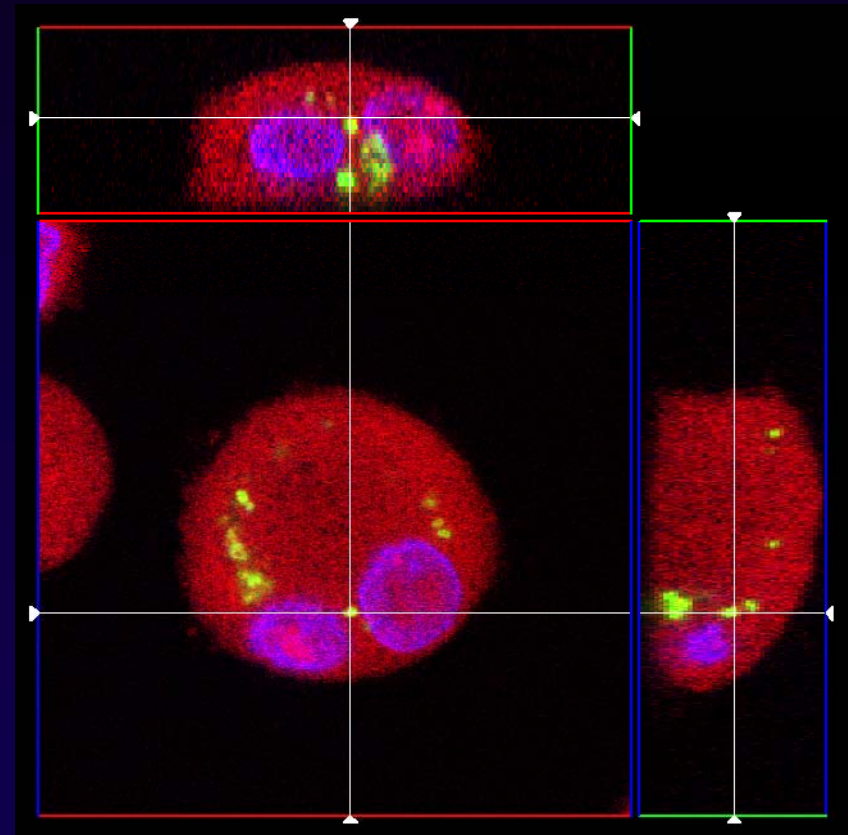


Confocal Microscopy of MDM

H. influenzae

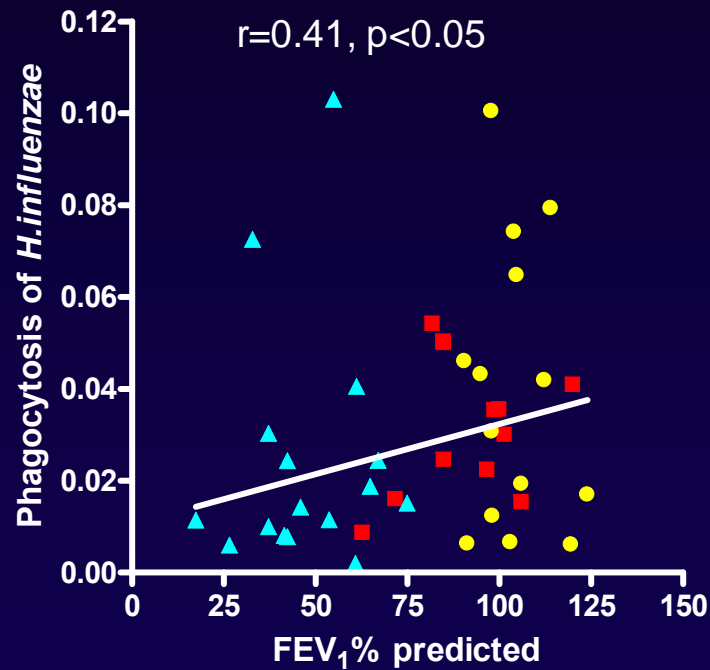


S. pneumoniae

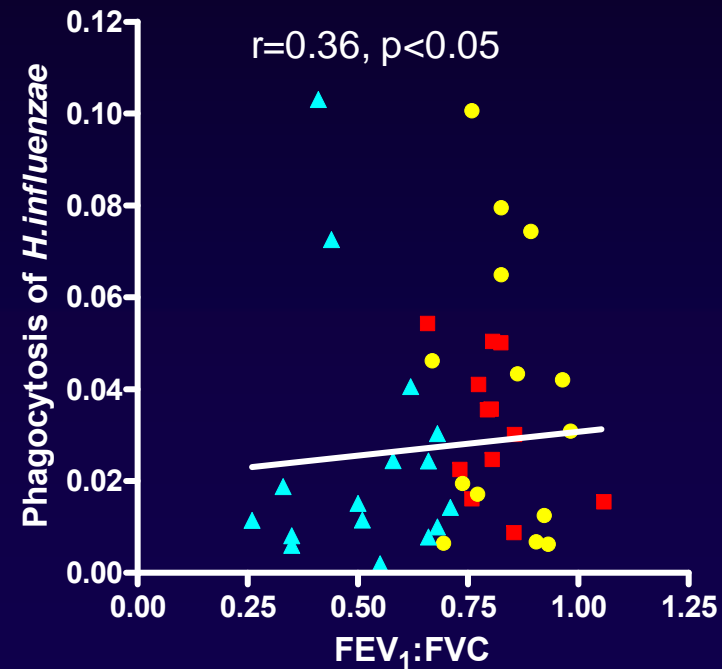


Relationship between Lung Function and Phagocytosis of *H.influenzae*

FEV₁% predicted



FEV₁:FVC

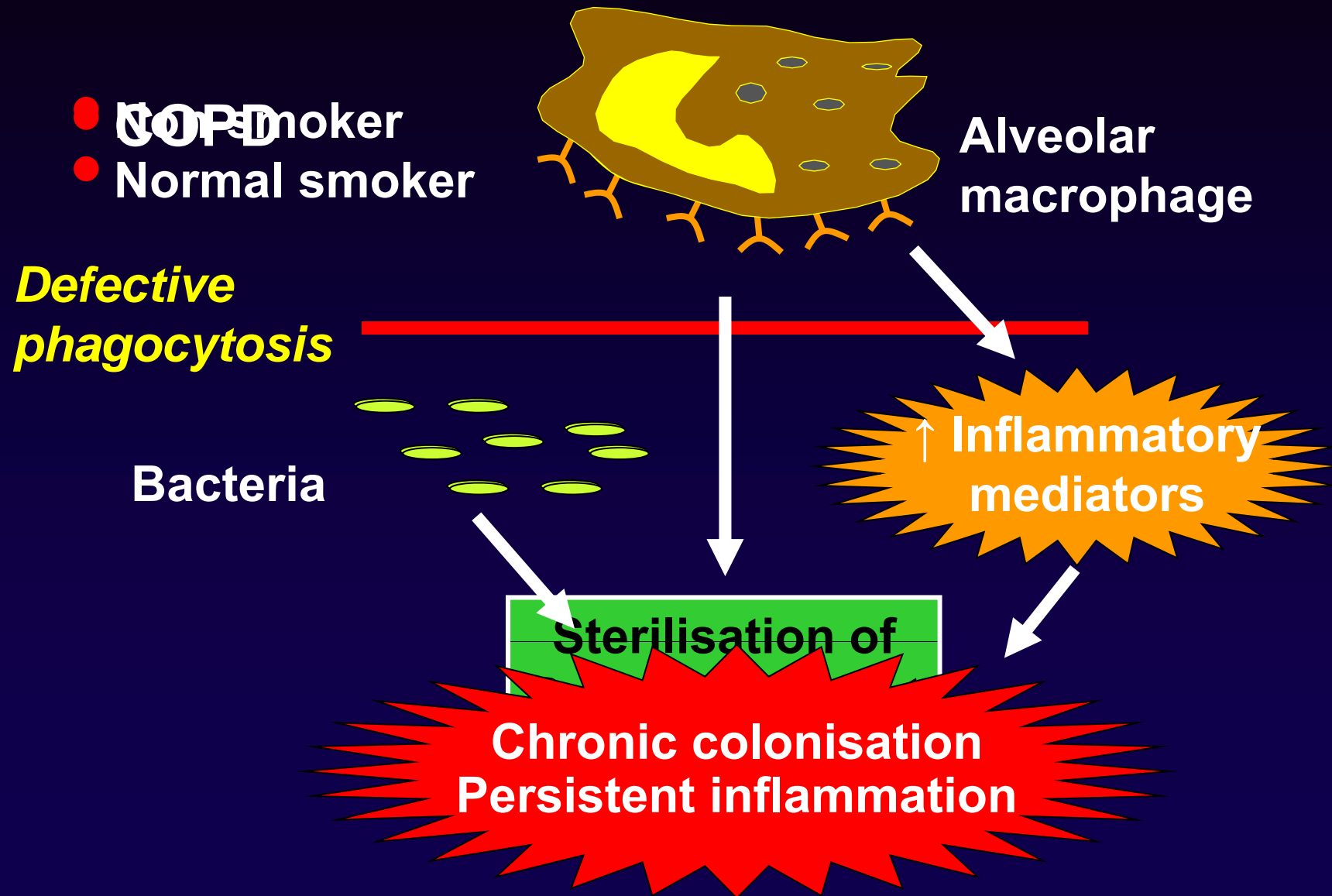


● Non-smokers

■ Smokers

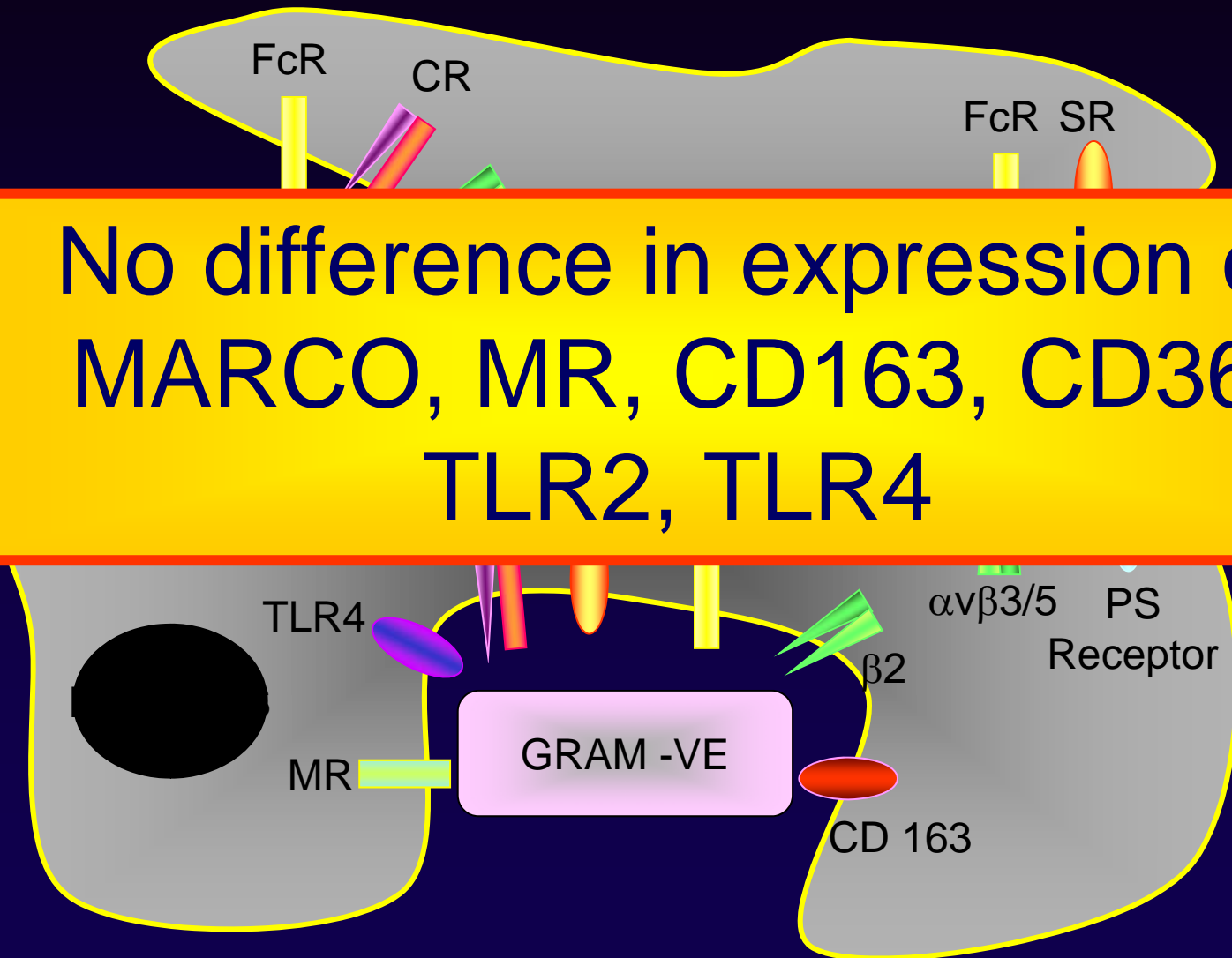
▲ COPD

Macrophage Phagocytosis

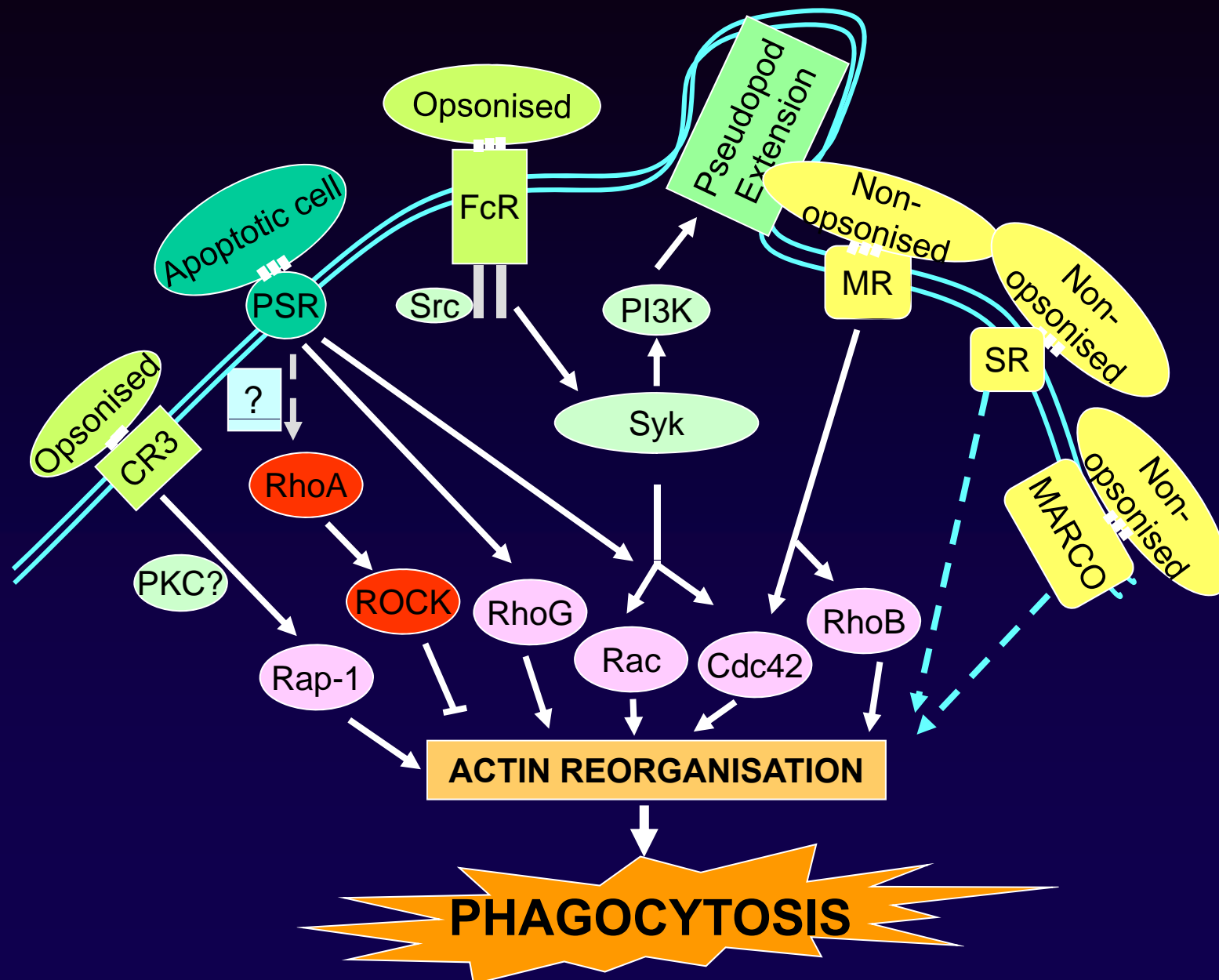


Recognition of Particles

No difference in expression of
MARCO, MR, CD163, CD36,
TLR2, TLR4

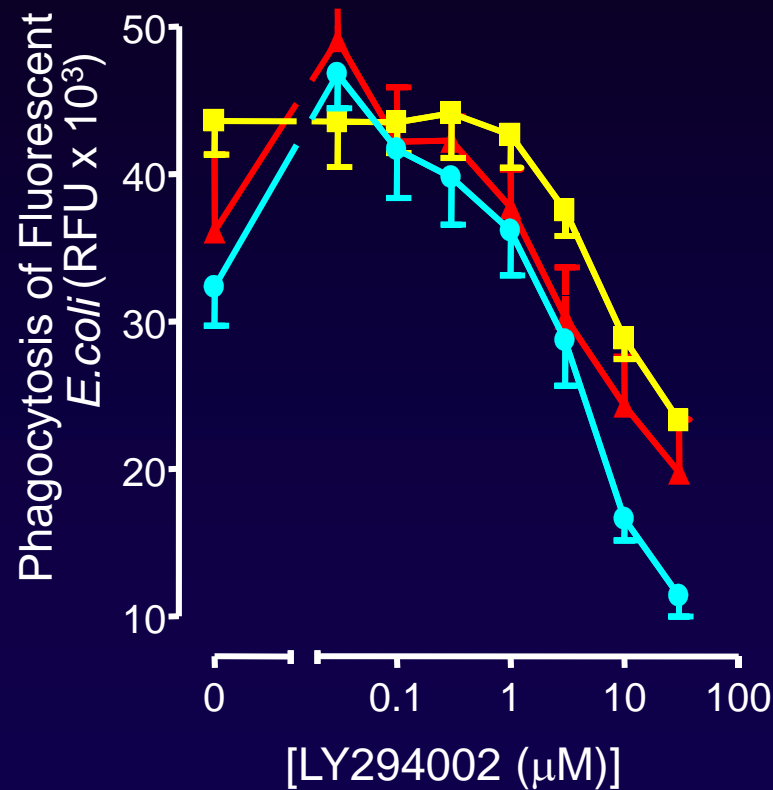


Signal Transduction Pathways

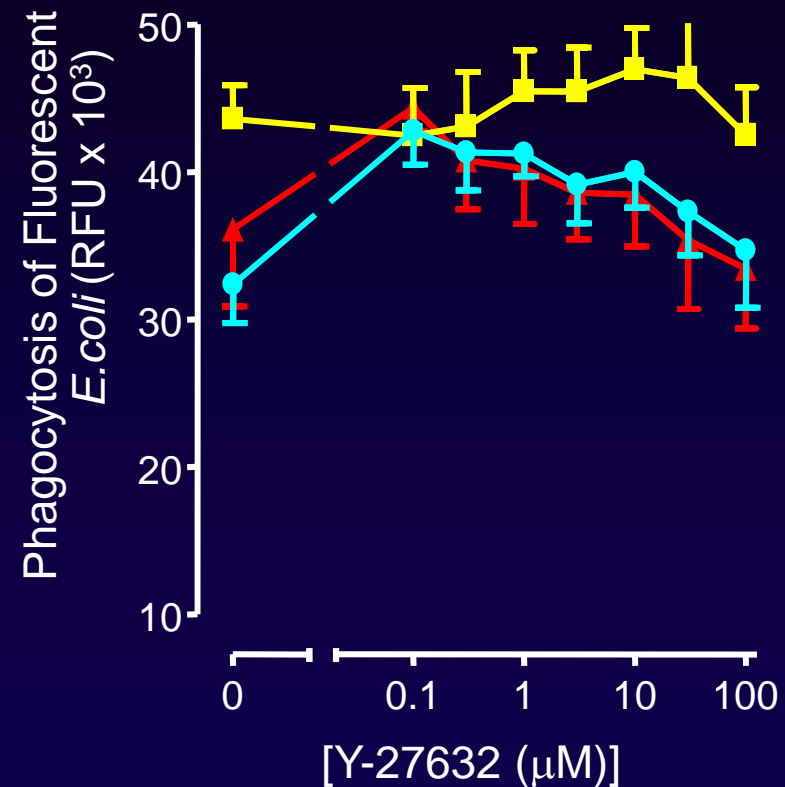


Effect of Kinase Pathway Inhibitors

PI3K Inhibitor

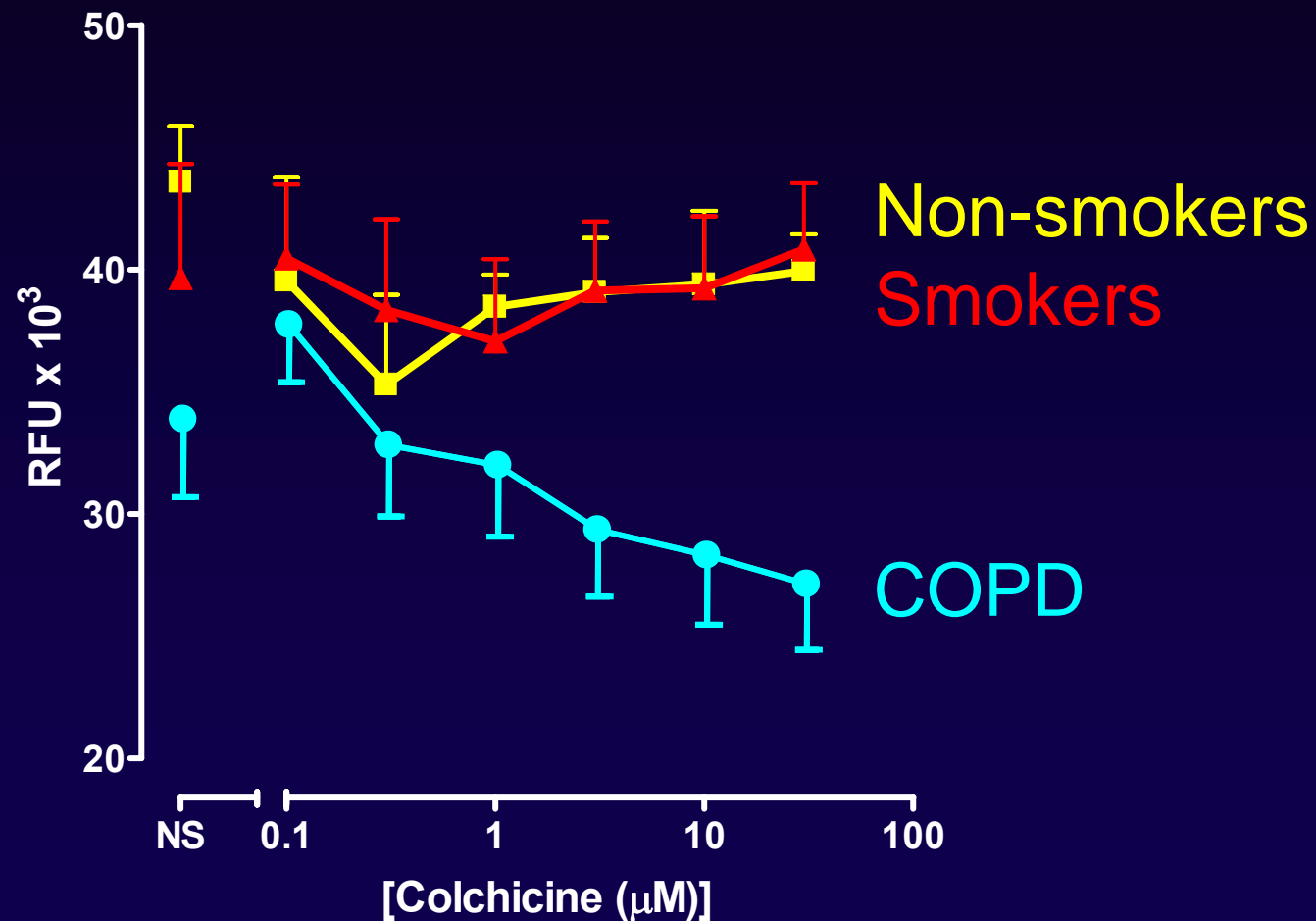


Rho kinase Inhibitor



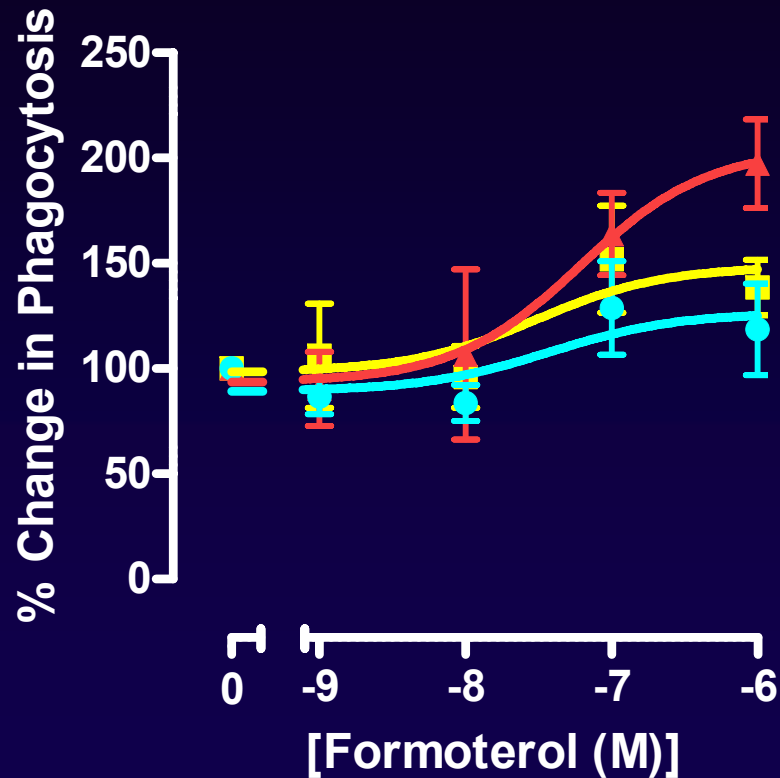
■ Non-smokers n=6 ▲ Smokers n=6 ● COPD n=9

Effect of Colchicine (microtubule disruptor) on *E.coli* Phagocytosis in MDM

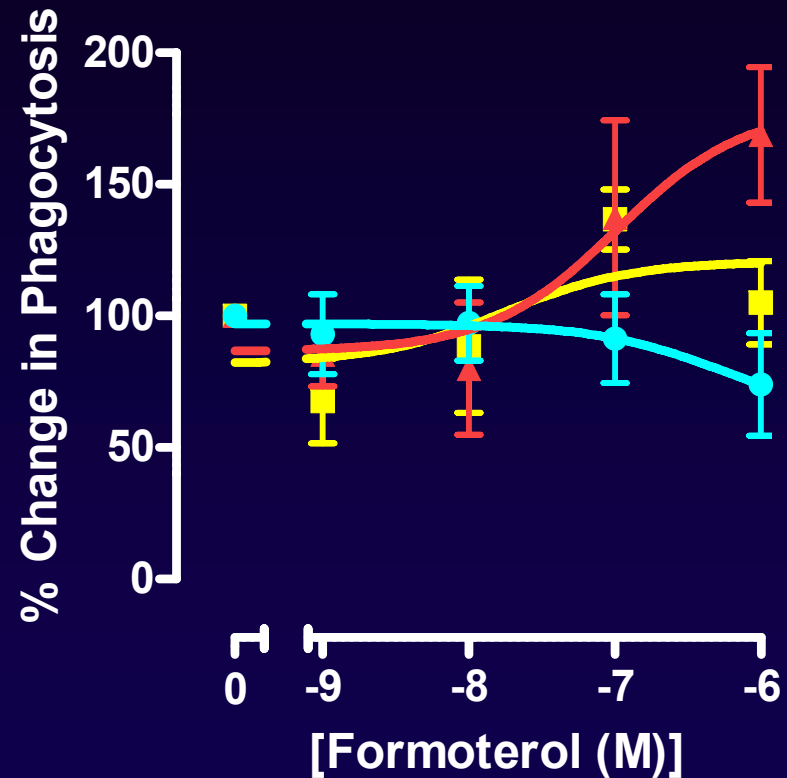


Effect of Formoterol on Phagocytosis

H. influenzae



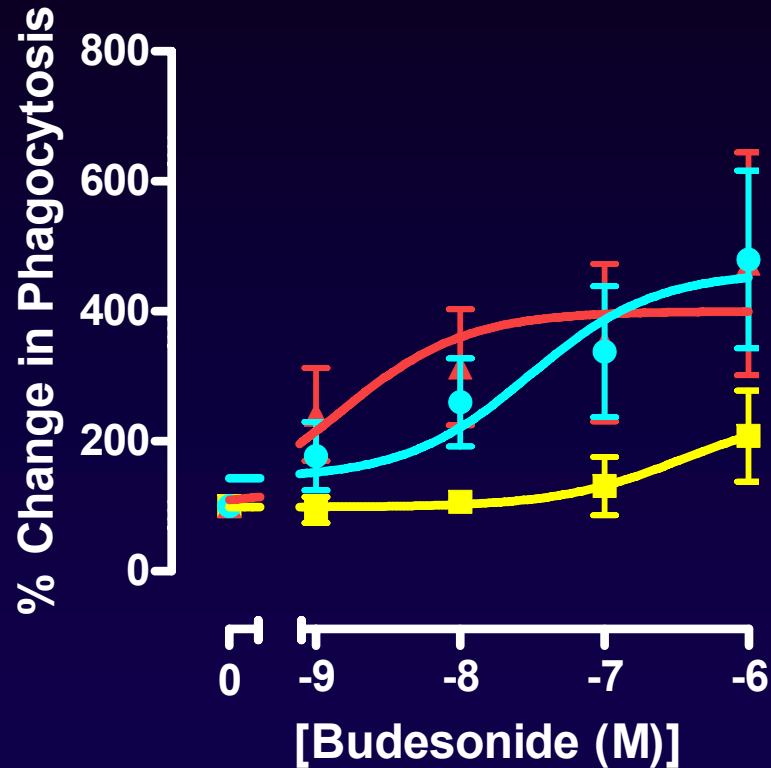
S. pneumoniae



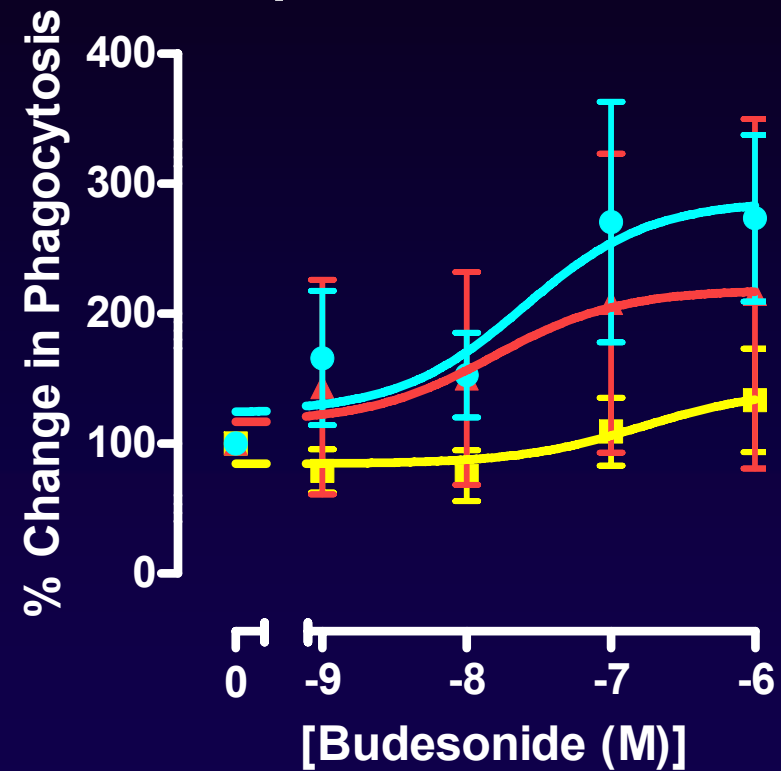
■ Non-smokers n=5 ▲ Smokers n=6 ● COPD n=5

Effect of Budesonide on Phagocytosis

H. influenzae



S. pneumoniae



■ Non-smokers n=5 ▲ Smokers n=6 ● COPD n=5

Summary

Monocytes/macrophages could be targets for asthma and COPD

Role – Dependent on environment/phenotype

Inhibit – monocyte migration
mediator release
protease activity

Promote – phagocytosis
immunomodulator release
e.g. IL-10