

Killing of Pathogens by Mice and Human Alveolar Macrophages:

Involvement of SP-A and Reactive Oxygen-Nitrogen Intermediates

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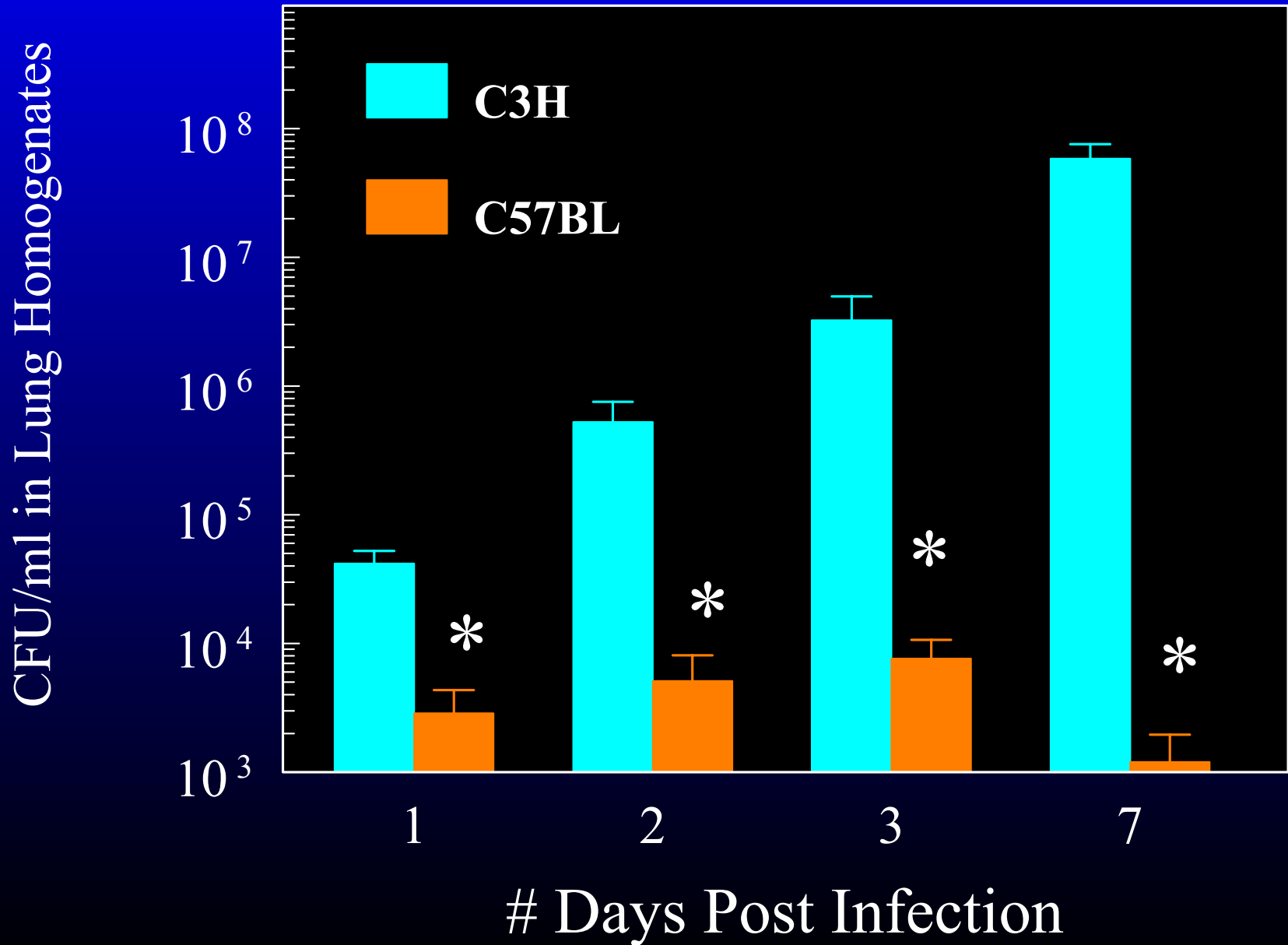
Judy Hickman-Davis, DVM, PhD



M. pneumoniae: The Problem

- *M. pneumoniae* are a leading cause of pneumonia worldwide
- In the U.S., mycoplasmas account for up to 20-30% of the 4 million cases of pneumonia reported annually
- They exacerbate the course of asthma and COPD and cause extrapulmonary complications.

Mycoplasmas in Lungs of Mice

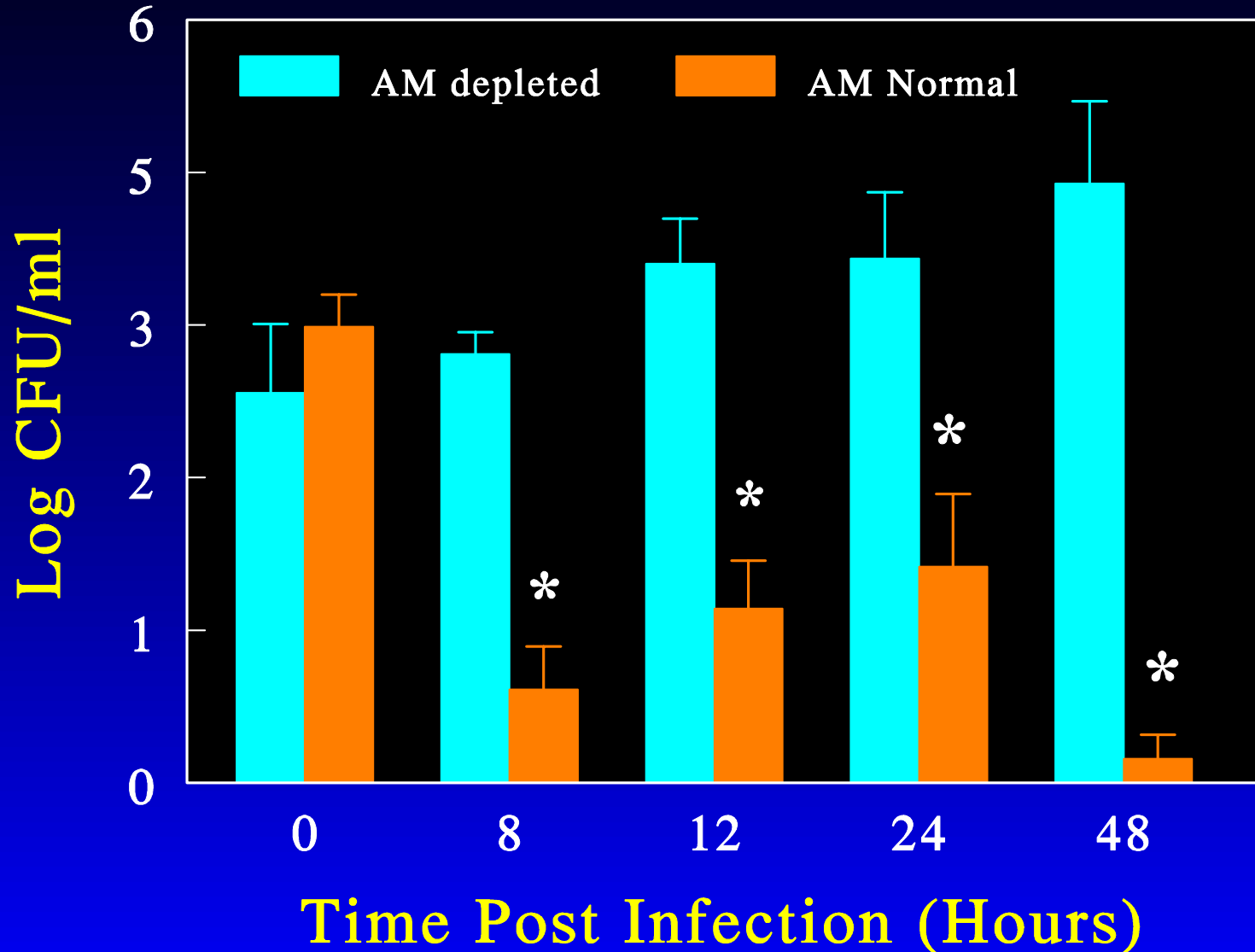


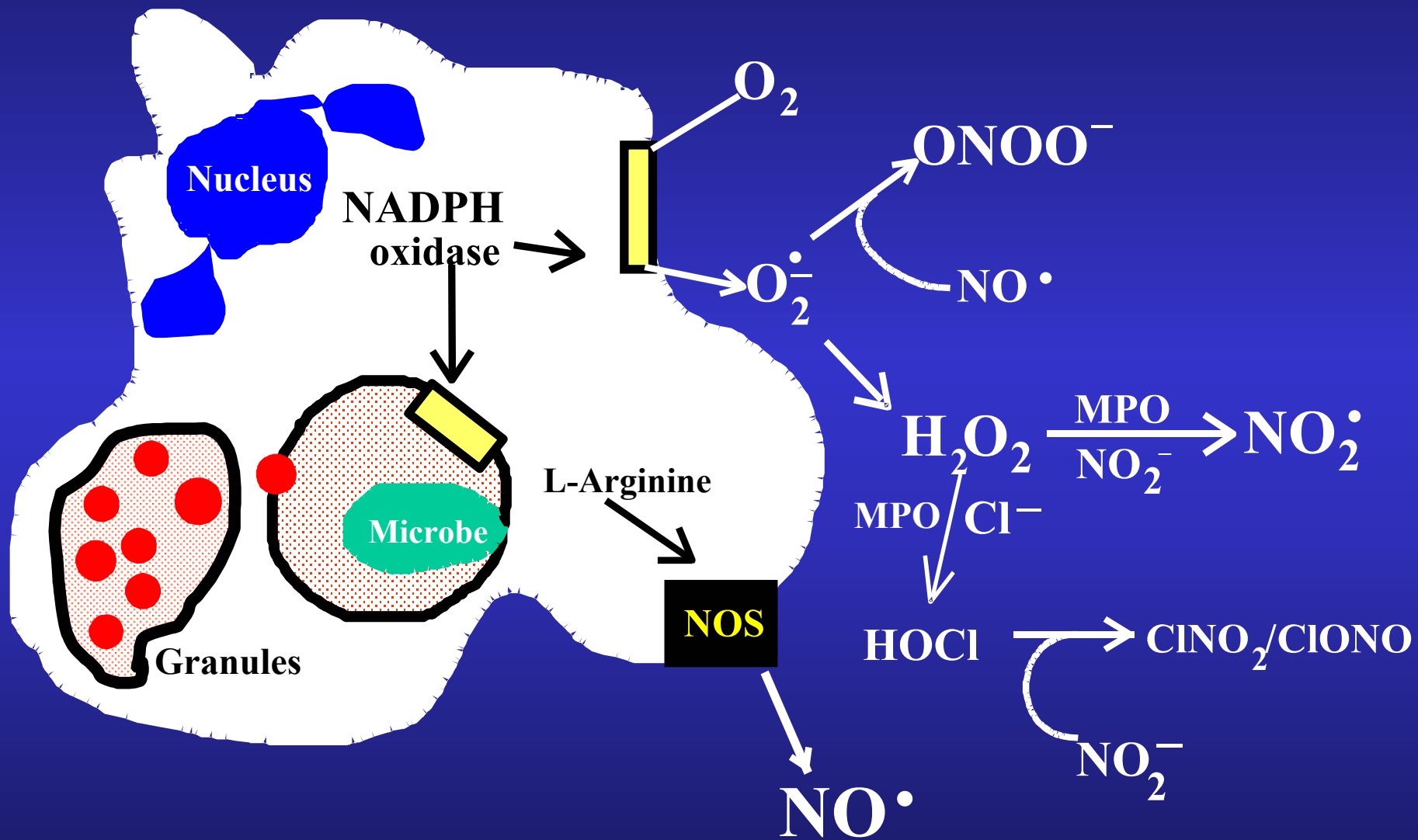
Innate Immunity

Early nonspecific phase of the immune response in the absence of specific antibody

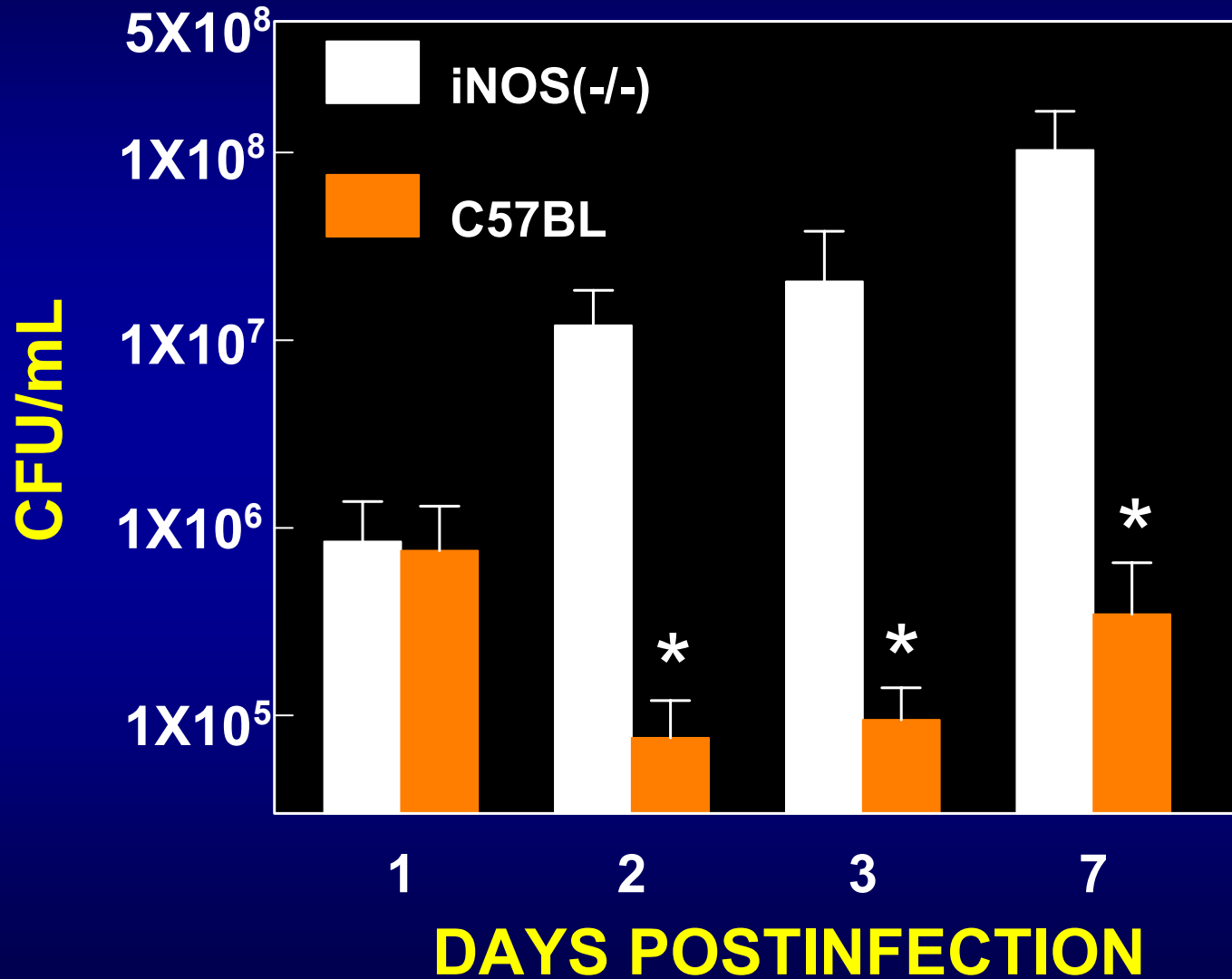
- Alveolar Macrophages
- Reactive Oxygen-Nitrogen Species
- Collectins

Mycoplasmas in Lung Homogenates Of C57BL/6 mice





MYCOPLASMAL CLEARANCE C57BL iNOS(-/-) MICE

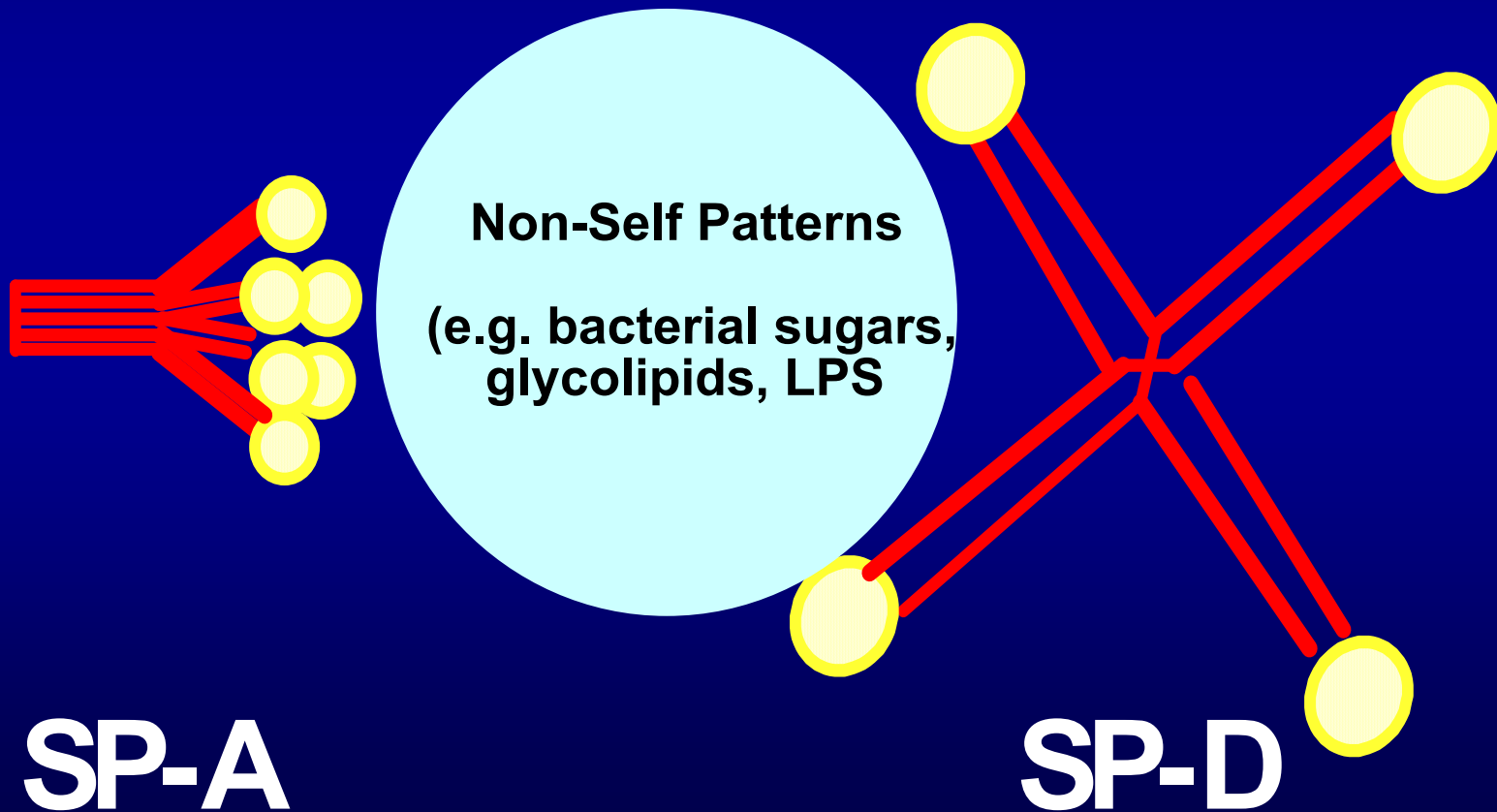


Collectins

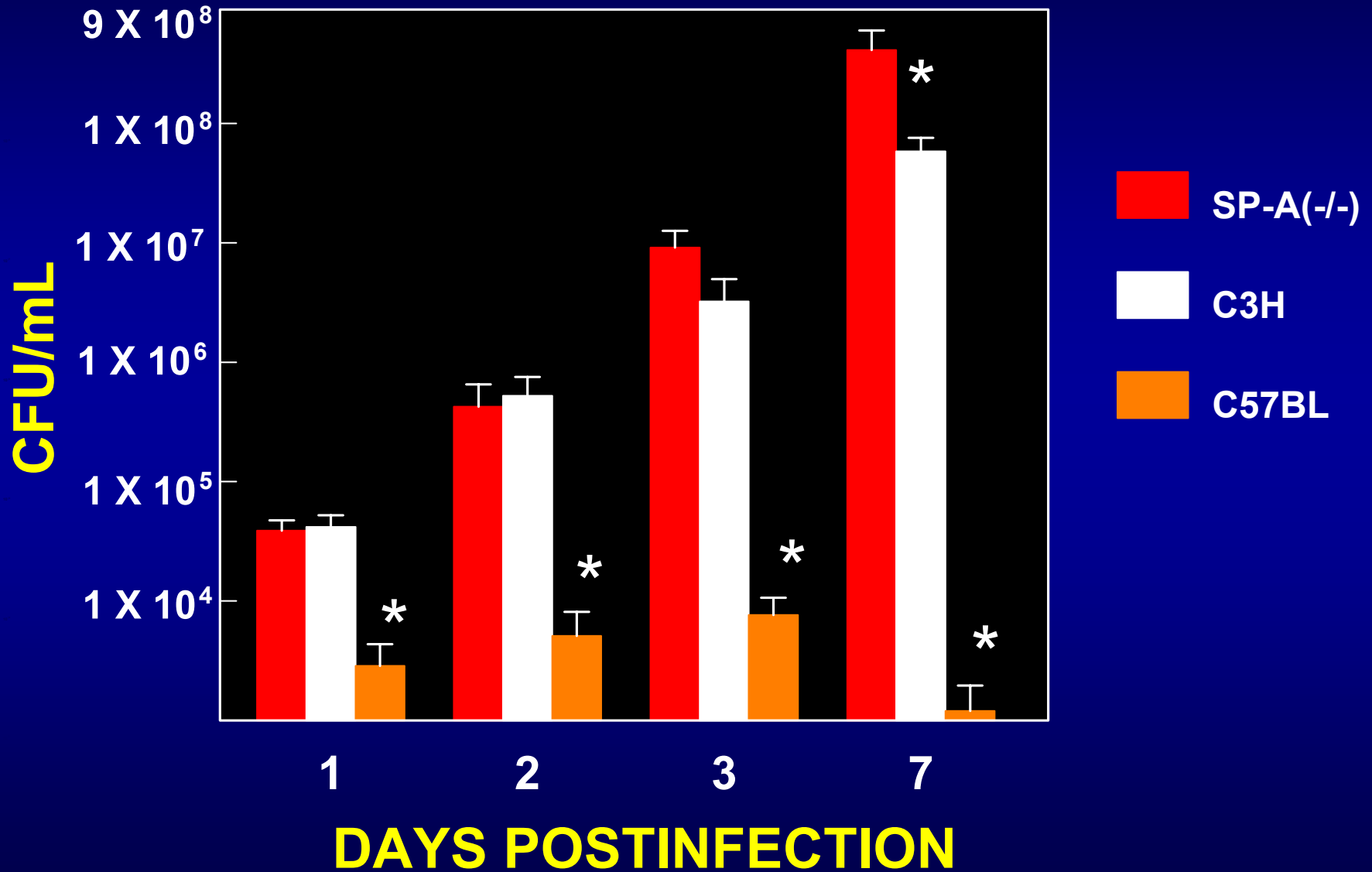
Collagen-like

Lectin

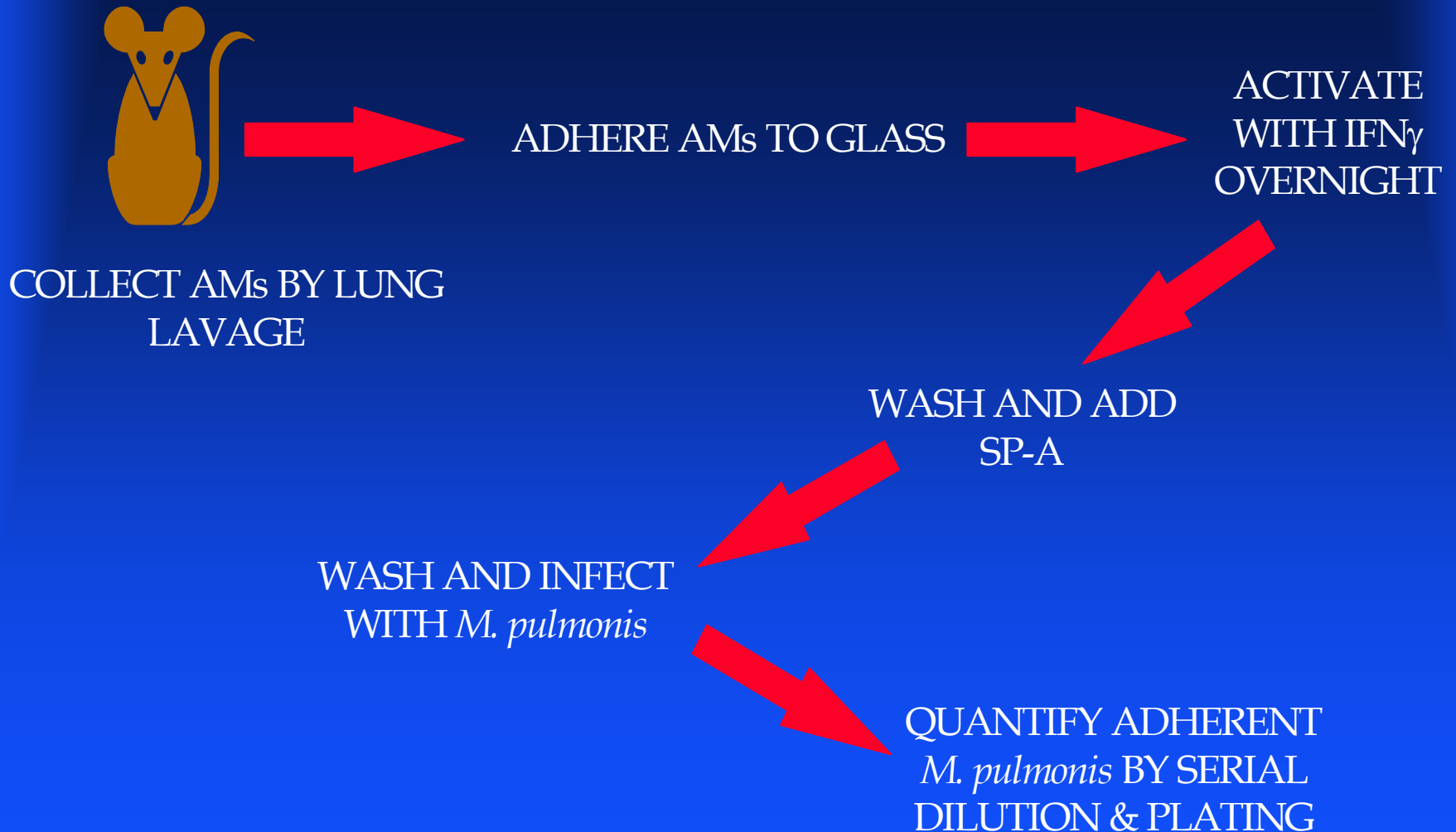
Pattern-recognition molecules



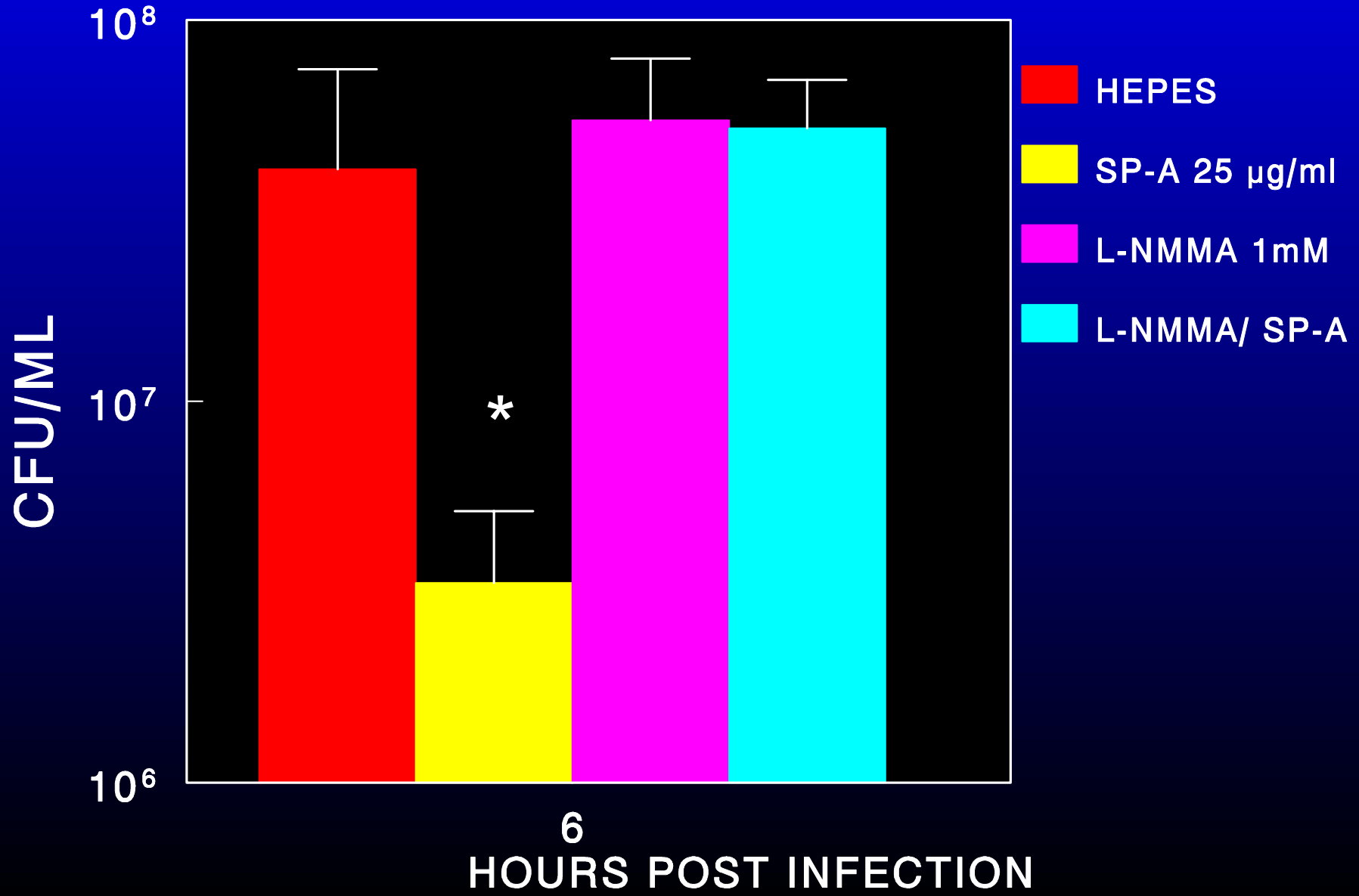
MYCOPLASMAL CLEARANCE SP-A(-/-) MICE



In vitro Killing Assay



AM Killing of Mycoplasmas in vitro: Effect of SP-A & L-NMMA



Pulmonary Host Defense and SP-A

Collectin: *Collagen-like Lectin* Superfamily

Clearance of bacterial, viral and fungal pathogens:

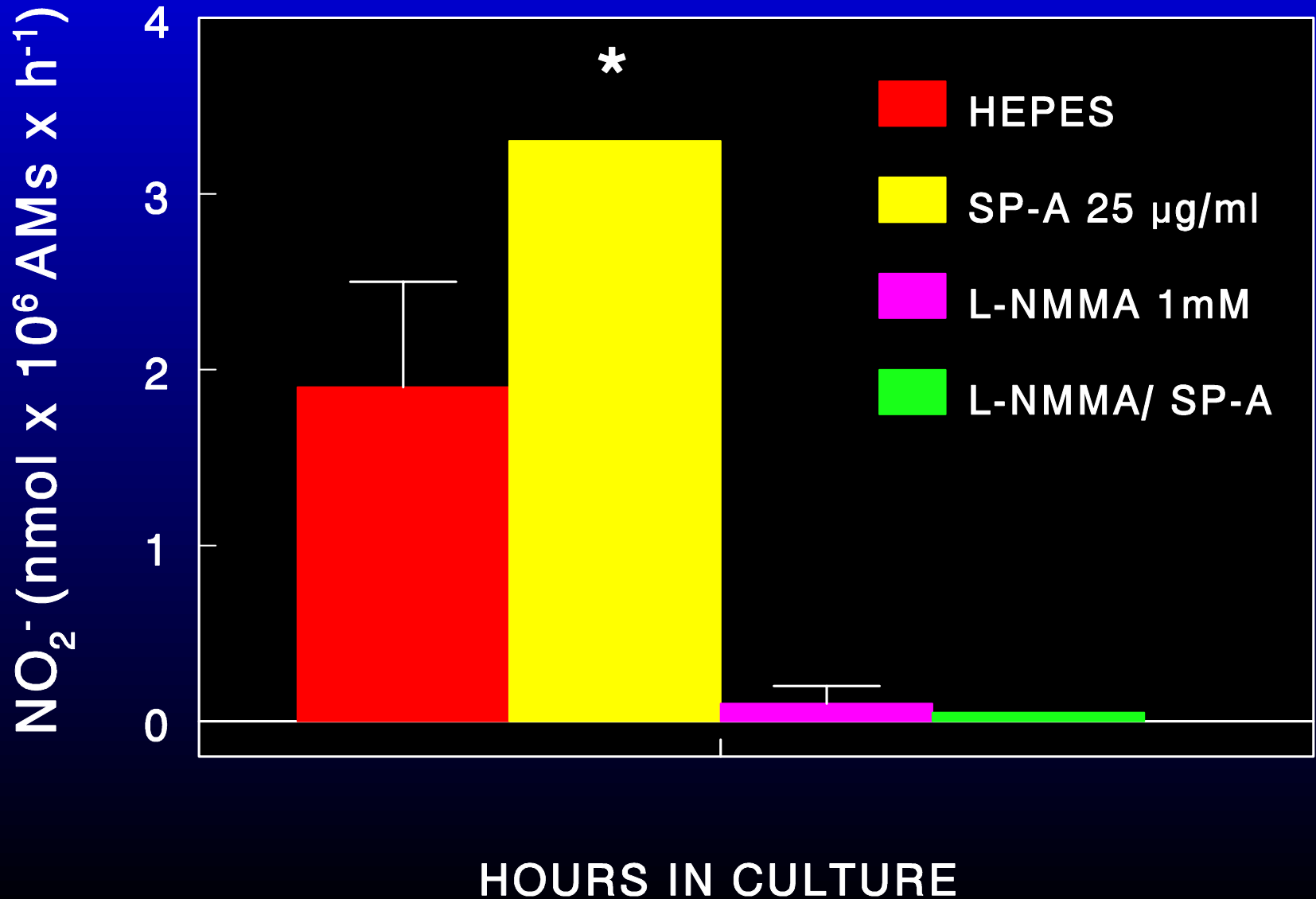
Opsonin

- Binding to pathogens and presentation to inflammatory cells

Cell Mediated Effects

- NO• production
- Superoxide production
- Phagocytosis

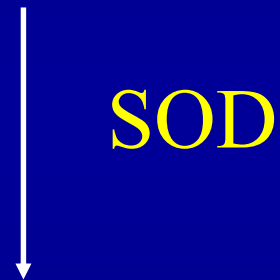
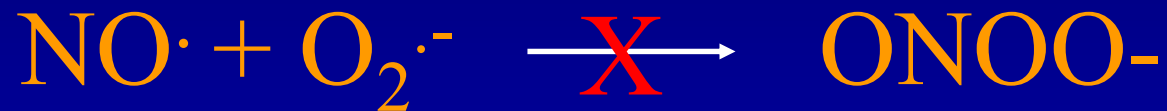
Nitrite Production by C57BL/6 AMs Infected with Mycopl. for 18 h



Production of Peroxynitrite



Production of Peroxynitrite



SOD Prevents Killing of Mycoplasmas by INF- γ stimulated AM



Mycoplasma pulmonis Infection in Mice

- Innate immune mechanisms determine the course of mycoplasmal disease
- AMs are the primary effector cell for early mycoplasmal clearance
- SP-A mediates mycoplasmal killing by enhancing the production of reactive oxygen-nitrogen species
- ONOO⁻ is the primary toxic NO• metabolite responsible for mycoplasmal killing

Do Human AMs

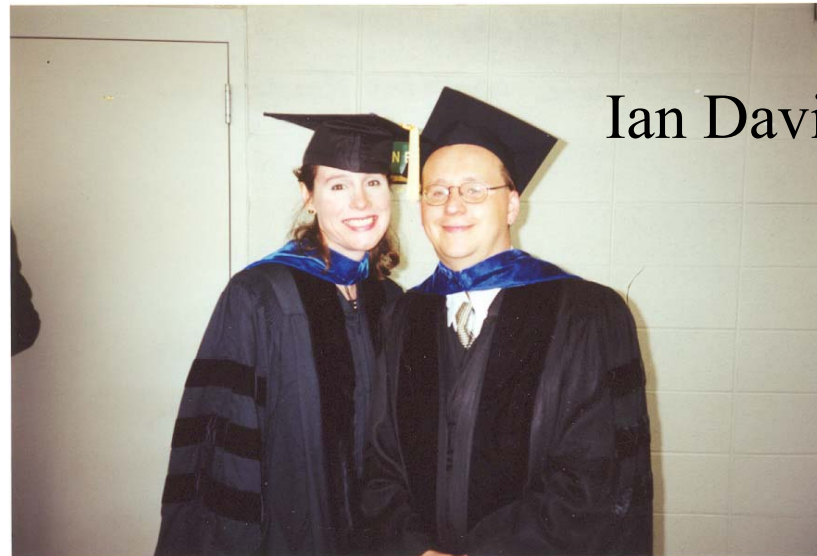
Produce •NO?

Does SP-A regulate
•NO production and
killing of pathogens
by AMs?

Judy Hickman-Davis, DVM, PhD



Philip O'Reilly, MD



Ian Davis, DVM,
PhD

Sources of Human AMs

- Normal healthy non-smoking volunteers
- Patients with lung transplantation that were undergoing routine surveillance lavage

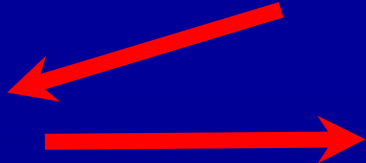
Killing of *Klebsiella* by AM

Adherence purification of 5×10^5 AMs

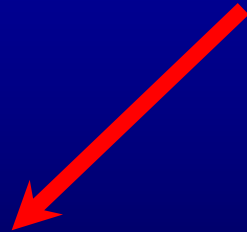


Add 25 μg /ml human SP-A + Infect with $\sim 10^8$
Klebsiella pneumoniae

Wash

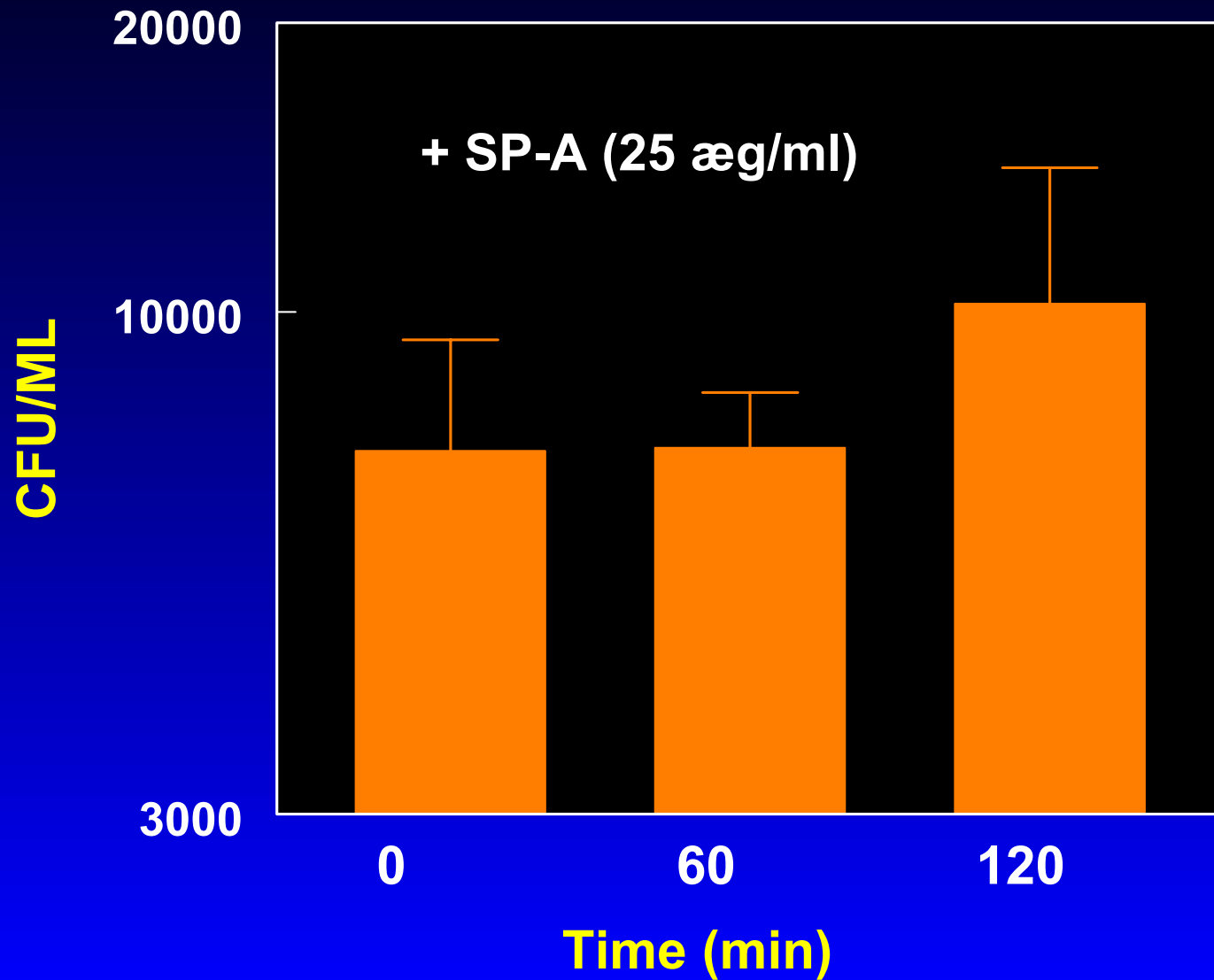


Incubate for 60 – 120 min
Gentamycin

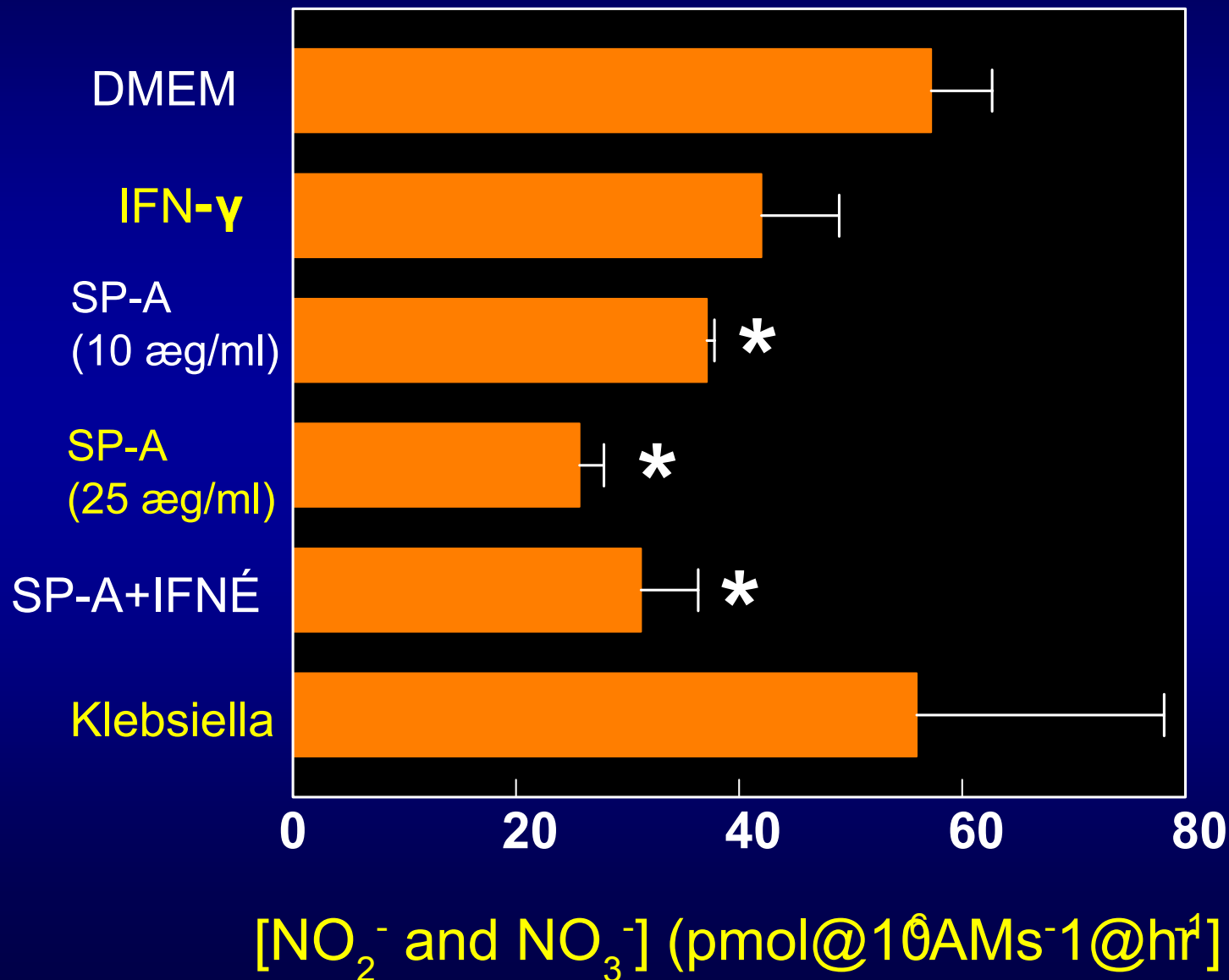


Enumeration by serial dilution and plating

Normal AMs do not kill Klebsiella



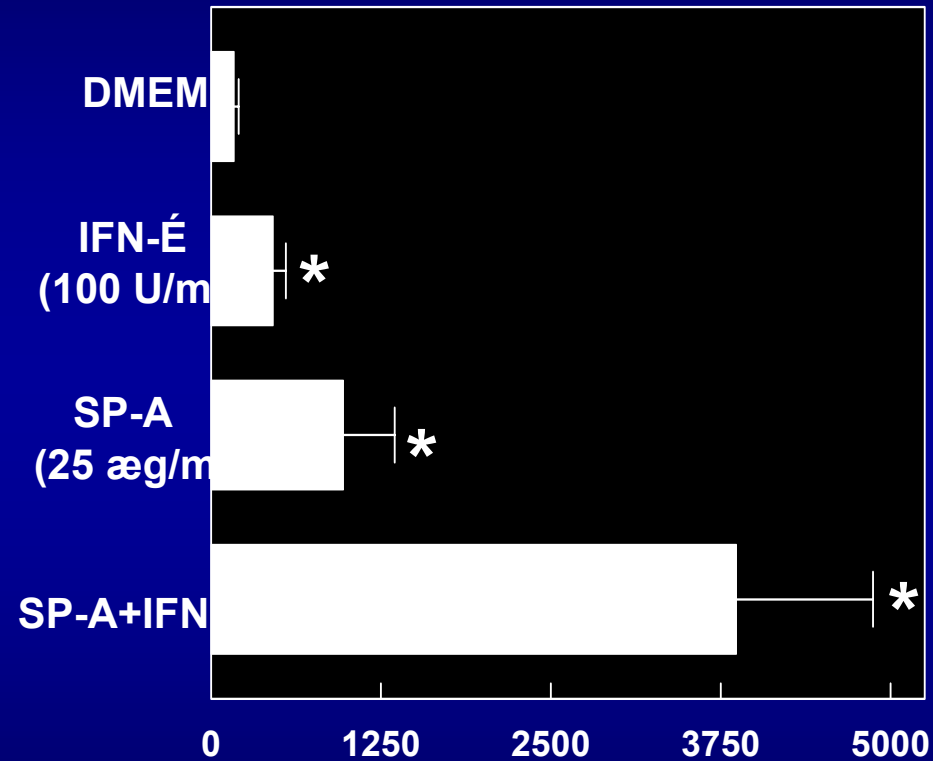
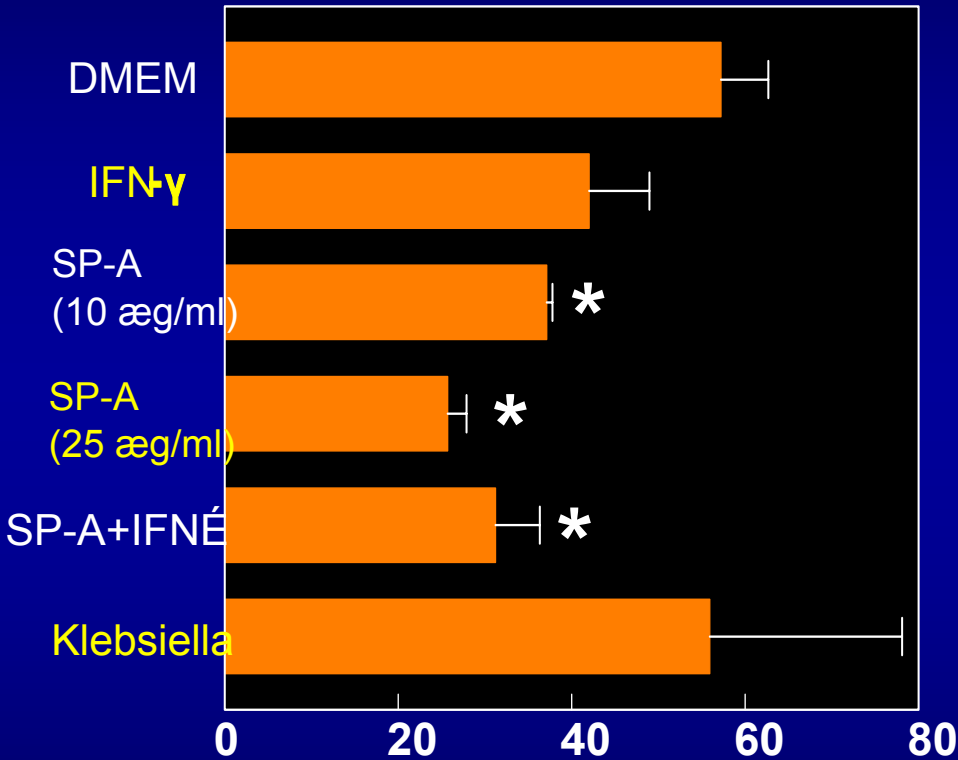
Production of NO_x by AM from Normal Humans



Production of NO by AMs

Humans

C57BL Mice

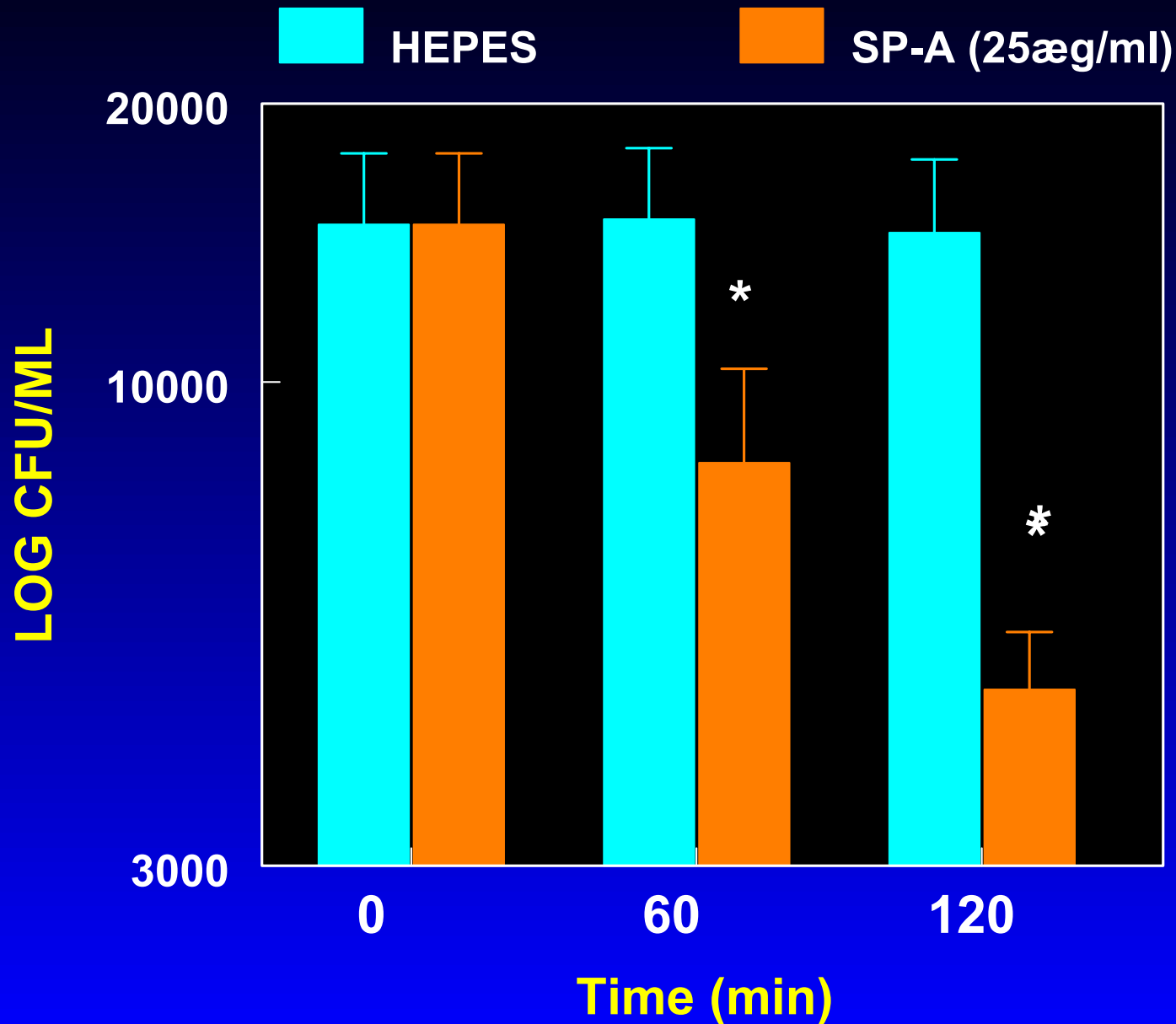


$[\text{NO}_2^- + \text{NO}_3^-]$ ($\text{pmol} \times 10^6 \text{ AMs} \times \text{h}^{-1}$)

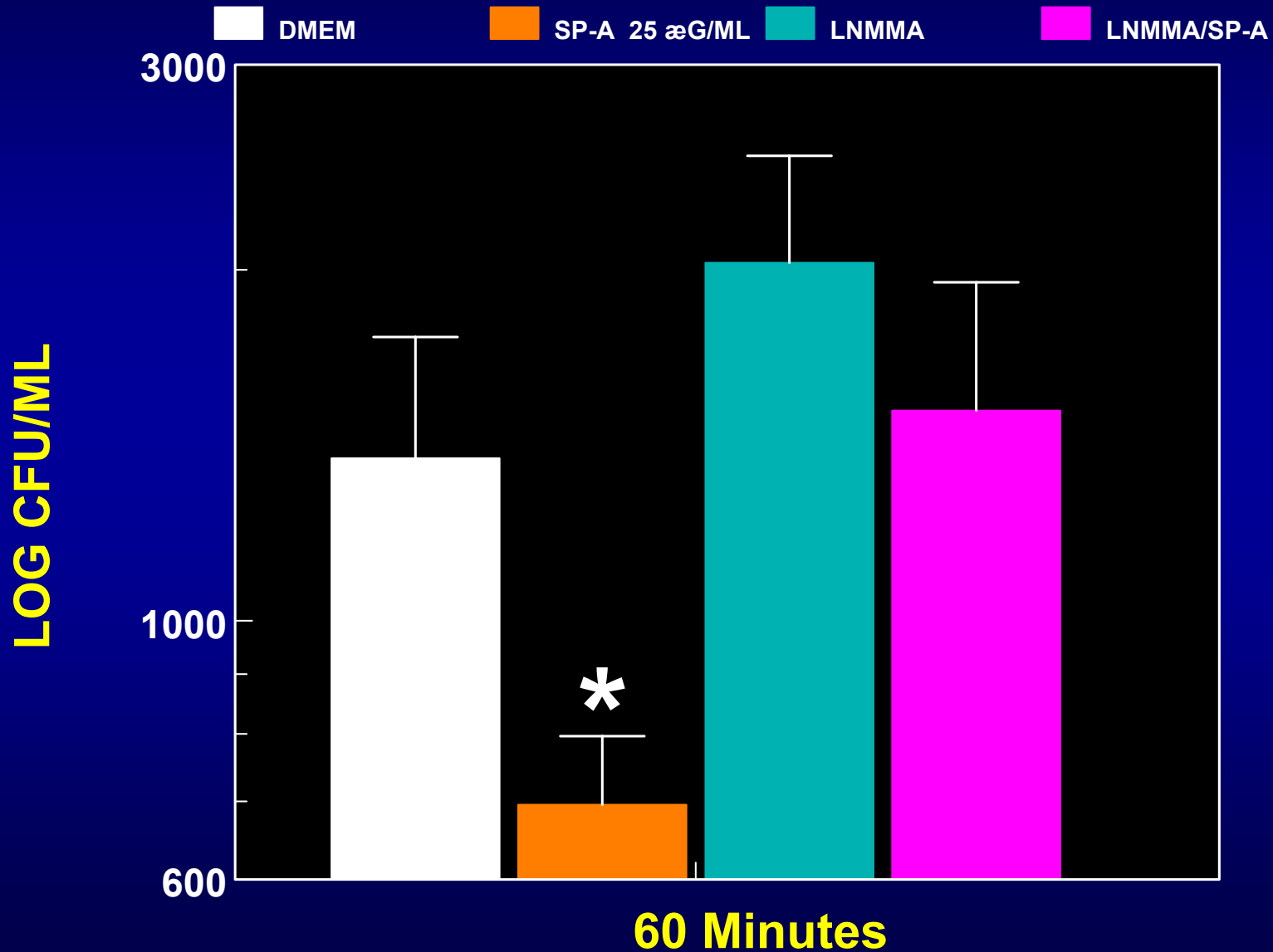
Sources of Human AMs

- Normal healthy non-smoking volunteers
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Killing of Klebsiella by Transplant AM



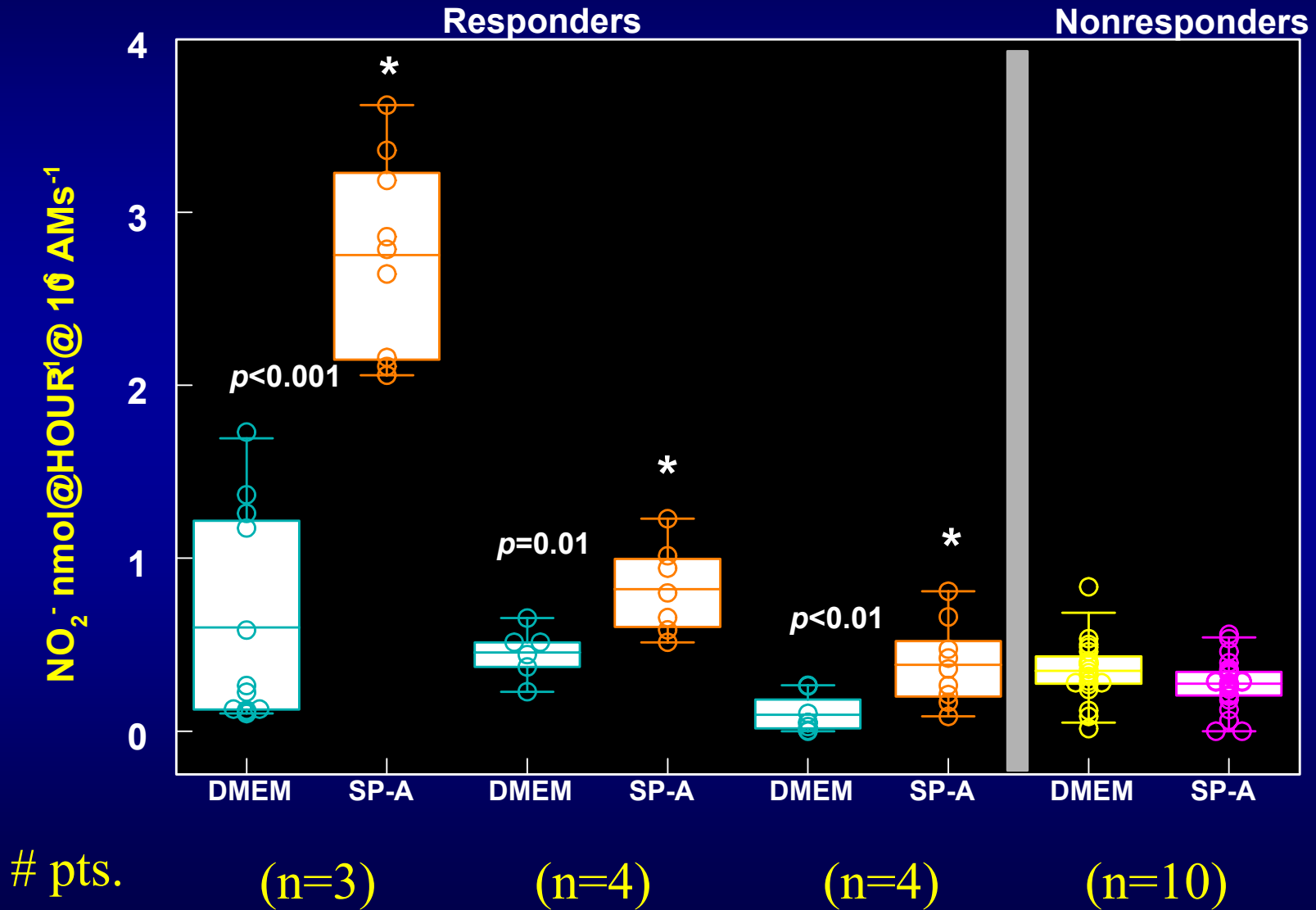
Klebsiella Killing by Transplant AMs: Involvement of SP-A and NO@



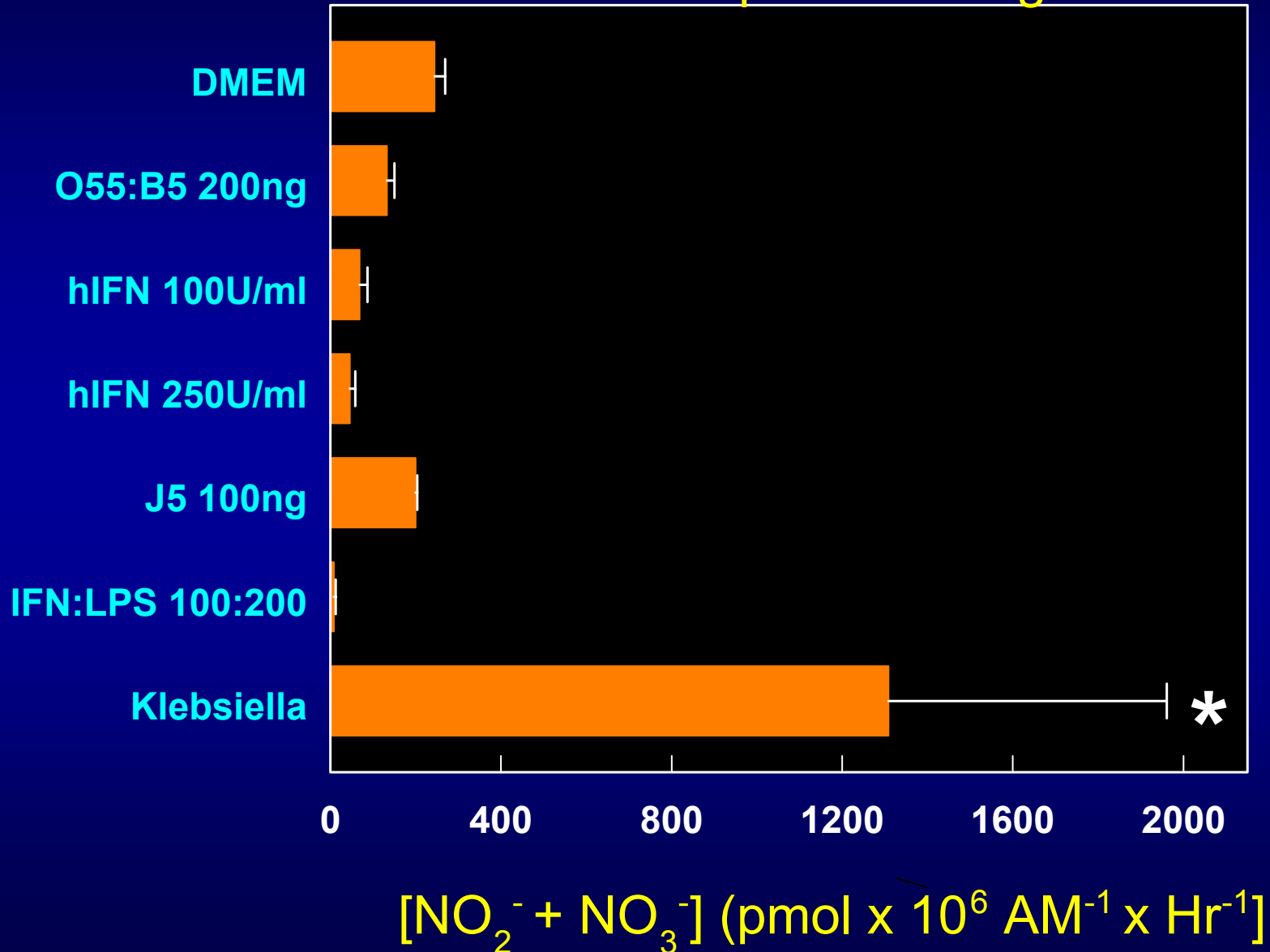
How does SP-A mediate killing of *Klebsiella* by Transplant AMs?

- Increases production of reactive oxygen-nitrogen intermediates by AMs

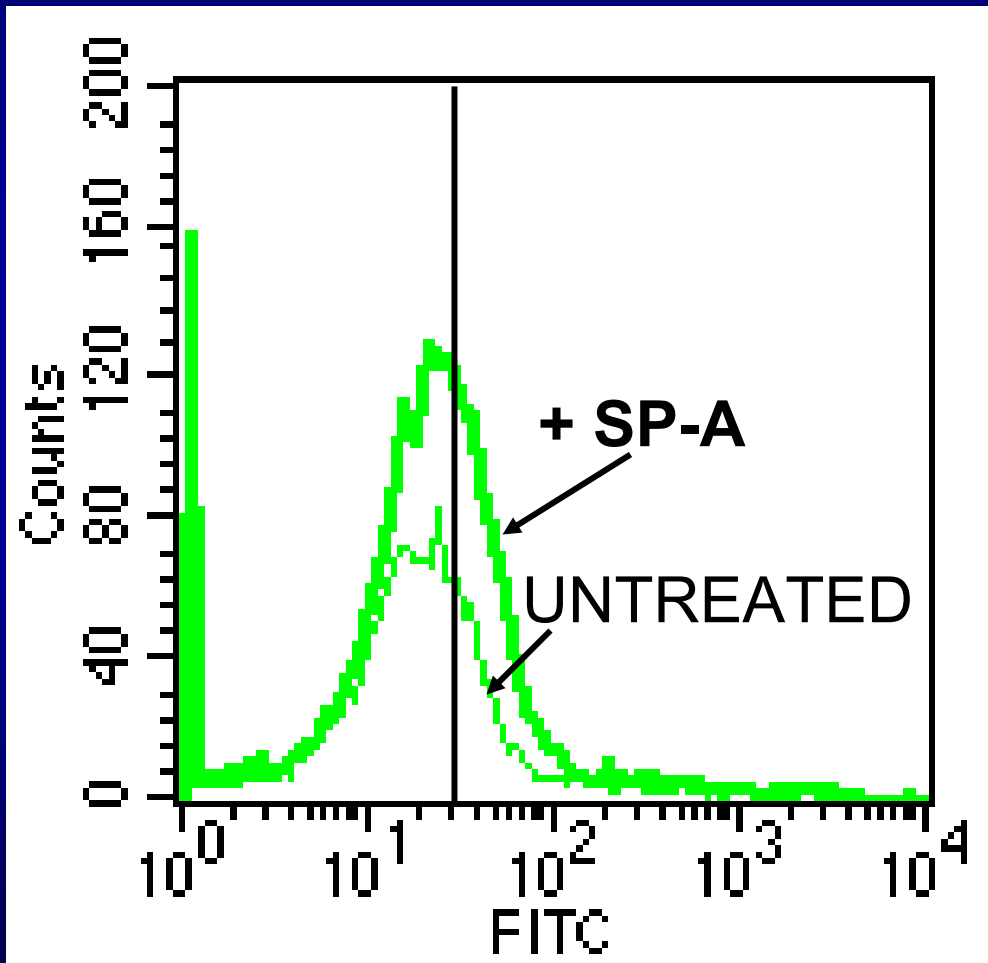
Production of NO by AM from Transplant Patients Incubated with SP-A



NOx Production by AM from Transplanted Lungs



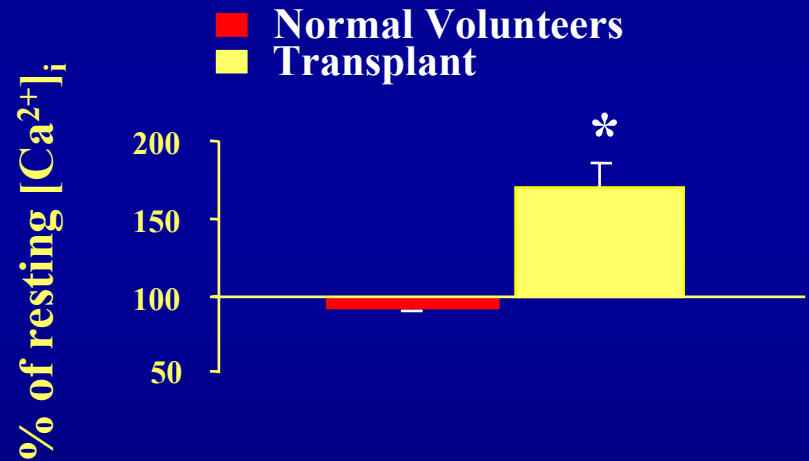
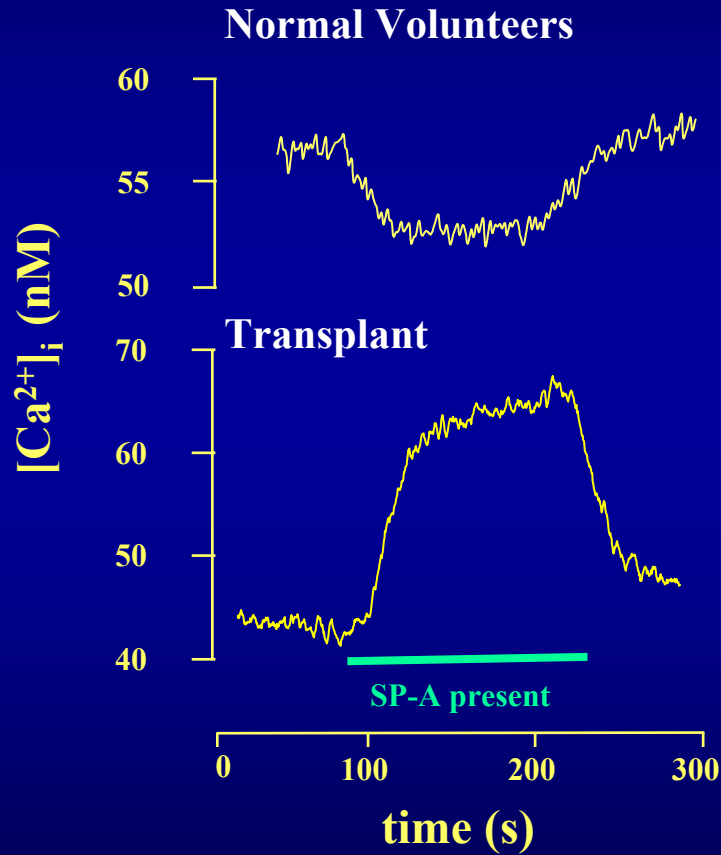
SP-A Increases Phagocytosis of FITC-Klebsiella



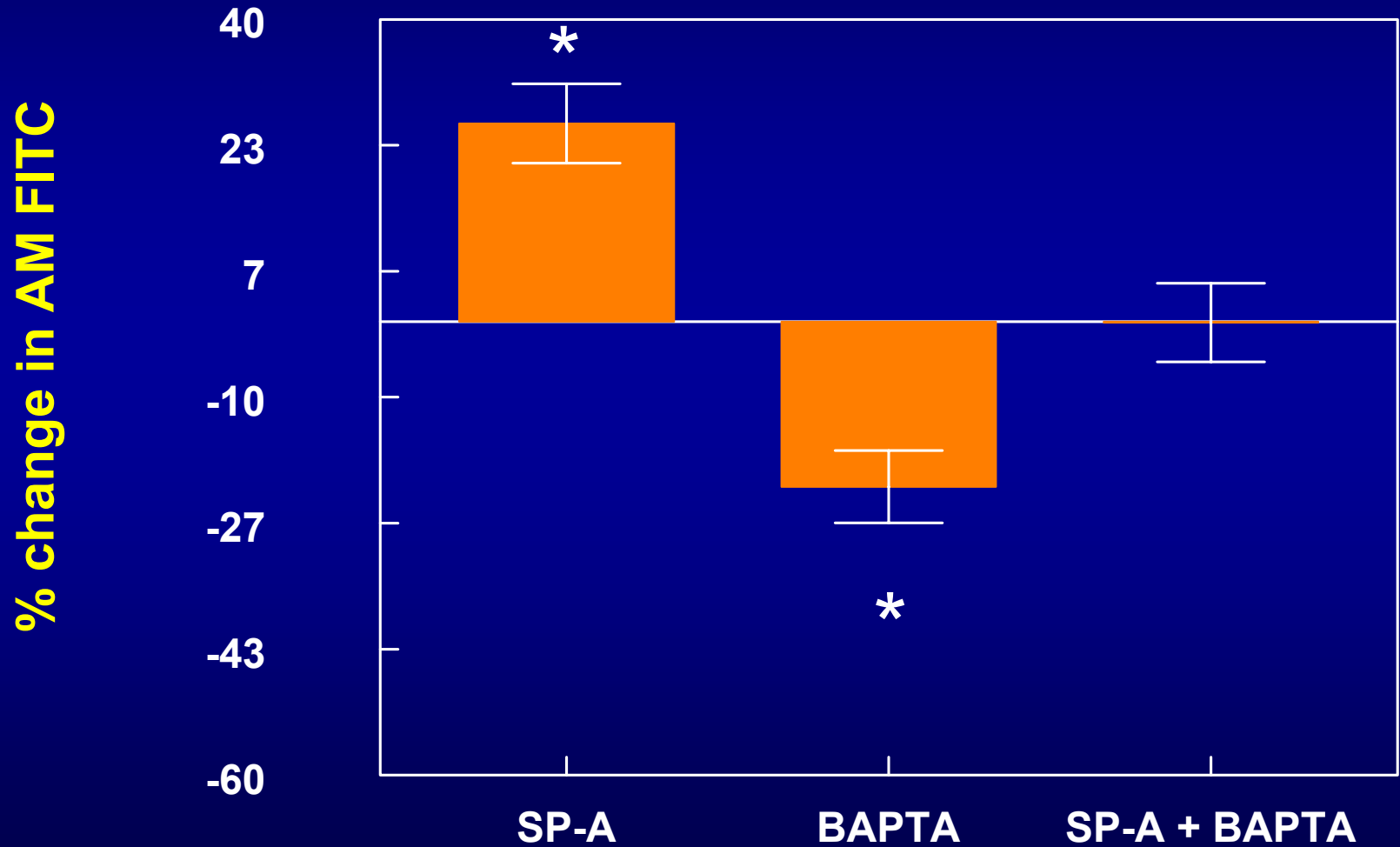
How does SP-A mediate killing of *Klebsiella* by Transplant AMs?

- Increases production of reactive oxygen-nitrogen intermediates by AMs
- SP-A increases phagocytosis of *Klebsiella* by human AMs

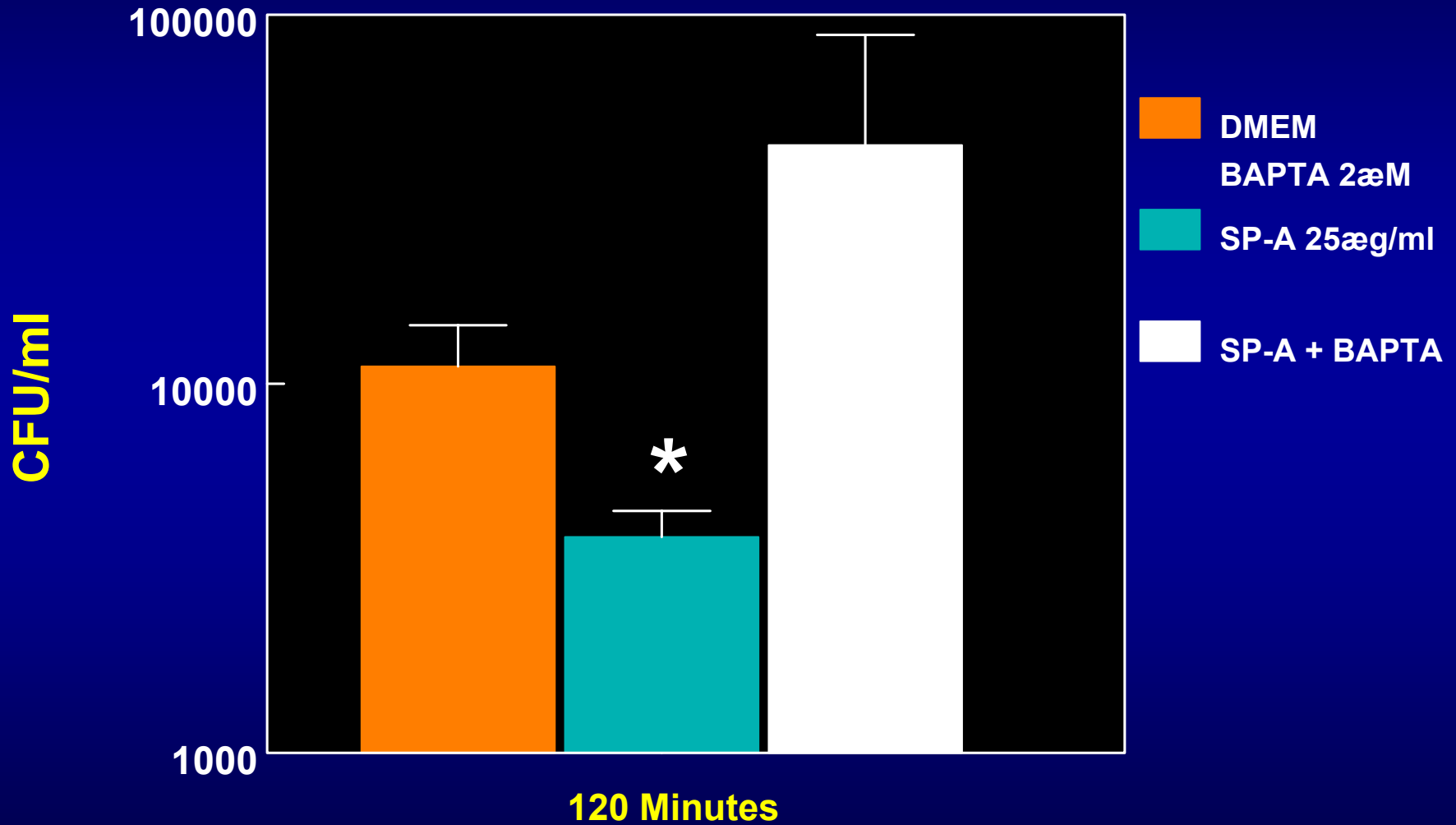
Measurements of Intracellular Ca^{2+} in Human AM During Perfusion with SP-A



Effect of BAPTA on Phagocytosis of FITC-labelled Klebsiella by AMs from Transplanted Patients



Effect of BAPTA on Klebsiella Killing by Transplant AMs



How do transplanted AMs kill *Klebsiella*?

- SP-A differentially stimulates increased NO• and O₂•⁻ production by AMs, **BUT** *Klebsiella* uniformly stimulates increased NO• production by AMs
- SP-A uniformly stimulates increased intracellular calcium levels by AMs
- SP-A uniformly stimulates calcium-dependent phagocytosis of *Klebsiella* by AMs

Acknowledgements

University of Alabama at Birmingham

- **Judy Hickman Davis, DVM, PhD**
- **Philip O'Reilly, MD**
- **Ian Davis, DVM, PhD**
- **Janos Peti-Peterdi, MD**
- **Ms. Glenda Davis**

Acknowledgements

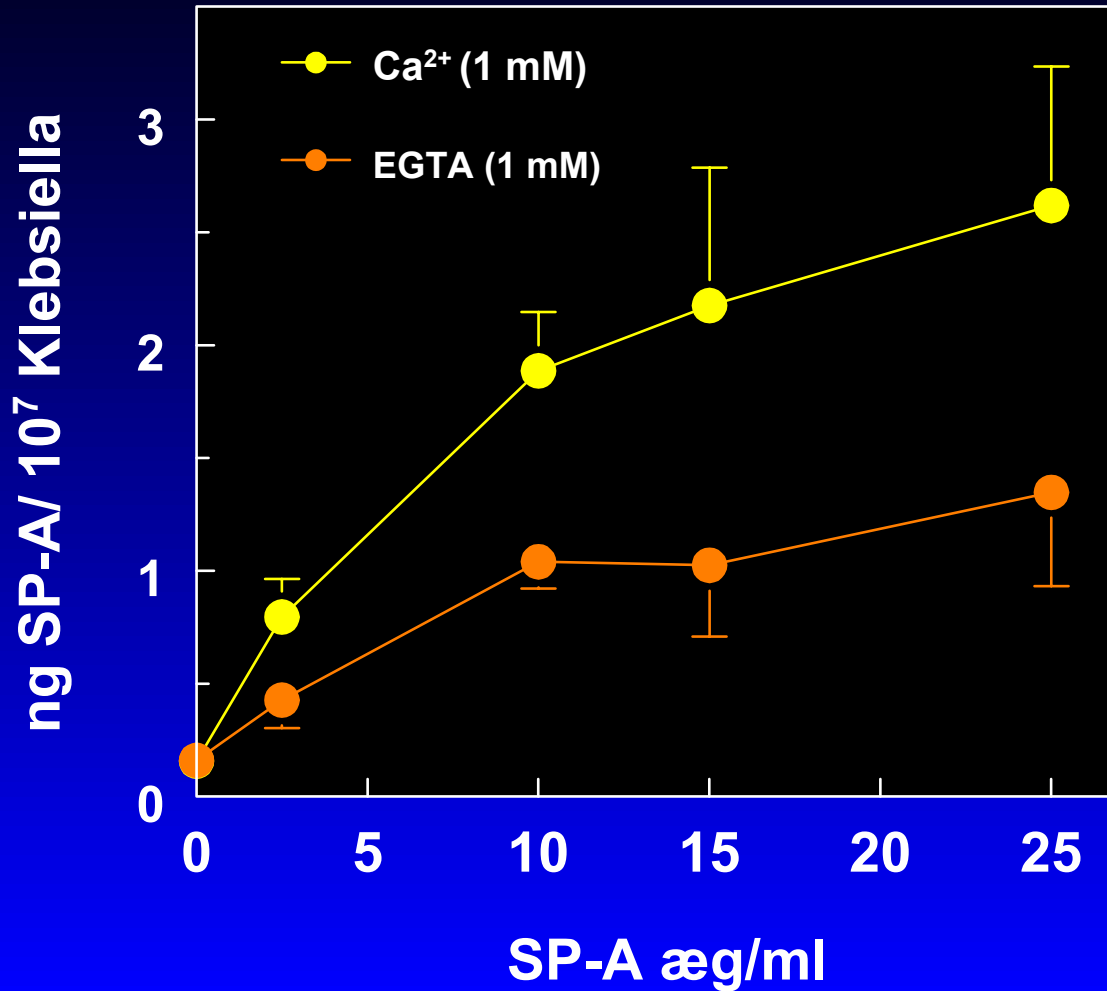
University of Alabama at Birmingham

- **K. Randall Young, MD**
- **Darwin Bell, PhD**
- **Russell Lindsey, DVM**

Environmental Protection Agency

Robert B. Devlin, PhD

Binding of Klebsiella to SP-A



Phagocytosis Assay

5×10^5 AMs



25 $\mu\text{g}/\text{ml}$ SP-A + $\sim 10^8$ FITC-*Klebsiella*



Shake X 60min @ 37°C



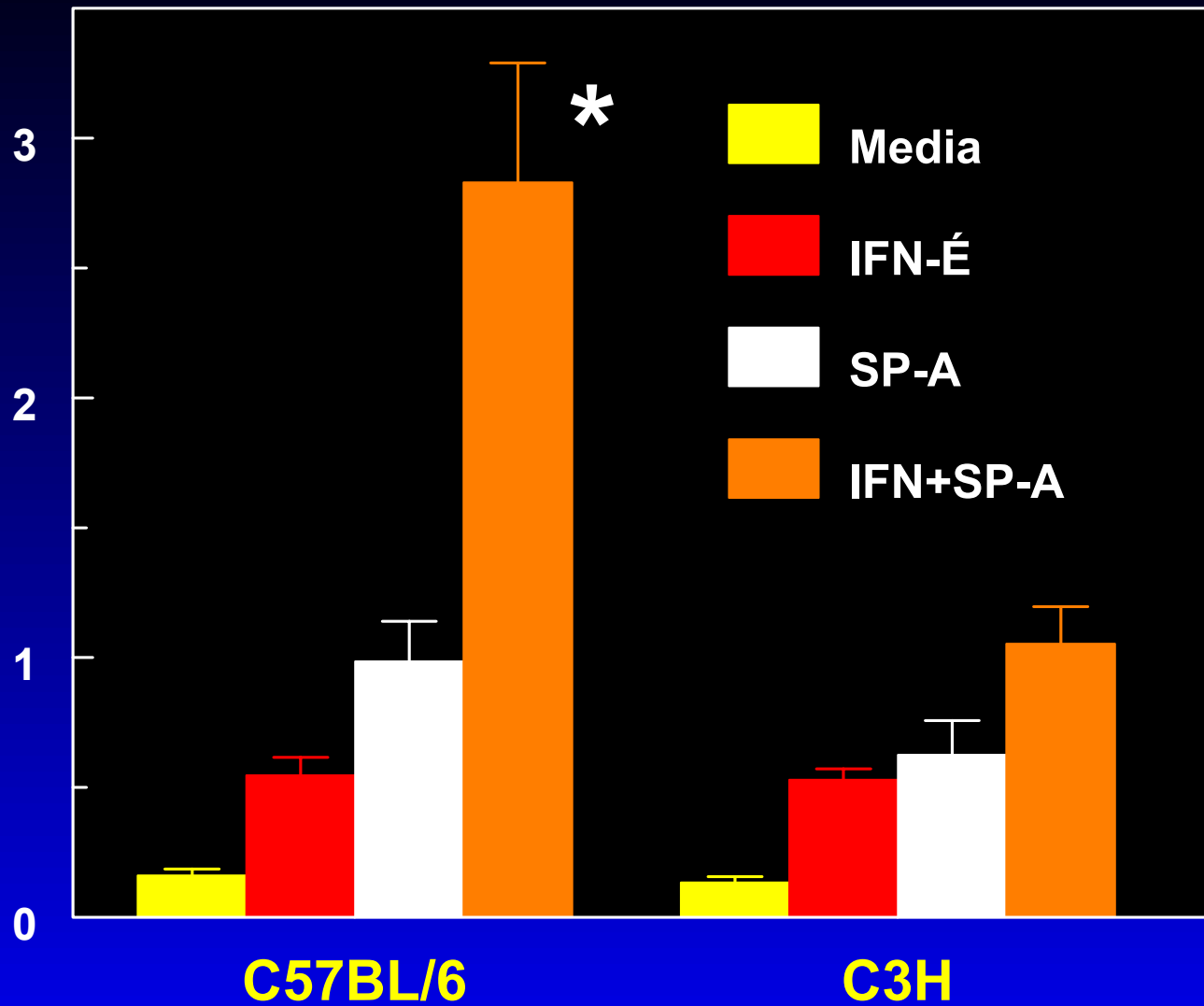
Wash ice cold PBS + 0.2% EDTA



10 $\mu\text{g}/\text{ml}$ EtBr

Production of NO_x by AMs

[NO₂⁻ and NO₃⁻] (nmol X10⁶ AMs⁻¹ @ h¹)



NOx Production by AM from Transplanted Lungs

