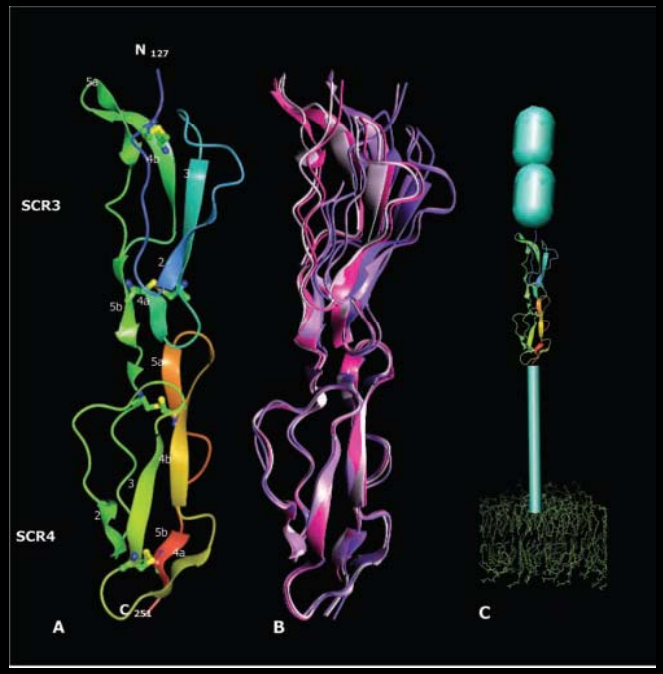
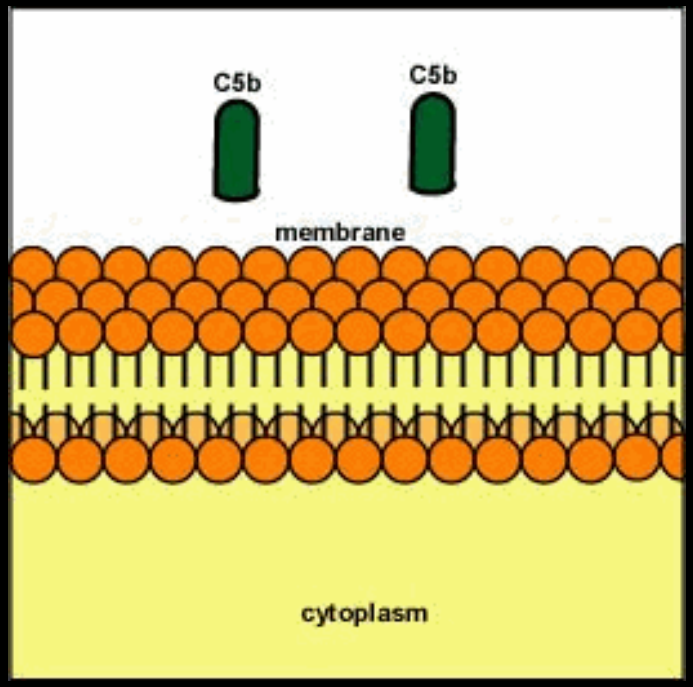
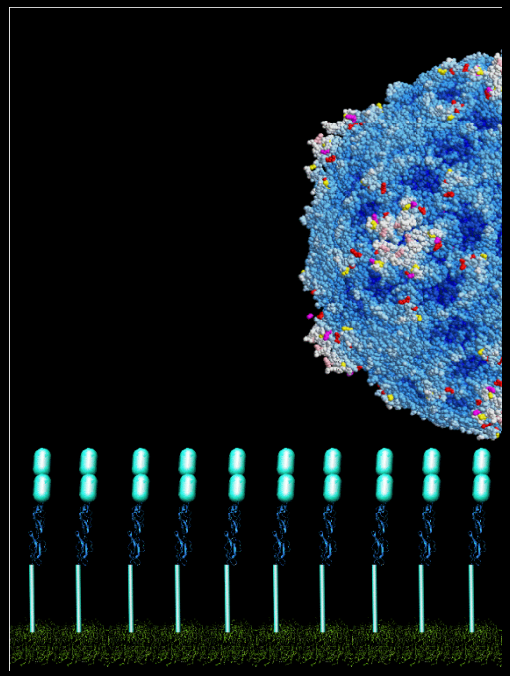
Current Status of Complement Activation in Trauma and Attempts to Manipulate the System to Prolong the Golden Hour







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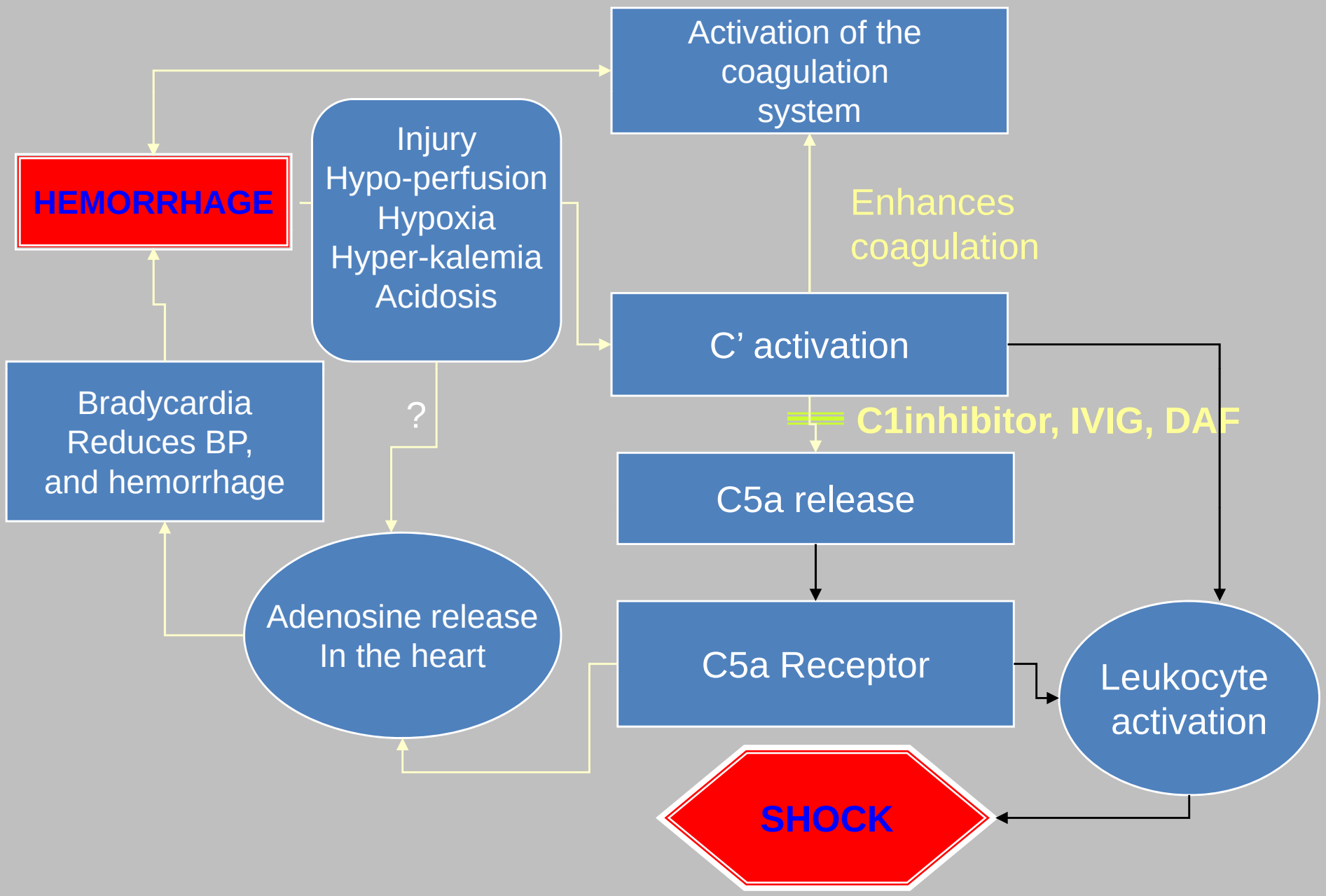
Division of Military Casualty Research
Walter Reed Army Institute of Research

OUTLINE

- Complement Activation in Trauma Patients – The Hungarian Study
- Complement Inhibition Therapy with hrDAF
  - Experimental Design
  - DAF Alone
  - DAF with Hextend
- What Is Next?

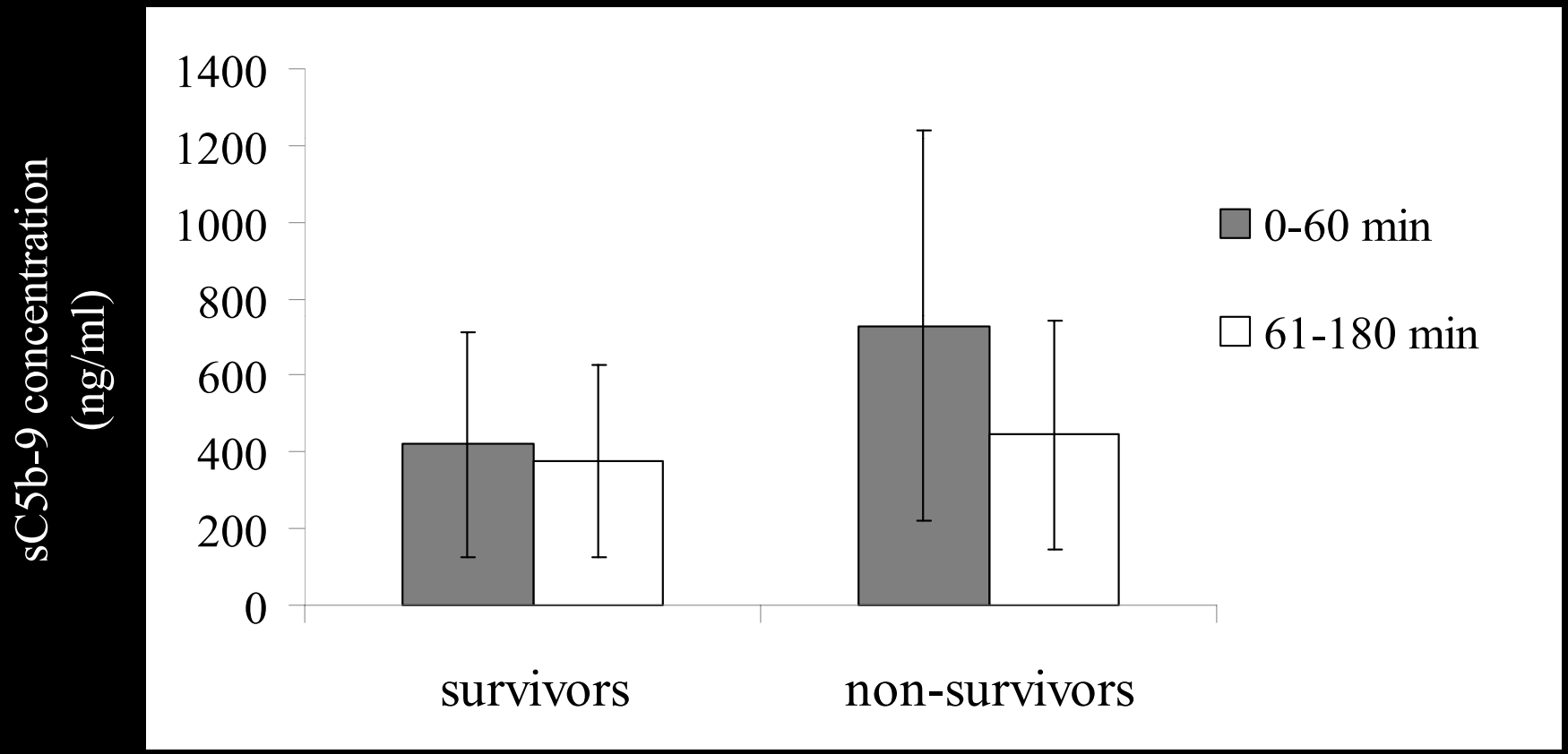
Events in the early phase of hemorrhage



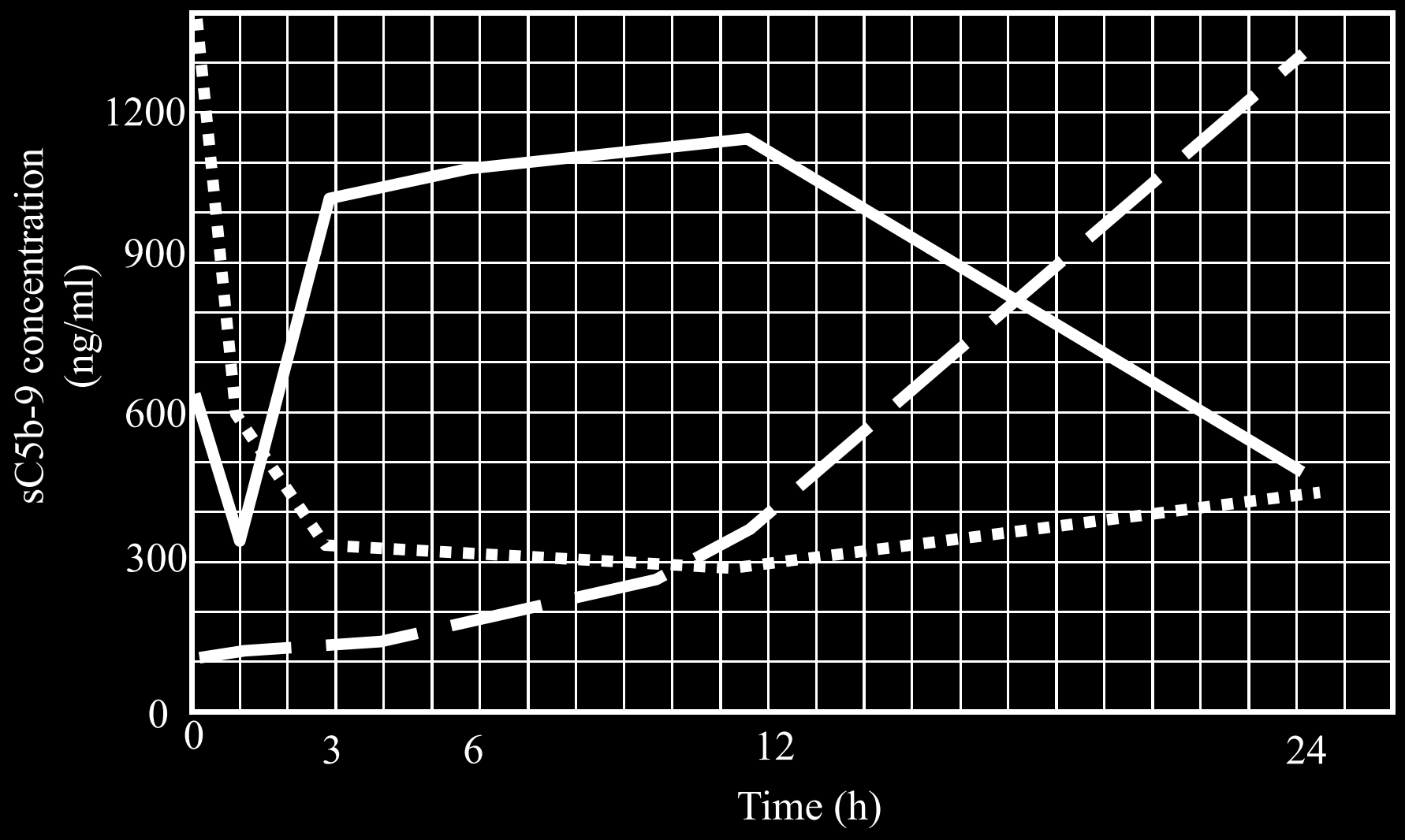
The Hungarian Study

- Adult patients with blunt multiple or severe injuries (ISS>12) admitted directly to the shock room then transferred to ICU
- Exclusion criteria: age<18 years; penetrating injury; time between injury and admission > 3 hours; transport from other hospital; presence of non-traumatic severe disease (eg: stroke, cancer)
- Complement measurements performed at admission, then 1,3,6,12,24 hours later

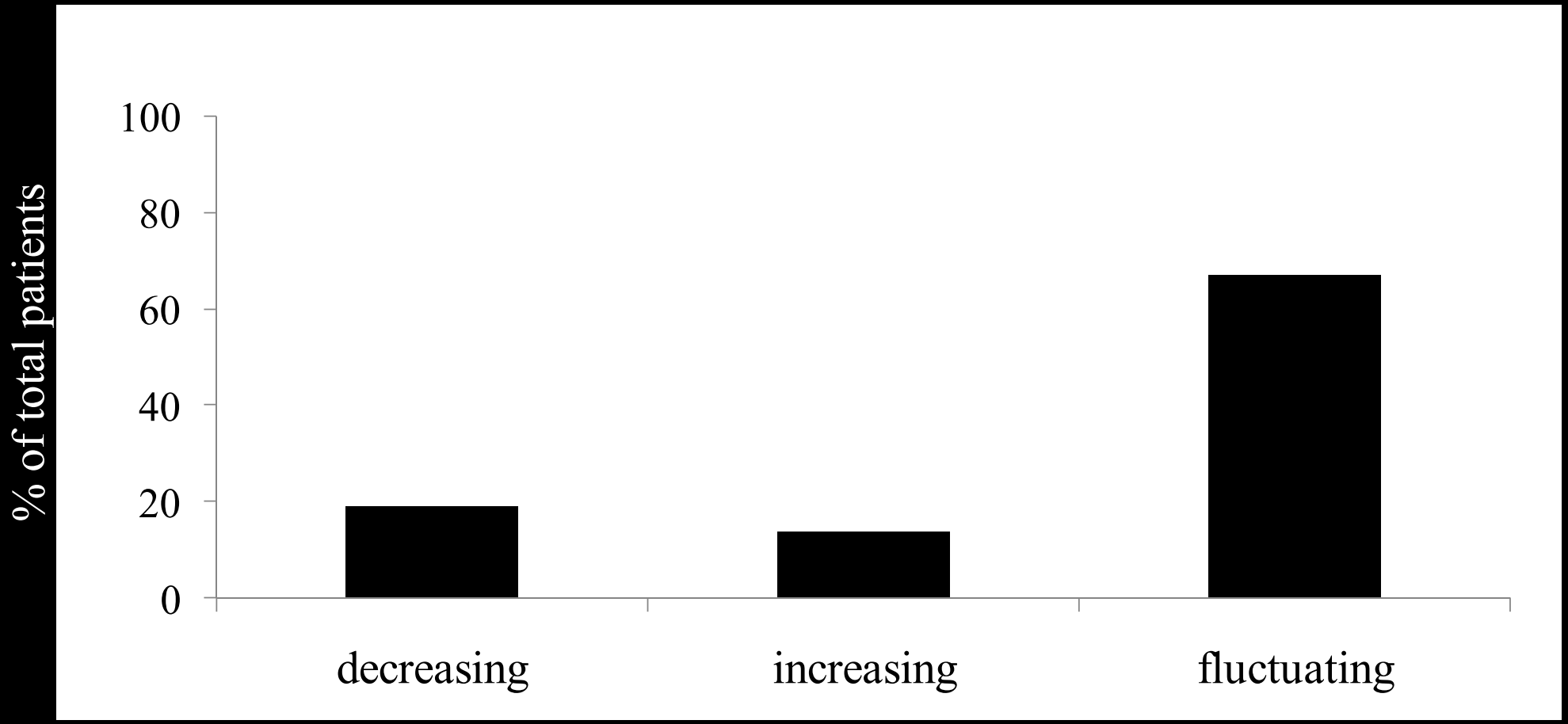
Higher sC5b-9 Levels at Admission Relates to Mortality

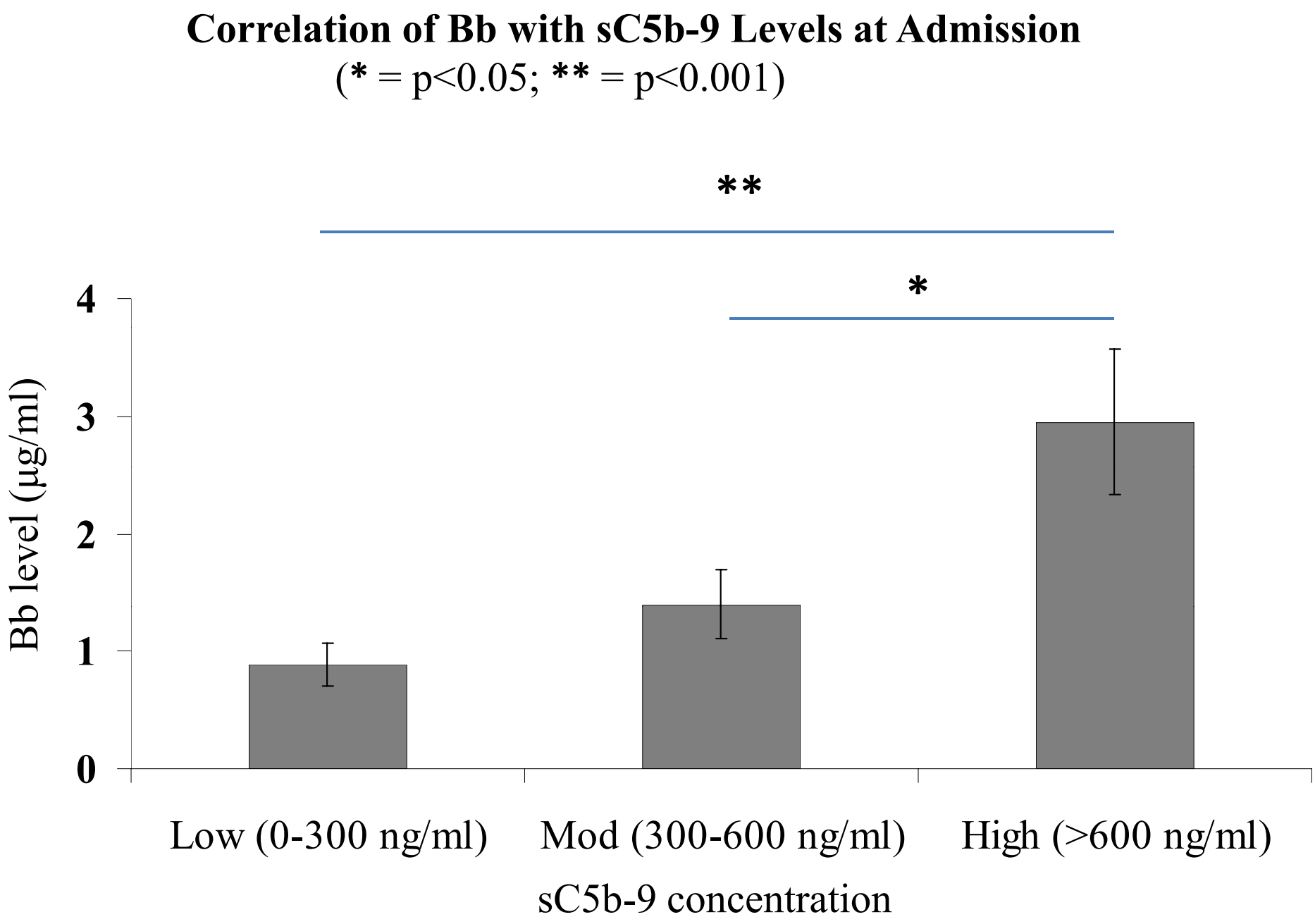


Typical Kinetic Curves of sC5b-9 Changes After Multiple Injury
Steadily decreasing (*dotted line*), steadily increasing (*broken line*), fluctuating (*solid line*)

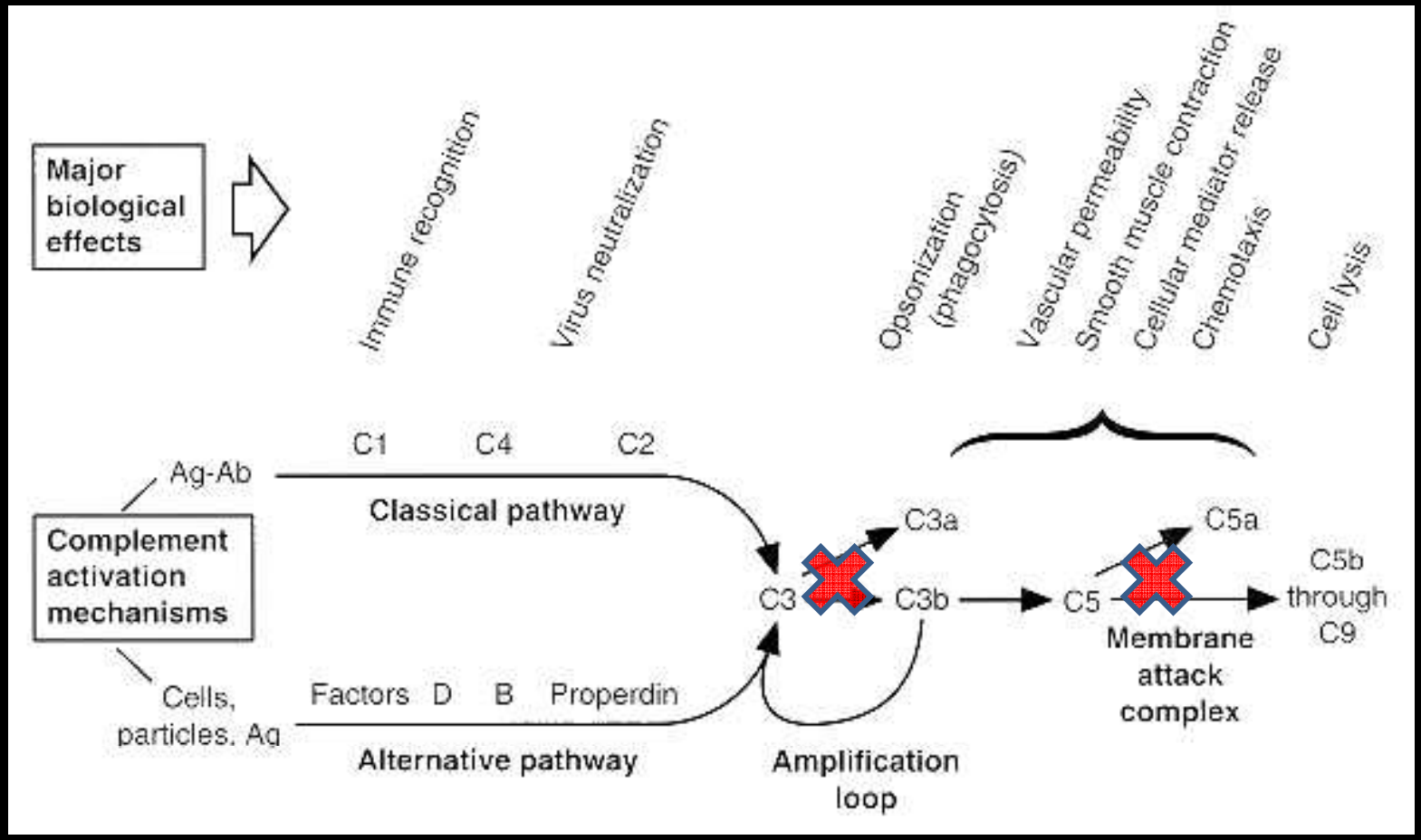


Occurence of Clinical Triad (hypotension, elevated serum lactate, coagulopathy) in Patients with Different Complement Activation Kinetics



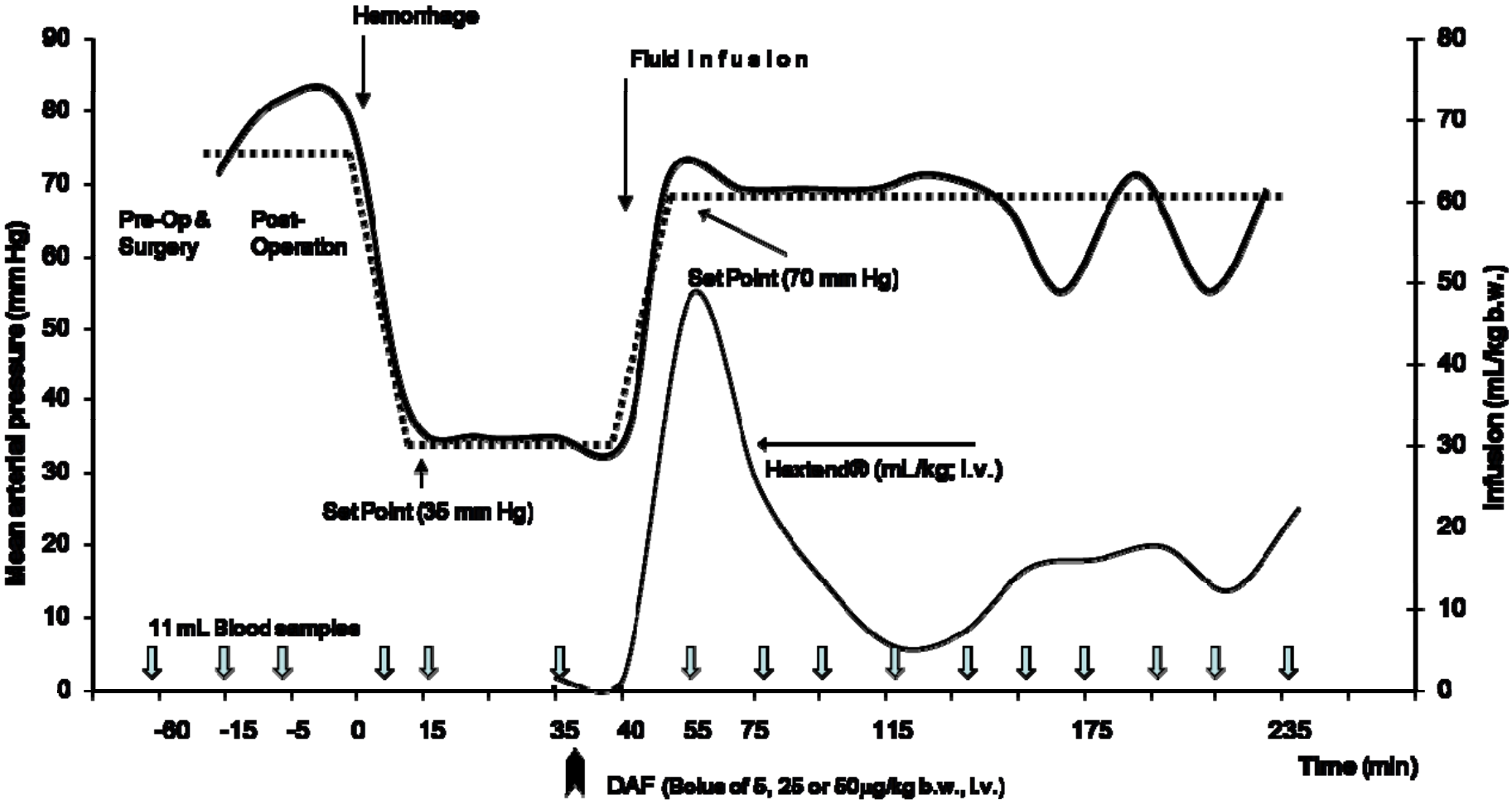

Correlation of Bb with sC5b-9 Levels at Admission
(* = p<0.05; ** = p<0.001)
**
*
4
3
2
1
0
Bb level (µg/ml)
Low (0-300 ng/ml)
Mod (300-600 ng/ml)
High (>600 ng/ml)
sC5b-9 concentration

Complement Inhibition Therapy with Human Recombinant Decay Accelerating Factor (hrDAF)


Major biological effects
Immune recognition
Virus neutralization
Opsonization (phagocytosis)
Vascular permeability
Smooth muscle contraction
Cellular mediator release
Chemotaxis
Cell lysis
Ag-Ab
C1
C4
C2
Classical pathway
Complement activation mechanisms
Cells, particles, Ag
Factors D B Properdin
Alternative pathway
C3
C3a
C3b
Amplification loop
C5
C5a
Membrane attack complex
C5b through C9

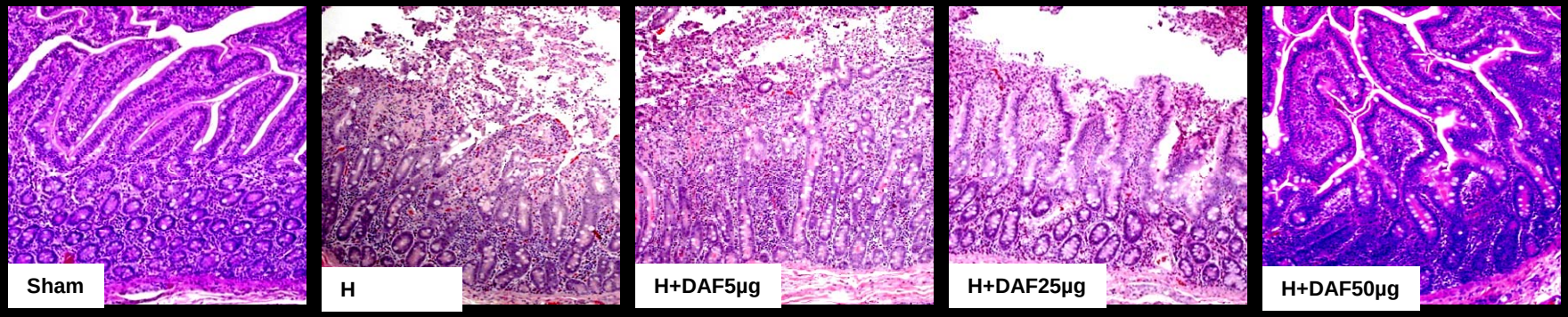
DAF

Experimental Design

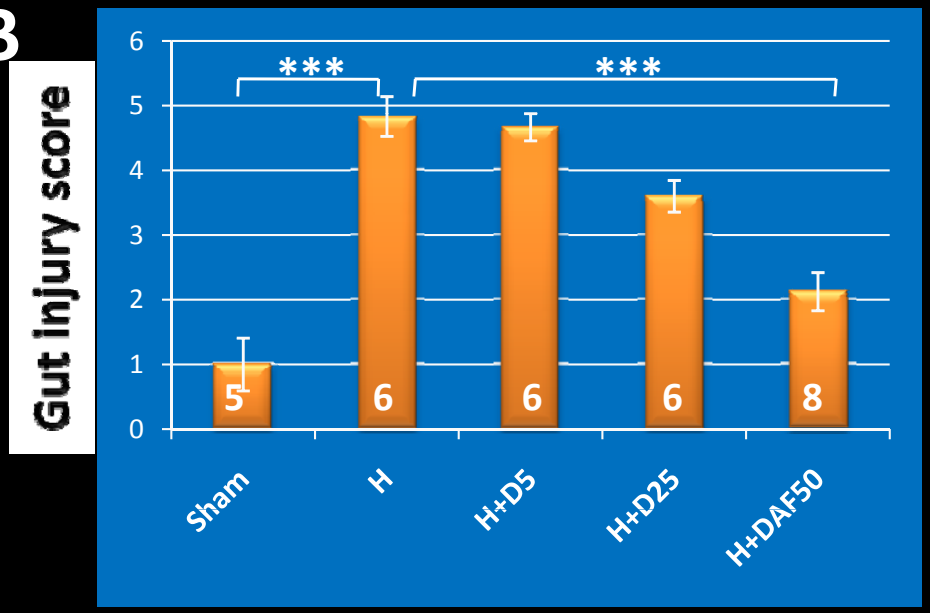


DAF Alone

A

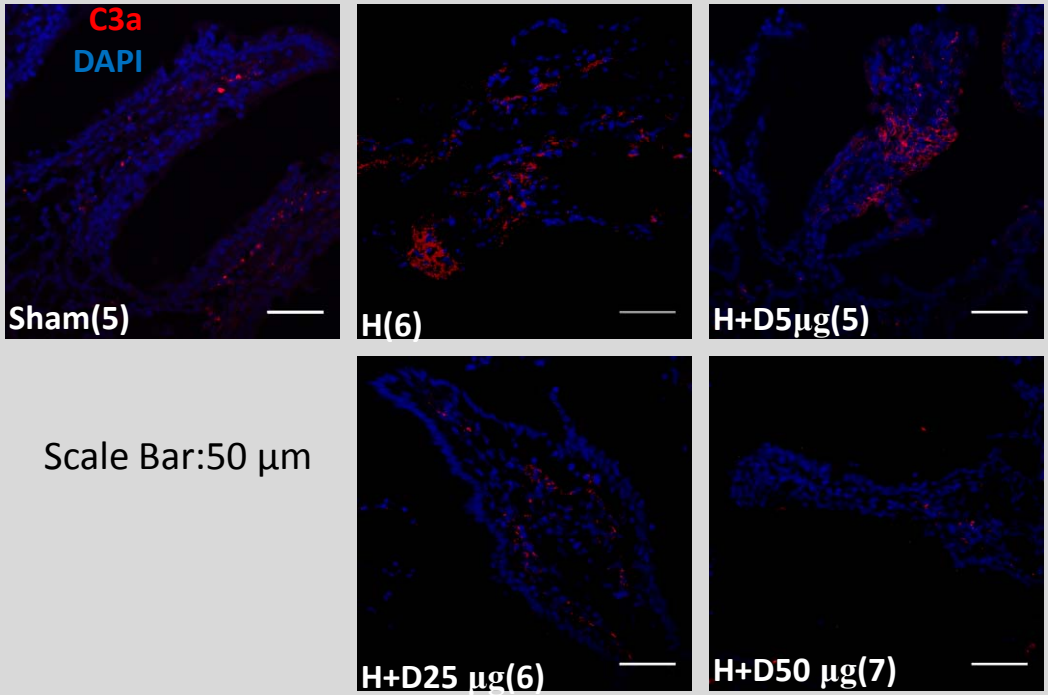

Sham
H
H+DAF5μg
H+DAF25μg
H+DAF50μg

B

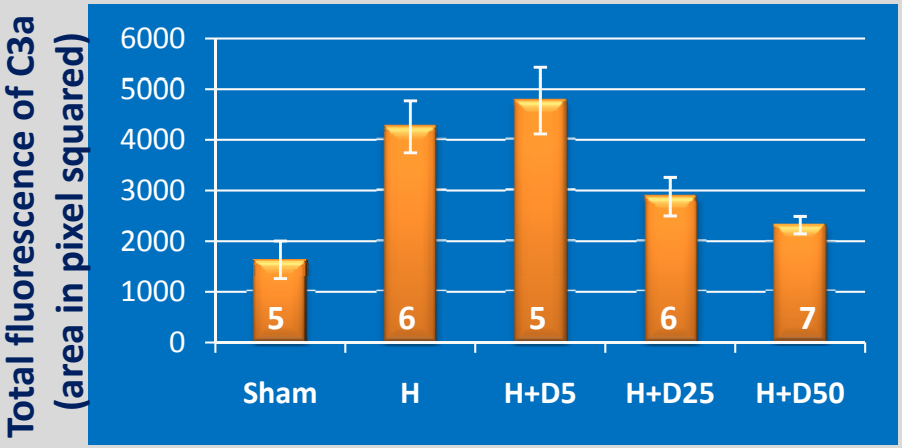

Gut injury score
6
5
4
3
2
1
0
***
***
5
6
6
6
8
Sham
H
H+D5
H+D25
H+DAF50

Gut

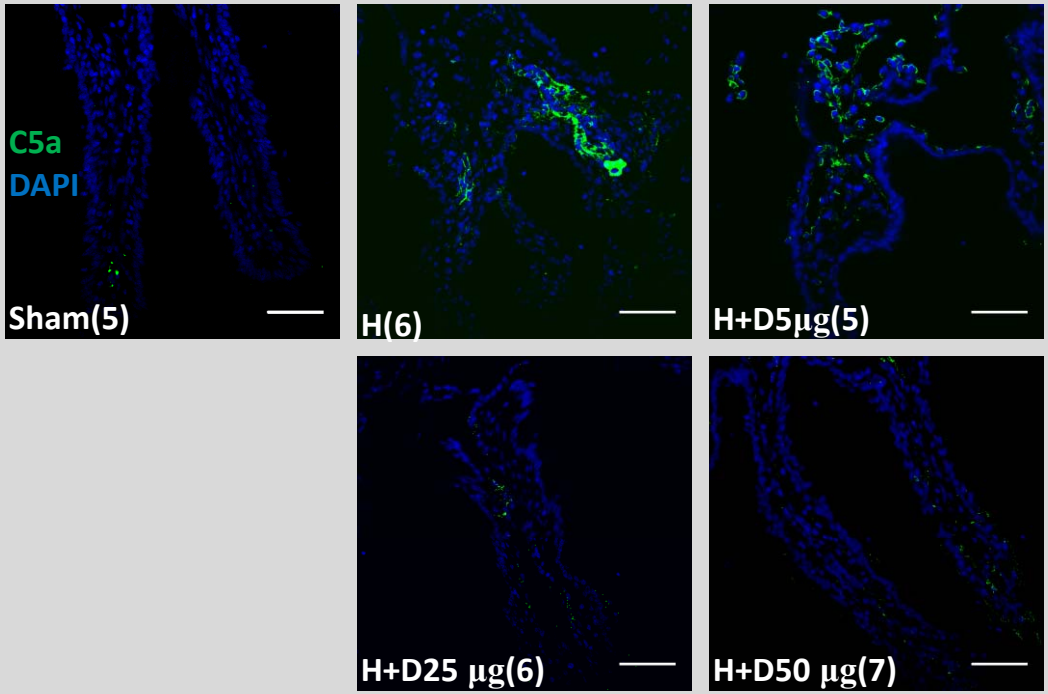
A

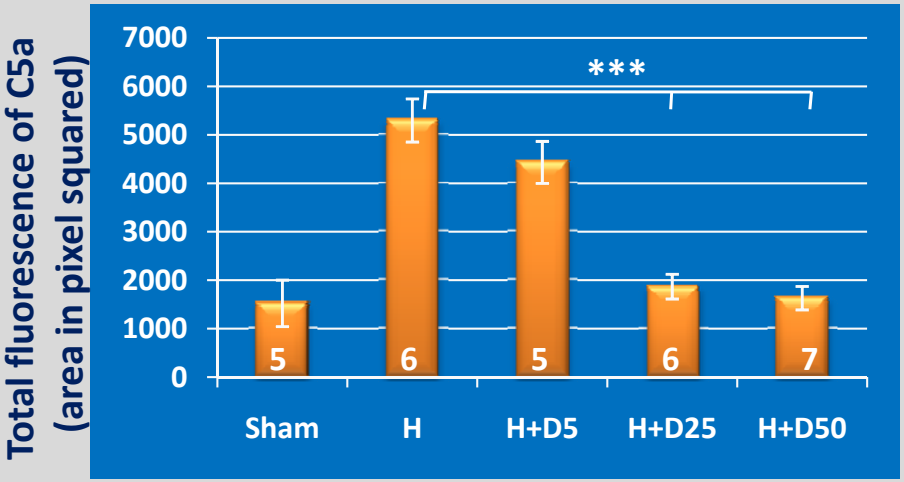


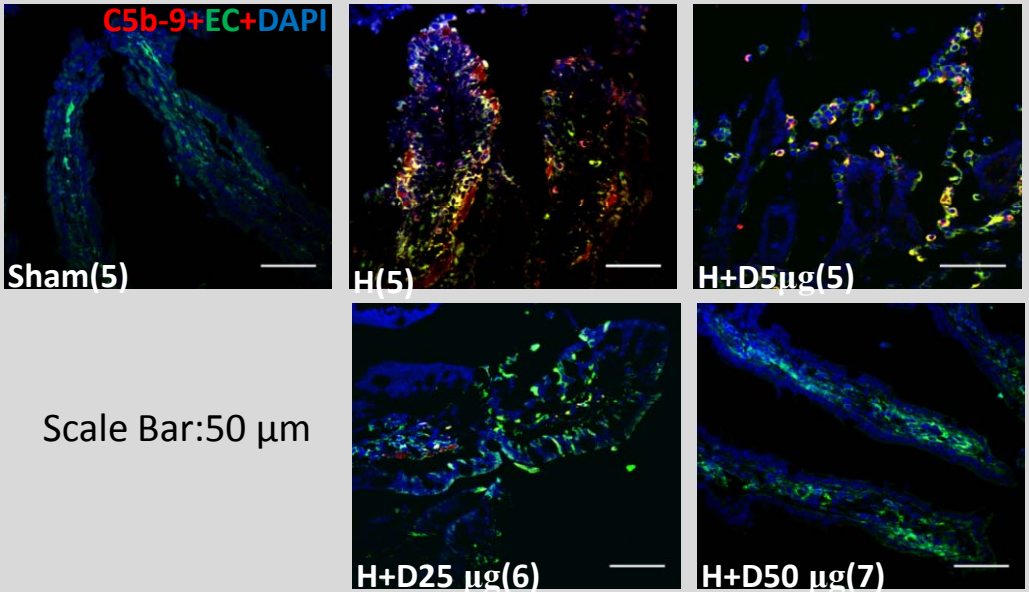
Scale Bar:50 µm



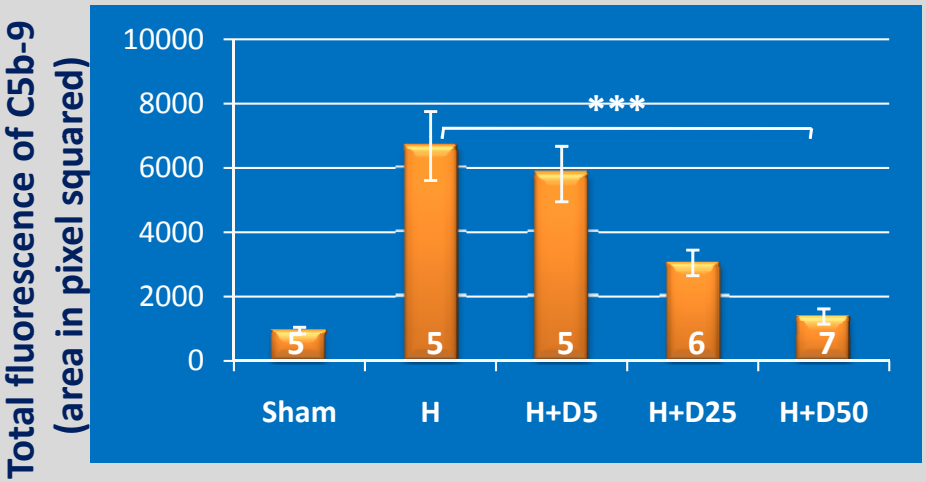
B



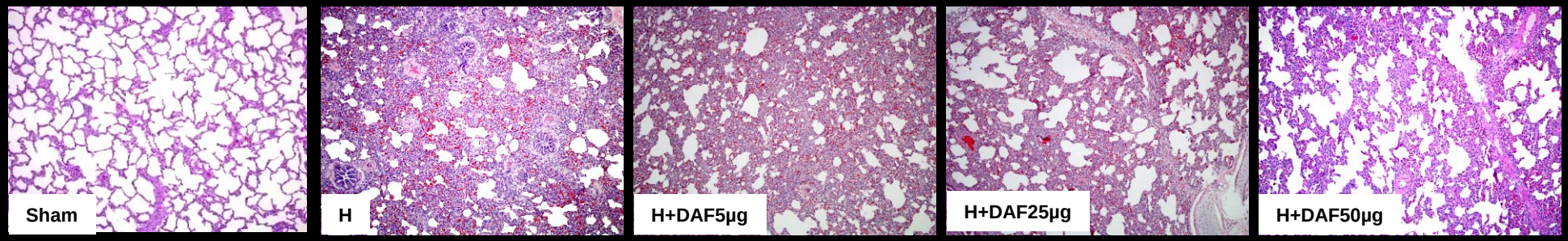




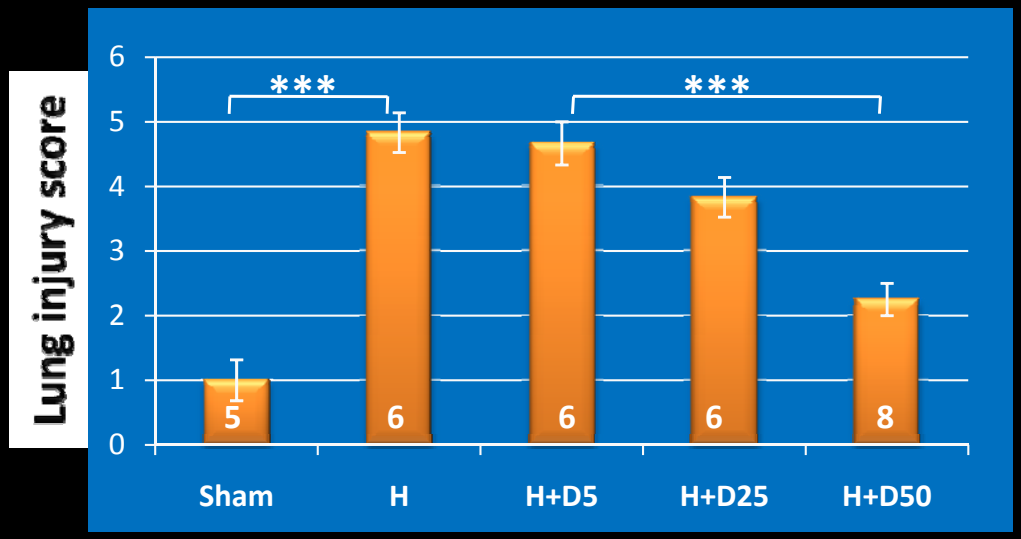
Scale Bar:50 µm



A

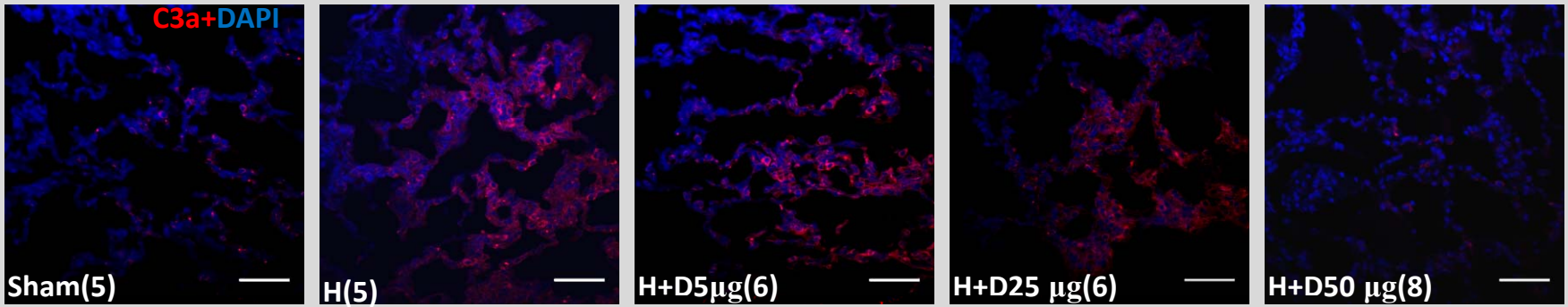

Sham
H
H+DAF5μg
H+DAF25μg
H+DAF50μg

B

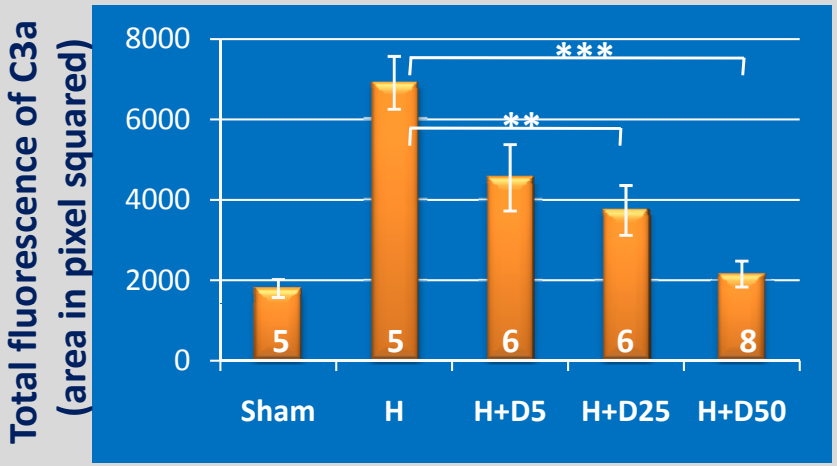

Lung injury score
6
5
4
3
2
1
0
***
***
5
6
6
6
8
Sham
H
H+D5
H+D25
H+D50

Lung

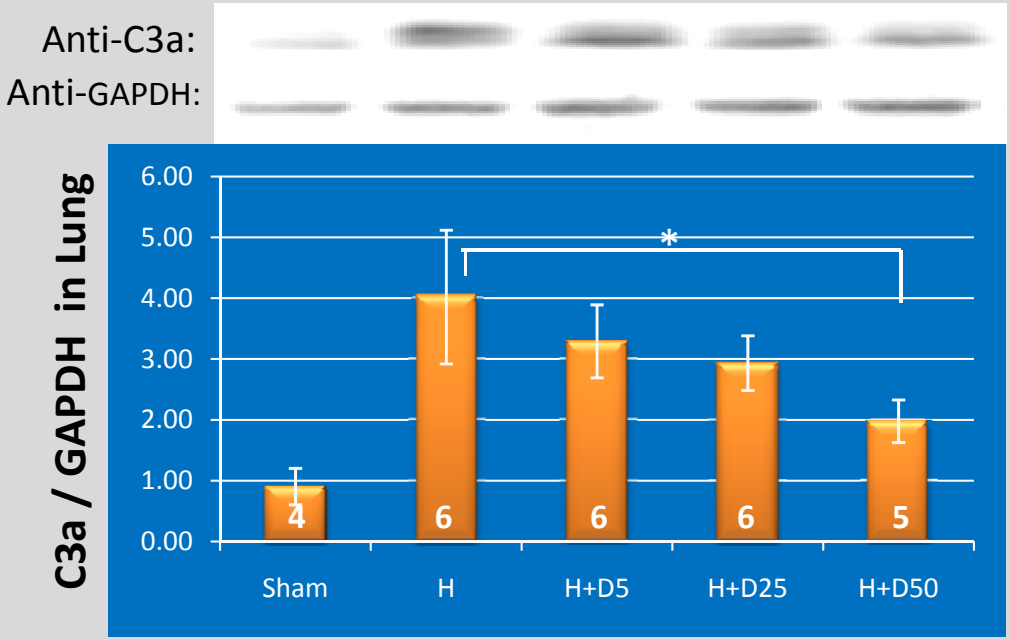
A

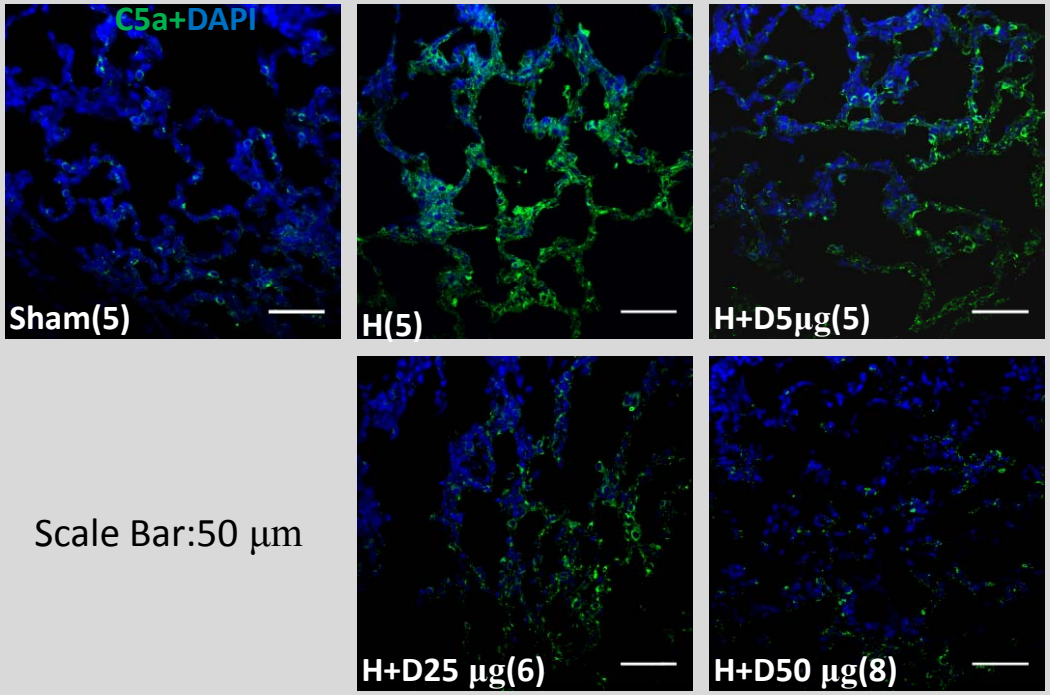


Scale Bar:50 μm

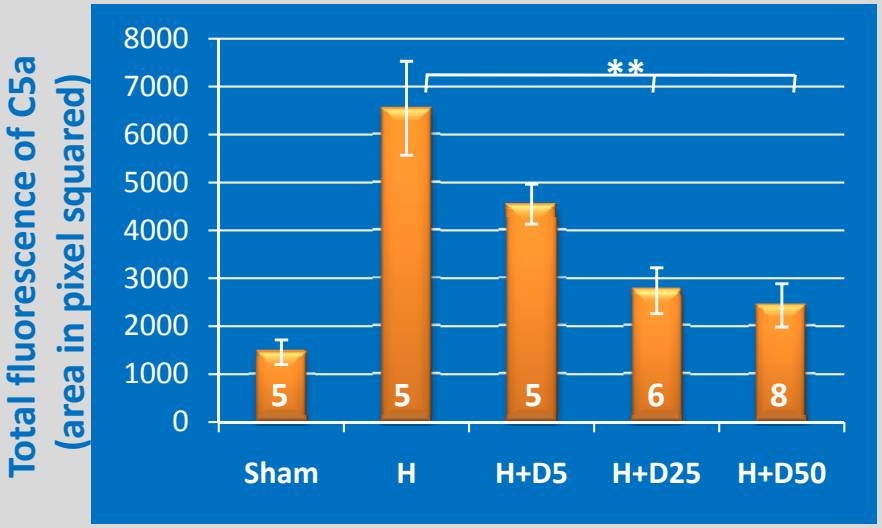


B

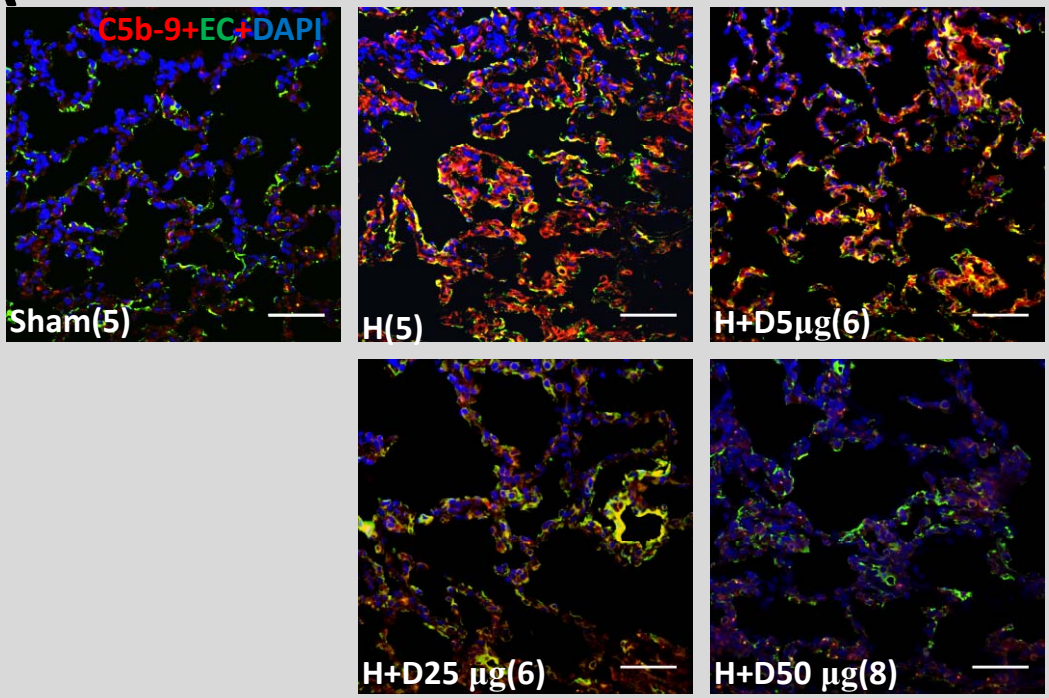


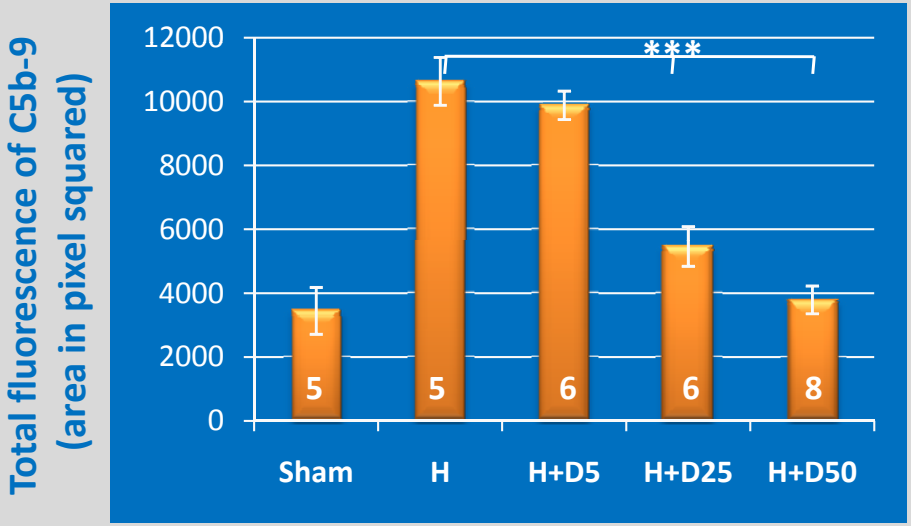


Scale Bar:50 µm

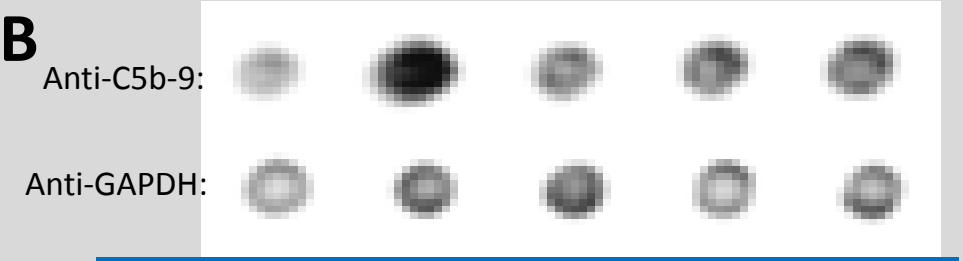


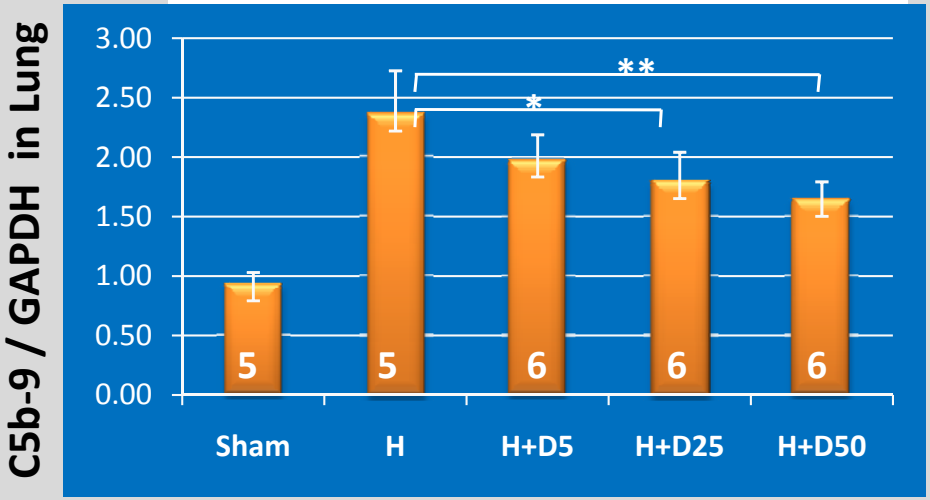
A


C5b-9+EC+DAPI
Sham(5)
H(5)
H+D5µg(6)
H+D25 µg(6)
H+D50 µg(8)

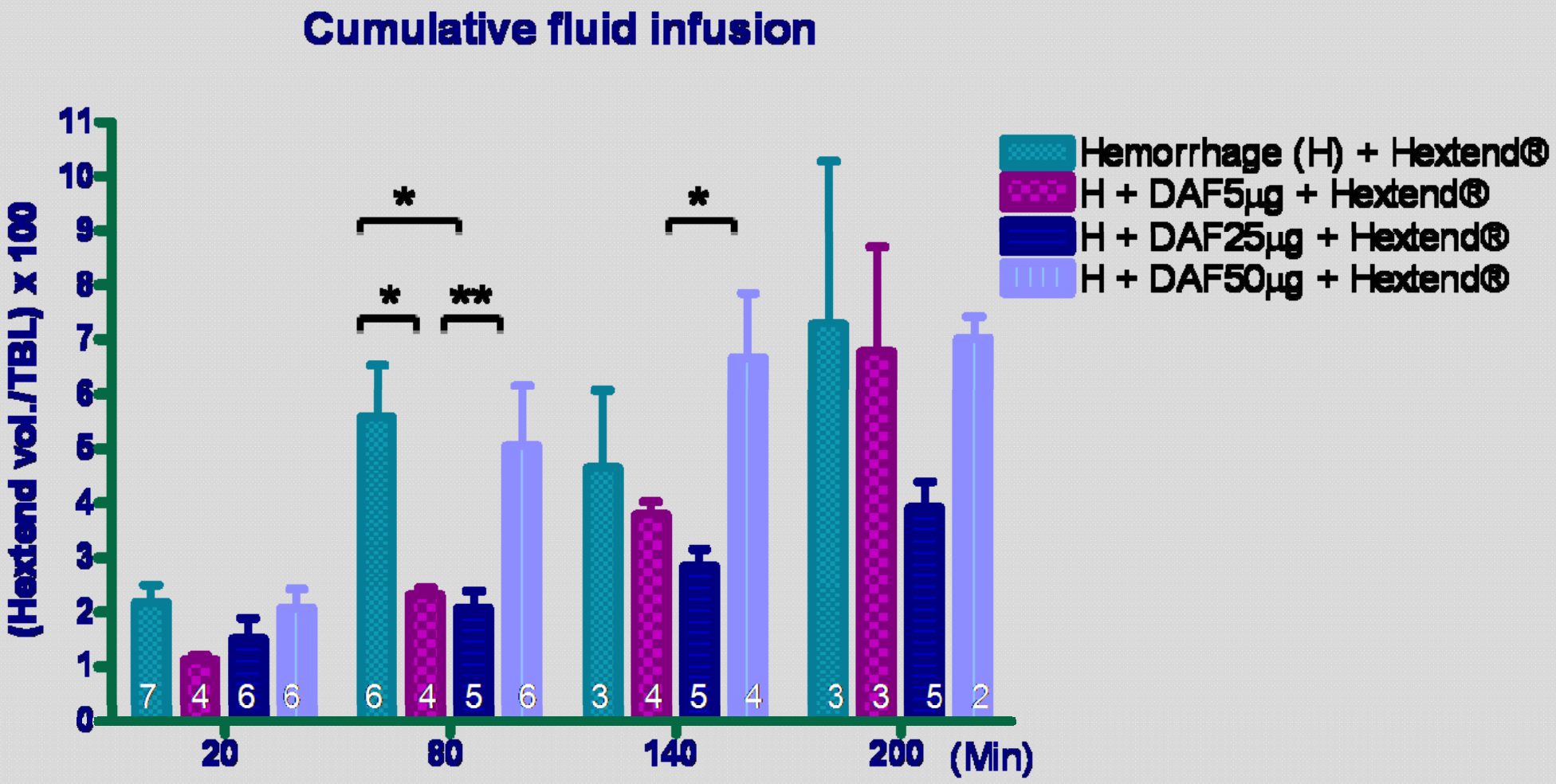

Total fluorescence of C5b-9
(area in pixel squared)
12000
10000
8000
6000
4000
2000
0
***
5
5
6
6
8
Sham
H
H+D5
H+D25
H+D50

B

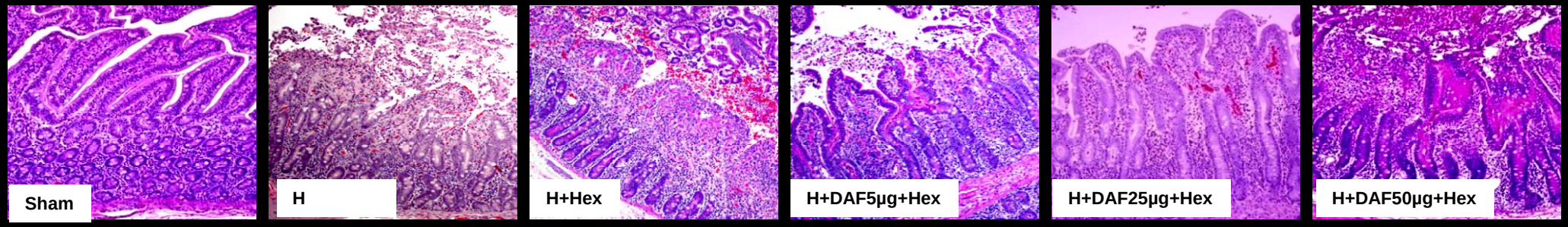

Anti-C5b-9:
Anti-GAPDH:


C5b-9 / GAPDH in Lung
3.00
2.50
2.00
1.50
1.00
0.50
0.00
**
*
5
5
6
6
6
Sham
H
H+D5
H+D25
H+D50

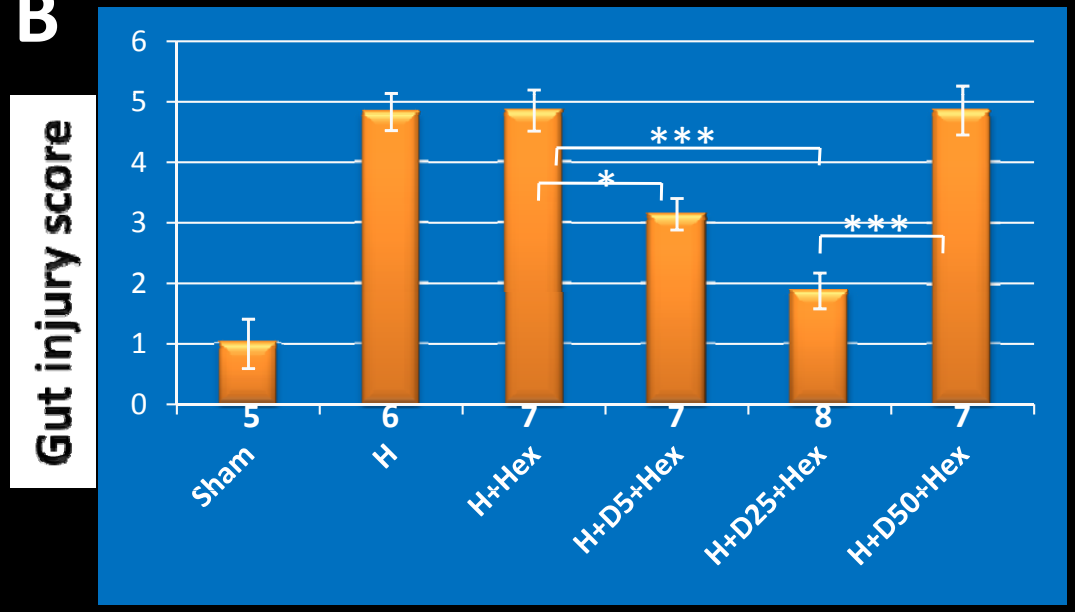
DAF with Hextend


Cumulative fluid infusion
(Hextend vol./TBL) x 100
0
1
2
3
4
5
6
7
8
9
10
11
20
80
140
200
(Min)
*
*
**
*
7
4
6
6
6
4
5
6
3
4
5
4
3
3
5
2
Hemorrhage (H) + Hextend®
H + DAF5µg + Hextend®
H + DAF25µg + Hextend®
H + DAF50µg + Hextend®

A

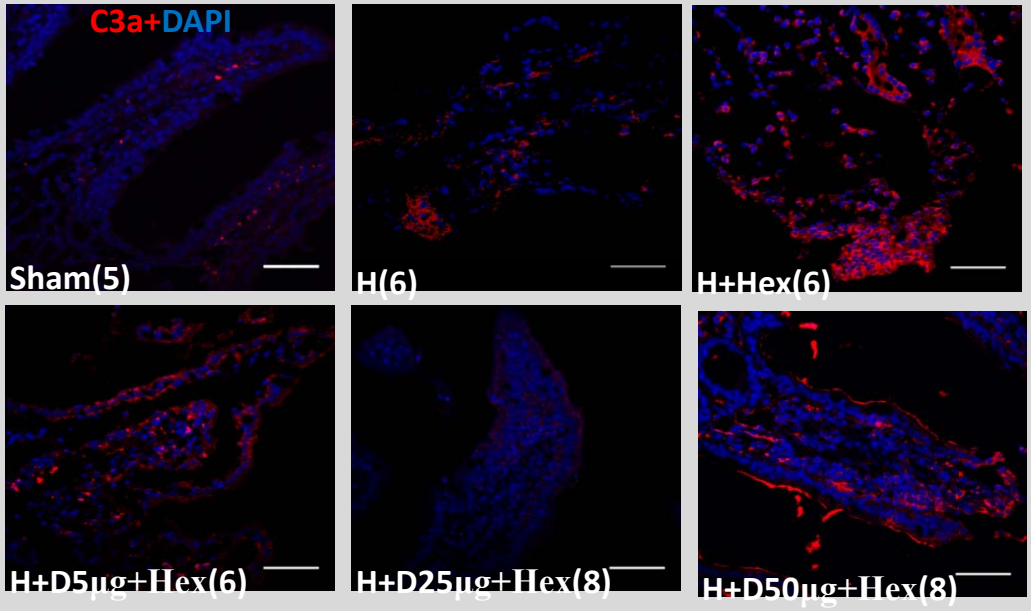

Sham
H
H+Hex
H+DAF5µg+Hex
H+DAF25µg+Hex
H+DAF50µg+Hex

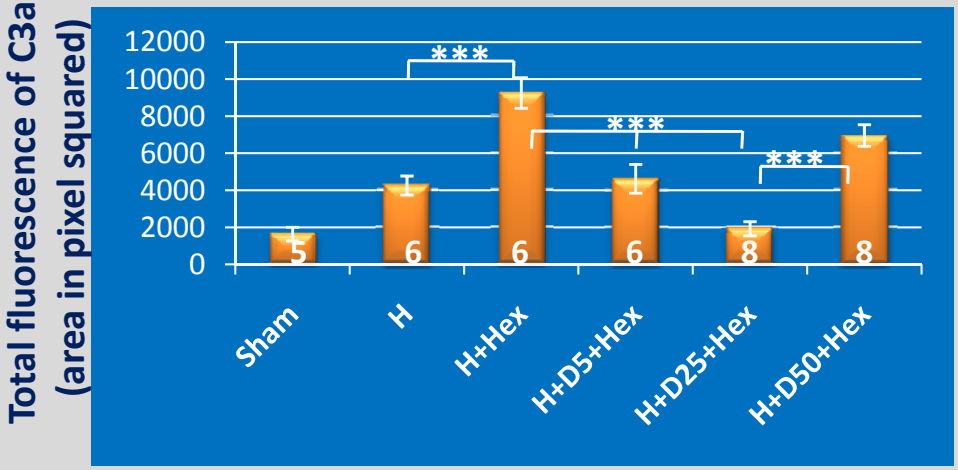
B


Gut injury score
6
5
4
3
2
1
0
***
*
***
5
6
7
7
8
7
Sham
H
H+Hex
H+D5+Hex
H+D25+Hex
H+D50+Hex

Gut

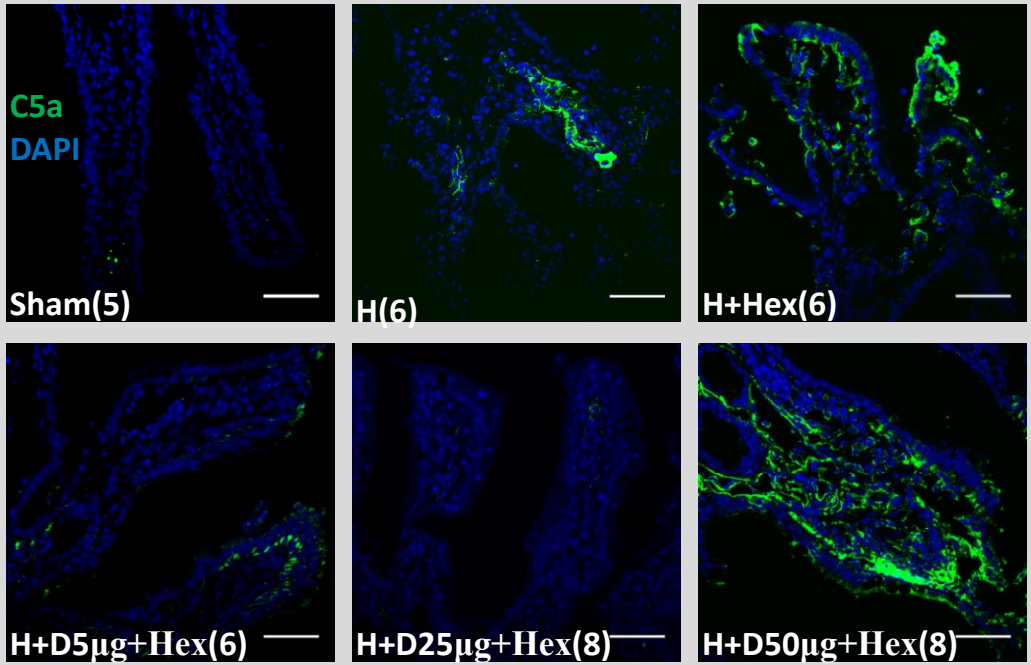
A

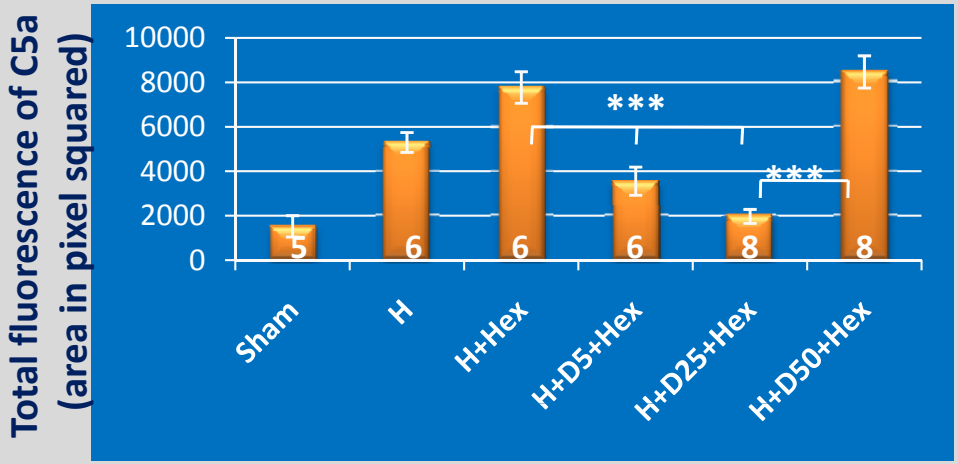


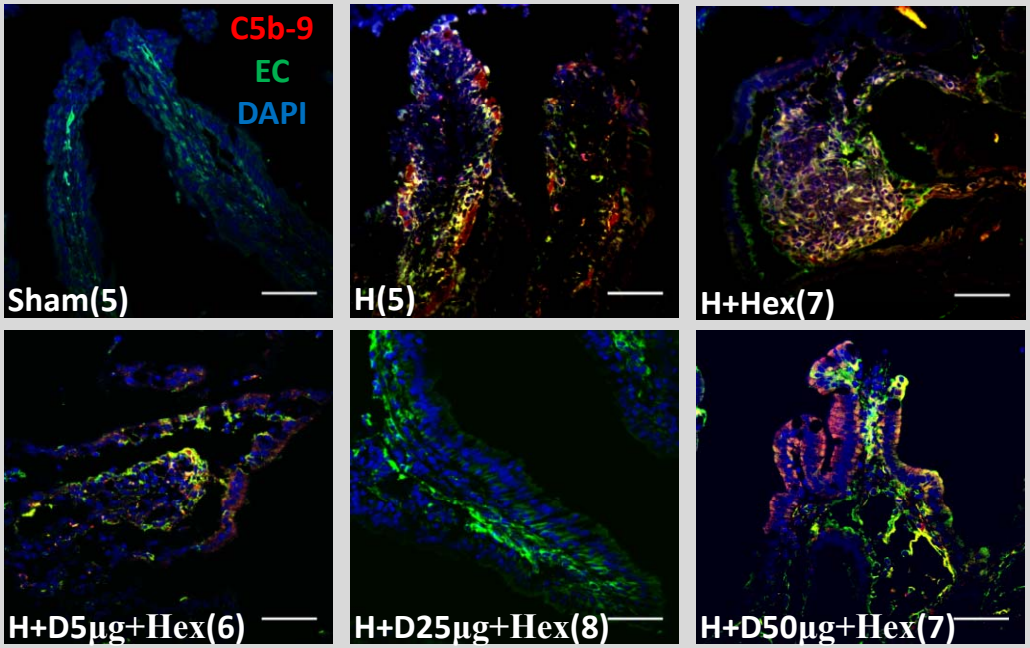


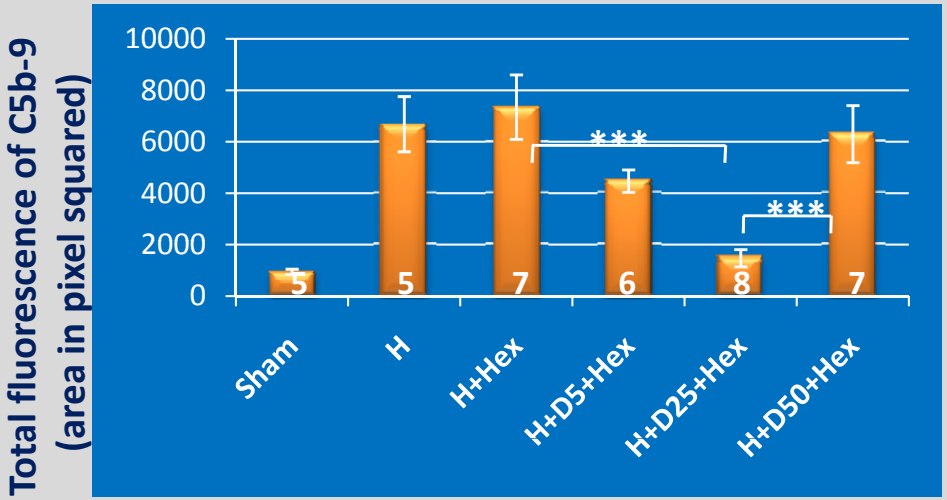
B

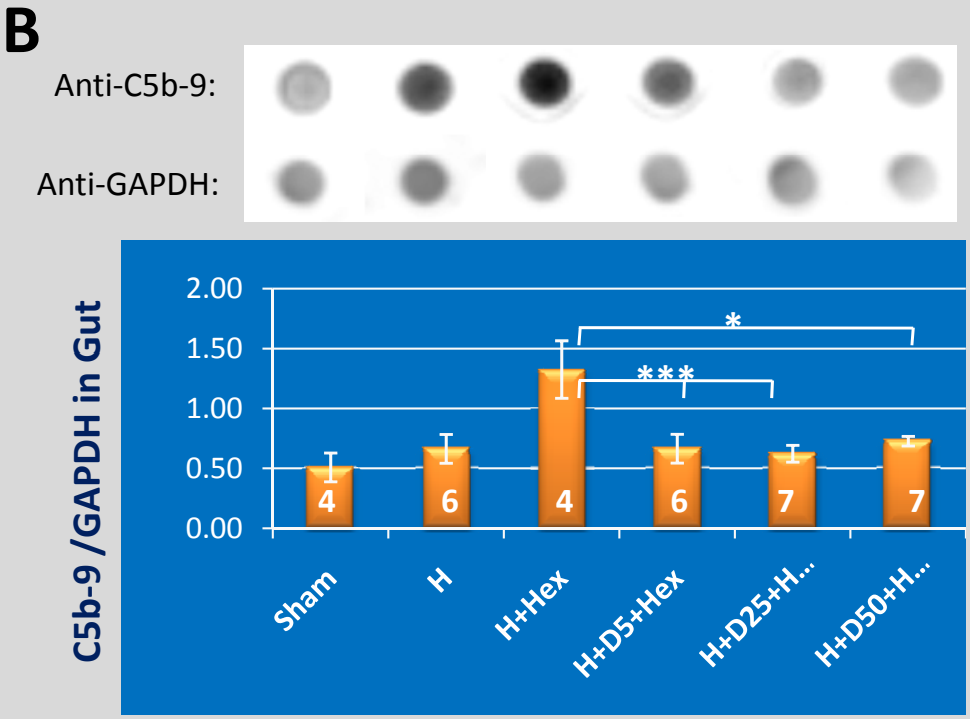
Scale bar:50 μm



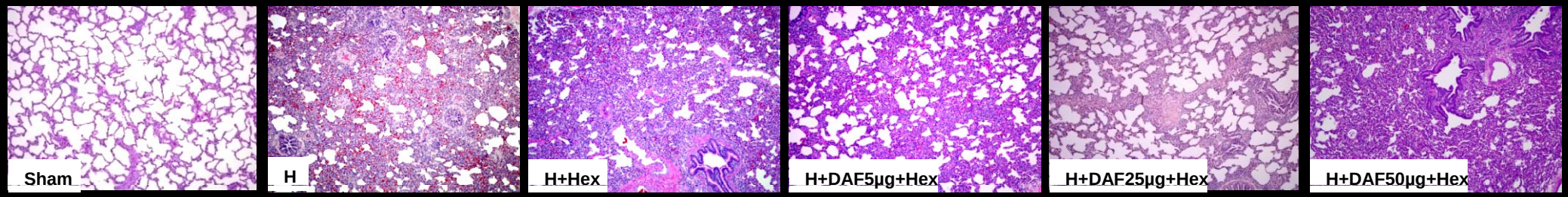



A
Scale Bar:50 µm
C5b-9
EC
DAPI
Sham(5)
H(5)
H+Hex(7)
H+D5µg+Hex(6)
H+D25µg+Hex(8)
H+D50µg+Hex(7)

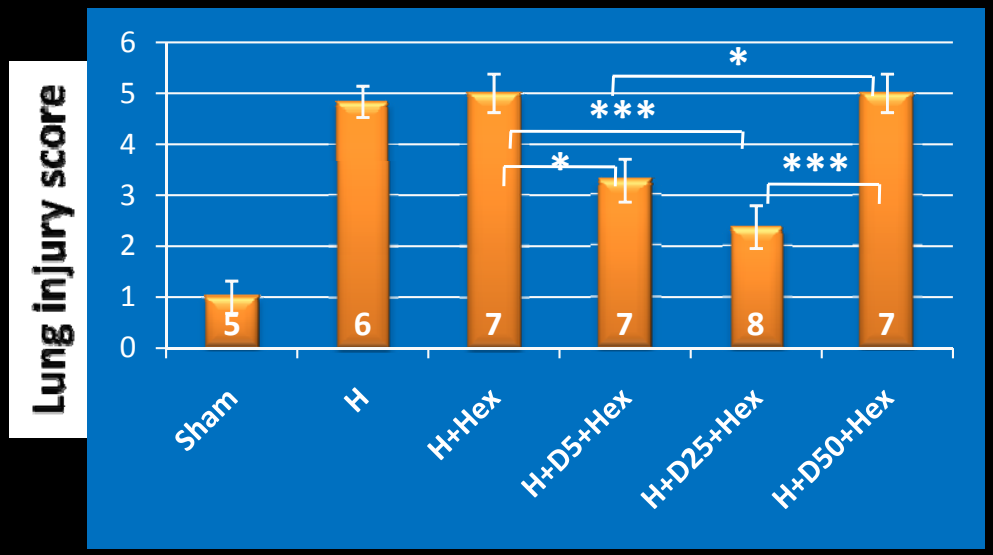

Total fluorescence of C5b-9
(area in pixel squared)
10000
8000
6000
4000
2000
0
***
***
5
5
7
6
8
7
Sham
H
H+Hex
H+D5+Hex
H+D25+Hex
H+D50+Hex


B
Anti-C5b-9:
Anti-GAPDH:
C5b-9 /GAPDH in Gut
2.00
1.50
1.00
0.50
0.00
*
***
4
6
4
6
7
7
Sham
H
H+Hex
H+D5+Hex
H+D25+H...
H+D50+H...

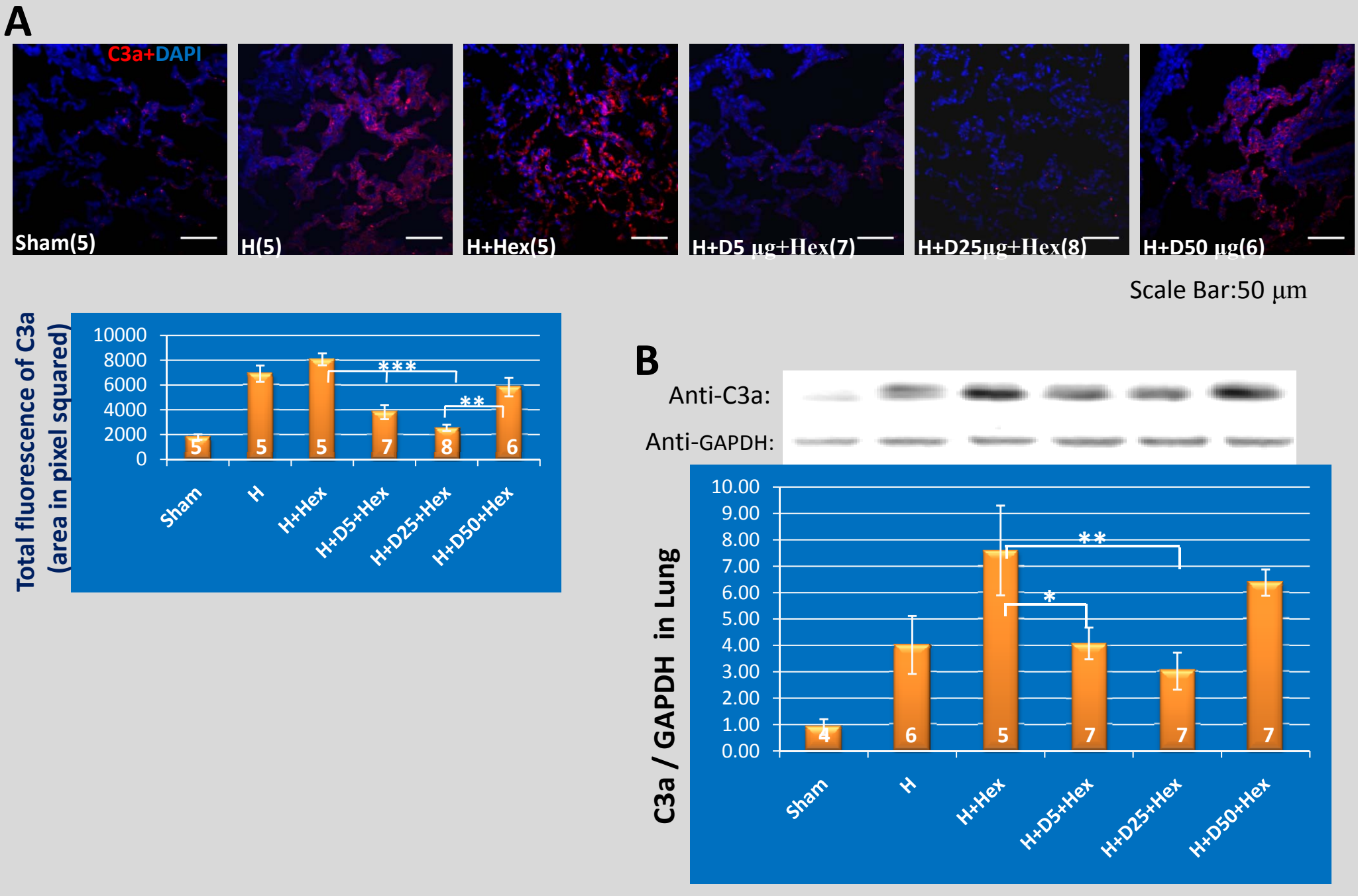
A

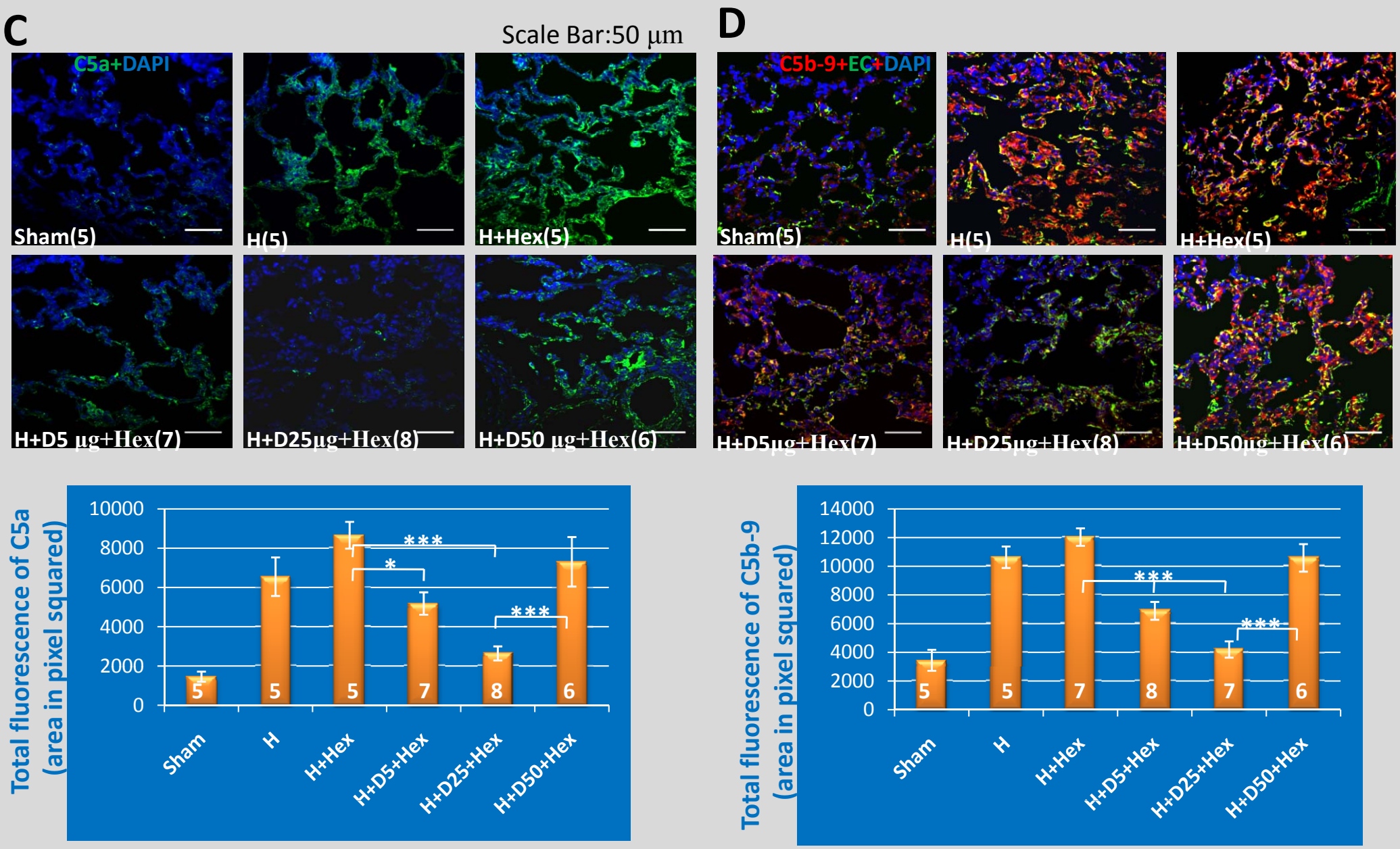

Sham
H
H+Hex
H+DAF5µg+Hex
H+DAF25µg+Hex
H+DAF50µg+Hex

B


Lung injury score
6
5
4
3
2
1
0
5
6
7
7
8
7
*
***
*
***
Sham
H
H+Hex
H+D5+Hex
H+D25+Hex
H+D50+Hex

Lung


A
C3a+DAPI
Sham(5)
H(5)
H+Hex(5)
H+D5 μg+Hex(7)
H+D25μg+Hex(8)
H+D50 μg(6)
Scale Bar:50 μm
Total fluorescence of C3a (area in pixel squared)
10000
8000
6000
4000
2000
0
***
**
5
5
5
7
8
6
Sham
H
H+Hex
H+D5+Hex
H+D25+Hex
H+D50+Hex
B
Anti-C3a:
Anti-GAPDH:
C3a / GAPDH in Lung
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
**
*
4
6
5
7
7
7
Sham
H
H+Hex
H+D5+Hex
H+D25+Hex
H+D50+Hex


C
Scale Bar:50 µm
C5a+DAPI
Sham(5)
H(5)
H+Hex(5)
H+D5 µg+Hex(7)
H+D25µg+Hex(8)
H+D50 µg+Hex(6)
D
C5b-9+EC+DAPI
Sham(5)
H(5)
H+Hex(5)
H+D5µg+Hex(7)
H+D25µg+Hex(8)
H+D50µg+Hex(6)
Total fluorescence of C5a (area in pixel squared)
10000
8000
6000
4000
2000
0
***
*
***
5
5
5
7
8
6
Sham
H
H+Hex
H+D5+Hex
H+D25+Hex
H+D50+Hex
Total fluorescence of C5b-9 (area in pixel squared)
14000
12000
10000
8000
6000
4000
2000
0
***
***
5
5
7
8
7
6
Sham
H
H+Hex
H+D5+Hex
H+D25+Hex
H+D50+Hex

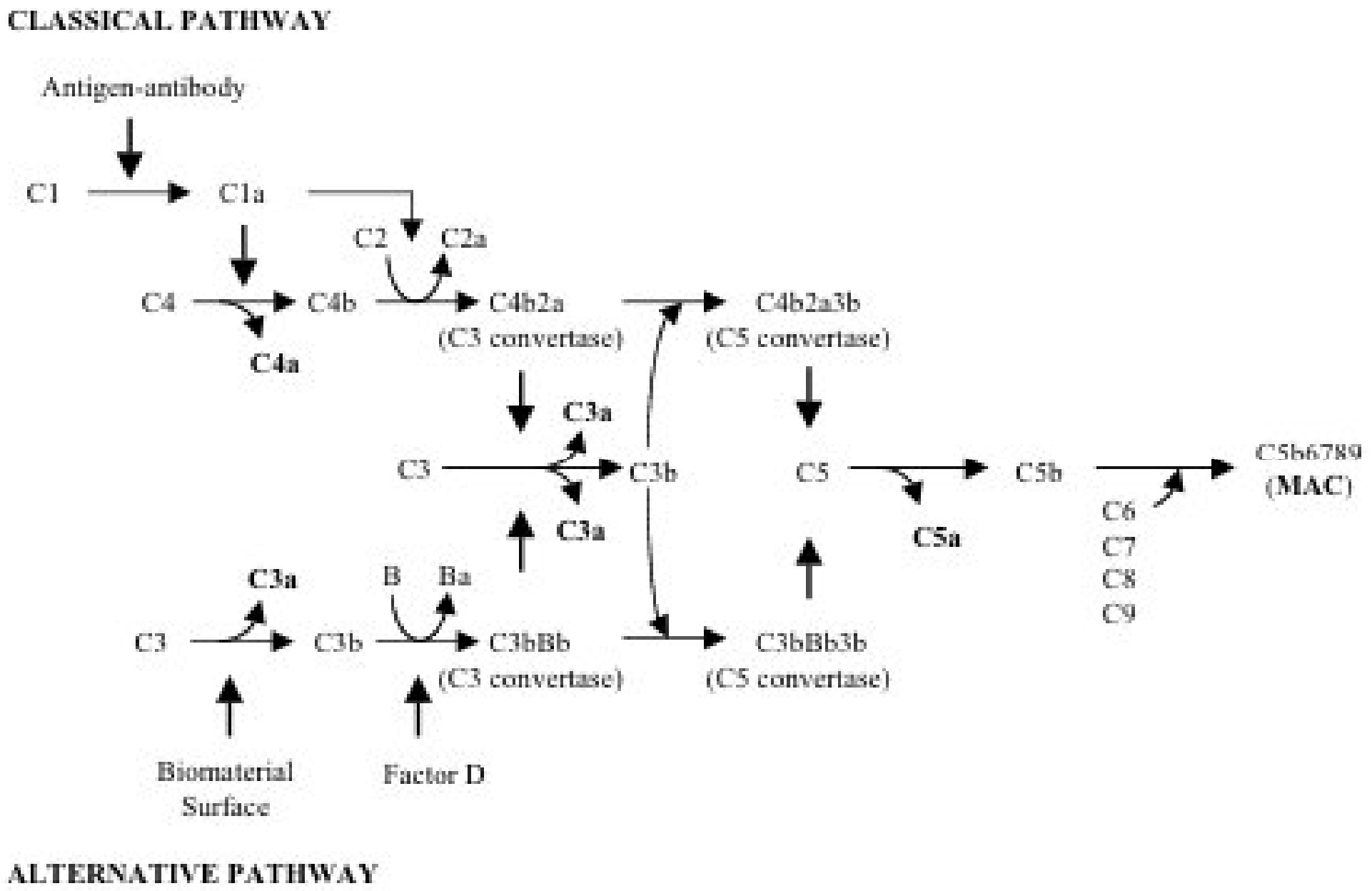
What Is Next?

1. DAF alone (w/o Hextend) afforded best protection against damage in gut and lung due to hemorrhage
2. High dose of DAF with Hextend - ?
3. Preliminary data on FDA approved C1inhibitor shows similar beneficial effects of DAF
4. FY10 - Testing of New Genentech inhibitor of the alternative pathway in trauma model at BHT

Core Team Members

- Yansong Li – WRAIR/ISR
- Milomir Simovic – WRAIR/ISR
- Tim Bentley - WRAIR
- Janos Szebeni – Zoltan Institute (Hungary)
- Peter Lapchack – BIDMC
- George Tsokos – BIDMC/Harvard

END


CLASSICAL PATHWAY
Antigen-antibody
C1
C1a
C2
C2a
C4
C4b
C4a
C4b2a
(C3 convertase)
C4b2a3b
(C5 convertase)
C3a
C3
C3b
C3a
C5
C5b
C5a
C5b6789
(MAC)
C6
C7
C8
C9
C3a
B
Ba
C3
C3b
C3bBb
(C3 convertase)
C3bBb3b
(C5 convertase)
Biomaterial
Surface
Factor D
ALTERNATIVE PATHWAY

Complement Inhibition Drug Discovery

Molecular Mechanisms of Action

Identify Target → **Test Feasibility In Vitro and Rodent Models** → **Pig models**
- **Efficacy**
- **Toxicity**

→ **First in Humans**

- Therapeutic window from clinical studies
- CR2-fH (human factor H)

- CRIg
- Hextend – DAF interaction
- HSD – DAF

- DAF (HSD, LR)
- Anti-C5a
- C1inh
- CRIg

- Activated platelets decorated with Complement
- Combined trauma / hemorrhagic shock model in pigs
- Use of non-human primates for POT-4 (compstatin)
- Survivability as endpoint