Hyaluronidase, Hyaluronan and Inflamma
Answers from the air pouch

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Halozym
THERAPEUTICS

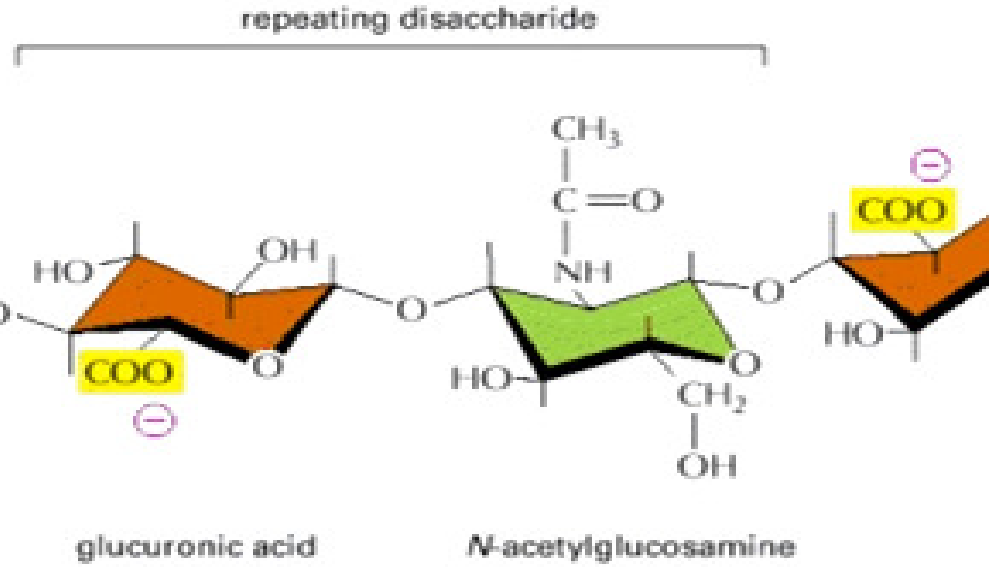
nda

- Background
  - HA, rHuPH20 & Enhanze technology for drug delivery

- Studies on HA and rHuPH20 in inflammation
  - In vitro: HA-TLR interaction
  - In vivo: Air pouch model

onan (HA) is a glycosaminoglycan (GAG) of up to 25,000 repeating haride units

HA synthase



Hyaluronidase
PH20)

Polysaccharide

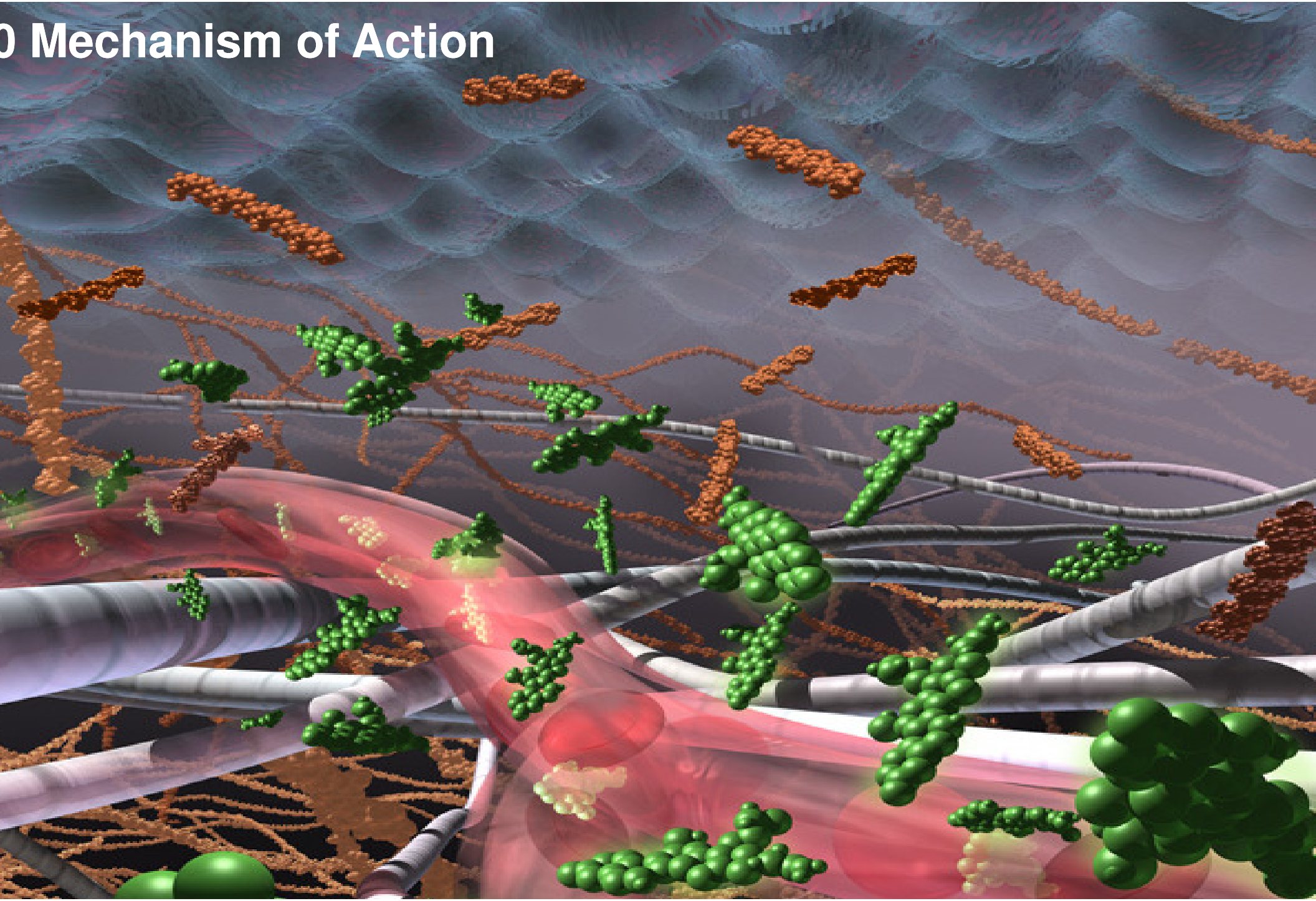
- Negatively charged and hydrophilic
- Forms viscous hydrated gel in ECM
- HA + collagens (and other ECM proteins) fo semi-permeable matrix between cells
- ~15 g HA in an adult human being, with ~5 turned over daily

Tissue distribution

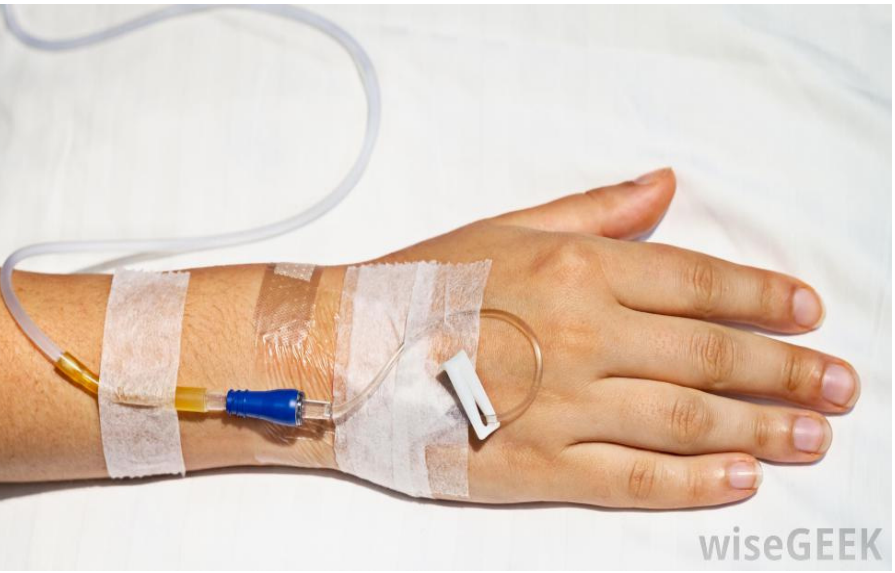
Tissue	% tot
Skin	50%
Skeleton and supportive tissue	25%
Muscle and Viscera	15%
Other tissues	10%

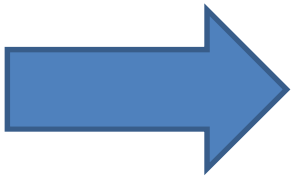
raser, 1992

0 Mechanism of Action



nze Technology: Subqutaneous Dosing of Biotherapeutics





IV

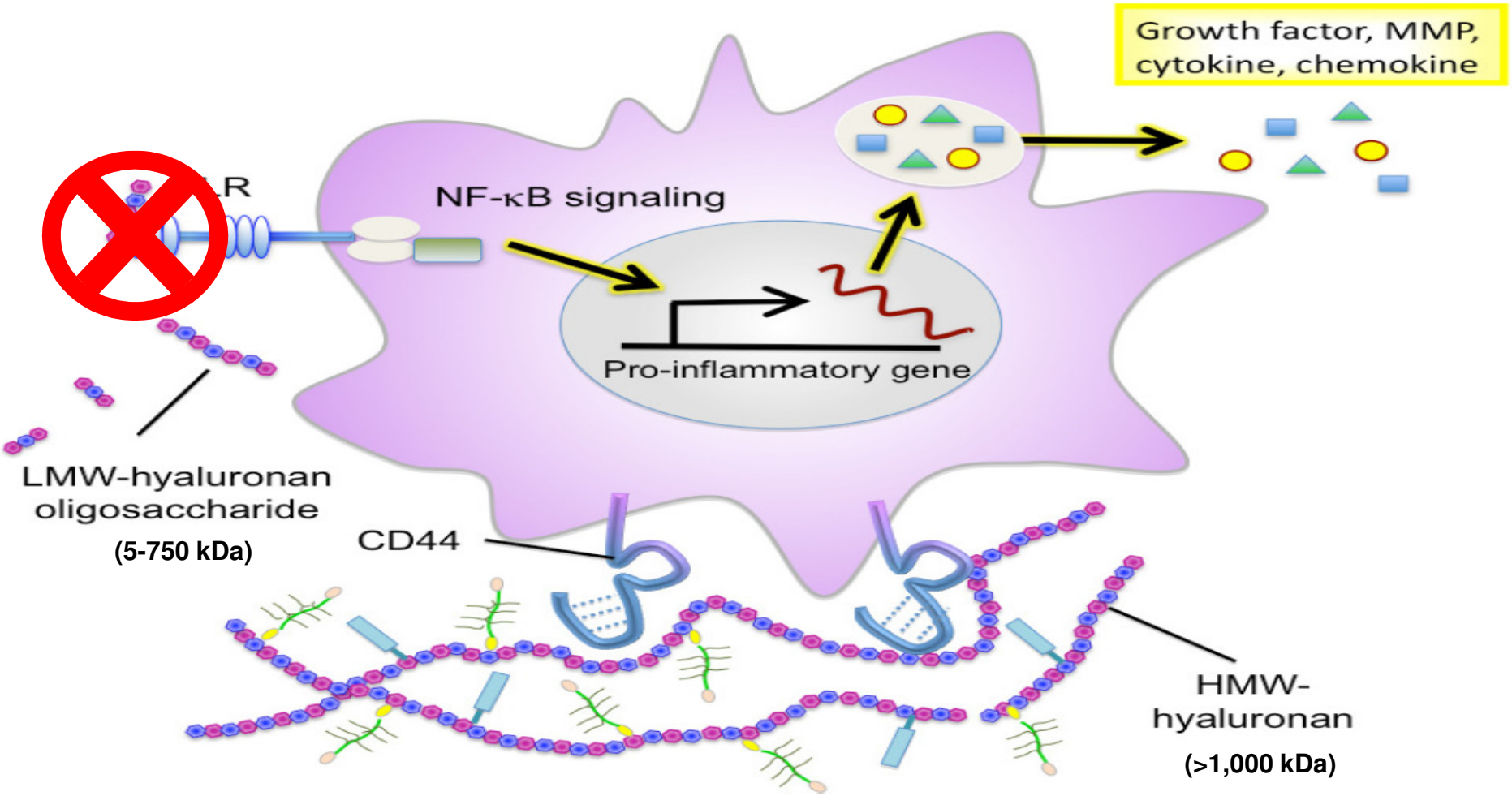
SC

nt Drugs using Enhanze Technology: Approved and Investigational

Proprietary Product Candidates	Therapeutic Area	Use/ Indication	Research/ Preclinical	Phase 1	Phase 2	Phase 3	Filed for Approval	Approved Product
Baxter HyQ (immunoglobulin with rHuPH20)	Immunology	Primary immunodeficiency						
Roche (up to 8 potential targets) Herceptin® SC	Oncology	Breast Cancer						
MabThera® SC	Oncology	Non-Hodgkin's lymphoma						
Actemra® SC	Anti-inflammatory	Rheumatoid arthritis						
Pfizer (up to 6 potential targets)	Primary & Specialty Care	3 specified 3 pending	Undisclosed					
ViroPharma Cinryze® with rHuPH20	Immunology	Hereditary angioedema						
Intrexon Alpha 1-antitrypsin with rHuPH20	Immunology	Alpha 1 antitrypsin deficiency						

IV → SC

ature Proposed Mechanism of Pro-Inflammatory Response by LMW HA
bolic product of PH20)

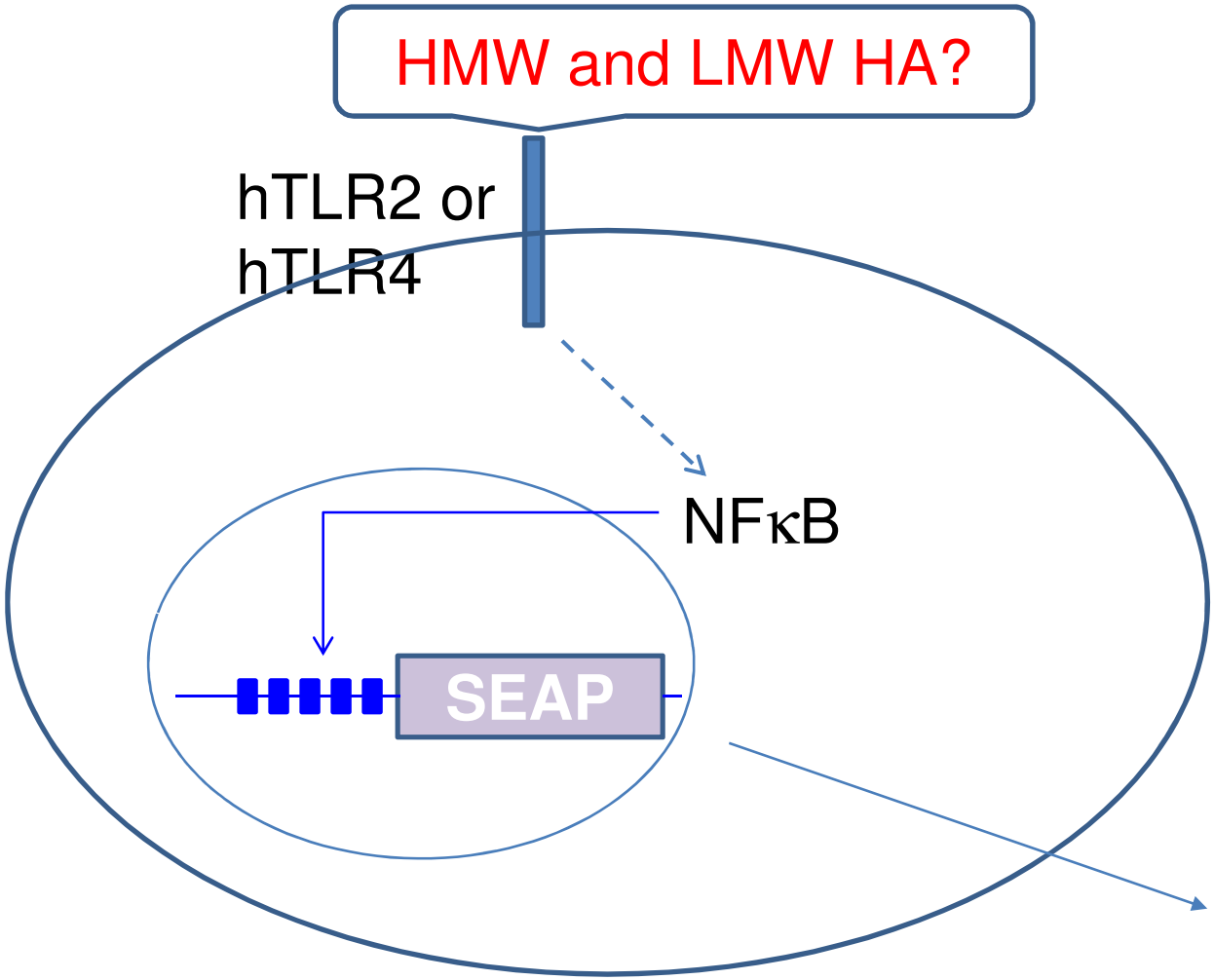


CCL2/MCP
CCL3/MIP
CCL4/MIP
CCL5/RAN
CXCL1/GR
CXCL2/MI
CXCL9/MI
CXCL10/IP
IL-1b
IL-8/LIX
IL12 p40/p
TNFa

ce rHuPH20 degrades HMW HA → LMW HA fragments, does it stimulate
mmation?

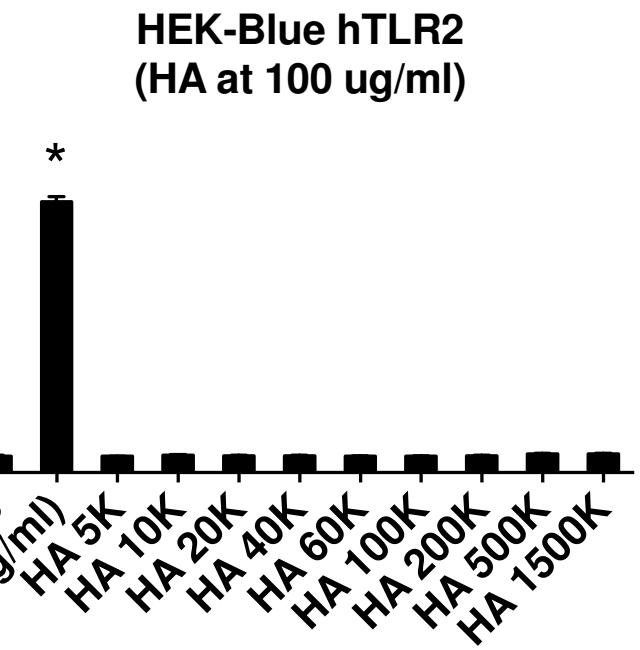
., 2011)

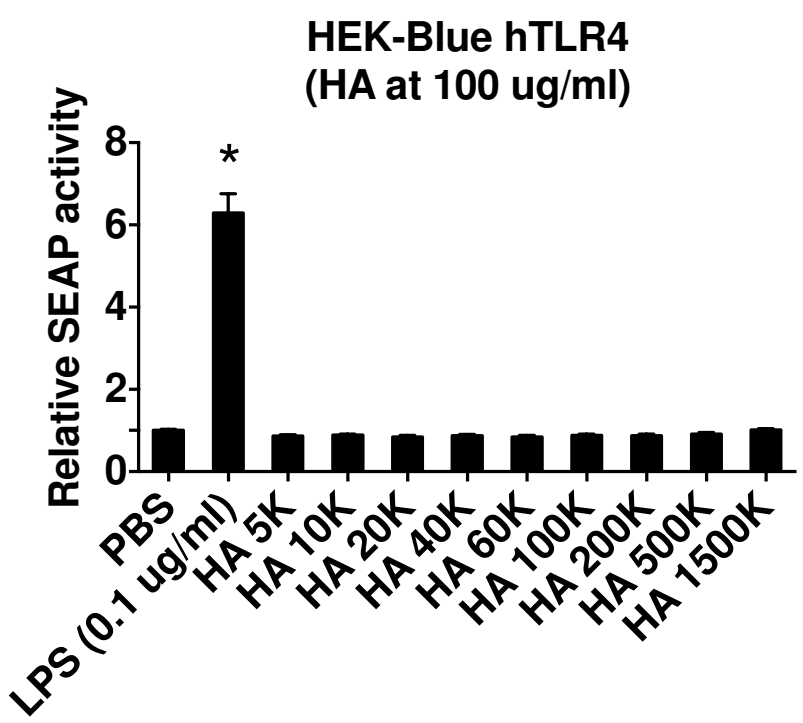
Blue hTLR2 and hTLR4 reporter system

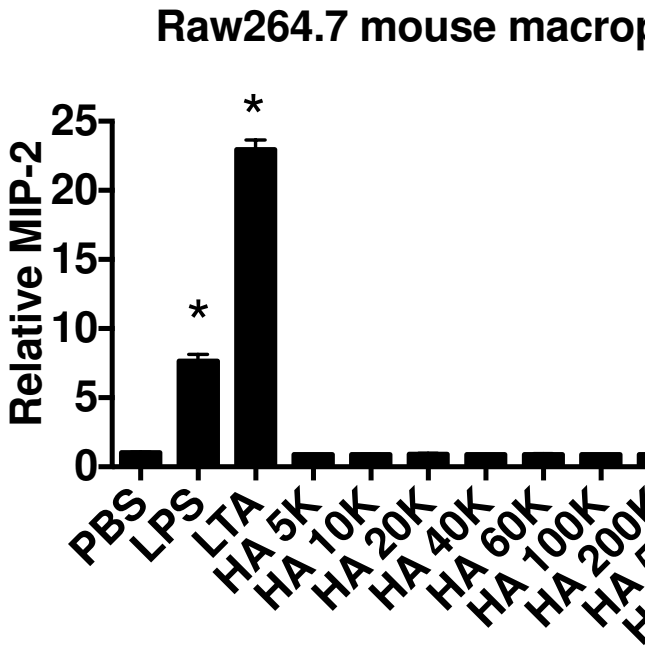


Alkaline Phosphat
activity assay

oes not activate TLR2/TLR4 in cell-based assays



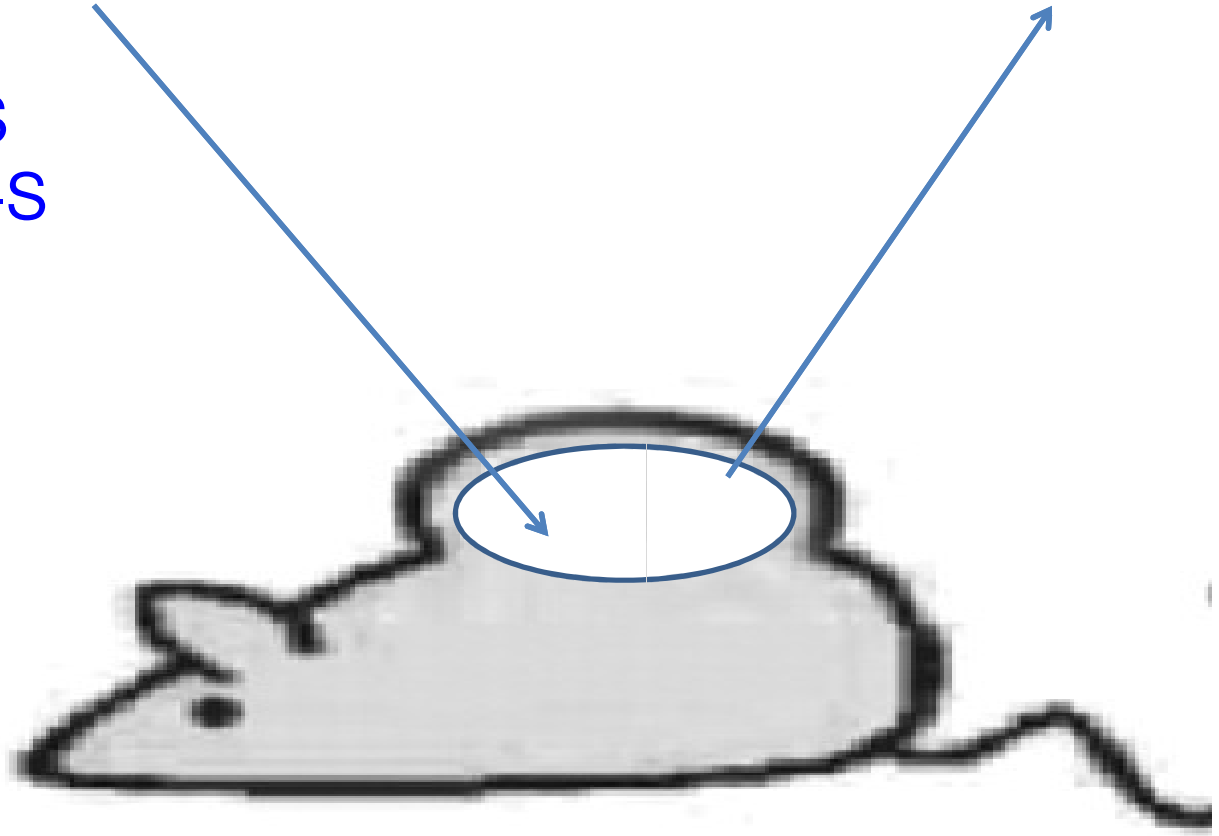




ouch Model of inflammation

timulation:

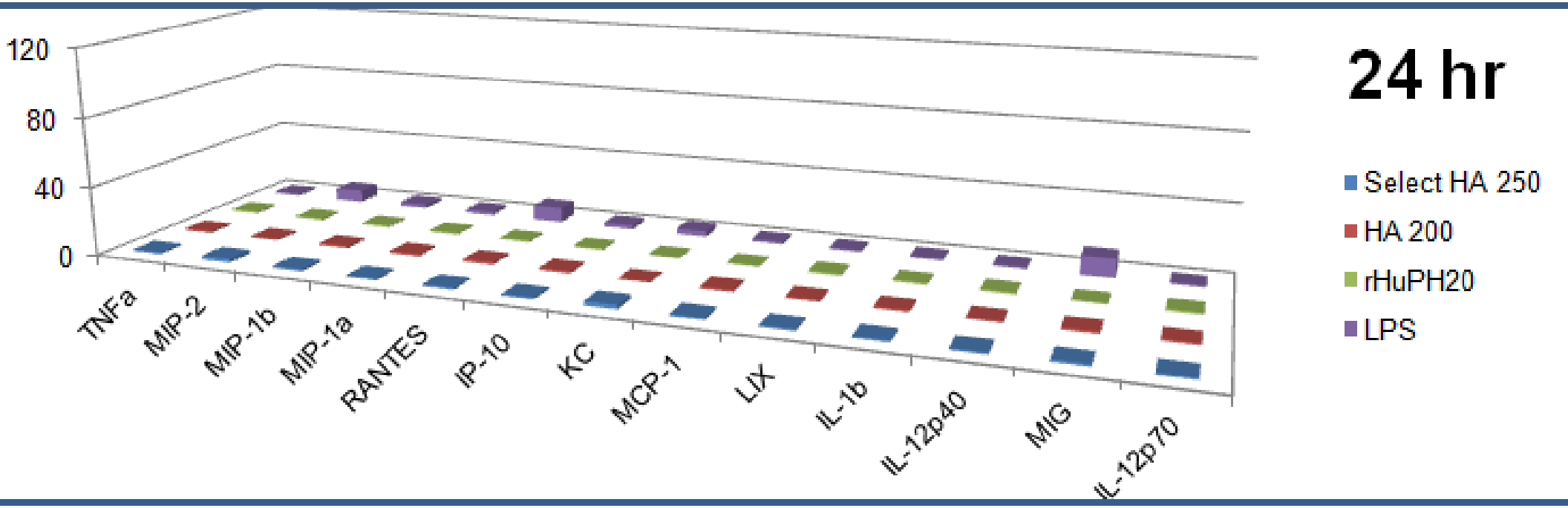
H20
HA
a Hyal type I-S
a Hyal type IV-S



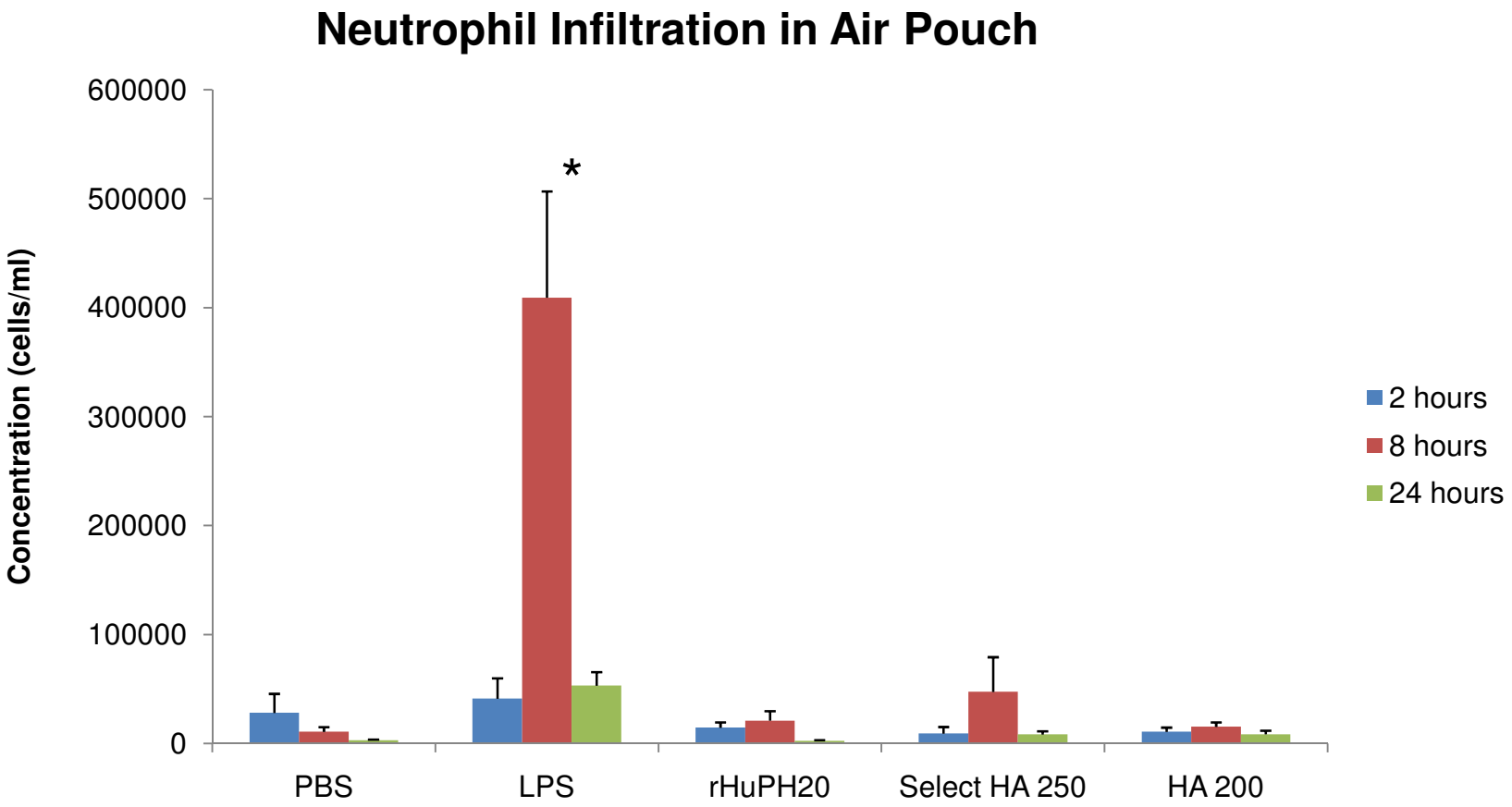
APE analysis:

- Cytokines/Chemokine
- AP infiltrating cells

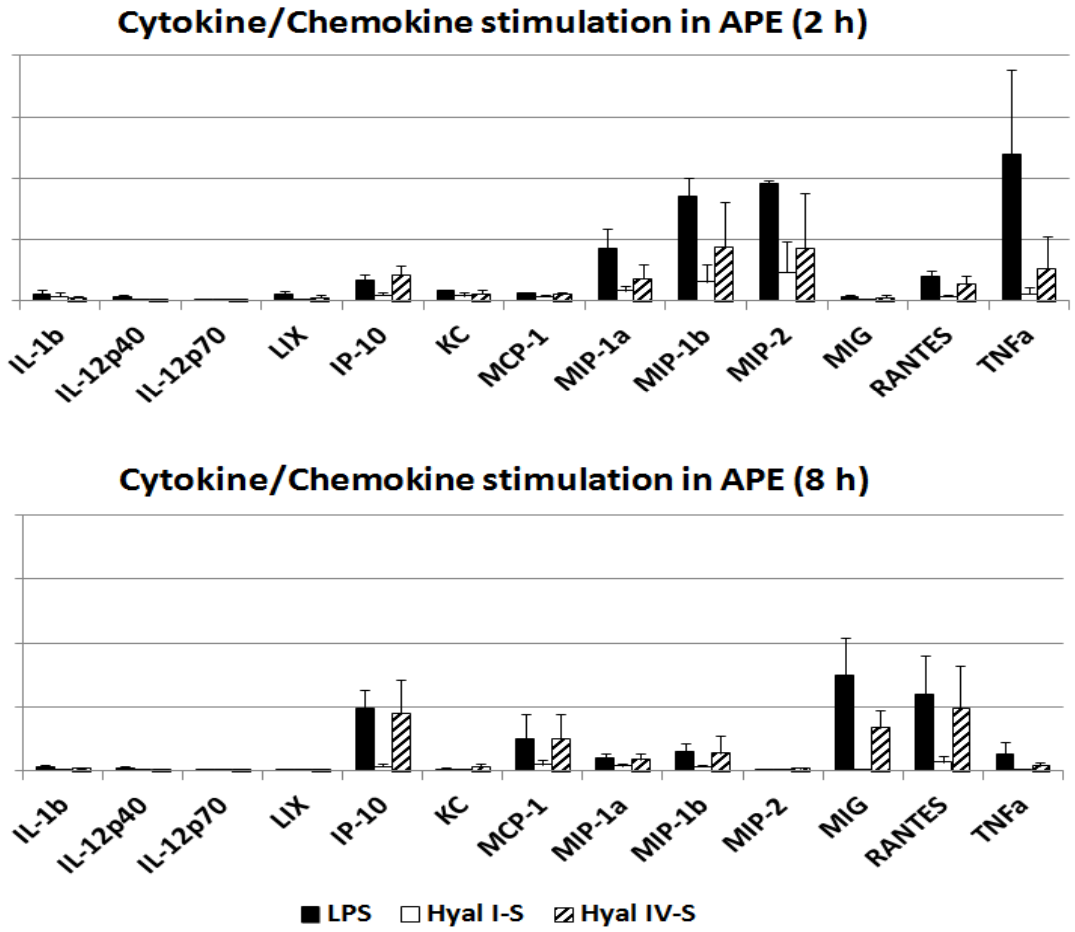
er LMW HA nor rHuPH20 stimulated cytokine/chemokine uction in the air pouch

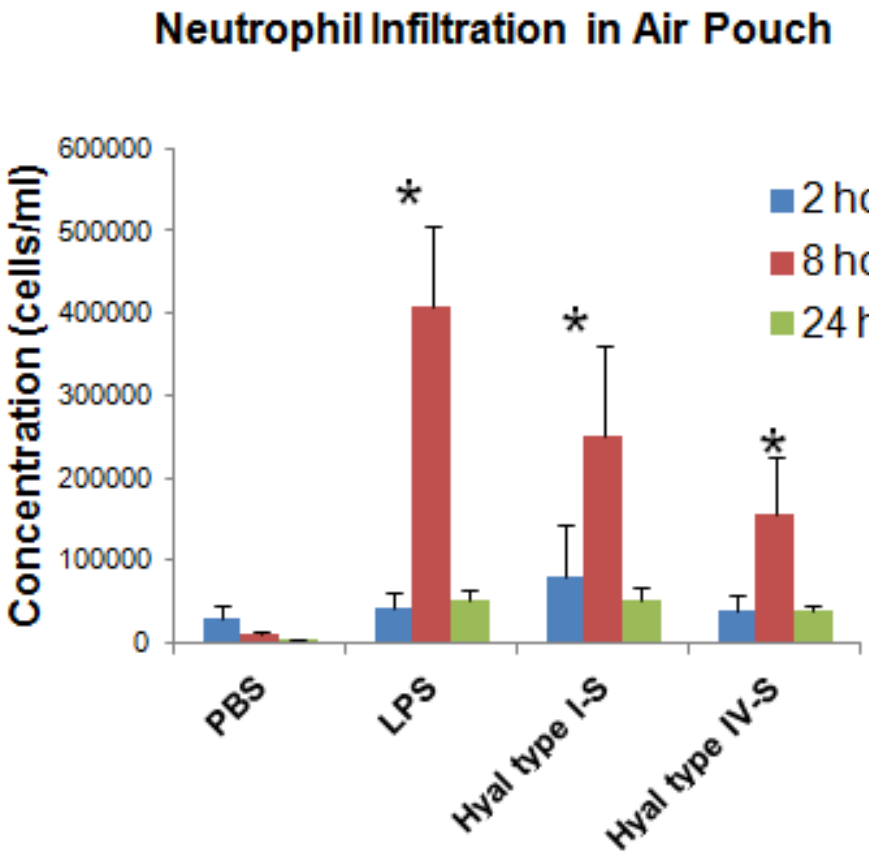


er LMW HA nor rHuPH20 induced neutrophil influx into the air pouch



type I-S and IV-S are pro-inflammatory in vivo

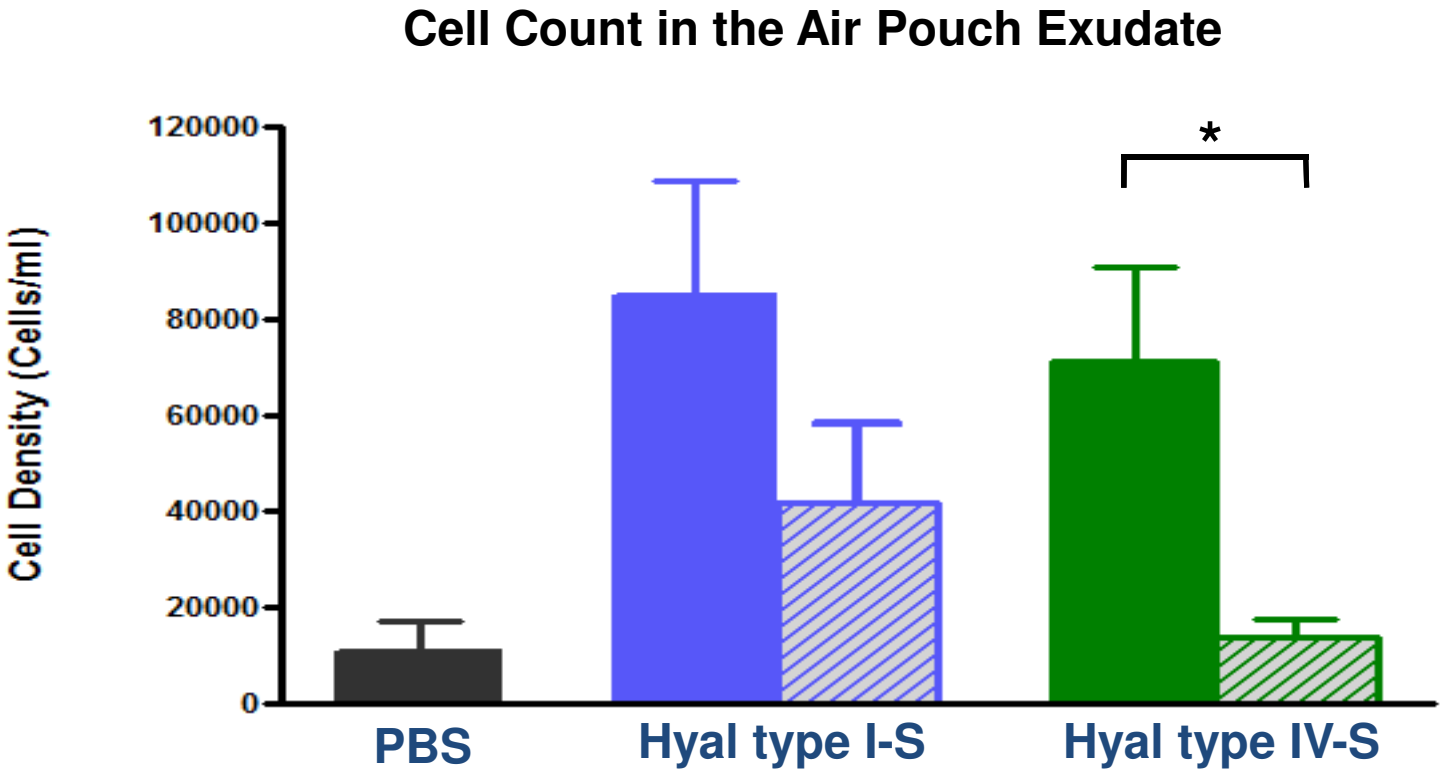




toxin Level in the Test Reagents correlates with inflammation

Reagent	Endotoxin (EU/ml)	Stimulation of Inflammation
PBS	<0.1	
LPS (1 ug/ml)	3905	+
rHuPH20 (2,400 U/ml)	<0.1	-
Hyal type I-S (2,400 U/ml)	34.7	+
Hyal type IV-S (2,400 U/ml)	234	+
Select-HA 250 (500 ug/ml)	0.7	-
HA 200 (500 ug/ml)	<0.1	-

tion of Endotoxin Reduced the Pro-inflammatory Activity of BTHs



BTH

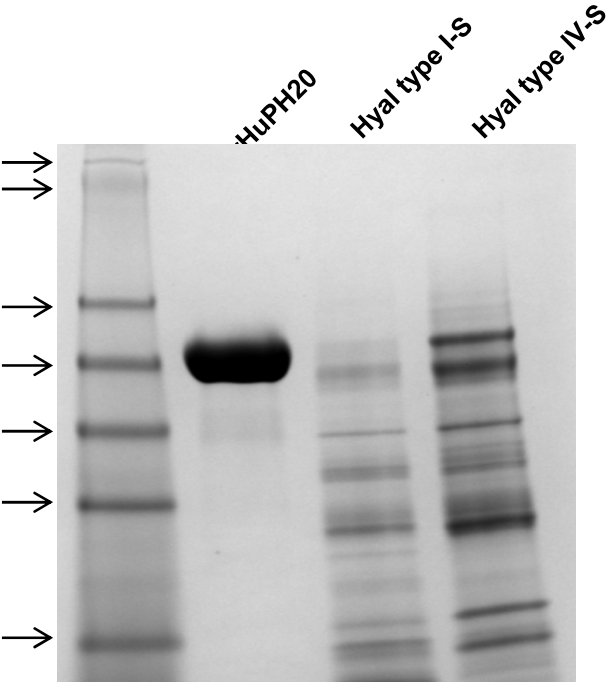
Endotoxin deplete

	Endo
Hyal type I-S	0.29
Hyal type IV-S	0.31

* p<0.05

- Endotoxin is one of the pro-inflammatory components in Hyal type I-S
- Endotoxin is the major pro-inflammatory component in Hyal type IV-S

other proteins may also contribute to the pro-inflammatory ty of Hyal type I-S and IV-S.



Summary

- rHuPH20, and its catabolic product LMW HA, do not stimulate inflammation in models evaluated
- Bovine testis hyaluronidases (commercial source) stimulate inflammation
- The endotoxin (and possibly other protein contents) but not the hyaluronidase activity ar the contributing components in bovine testicul hyaluronidases that induces inflammation

H20 and LMW HA are not pro-inflammatory

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MMUNOLOGY

nation is current as
2014.

Recombinant Human Hyaluronidase PH20 Does Not Stimulate an Acute Inflammatory Response and Inhibits Lipopolysaccharide-Induced Neutrophil Recruitment in the Air Pouch Model of Inflammation

Zhongdong Huang, Chunmei Zhao, Yanling Chen, Jessica A. Cowell, Ge Wei, Anne Kultti, Lei Huang, Curtis B. Thompson, Sanna Rosengren, Gregory I. Frost and H. Michael Shepard

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