Inflammation: the Good, the Bad, and the Ugly

W. Forrest Johnston, MD
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Disclosures

• No financial conflicts of interest
• Will discuss off label use of medication
Outline

• Reflects my understanding of inflammation over the course of residency
• PGY 1-2 → inflammation was good
• Lab years → inflammation could be bad
• PGY 5-7 → inflammation is a balance applicable to diseases in all organ systems
  – Even those that some consider to be “ugly” (i.e. colorectal)
Outline

• Good inflammation
• Bad inflammation
  – Interleukin-1β (IL-1β)
  – Aortic aneurysms
• Ugly part
  – Application of altering inflammation signaling in inflammatory bowel disease
Cardinal Signs of Inflammation

- Described by Celsus (25 B.C. – 50 A.D)
  - Calor, Rubor, Tumor, Dolor
- 5\textsuperscript{th} sign added by Virchow in 19\textsuperscript{th} century
  - Loss of function/restricted function
Inflammation is Good

Functions:
• Fight infection
• Aid in wound healing
• Clean cellular debris

Without Inflammation:
• Opportunistic infections
• Poor wound healing

First Day: Jun 2009
Inflammation Can be Bad

- Inflammation is a complex biological response involving immune system and vasculature.
- Too much inflammation → disorders.
- Inflammatory Disorders
  - Asthma
  - Autoimmune disease
  - Rheumatoid arthritis
  - Inflammatory bowel disease
  - Reperfusion injury
  - Aortic aneurysms
Interleukin-1 (IL-1)

• Major cytokine of inflammation
• Plays central role in inflammation and immune response to both infectious and sterile insults
• Produced by macrophages, monocytes, dendritic cells
IL-1 Mechanism

IL-1 Mechanism

IL-1β

MyD 88

IL-1R

IL-1α and β

IRAK 1 + IRAK 4

TRAF 6

NF-κB

iNOS

IL-1 (α and β)

IL-6

IL-8

TNFα

PGE2

IL-1α and β

IL-6

IL-8

TNFα

PGE2
IL-1 Mechanism

- Interleukin-1 (IL-1) is a proinflammatory cytokine that induces monocytes/macrophages to release more proinflammatory cytokines.
IL-1 Mediated Effects

IL-1 is gatekeeper for inflammation

- IL-1 components
  - IL-1α and IL-1β
    - Two distinct proteins
    - Bind IL-1 Receptor (IL-1R)
- IL-1α is active intracellularly and represents small fraction of overall IL-1
- IL-1β is predominant IL-1
  - Amplifier of inflammation, recruits macrophages
  - Active extracellularly and is in serum
IL-1β Production

• IL-1β activation is controlled by caspase-1
  – Caspase-1 cleaves pro IL-1β into active IL-1β

• Caspase-1 activation is tightly controlled by the **inflammasome**
  – Inflammasome activates caspase-1
  – Caspase-1 activates IL-1β
  – IL-1β triggers inflammatory response
Inflammation in Aortic Aneurysms

• Inflammation contributes to aortic wall degradation

• Human AA samples:
  – Increased inflammatory cytokines, including interleukin-1β
  – Increased proteases

• Aneurysm pathogenesis is not well understood
Clinical Need

• No specific medical treatments exist for aortic aneurysms
• Role of inflammation and IL-1β signaling in aneurysm formation was unknown
Mouse Elastase AAA Model

Day 0

Day 14

Aorta

Iliac bifurcation

Left renal vein

Control section

IVC

Aortotomy repair

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Size Comparison
IL-1β in mouse AAA model

- Mouse AAAs had increased IL-1β

- To evaluate IL-1β:
  - Mouse AAA model
    - Wild type (WT)
    - IL-1β knockout (KO)
    - IL-1 receptor (IL-1R) KO
IL-1β Genetic Deletion Prevented AAA Formation

- Genetic deletion of IL-1β reduced aortic dilation by 52%
- Genetic deletion of IL-1R reduced aortic dilation by 37%
- IL-1β and IL-1R KO aortas had decreased proteases (MMPs), less proinflammatory cytokines, and preserved aortic architecture

Preserved Aortic Structure

Elastin Staining:

WT  IL-1β KO  IL-1R KO

Verhoeff-Van Gieson (VVG) Stain for Elastic Fibers
Pharmacologic IL-1R Antagonism

- **Anakinra** (brand name Kineret):
  - Recombinant human IL-1 receptor antagonist
    - Competitively binds IL-1R to prevent downstream effects of IL-1 signaling
  - FDA approved
  - Proven beneficial in autoimmune diseases
    - Rheumatoid arthritis
    - Gout
    - Osteoarthritis
    - Type 2 diabetes
Decreases in both aortic IL-1β levels and aortic diameter with increasing doses of anakinra significantly correlated.

- \( R = 0.575 \) (95% CI: 0.203 to 0.802), \( p < 0.01 \)
Anakinra Treatment Decreases Aortic Diameter

**Day 3 Anakinra Treatment**

- Vehicle Only (n=7)
- Anakinra (n=7)

**Day 7 Anakinra Treatment**

- Vehicle Only (n=5)
- Anakinra (n=5)

43% reduction in aortic dilation

40% reduction in aortic dilation

From Johnston *et al.* Genetic and Pharmacologic Disruption of Interleukin-1β Signaling Inhibits Experimental Aortic Aneurysm Formation. *Arteriosclerosis Thrombosis and Vascular Biology.* 2013; 33:294-304
AAA and IL-1β Conclusions

• Human and mice AAA have increased IL-1β
• Genetic inhibition of IL-1β prevented experimental AAA formation
• IL-1R antagonism with anakinra prevented AAA formation and halted AAA progression
Lessons Learned in Lab Year #1

• Inflammation is way more complex than originally believed
• Teamwork needed if anything is going to be accomplished in two years in lab
• Timing is everything
  – Key to finding a good mentor
  – Key to finding a good project

June 2012: end 1st year lab
Opportunity

• What about the rest of the aorta?
• The thoracic aorta is genetically distinct from abdominal aorta\(^1-3\)
  – Increased elastin and collagen content
  – More prevalent vasa vasorum should have less wall ischemia
• Thoracic aorta is prone to aneurysm formation and thoracic aortic aneurysms are increasing in prevalence\(^4\)

IL-1β Upregulated in Human TAAs

- Normal thoracic aortic (TA) samples from organ transplantation donors
- TAA samples from pts undergoing open descending TAA repair

Available Models of Aortic Aneurysm Formation

- Marfan models
  - Topical Calcium Chloride ≈ 25-40% in 4 wks
  - Angiotensin II models → dissections in 4 wks
  - Elastase Perfusion ≈ 100% dilation in 2 wks
  - Topical Elastase ≈ 100% dilation in 2 wks
  - Calcium Chloride ≈ 50-100% dilation in 4 wks
New TAA Model

• Exposure techniques adapted from Ikonomidis/Jones lab at MUSC\(^1\)
  1. Orotracheal intubation
  2. Left thoracotomy for exposure of thoracic aorta
  3. Reflect lung anteriorly
  4. Strip pleura over aorta
  5. Application of undiluted purified porcine elastase for 5 minutes\(^2\)
  6. Expand lung and close thoracotomy
  7. Recovery and evaluation at 3, 7, 14, 21, and 28 days postoperatively

1. Ikonomidis et al, *Journal of Surgical Research* 2003
2. Bhamidipati, Upchurch, Ailawadi *et al.*, *Surgery* 2012
Murine Elastase TAA Model

Aortic Dilation

WT TAA (Elastase)
WT Control (Saline)

n = 5/group/timepoint
Murine Elastase TAA Model

WT Control (Saline Sponge)  WT TAA (Elastase Sponge)

Elastin
Smooth Muscle Cells
Macrophages
IL-1β
IL-1β and IL-1R Deletion Prevent TAAs

IL-1R antagonism prevented and treated experimental TAAs

**Prevention:**

- **Experimental Design:** Osmotic Pump Insertion
  - Vehicle (control)
  - Anakinra

- **Day 0**
- **Day 6**
- **Day 14**

- Topical Elastase to Thoracic Aorta
- Osmotic Pump Change
- Harvest Aorta

**B**

- **Anakinra Prevention**
  - p=0.002
  - Vehicle n=8
  - Anakinra n=11

**Treatment:**

- **Experimental Design:** Osmotic Pump Insertion
  - Vehicle (control)
  - Anakinra

- **Day 0**
- **Day 3**
- **Day 7**
- **Day 14**

- Topical Elastase to Thoracic Aorta
- Osmotic Pump Change
- Harvest Aorta

**B**

- **Anakinra Treatment**
  - p=0.01
  - Vehicle n=8
  - Anakinra n=10
TAA Conclusions

• Periaortie application of elastase generated reproducible and robust TAAs with similar histology and cytokines to human TAAs

• Genetic or pharmacologic inhibition of IL-1β decreased TAA formation

• Delayed antagonism of IL-1β limited TAA growth

• Suggests a fundamental role for IL-1β during both formation and progression of TAAs
Application

- IL-1β is increased in human AAA and TAAs
- IL-1β signaling is needed for both AAA and TAA initiation and progression
- Safe application to humans
  - long term daily use of anakinra is safe and well tolerated for up to 36 months

1. Ann Rheum Dis. 2006;65:1006-1012
# Future Possible Therapies in Patients with Descending TAA or AAAs

Currently available therapies:

- Anti IL-1β (canakinumab & Xoma 052)
- IL-1Ra (anakinra)
- Anti-soluble IL-1R1
- IL-1 trap (rilonacept)
- Anti IL-1R1

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**EudraCT Number:** 2013-002088-25  
**Sponsor Protocol Number:** CACZ885X2201  
**Start Date:** 2013-12-23  
**Sponsor Name:** Novartis Pharma Services AG

### Most Recent Events

**04 Feb 2016**  
Novartis terminates a phase II trial for Abdominal aortic aneurysm in USA, Denmark, Germany, the Netherlands and the United Kingdom (NCT02007252)

### Population Details

- **Population Age:** Adults, Elderly  
- **Gender:** Male, Female

### Trial Protocol

- **Trial protocol:** NL (Prematurely Ended) DK (Ongoing) GB (Ongoing) DE (Prematurely Ended)

### Trial Results

- **Trial results:** (No results available)
Lessons Learned in Lab Year #2

• Apply knowledge in one area to another and sometimes it works
• Developing a model can be more difficult than the experiment
• Be careful with who you share your research results with
• Presenting at meetings is helpful to get published
What have we covered so far?

• Inflammation is good for wound healing
• Inflammation can be bad
  – Contributes to aortic aneurysm formation and progression
  – Useful target for pharmacologic therapy
Now for the “Ugly” Part

IL-1β
Inflammatory Bowel Disease

- Inflammation in the colon is a delicate equilibrium of providing adequate protection from noxious stimuli and allowing tissue healing/regeneration

- Equilibrium is altered in IBD
Recent Investigation in IBD

Chemokine and cytokine levels in inflammatory bowel disease patients

Udai P. Singh, Narendra P. Singh, E. Angela Mu, and Mitzi Nagarkatti, Prakash S. Nagarkatti

![Graphs showing cytokine levels in IBD patients compared to controls](image-url)
• Conclude that IBD is a imbalance of intricate interactions of signaling pathways and immune cells mediated by cytokines and chemokines

• IMPLICATION: since nearly all inflammatory cytokines are elevated, site of dysregulation must be proximal in the inflammatory pathway
IL-1 triggers inflammation

- Increased production of inflammatory cytokines
- Imbalance of inflammation may contribute to inflammatory bowel disease
• Experiment A: Looked at IL-1, IL-Ra, and ratio of IL-1:IL-1Ra in colectomy samples
  – Control samples (malignant and nonmalignant, n=16)
  – Crohn's disease (CD, n=12)
  – Ulcerative colitis (UC, n=12)
IBD had lower IL-1:IL-1Ra Ratios

Controls =
- neoplasm n=10
- FAP n =4
- diverticulitis n=1
- sigmoid stricture n=1
CD = Crohn's disease
UC = Ulcerative colitis

• Experiment B: compared colonoscopy mucosal biopsies
  – Normal (N controls, n=16)
  – Inflammatory controls with self limited colitis (I controls, n=16)
  – Crohn’s disease (CD, n=12)
  – Ulcerative Colitis (UC, n=18)
IL-1β increased in Inflammatory Bowel Disease (IBD)

N = normal controls
I = inflammatory controls
  (self-limited colitis)
CD = Crohn's disease
UC = Ulcerative Colitis

IBD Severity Related to IL-1:IL-1Ra Ratio

MILD = mild IBD severity
MODERATE = moderate IBD severity
SEVERE = severe IBD severity

Could Altering IL-1:IL-1Ra Ratio Be Helpful?

- IL-1R antagonist (anakinra) reduced inflammation in rabbit model of immune complex colitis

Case Reports of Patient Application

- Canakinumab (anti IL-1β antibody) used to treat severe Crohn’s related arthritis that was refractory to traditional medications
Anakinra (IL-1R antagonist) given to patient with Crohn’s disease and arthritis → worsening abdominal pain, fever, diarrhea, arthralgias, and depression

Future Directions

• Application of anti IL-1β signaling agents in IBD patients
  – may alter IL-1/IL-1Ra ratio

• Application of inflammatory modulation in malignancy
  – Alterations in immune surveillance and inflammation may play a role in malignancy
  – Ex: IL-1 from local epithelial cells induces growth factor secretion → cell proliferation → malignancy
Conclusions

1. Inflammation is multifaceted, intricate, and has lots of opportunities for future research

2. Inflammation plays a major role in many disease processes
   - Recent success in manipulating the inflammatory balance
   - Application of that knowledge is still under investigation
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Thank you
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