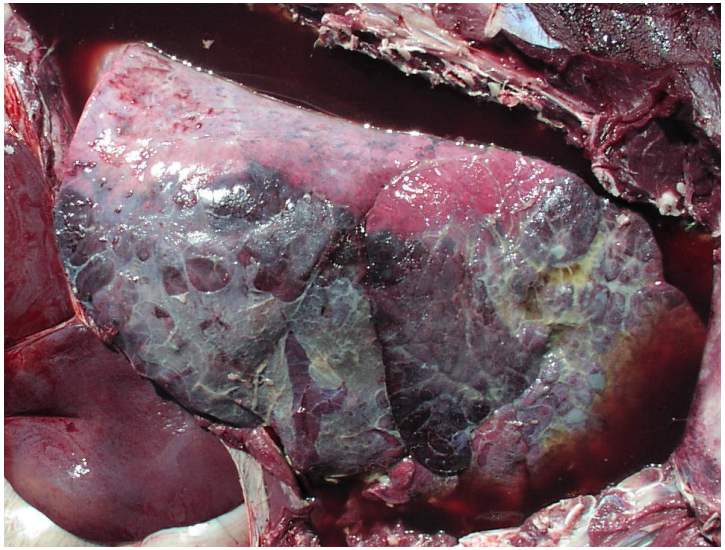
The role of inflammation in bovine respiratory disease: blessing or curse?



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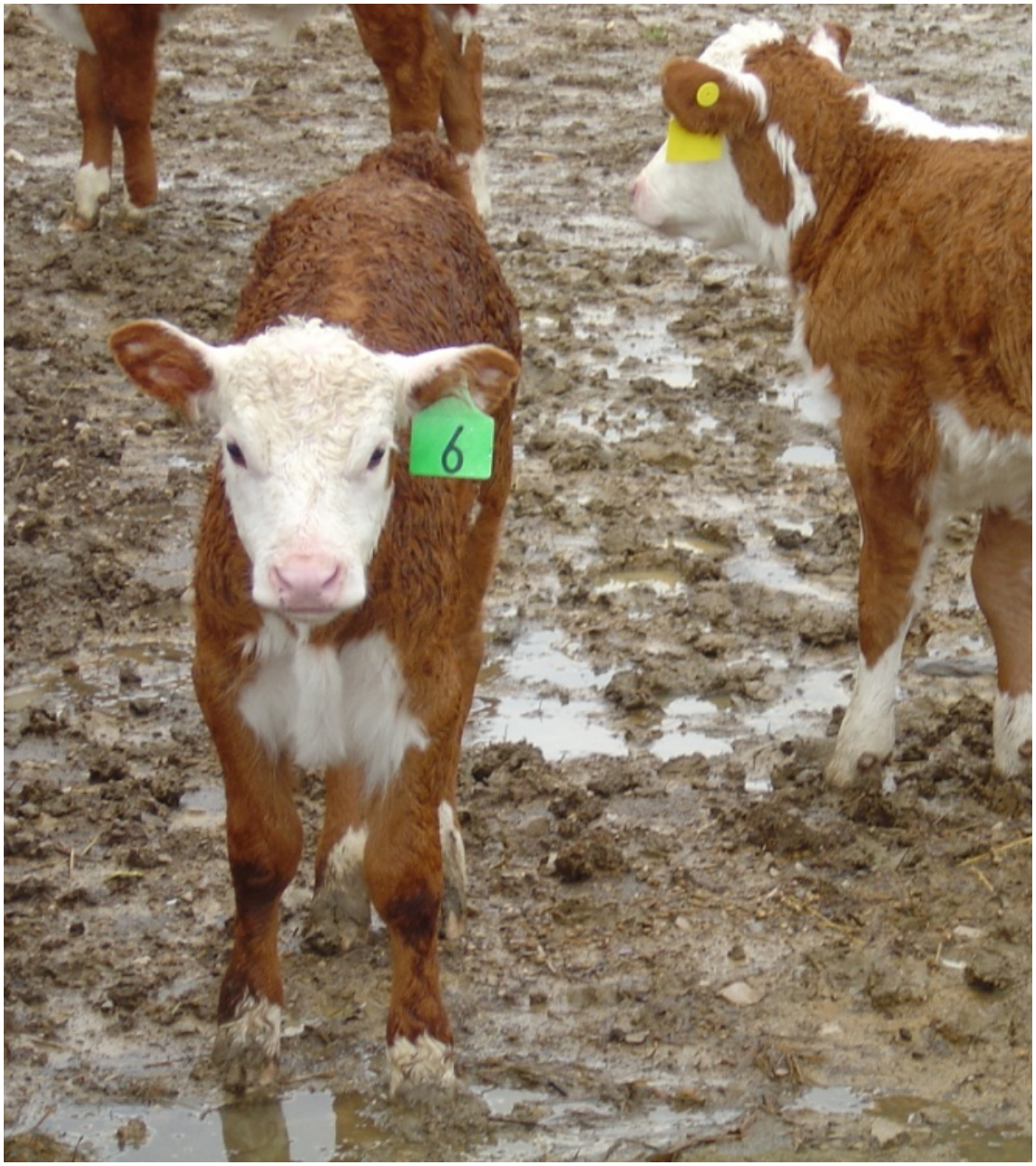
Outline of this presentation

- Genesis of the inflammatory response
- Infection and inflammation in BRD
- Benefits of anti-inflammatory therapy in BRD
  - Experimental challenge studies
  - Field trials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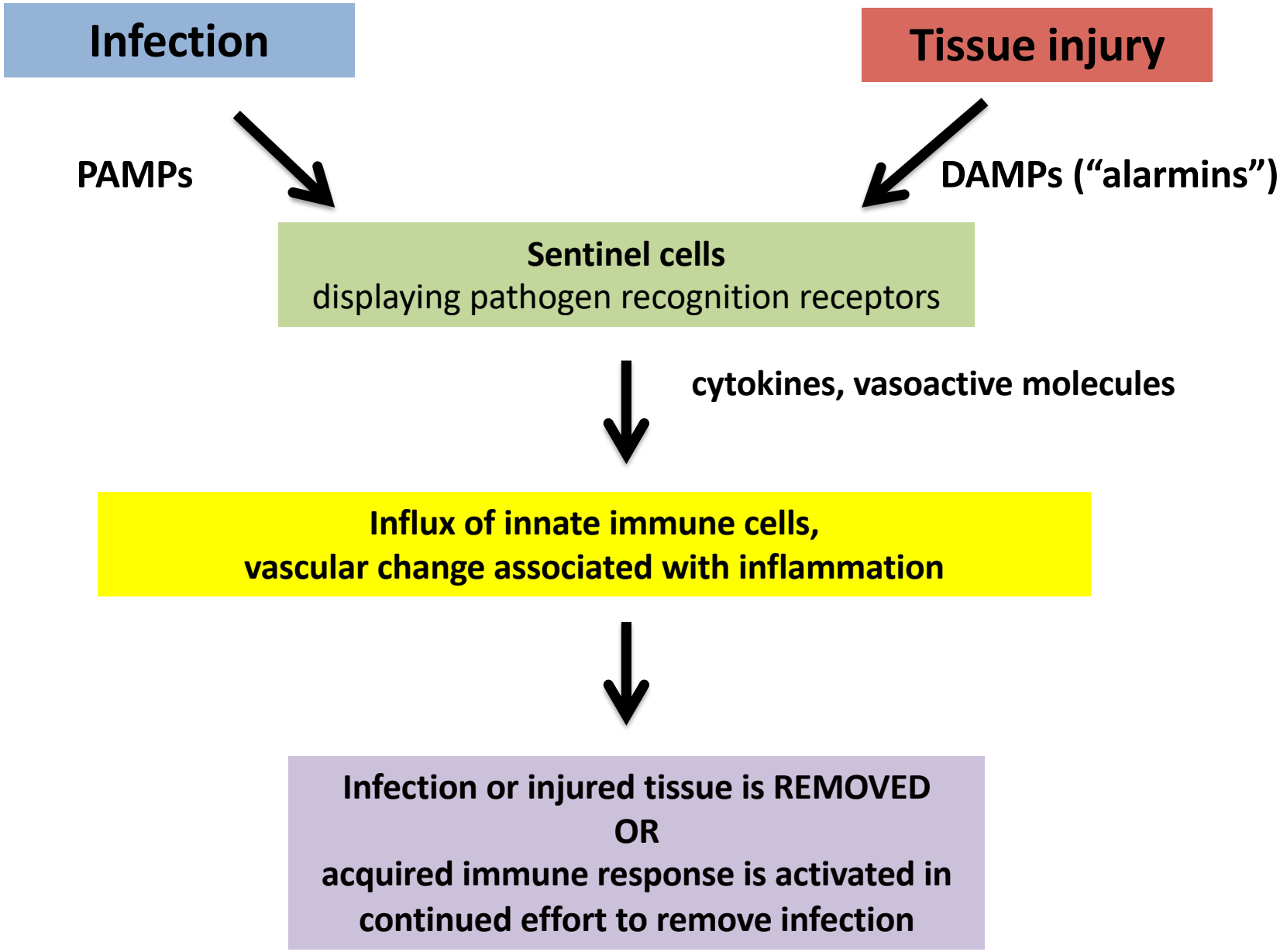
Immune System: Overview

- Innate immune response
  - Immediately and always active
  - Responds to a broad variety of agents

- Acquired (aka adaptive) immune response
  - Takes several days-weeks to be fully active
  - Reacts specifically to a single agent
  - Improves with repeated exposure: “memory”

- Mechanisms important in the initial response to infection are also involved in the response to non-infectious tissue injury
  - e.g., tissue trauma
- All induce inflammation
- Inflammation activates the immune response


Infection
Tissue injury
PAMPs
DAMPs ("alarmins")
Sentinel cells
displaying pathogen recognition receptors
cytokines, vasoactive molecules
Influx of innate immune cells,
vascular change associated with inflammation
Infection or injured tissue is REMOVED
OR
acquired immune response is activated in
continued effort to remove infection

Pathogen-associated molecular patterns (PAMPs)

- Highly conserved molecules found in many different microorganisms
- Host response evolved to recognize these
  - relatively few molecules initiate immunity to the limitless microbial world
- Cells recognize PAMPs with their pathogen recognition receptors (PRRs)

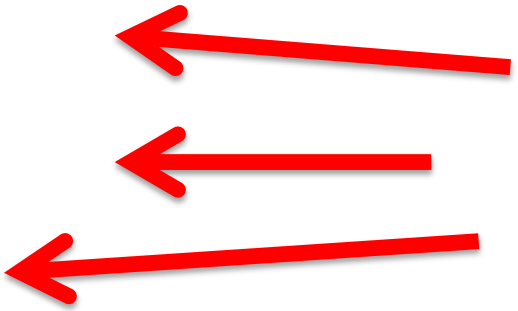
Important PAMPs

- Peptidoglycan
  - Gram positive bacteria (*Staph*., *Strep*., and others)
- Lipopolysaccharide
  - Gram negative bacteria (*E. coli*, *Salmonella*, and others)
  - fungi (*Aspergillus* and others)
- Unmethylated CpG nucleotide motifs
  - bacteria and viruses
- dsRNA
  - viruses

Important damage associated molecular patterns (DAMPs)

- DAMPs are components of host tissues
- **Extracellular** DAMPs
  - Small fragments of hyaluronic acid, fibronectin, collagen
- **Intracellular** DAMPs
  - Unmethylated CpG DNA **from mitochondria**
  - Adenosine from ATP and nucleic acids
  - Uric acid
    - Breakdown product of purines (e.g. adenine, guanine)

- Sentinel cells in tissues are the first cells to “see” PAMPs or DAMPs
  - dendritic cells
  - macrophages
  - mast cells
  - epithelial cells
  - fibroblasts



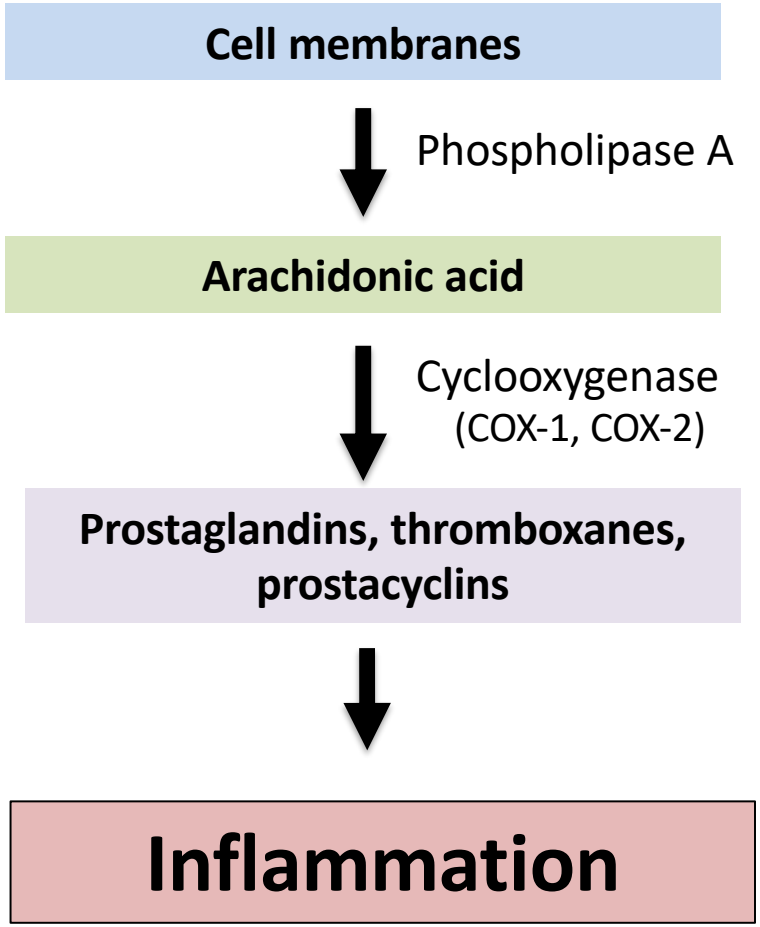
These cells can also migrate into regions of inflammation

Macrophages

- Identify and kill pathogens via many PRRs and other surface receptors
  - phagocytosis
  - secrete antimicrobial products
- **Produce molecular mediators of inflammation**
  - Prostaglandins
  - Proinflammatory cytokines: IL-1, TNF-a, and IL-6
    - Activate inflammation
      - Inflammation activates the immune response

Prostaglandins and other lipid mediators of inflammation

- Result from activity of enzymes on cell membranes
  - **Cyclooxygenase (COX)**
- Produced by many different types of cells
- Prostaglandins
  - Increased blood flow
  - Vascular permeability
  - Inflammatory cell influx



Cytokines

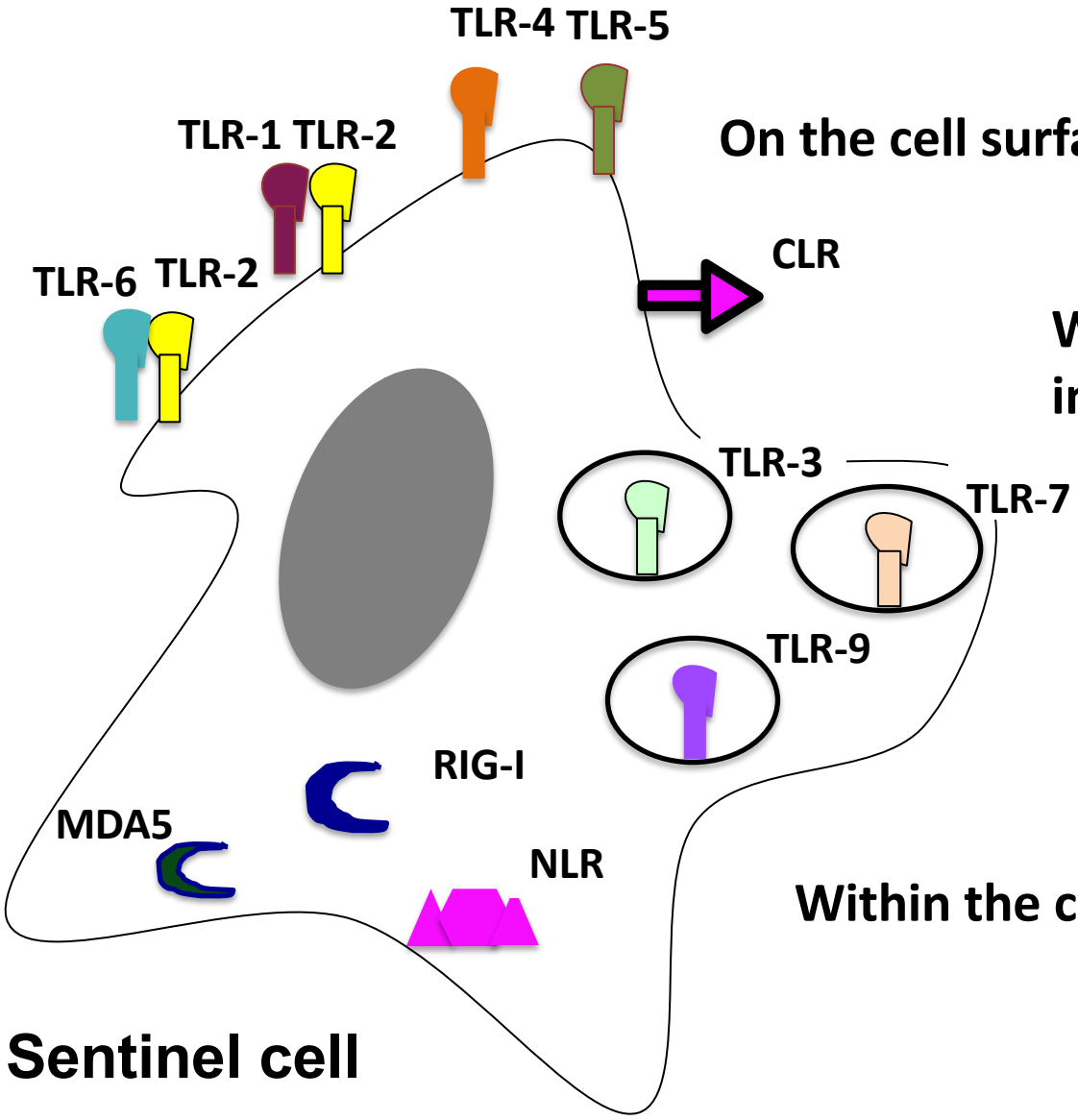
- All cells influence other cells by release of cytokines
- Cytokines...
  - Activate the immune response
  - Direct specific types of responses
    - Anti-viral, anti-bacterial, anti-parasitic
  - Contribute to inflammation and sometimes death
    - “Septic shock”

Pathogen recognition receptors (PRR)

- Macrophages and other sentinel cells activate the inflammatory/immune response when their PRR bind PAMPs or DAMPs

- PRR are found
  - On cell surface
  - Inside endosomes inside the cell
  - In cell cytoplasm

Sites where cellular pathogen recognition receptors (PRR) can bind to PAMPs or DAMPs

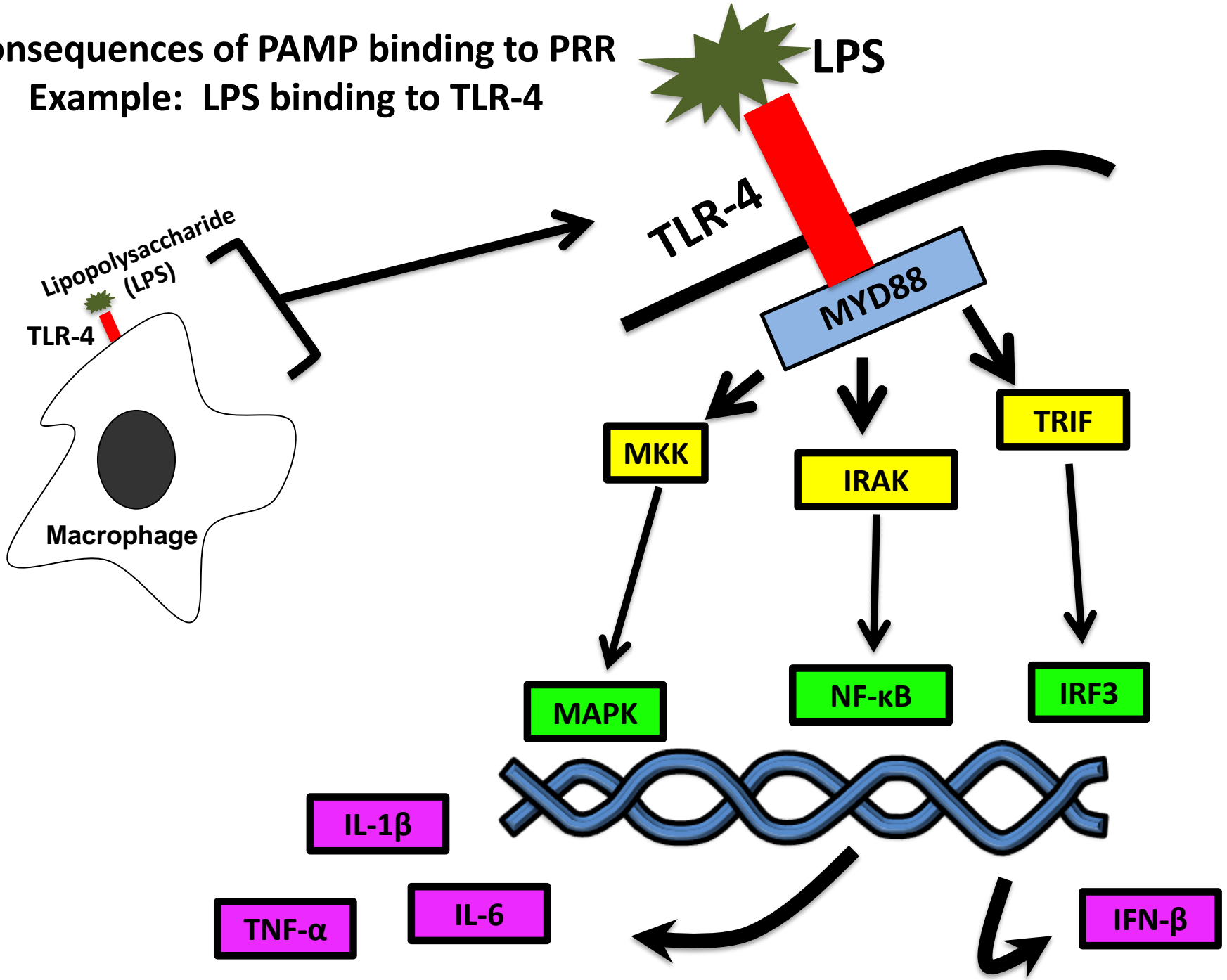


- Binding of PRR by a PAMP or DAMP initiates a signal transduction sequence in the cell

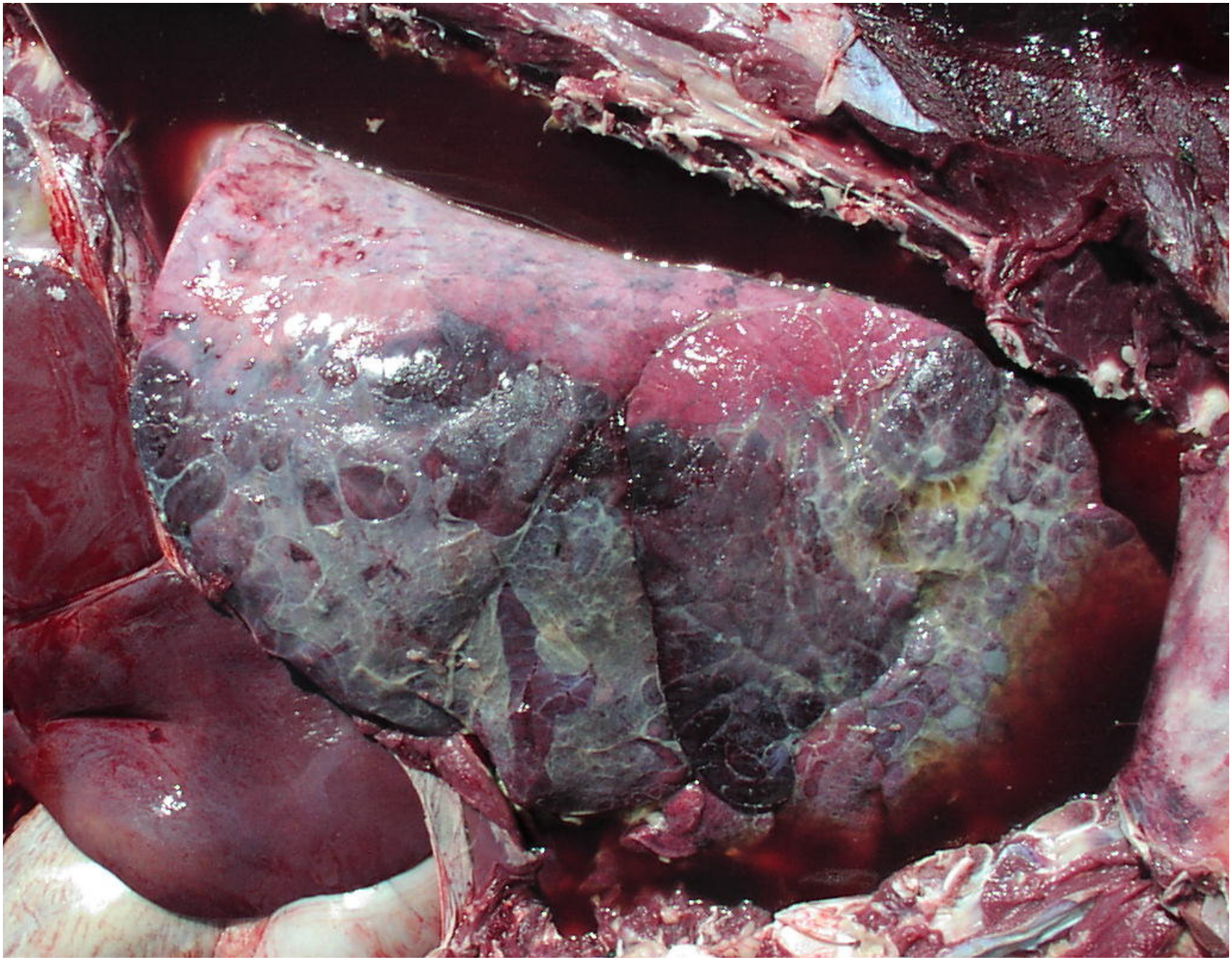
- This will cause the cell to produce cytokines that will in turn activate the inflammatory/immune response

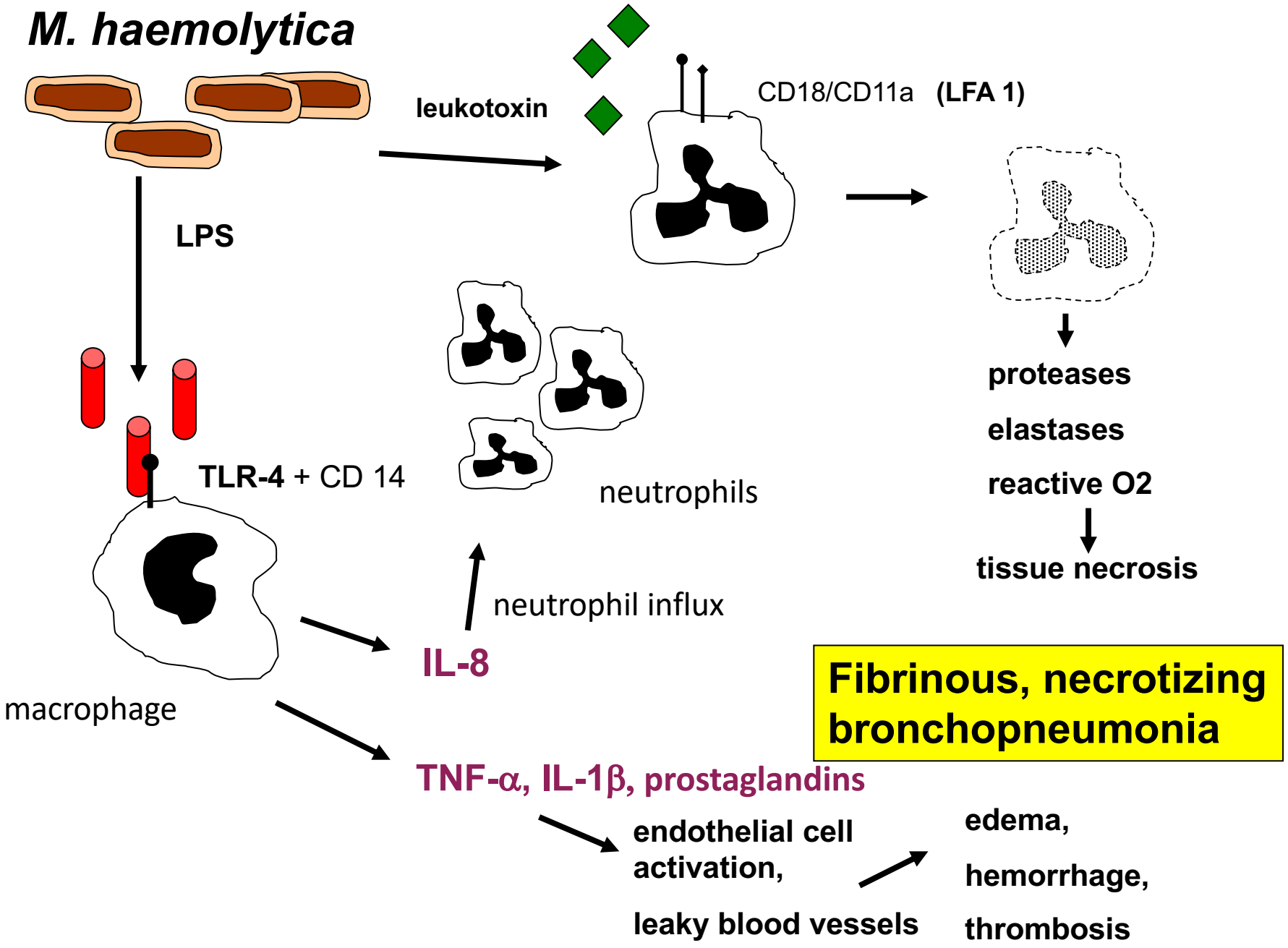
- The mixture of cytokines produced will determine the kind of immune response activated

Consequences of PAMP binding to PRR
Example: LPS binding to TLR-4



So…*why* does this matter??




M. haemolytica
leukotoxin
CD18/CD11a (LFA 1)
LPS
proteases
elastases
reactive O2
tissue necrosis
TLR-4 + CD 14
neutrophils
neutrophil influx
IL-8
macrophage
Fibrinous, necrotizing bronchopneumonia
TNF-α, IL-1β, prostaglandins
endothelial cell activation,
leaky blood vessels
edema,
hemorrhage,
thrombosis

- Temporary depletion of neutrophils prior to experimental *Mannheimia haemolytica* infection significantly decreased clinical signs of disease and lung pathology

  Slocombe et al., 1985; Weiss et al., 1991

- This demonstrated the central role of inflammation mediated by neutrophils in disease due to *M. haemolytica*

Inflammation in BRD: another example

- Bovine respiratory syncytial virus
  - Virus infects epithelial cells of bronchi and alveoli
  - Infected epithelial cells release interferons, interleukin 8
    - Interferons activate natural killer cells and T lymphocytes to kill infected epithelial cells
    - Interleukin 8 induces neutrophil chemotaxis
  - Lung mast cells release histamine: bronchoconstriction
  - Bronchiolitis, alveolitis, and respiratory distress

Photo: Dr. J. VanDonkersgoed

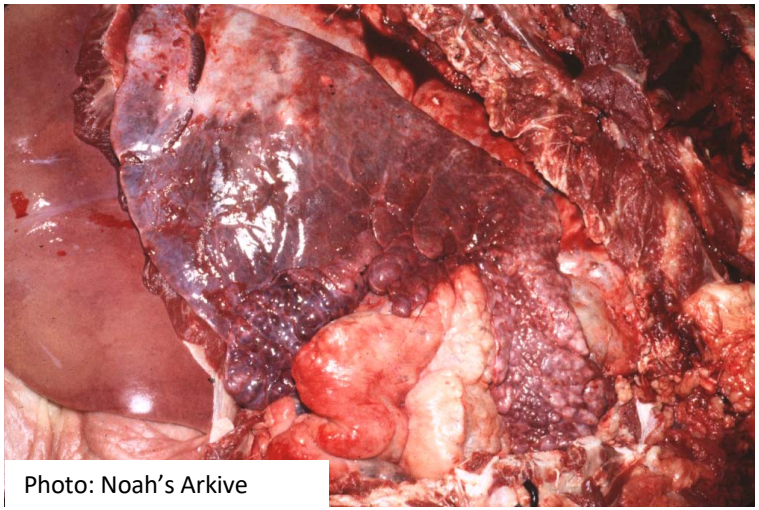
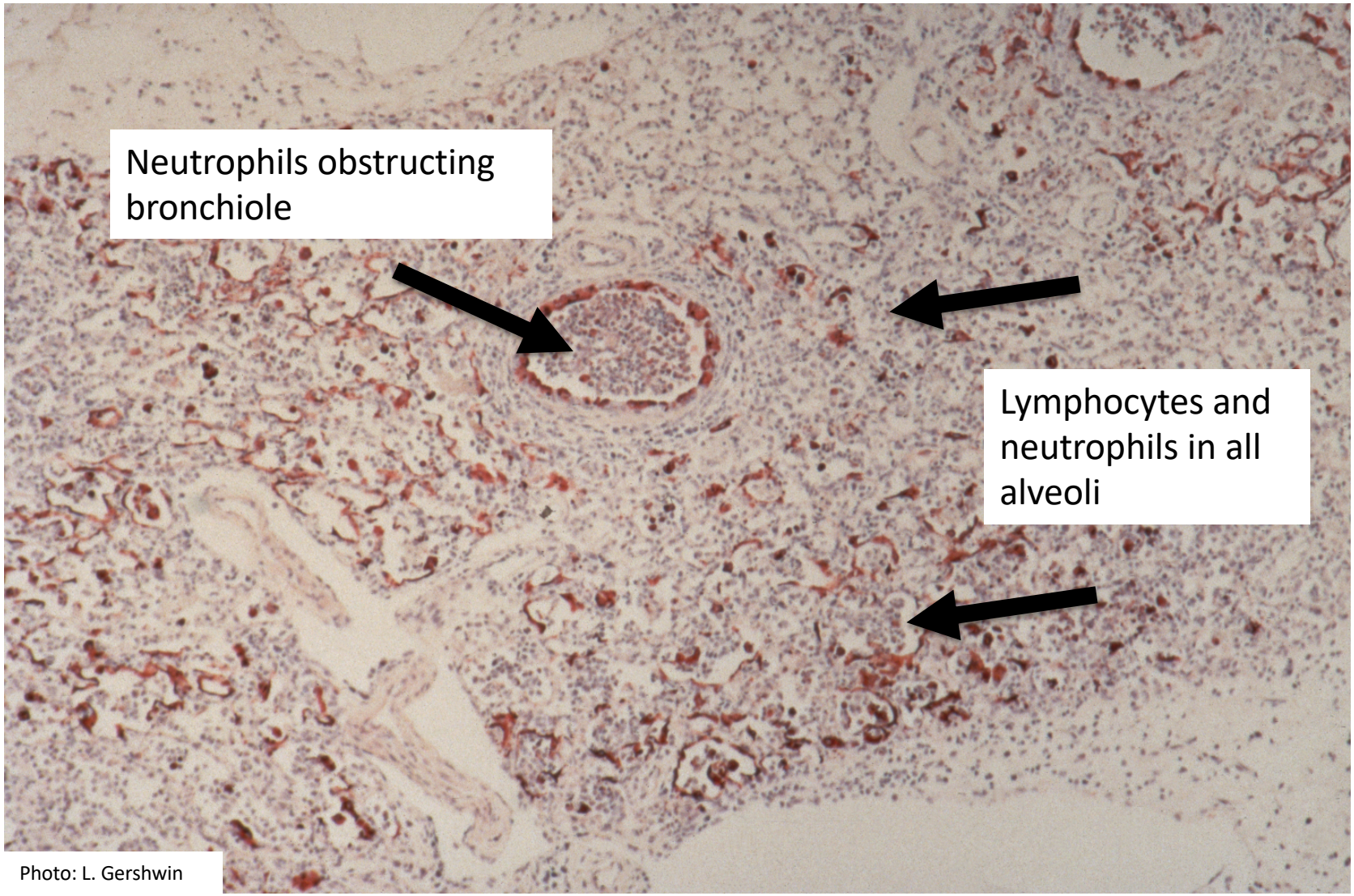


Photo: Noah's Arkive



Microscopic view of lung inflammation, BRSV (brown stain: virus)

- Infection with viruses and bacteria that contribute to BRD induces inflammation
  - Inflammation is necessary to activate immunity

    BUT
  - Inflammation contributes to morbidity
  - Excessive inflammation can lead to death

- Given this, it is logical to consider treating BRD with anti-inflammatory drugs
  - Corticosteroids and non-steroidal anti-inflammatory drugs (NSAID) have been used to treat BRD

Corticosteroids: anti-inflammatory effects

- Corticosteroids inhibit inflammation through multiple mechanisms
  - Decrease expression of COX-2
  - Decrease expression of proinflammatory cytokines
  - Increase expression of annexin A1
    - Annexin A1 inhibits phospholipase A_2
      - Decreased breakdown of cell membranes to form arachidonic acid
        - » Decreased production of prostaglandins etc.
  - Decrease neutrophil movement out of blood vessels

Chatham, 2014; Girol et al., 2013;
Perretti and D'Acquisto, 2009

- In an **experimental challenge study**, corticosteroids decreased disease and pathology due to *M. haemolytica*
  - Calves treated with dexamethasone before and during experimental *M. haemolytica* infection
    - 6 hours before and immediately before (2 mg/kg)
    - Every 12 hours after infection (1 mg/kg)
  - After infection, clinical signs significantly less severe in dexamethasone-treated calves ($P < 0.05$)
  - At 48 hours after infection, treated calves had significantly less lung pathology ($P < 0.05$)
    - Dexamethasone treated: 6% abnormal lung
    - No dexamethasone: 69% abnormal lung

Malazdrewich et al., 2004

- In contrast, corticosteroids were not beneficial in one **large clinical trial**
  - 2,184 yearling cattle with BRD
  - Treated with tetracycline, pyrilamine +/- dexamethasone
    - 1113 cattle received 20 mg dexamethasone SID x 3 days
    - 1071 cattle received placebo SID x 3 days

	% cattle dead (nsd)	**% cattle requiring additional treatment (relapse) *P* < 0.05**
dexamethasone	6.9%	23.8%
placebo	5.7%	18.0%

Conclusion: cattle treated with dexamethasone for 3 days after BRD diagnosis were more likely to require subsequent additional treatment

Christie et al., 1977

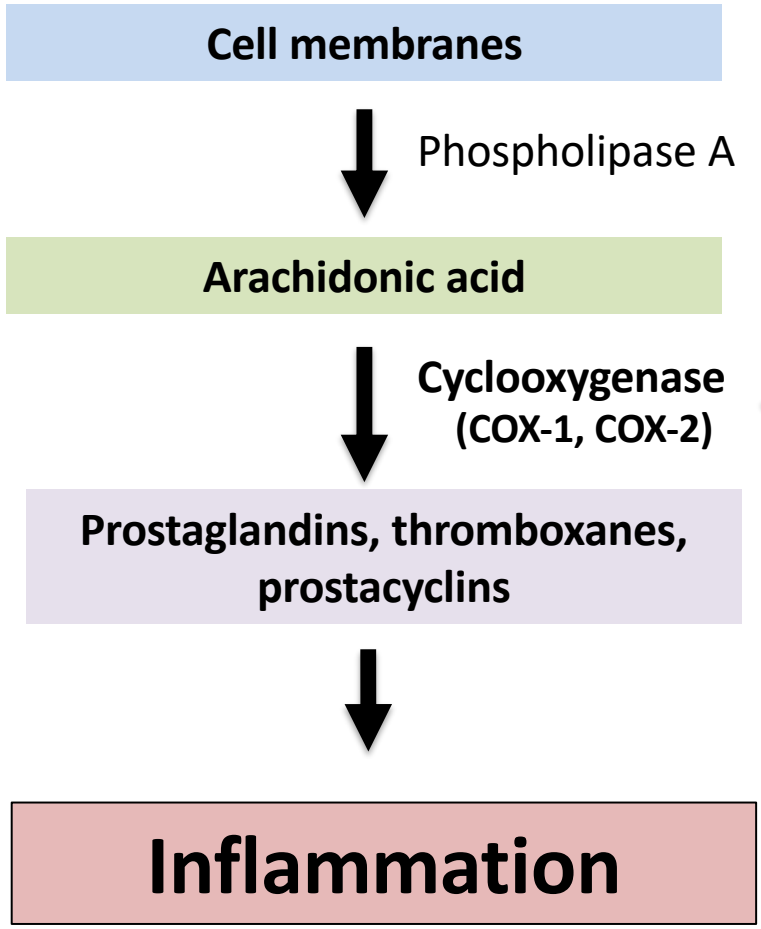
Corticosteroids and BRD

- Little additional published research evaluating corticosteroids in infectious BRD

- Corticosteroids are used for individual cattle with severe acute respiratory disease with life-threatening inflammation
  - Acute bovine pulmonary edema/emphysema (“fog fever”)
  - Anaphylaxis
  - Tracheal edema syndrome

- While logical, these applications are not evidence-based

NSAIDs and inflammation



- NSAIDs inhibit function of cyclooxygenase
- May be non-selective
  - Inhibit COX-1 and COX-2
  - Anti-inflammatory
  - Toxicity (COX-1 inhibition)
- May be selective
  - more COX-2 inhibition
  - Anti-inflammatory with less toxicity

NSAID treatment of BRD

- NSAIDs commonly given to cattle with BRD
  - Flunixin meglumine
    - Licensed in some countries for BRD
  - Meloxicam
    - Licensed in some countries for BRD

- Florfenicol + flunixin meglumine combination (Resflor, MSD)

Meloxicam for BRD in feedlot cattle

- Two hundred female feedlot cattle treated for BRD
- Masked, controlled, randomized study
  - One hundred cattle: oxytetracycline + meloxicam
  - One hundred cattle: oxytetracycline + placebo (saline)
- Rectal temp and clinical score: days 0, 1, 2, 3, 7
- Body weight: days 0, 7, 35, 70, and 105
- Lung lesions evaluated at slaughter

Friton et al., 2005

Meloxicam with oxytetracycline for BRD

	Meloxicam	Placebo
Percent of cattle with fever on day 2	**49%***	**62%**
Average daily gain (kg/d) day 70	1.34*	1.22
Average daily gain (kg/d), day 172	1.23**	1.12
Carcass weight (kg)	282*	270
Percent abnormal lung at necropsy	0.5%*	1.18%
		* P < 0.05, ** P < 0.01

No significant difference for clinical scores on any day, percent of cattle suffering relapse, or percent of cattle requiring future BRD treatment.

Friton et al., 2005

Florfenicol-flunixin and veal calf BRD

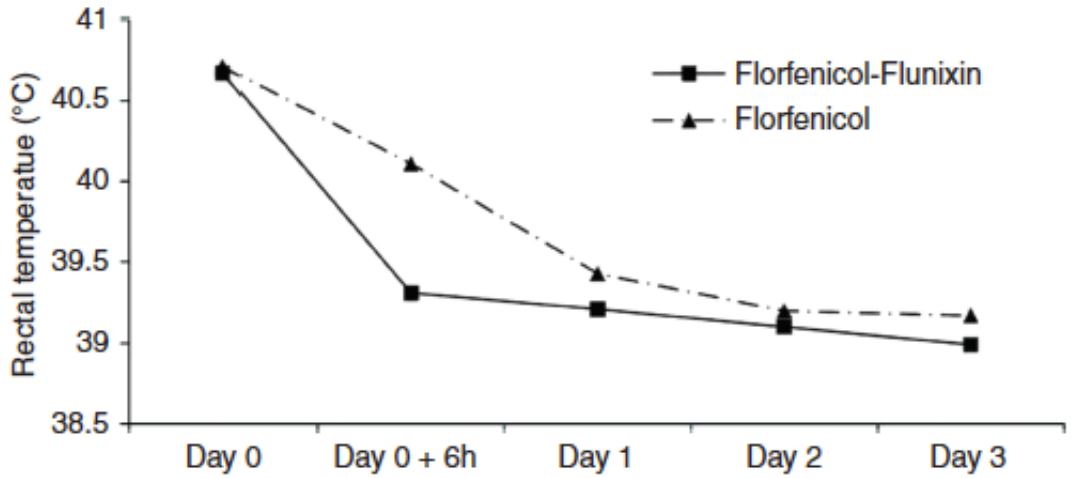
- "Non-inferiority" trial of florfenicol + flunixin vs florfenicol only for calf BRD
- 9 different veal operations in Belgium, France, and Spain
  - Calves purchased from local auction markets
  - No vaccinations, antimicrobials, or anti-inflammatories given at arrival
  - Calves with BRD randomly assigned to treatment
  - Calves examined for 10 days; evaluators masked to treatment groups

Thiry et al., 2014

- 106 calves treated with florfenicol-flunixin
  - Weight at enrollment: 52.4 +/- 9.6 kg
- 104 treated with florfenicol
  - Weight at enrollment: 51.2 +/- 9.2 kg

Decrease in rectal temperature for both groups

P < 0.05 for florfenicol-flunixin group



Thiry et al., 2014

Florfenicol-flunixin was non-inferior for number of calves with treatment success on day 4 or day 10 post treatment

TABLE 3: Summary of efficacy results

Variable	Florfenicol-flunixin	Florfenicol
N enrolled animals	106	104
Age (days) at enrolment	29.08±6.48	28.07±6.47
Body weight (kg) at enrolment	52.38±9.62	51.21±9.19
Drop in temperature six hours after treatment*	–1.36°C	–0.6°C
N treatment success on day four	99 (93.4%)	93 (90.3%)
N treatment success on day 10	71 (67%)	62 (60.2%)

*The florfenicol-flunixin formulation was significantly superior to florfenicol for the change in rectal temperature ($P<0.0001$)

Thiry et al., 2014

- In this study, no significant difference in performance and health outcomes
- Weights only measured for 10 days after treatment

Photo: Dr. J. VanDonkersgoed

- In this comparison of florfenicol and florfenicol-flunixin in veal calves, flunixin did not apparently improve outcomes

Florfenicol-flunixin and feedlot BRD

- Objective: compare 3 AM for BRD treatment
  - Florfenicol-flunixin (Resflor Gold)
  - Tulathromycin (Draxxin)
  - Ceftiofur crystalline free acid (Excede)
- Cattle: auction market derived
  - Treated with long acting oxytetracycline at arrival
  - Other standard processing
- At BRD diagnosis ("undifferentiated fever")
  - Transported 7 – 133 km to research feedlot
  - Randomly allocated to treatment group

Hannon et al., 2009

Florfenicol-flunixin and feedlot BRD

- Outcomes evaluated:
  - 1st, 2nd, and 3rd BRD relapse rate
  - BRD mortality
  - Overall mortality
  - Average daily gain
  - Dry matter intake
  - Slaughter weight
  - Dressing percentage
  - Carcass yield and quality grade
  - Financial returns

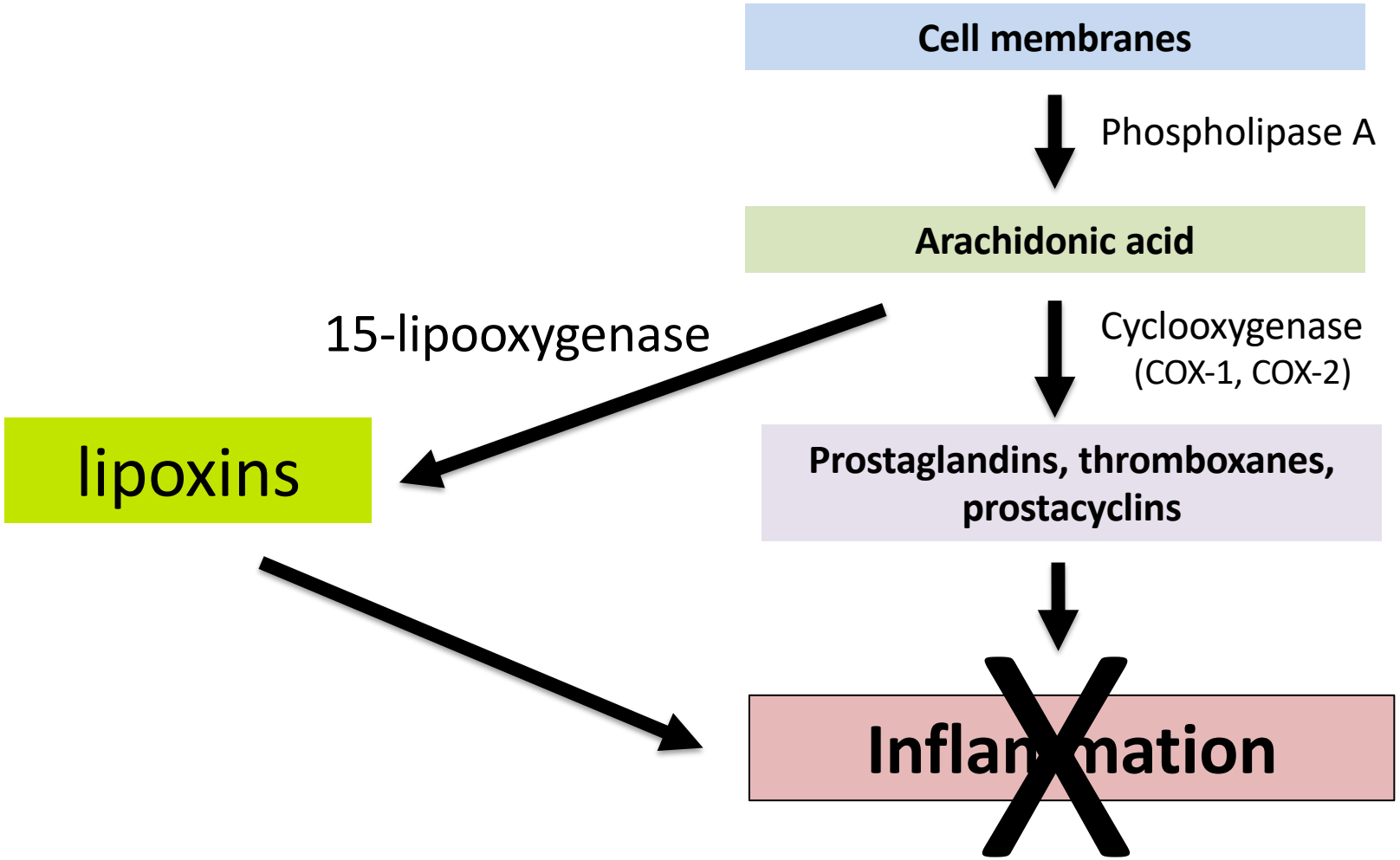
- First BRD relapse:
  - cattle given FLOR-FM had 3.5x greater risk vs cattle given TUL (*P* = 0.004)
- Overall mortality:
  - cattle given TUL had 5x greater risk than cattle given FLOR-FM (*P* = 0.032)
  - Cattle given CEF had 10X greater risk than cattle given FLOR-FM (*P* = 0.018)
- Unlike earlier meloxicam trial, no effect on ADG or lung lesions

Total economic advantage for FLOR-FM per treated animal

Vs TUL: Can$46.23 Vs CEF: Can$108.77

- Important caveat: positive effects of FLOR-FM may be have been due to FLOR, FM, or both together
  - Cannot distinguish with this experimental design

Something new?



- Lipoxins and related “specific pro-resolving mediators” (SPM) are lipid molecules that suppress inflammation
  - Production is activated near the beginning of the inflammatory response
  - Modulate inflammation, presumably to limit tissue damage

- Lipoxins and other SPM associated with
  - Decreased mortality in mouse models of sepsis

    Spite et al., 2009
  - Resolution of airway inflammation in mouse models of asthma

    Rogerio et al., 2012
  - Decreased asthma severity in humans

    Vachier et al., 2005

- Recent small study in high risk beef cattle
  - Cattle that resisted BRD had significantly increased activation of SPM pathways at arrival, compared to cattle that developed BRD

    Scott et al., 2020
  - Resistance to BRD may be due to improved ability to limit inflammation?
  - Watch for more research

Inflammation and BRD, Summary

- Infection and injury activate inflammation
  - PAMPs and DAMPs interacting with immune cells
- Inflammation is necessary to induce immunity
- Too much inflammation can be lethal
- Inflammation causes the lesions of BRD

- Treatment of BRD with NSAIDs decreases morbidity and improves growth in some cases
  - Inconsistent effects may be due to
    - Wrong time of administration?
    - Poor understanding of benefits vs harms of lung inflammation in BRD?
- Endogenous lipoxins and SPM may have beneficial effect to mitigate BRD
  - Possibility of new therapies or preventives??

- Improved understanding of inflammation in BRD is needed!