New Genus of Pro-Resolving Lipid Mediators from EFA

Resolvins & Protectins: SPM in Inflammation & Organ Protection

Disclosures for Charles N. Serhan

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RvE1, RvD1, P1
- Eyes
  - Anti-inflammatory and neuroprotection (Retinopathy)
  - Wound healing (Cornea thermal injury)
- Cardiovascular
  - Platelet aggregation
- Kidney
  - Renal ischemic injury
- Lungs
  - Alveolar inflammation (asthma)
- GI tract
  - PMN and weight loss
  - Pancreatitis
  - Survival (Curflax)

RvD1, PD1
- Brain
  - Stroke damage and PMN entry into the brain
  - Neuroprotective (Stem Cell)
- Oral Medicine
  - Inflammation reduced tissue and bone loss (Periodontitis)
- Anti-Inflammation Pro-Resolution
  - nano - micrograms

Human Deficiencies (n-3 EFAs)

- Neural Protection
- Severe Depression, postpartum depression
- Acute coronary syndromes
- Chronic Alcoholism

DHA

Brain = Exudate?

Genomics

Lipidome

Proteomics

Lipidomics

Structural Lipids

Metabolomics

Autacoids: Chemical Mediators

short-lived paracrine / autocrine signals

Lipid Mediator-Informatics

Today's Outline

- Structural Elucidation of Novel Specialized Pro-Resolving Chemical Mediators (SPM)
- Pro-Resolving Actions: Resolvins & Protectins
- Biosynthesis and Actions of Resolvins & Protectins in Disease Models
- MOA of Resolvins & Protectins
- Gaps in knowledge

Clinical Implications

Is pus "good or bad" for you?
Decision Paths in Acute Inflammation: Resolution or Chronic Inflammation?

Cardinal signs of inflammation:
- Calor (heat)
- Rubor (redness)
- Tumor (swelling)
- Dolor (pain)

Inflammation types:
- Acute Inflammation
- Chronic Inflammation

Injury

Cardinal signs
- Loss of function

Acute Inflammation

Chronic Inflammation

Wound Healing
Scarring

Diabetes
Cardiovascular diseases
Asthma

New concept:
Active process

WAR: Even if Defensive
Unwanted Side Effects

Human Neutrophils
1st line host defense
Protective

Resolution

 fadeIn

Inflammation

Resolution

 fadeIn

Rheumatoid Arthritis
Periodontal Disease

Resolution

Chronic Inflammation

Proteases
O2 radicals

Systems Approach Mapping Resolution
Temporal-Differential Analyses of Resolution

TNF-a

Air pouch skin
Oral Inflammation
Peritonitis
Airway Lung
Renal I/R
Stroke

Sample collection
Cellular composition
Differential counting
FACS analysis

LM-Lipidomics
Proteomics

Solid phase extraction
LC-UV-MS-MS
Informatics
Profiling of lipid mediators

Proteins of interest
Novel proteins

Bioinformatics/ Data analysis

Problems of interest
Novel pathways

Cellular composition

Cavity

Proteomics

Profiling of lipid mediators

Proteins of interest

Novel proteins

Sample collection

Cellular composition

Differential counting

FACS analysis

LM-Lipidomics

Proteomics

Solid phase extraction

LC-UV-MS-MS
Informatics

Bioinformatics/ Data analysis

Problems of interest

Novel pathways
Lipid Mediator-Lipidomics Informatics

**Chemical & Chemical Progenitor Lipid Mediators**

- **Identification & Search Algorithm**
  - Lipidomic Databases
  - Unbiased Approach

**Programmed Resolution**

**Acute Inflammation**
- PMNs
- Monocytes/Macrophages

**Resolution**
- Specialized Pro-Resolving Mediators (SPM)
- A Novel Class of Endogenous Lipid Mediators
- Edema

**Return to Homeostasis**

**Alpha signals Omega**
- Reduce Further PMN Influx
- Efferocytosis
- Non-fever causing

**Eicosanoid Class Switching**
- prostaglandins
- leukotrienes
- mono- 
- di
- tri

**Ideal Outcome of Inflammation**
- Complete Resolution
- Systems Approach to Mapping Resolution
- Programmed Resolution
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**Families Specialized Pro-Resolving Mediators (SPM):**
- A Novel Class of Endogenous Lipid Mediators

- RvE1
- LXA4
- PD1

**Lipid Mediators**

- Lipoxins
- Protectins
- Resolvins
- D-series
- E-series

**Monocyte/Macrophages**
- Chemotaxis
- Proinflammatory
- Anti-inflammatory

**EDTA**
- blood

**Acute phase**
- mins to hrs

**Resolution phase**
- hrs to days

**Reduce Further PMN Influx**

**Efferocytosis**
- Non-fever causing
Protectins

Neuroprotectin D1
Protectin D1

Anti-Inflammatory in vivo
PMN "STOP" infiltration
air pouch, airway inflammation
peritonitis, renal I/R, Liver Protection

Glial cells
Ligand specific cell signaling
Reduces cytokine expression

Brain & Retina
Bazan et al JBC, PNAS
Reduces Stroke Damage & Retinal Injury
Corneal Injury & Wound Healing
Gronert et al JBC

Protectins

Murine Brain, Human Blood and Gliial Cells
Autacoids that counter inflammation

Neuroprotectin D1
/ Protectin D1

Reduce Stroke Damage & Retinal Injury
Corneal Injury & Wound Healing
Bazan et al JBC, PNAS

Bazan et al JBC, PNAS

Protectins

Brain Ischemia/Reperfusion
• Neuroprotective actions in ischemia/reperfusion injury
• Dampens NFκB expression and COX-2 induction
• Reduces PMN influx

Kidney Ischemia Injury
• Mitigates acute kidney injury
• Reduces tubulointerstitial fibrosis
• Blocks TGFβ-mediated activation of macrophages
• Anti-fibrotic action

Biosynthesis of Protectin D1 / Neuroprotectin D1

• Human Neutrophils
• Human T-cells
• Murine Exudates
• Murine Brain

Increased Activities
• Blocks T-cell and PMN migration
• Inhibits TNF & IL-1α secretion
• Promotes TGFβ-1 expression (inhibited by CCL3 and CXCR3)
• Reduces peritonitis and airway inflammation, etc.
• Upregulates CCR5 expression on apoptotic PMN, as scavenger for CCL3 and CCL5

Brain Ischemia/Reperfusion
• Neuroprotective actions in ischemic neuronal injury
• Reduced IL-1β, IL-6, and TNFα secretion in rat brain slices treated with CCL3 and CXCR3
• Reduced TNF-α mRNA

© 2010 Serhan et al.
Metabolic oxidation regulates embryonic stem cell differentiation

Organ Protection: SPM Accelerate Epithelial Wound Healing

Gronert et al, J Biol Chem, 2005

topical treatment 1 μg/tid
Fat-1 Transgenic Mice Rich in Endogenous Omega-3 increase Resolvins & Protectins Organ Protection

Elevated levels of n-3 Rv PD1 decreased vaso-oblitration & retinal neovascularization

Resolvins & Protectins


Omega-3 EPA and DHA

PNAS

What is pro-resolving?
A new bioaction of SPM

Endogenous anti-inflammation xx pro-resolution

Reduce further PMN infiltration

Anti-inflammatory
Pro-resolving
- promote sequestration of pro-inflammatory cytokines
- promote PMN clearance from epithelial surfaces
- induce pro-resolution M1 macrophages
- Direct removal of infiltrates from sterile inflammation

Pro-Resolving Mechanisms

Anti-inflammation

LXA₄, RvE1 and PD1 Promote Macrophage Phagocytosis
Non-phlogistic Activation of Macrophages

Apoptotic PMN

F4/80+ Gr-1+ macrophage (%) increase

LXA₄ RvE1 PD1 Vehicle

Apoptotic PMN

IL-10 (anti-inflammatory)

IL-6 (pro-inflammatory)

IFN-γ

LXA₄, RvE1 and PD1 Promote Macrophage Phagocytosis
Non-phlogistic Activation of Macrophages

F4/80+ Gr-1+ macrophage (%) increase

LXA₄ RvE1 PD1 Vehicle

Apoptotic PMN

IL-10 (anti-inflammatory)

IL-6 (pro-inflammatory)

IFN-γ

No apparent changes: TNF-α, KC, JE, MIP-2

* P<0.05, ** P<0.01, *** P<0.001 (versus vehicle)
Cytokines & Chemokine

Self-Limited Evolving Exudate

CN Serhan in press 2010

n-3

Microscale Valves

Chemotaxis Assay Chamber

Microscale Valves Gradient Generator

Novel Microfluidics Chamber & Single Cell with 1 Drop of Blood <5min:
Actions of Resolvin D1 and DHA [2-3 hours]

Human Neutrophil
**Functional Decoding Metabolomics**

*Rare & Transient Mediators / Intermediates*

Chamber Anatomy & Design

Point of Care Medicine

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**Biosynthesis of RvD2**

Matching of Leukocyte-derived and Synthetic RvD2

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**RvD2 Reduces PAF-Stimulated Leukocyte Recruitment**

Intravital microscopy

PAF 100nM  
After RvD2 1nM

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Mouse Cremaster Muscle  
Post-Capillary Venule

Prof. M. Paredi et al  
WHRI London
Key Concepts & Points

- Resolution is an **active** not passive

- Resolvins & SPM **genus**
  are **Anti-inflammatory and Pro-resolving**

- **Organ Protective**: Renal, Lung & Neural Tissues

- SPM are not immunosuppressive
  stimulate containment: Sepsis (CPL)

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**GAPS in Current Knowledge**

1) Are EFA n-3 (DHA, EPA) vitamins? Can we consider the "Green Pro-drugs"?

2) What is the relationship between serum DHA/EPA to local resolvin & protectin (SPM) levels & actions?

3) What are the physiologic sources of DHA? Edema

4) Relevant Biomarkers: SPM receptor expression
   pathway metabolome
   pathway markers 17-HDHA
   serum 14-HDHA, RvE2
SPM deficiencies in production and/or actions may allow local inflammation to go on beyond its normal self-limiting process. If local tissue injury in Brain Trauma is indeed a non-resolving form of local inflammation then these results may have implications for nutrition & treatment.

**Conclusions**

- Chronic Inflammation
- Host Defense
- Acute Inflammation
- Resolution
- Injury / trauma
- Microbial infection
- Lipoxins (LXs)
- Resolvin D-series (RvDs)
- Aspirin-triggered LXs (ATL)
- Specialized pro-resolving mediators
- Resolvin E-series (RvEs)
- Neuroprotectins (NPD1/PD1)

**Chemical Mediators of Resolution**

- Lipoxins (LXs)
- Resolvin D-series (RvDs)
- Aspirin-triggered LXs (ATL)
- Specialized pro-resolving mediators
- Resolvin E-series (RvEs)
- Neuroprotectins (NPD1/PD1)

**Fighting off pain with resolvins**

Pain caused by chronic inflammation is a vexing health problem. But the currently available analgesic drugs cause major side effects when taken long-term. A new study points to a class of molecules, resolvins, which not only provide analgesia and are well-tolerated but may also reduce inflammation (pages 592–597).