Novel Lipid Mediators in Resolution of Inflammation

Prof. Charles N. Serhan

Director, Center for Experimental Therapeutic BWH
Dept Oral Medicine, Infection and Immunity
Harvard Medical School

1. Autacoids:
   - Local acting short lived paracrine / autocrine signals
   - Identification in humans
   - Lipid mediator profiling
   - Novel lipid mediators
   - Structural elucidation
   - Cell signaling

2. Genomics
   - Proteomics
   - Lipidome
   - Metabolomics
   - Lipidomics
   - Structural lipids
   - Identification biosynthesis and metabolism
   - Molecular species LC-MS-MS profiling

3. Resolution of acute inflammation
   - is an active process
   - Alpha signals Omega

Molecular events in Catabasis as defined decline of a disease
Local tissue programs that promote the return to homeostasis
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Schematic and histological sequence of events

Resolution of inflammation: state of the art, definitions and terms
The FASEB Journal
Charles N. Serhan, Sue D. Brain, Christopher D. Buckley, Derek W. Gilroy, Christopher Haslett, Luke A. J. O'Neill, Mauro Perretti, Adriano G. Rossi and John L. Wallace

Glossary: PUFA precursors to bioactive lipid mediators

“Classic” lipoxin-generating pathways
Pivotal role of the 5-LO
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Lipoxins & ATL in resolution of inflammation
- Reduction in PMN
- Generated during resolution
- Return of vascular permeability
- Monocyte Infiltration
- Stimulate macrophages to take up apoptotic PMN

Blood

LXA₄

Lipoxins / ATL

Limit PMN Infiltration

LXA₄

Neutrophil

Neutrophil apoptosis

Lipoxins counter-regulate cardinal signs of inflammation:
Reduce inflammation and promote resolution
- Regulate leukocyte traffic
  Stop PMN and Eosinophil infiltration
  Stimulate non-philagistic monocyte recruitment
- Redirect chemokine-cytokine axis
  i.e. block IL-8, IL-1 gene expression;
  TNFα action and secretion
- Reduce edema
- Reduce pain signals
- LX/LT neuronal stem cells

Colgan et al., 1993
Lee et al., 1989
Madison & Serhan 1996
Gedson et al., 2000
Gawrilz et al., 1998
Hachicha et al., 1999
Takano et al., 1997
Bandiera-Melo et al., 2000
Pouliot et al., 2000
Serhan et al., 2001
Svensson et al., 2007
Wada et al., 2006

Vascular endothelial mucosal epithelial cells

Down regulate
- PMN transmigration
- Vascular leakage
- Cytokine release & function
- Uptake & removal PMN

Claria & Serhan, PNAS

The screen versions of these slides have full details of copyright and acknowledgements
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Rapid screening: fast pace to stable analogs

LX & ATL stable analogs criteria
- Delay inactivation
- Increase / retain potency
- < 500 MW

Bioactive stable analogs

Vehicle + LTB4 + 15(S,R)-methyl-LXA4

LX & ATL deficiencies in human disease
LX analog pro-resolving treatments in animal disease models *

- Cardiovascular disease  LX/LT  ★
- Asthma  ★
- Aspirin sensitive asthma  ★
- Cystic fibrosis (Classic non-resolving)  Mouse
- Scleroderma  ★
- Rheumatoid arthritis  ★
- Gastrointestinal disease  IBD  ★
- Periodontal disease  LAP  ★

C.N. Serhan et al., 2006
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Outcome of inflammation: complete resolution
Images in clinical medicine
Lipid mediators

Specialize chemical mediators?
Complete RESOLUTION

Something fishy going on in the heart

Murine dorsal air pouch

Treated aspirin 3.5h and 4h n-3 PUFA

Reduction in number 25-60%
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Lipid mediators in inflammation – resolution
Lipid mediator informatics: LC-MS-MS based profiling

- Exudate tissues
- Solid phase extraction (IS)
- LC-MS-MS
- Tandem UV
- Lipid mediator profiles

- Functional analyses
- Cells – PMN Transmigration
- Cytokine gene regulation in vivo
- Physical properties
- Data base library identification
- “mining” know mediators

- Scale-up
- Establish actions
- PMN Transmigration
- Cytokine gene regulation in vivo
- Physical properties
- Data base library identification
- “mining” know mediators

- Biogenic Synthesis
- Cells – PMN Transmigration
- Cytokine gene regulation in vivo
- Physical properties
- Data base library identification
- “mining” know mediators

- Total organic synthesis
- Structural elucidation
- Novel compounds and actions

C.N. Serhan et al., Nature Immunol 2002

LC-MS-MS:
Exudates from ASA treated murine dorsal air pouches

Resolvin E1 Stops PMN infiltration

Vehicle
Resolvin E1 100 ng
ASA 1.0 mg
Dex 10 ug

PMN/air pouch exudate (x10^6)

The screen versions of these slides have full details of copyright and acknowledgements
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Biosynthesis of resolvin E1

Data base and search algorithms for identification of novel lipid mediators

RvE1 in human plasma: LC-MS/MS mediator lipidomics

Healthy individuals taking EPA (1g) and aspirin (160mg)

RvE1 in human plasma: LC-MS/MS mediator lipidomics

Healthy individuals taking EPA (1g) and aspirin (160mg)

The screen versions of these slides have full details of copyright and acknowledgements.
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Resolvins: actions of E series resolvins
Resolution phase interaction products

Resolvin E1

- PMN “stop” infiltration
  - Air pouch, PMN transmigration, peritonitis
- Biogenic synthesis
  - Scale-up, confirm MS, biosynthesis
- Total organic synthesis
  - Confirm structural assignments
  - Bioactions, expand
  - Regulates DC migration & inhibits IL-12
  - Label for receptors studies

Potent actions ng range
Dendritic cell

Resolvin E1: stereospecific total organic synthesis

Localized aggressive periodontitis: LAP
- Neutrophil functional abnormalities
- Familial aggregation with genetic predisposition
- Rapid destructive form of periodontal disease
- Alveolar bone loss

The screen versions of these slides have full details of copyright and acknowledgements
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Ligature + P. g. RvE1 treated

RvE1 protects from inflammatory bone loss

Percent bone loss

FASEB J., Dec. 2005

Receptor screening for counter-regulatory signals
NF-κB reporter gene system

The screen versions of these slides have full details of copyright and acknowledgements
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Specific GPCRs for resolvins E1 actions
- [3H]-RvE1 specific binding ~ 11nM Kd
- RvE1-dependent signals:
  - G-protein activation
  - ERK phosphorylation
  - Block NF-κB activation
  - Block DC IL-12 production
  - siRNA knock down

Resolvins E1 acts via two distinct leukocyte GPCRs

Resolvin E1 transformation

The screen versions of these slides have full details of copyright and acknowledgements
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

19-para-fluoro-phenoxyl RvE1

- Protects from rapid local inactivation
- Retains & prolongs bioactivity

DHA precursor to resolvins & protectins

CN Serhan et al., JEM 2002

Resolvins: actions of D series
Resolution phase interaction products

Resolin D1

PMN “stop” infiltration
Air pouch, PMN transmigration, peritonitis

Microglial cell
Reduces cytokine expression

Potent actions ng range

Biogenic & total organic synthesis
Scale-up, matching JBC 2007

CN Serhan et al., JEM 2002
Hong et al., JBC 2003, JASMS 2007

The screen versions of these slides have full details of copyright and acknowledgements
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Resolvin D1 and AT-resolvin D: human PMN transmigration

Resolvins E and D series reduce murine peritonitis

Protectins: DHA derived 17S-series
Murine brain, human blood and glial cells

The screen versions of these slides have full details of copyright and acknowledgements
**Novel Lipid Mediators in Resolution of Inflammation**
Prof. Charles N. Serhan

**PD1 stops leukocyte infiltration**
*Zymosan-induced peritonitis*

Treatment 2 hours after initiation

% PMN inhibition

- Zymosan (1mg/ml, i.p.) 51%
- Zymosan + PD1- free acid (1mg dose, i.p.) 46%
- Zymosan + PD1- methyl ester (1mg dose, i.p.)

Serhan et al., JI 2006

**Temporal-differential analyses of resolution**
Mediator lipidomics and proteomics

- Acute inflammation
- Temporal differential
- Sample collection (Peritoneal exudate)
- Cellular composition
  - Differential counting
  - FACS analysis
- Lipidomics
  - Solid phase extraction
  - LC-UV-MS-MS
- Proteomics
  - 2D gel electrophoresis
  - LC nanospray MS-MS
- Informatics
- Data analysis
- Profiling of lipid mediators
- Proteins of interest
- Novel proteins
- Local treatment

Informatics

Bioinformatics

Data analysis

Proteins of interest

Novel proteins

**Lipidomics**

**Proteomics**

**Informatics**

**Profiling**

**of lipid mediators**

**Proteins of interest**

**Novel proteins**

**Liquid chromatography**

**Tandem mass spectrometry**

**2D gel electrophoresis**

**LC nanospray**

**MS-MS**

**Solid phase extraction**

**LC-UV**

**MS-MS**

**2D gel electrophoresis**

**Profiling**

**Lipid mediators**

**Proteins of interest**

**Novel proteins**

**Local treatment**

**Pathway**

**ATL1**

**RVE1**

**NPD1**

**ATL2**

**Differential analyses of resolution**

**Mediator lipidomics and proteomics**

**Cellular composition**

**Differential counting**

**FACS analysis**

**Lipidomics**

**Proteomics**

**Informatics**

**Profiling**

**of lipid mediators**

**Proteins of interest**

**Novel proteins**

**Liquid chromatography**

**Tandem mass spectrometry**

**2D gel electrophoresis**

**LC nanospray**

**MS-MS**

**Solid phase extraction**

**LC-UV**

**MS-MS**

**2D gel electrophoresis**

**Profiling**

**Lipid mediators**

**Proteins of interest**

**Novel proteins**

**Local treatment**

**Pathway**

**ATL1**

**RVE1**

**NPD1**

**ATL2**
**Resolution Indices:**
- $\Psi_{\text{max}}$ - maximal PMN numbers
- $T_{\text{max}}$ - The time point when PMN numbers reach maximum
- $R_{50}$ - 50% of $\Psi_{\text{max}}$
- $T_{50}$ - The time point when the PMN numbers reduce to 50% of $\Psi_{\text{max}}$
- $R_{15}$ (Resolution Interval) - The time interval from the maximum PMN point ($\Psi_{\text{max}}$) to the 50% reduction point ($R_{50}$) [$T_{50} - T_{\text{max}}$
- $R_{\text{PMN}=\text{mono}}$ (Point of Intersection) - when the increase in mononuclear cells intersects the decrease in PMN (i.e., PMN numbers $\rightarrow$ mononuclear cell numbers)

**Resolution in quantitative terms:**

- (Magnitude)
  - $\Psi_{\text{max}} = 1.4 \times 10^6$
  - $T_{\text{max}} = 12$ h
- (Duration)
  - $R_{50} = 7 \times 10^6$
  - $T_{50} = 23$ h

**Molecular circuits of resolution: formation and actions of resolvins and protectins.**

Gerard L. Bannenberg, Nan Chang, et al., Journal of Immunology 2005: 4345

**Resolvins & protectins**

- Brain
- Oral
- Skin
- Ocular/retina
- Airway
- Liver
- GI

**Resolvins & protectins**

- Brain
- Oral
- Skin
- Ocular/retina
- Airway
- Liver
- GI

**Resolution of Inflammation**

Prof. Charles N. Serhan
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Resolvins & protectins D:
Identified in murine kidney after iARF

Mouse received 15 μg DHA/kg body weight or an equivalent volume of its vehicle
IARF was induced on FVB male mice (~22 g) by clamping both renal pedicles for 30 min.

Kidneys:
• Partially functional protection
• Reduced fibrosis
• Improved behavior

Mouse activity score following ischemia in mice treated with PD1 or RvDs

I/R plus Vehicle   PD1   RvDs
Score  2.1 ± 0.2*  2.8 ± 0.3  2.7 ± 0.4

Activity scores were determined at 24 hours
Dose of PD1 was 35μg and RvDs was 35μg.
Sham operated mice had activity scores of 3.0 irrespective of treatment;
*P = 0.03 (ANOVA)
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

A new deficiency disease produced by the rigid exclusion of fat from the diet

<table>
<thead>
<tr>
<th>No.</th>
<th>Tail of Rat 28113</th>
<th>Tail of Rat 28114</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>diet 550 + 5 drops of lard daily</td>
<td>diet 550 + 5 drops of glycerol daily</td>
</tr>
<tr>
<td>2</td>
<td>entirely normal in appearance</td>
<td>Glycerol gives no protection</td>
</tr>
</tbody>
</table>

Tail of Rat 28113: diet 550 + 10 drops of lard daily; entirely normal in appearance
Tail of Rat 28114: diet 550 + 5 drops of glycerol daily; Glycerol gives no protection

Summation: some key points to remember

- Anti-Fibrotic Amplification
- Anti-Inflammation Amplification
- Cellular Responses
- GPCRs
- Resolvins & protectins
- Summation: some key points to remember
- Omega-3 PUFA
- DHA & EPA

The screen versions of these slides have full details of copyright and acknowledgements.
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan

Resolvins & protectins
Novel families of chemical mediators

- Resolvins = Resolution phase interaction products
- Novel structures & signals that are protective
- Generated during resolution stage of inflammation
- Dampen inflammation & PMN injury “from within”
- Pg to ng range of actions in vivo lung, kidney, GI
- Promote resolution: “stop PMN infiltration”
- Activate pro-resolving circuits
- Resolution is an active process
- Likely active endogenous mediators of essential omega-3 PUFA
Novel Lipid Mediators in Resolution of Inflammation
Prof. Charles N. Serhan