Inflammation:

Novel Pro-Resolving Mediators and Mechanisms in Inflammatio: Immunoresolvents

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Metagenics

Providing Educational Support for Healthcare Providers

Novel Pro-Resolving Mediators and Mechanisms in Inflammation: Immunoresolvents

Jesmond Dalli and Charles N. Serhan

Instructor
Harvard Medical School

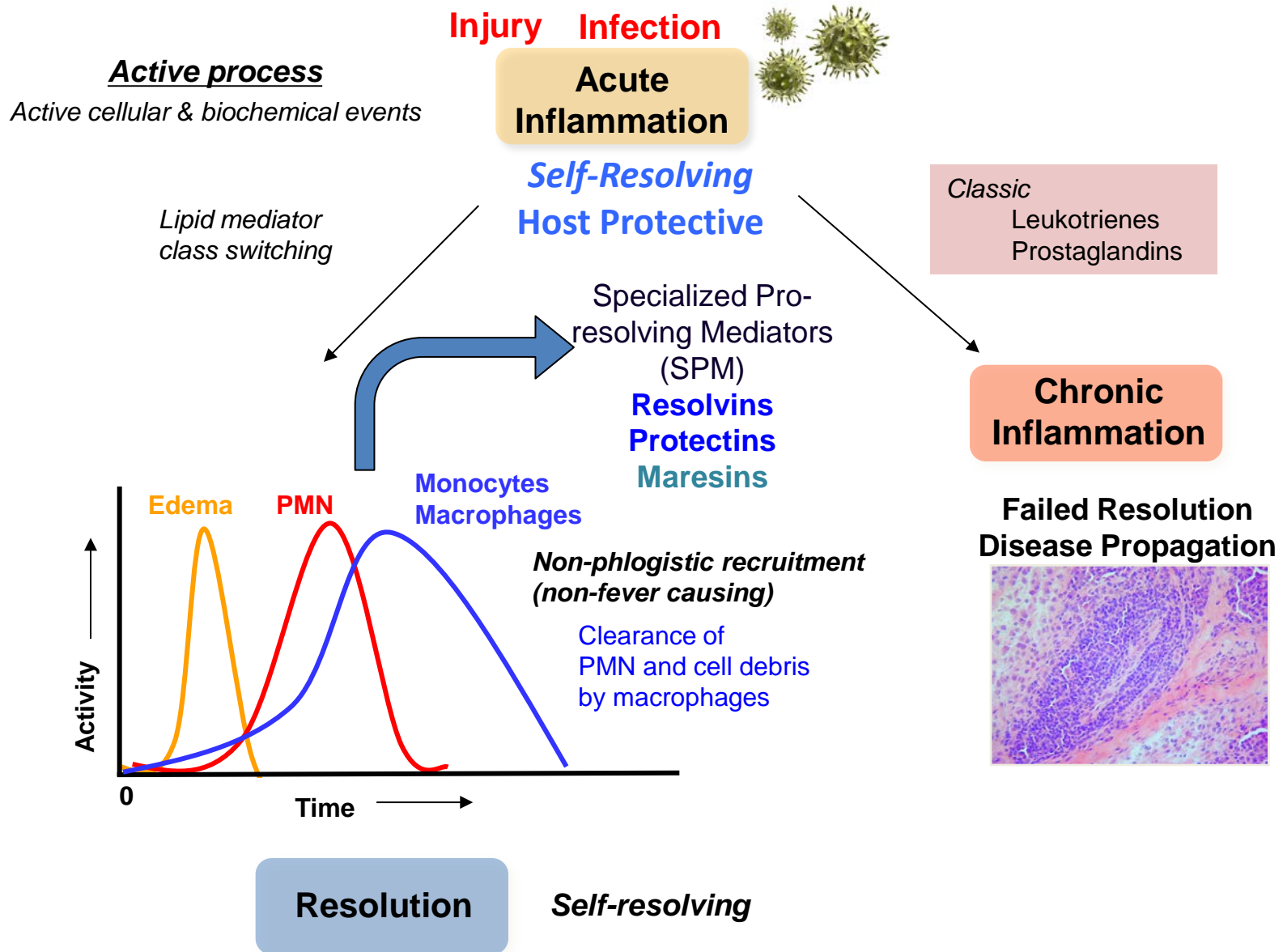*Lipid Mediator Metabololipidomics Co-leader*
Center for Experimental Therapeutics
Brigham and Women's Hospital

Today's Outline: Focus on Human Translation

- **What controls excess Inflammation & Infection ?**

- **Structural Elucidation of Novel Specialized Pro-Resolving Chemical Mediators (SPM)**

- **Functional Decoding Metabolomics of Novel Bioactive Mediators (**Live Infections, Receptors**)**

- **New Approach for Functional LM/SPM Profiling**

Decision Paths in Acute Inflammation: Ideal Outcome is Resolution

Injury   **Infection**

Acute Inflammation

Active process

Active cellular & biochemical events

Self-Resolving
Host Protective

Classic
   Leukotrienes
   Prostaglandins

Lipid mediator class switching

Specialized Pro-resolving Mediators (SPM)
Resolvins
Protectins
Maresins

Chronic Inflammation

Monocytes Macrophages

Non-phlogistic recruitment (non-fever causing)

Clearance of PMN and cell debris by macrophages

Failed Resolution Disease Propagation

Edema   **PMN**

Activity

0

Time

Resolution   *Self-resolving*

From Taber's Cyclopedic Medical Dictionary:

resolution *1. Decomposition; absorption or breaking down of the products of inflammation. 2. Cessation of inflammation without suppuration. The return to normal.*
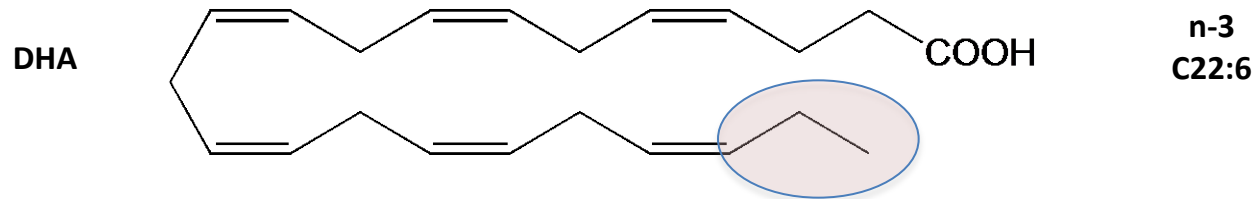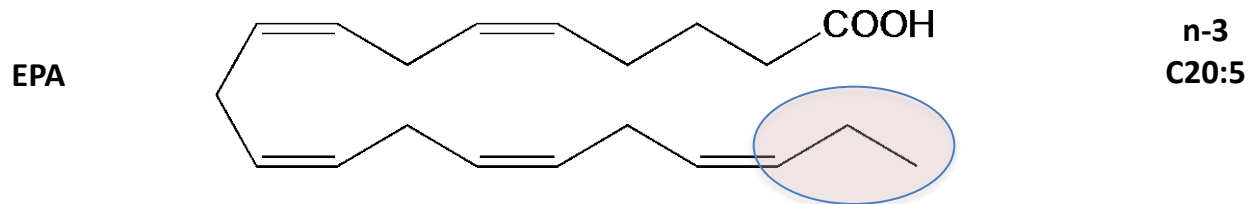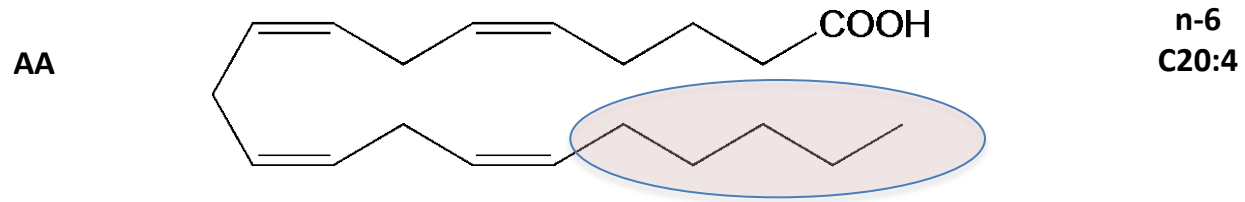
resolvant *1. Promoting disappearance of inflammation. 2. That which causes dispersion of inflammation.*

Immunoresolvent: endogenous mediator or agent that stimulates resolution

The resolution of inflammation:
the devil in the flask and in the details
Serhan CN. *FASEB J* 2011;25:1441-1448.
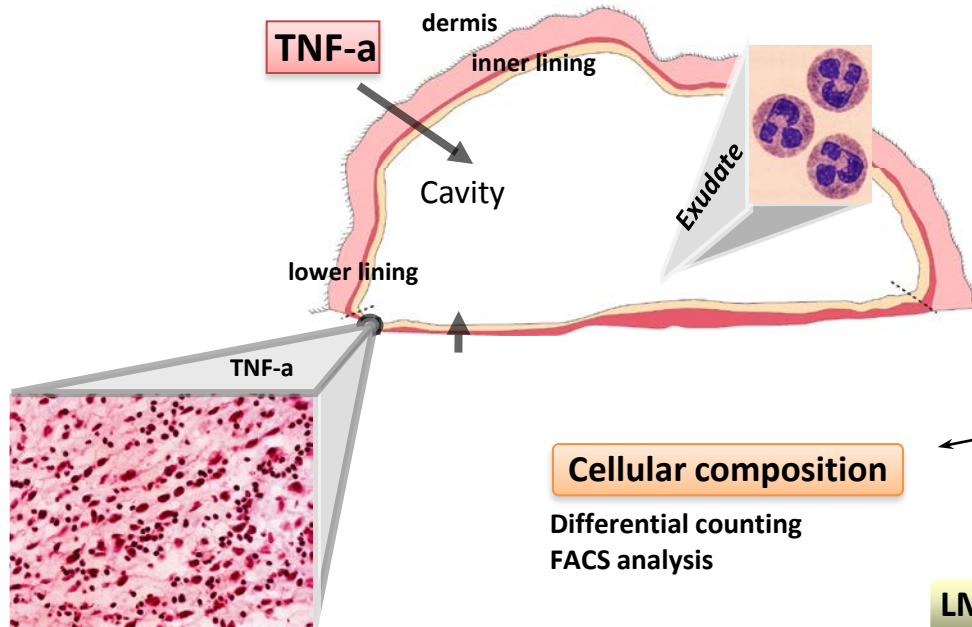
PUFA n-6 & n-3

AA

COOH

n-6
C20:4

EPA

COOH

n-3
C20:5

Theory Compete for AA

DHA

COOH

n-3
C22:6

Essential fatty acids: They exert critical functions in human health
Not produced by human cells
Obtained from our diet

Systems Approach Mapping Resolution
Temporal-Differential Analyses of Resolution

Spatial & Temporal Relationships

TNF-a

dermis
inner lining
Cavity
lower lining
TNF-a
Exudate

self-limited

Acute inflammation

Temporal   *Differential*

Sample collection exudate

Cellular composition

Differential counting
FACS analysis

LM-Lipodomics   Proteomics

Resolution miRNA
Resolvins

Lipid Mediator Class Switching
Air pouch skin
Oral Inflammation
Peritonitis
Airway Lung

Renal I/R
Stroke
Human Adipose
Human Lymph Nodes

Serhan CN. *Ann Rev Immunology* 2007;25:101-137.

Resolvin Biosynthesis by Human Leukocytes

E series Resolvins

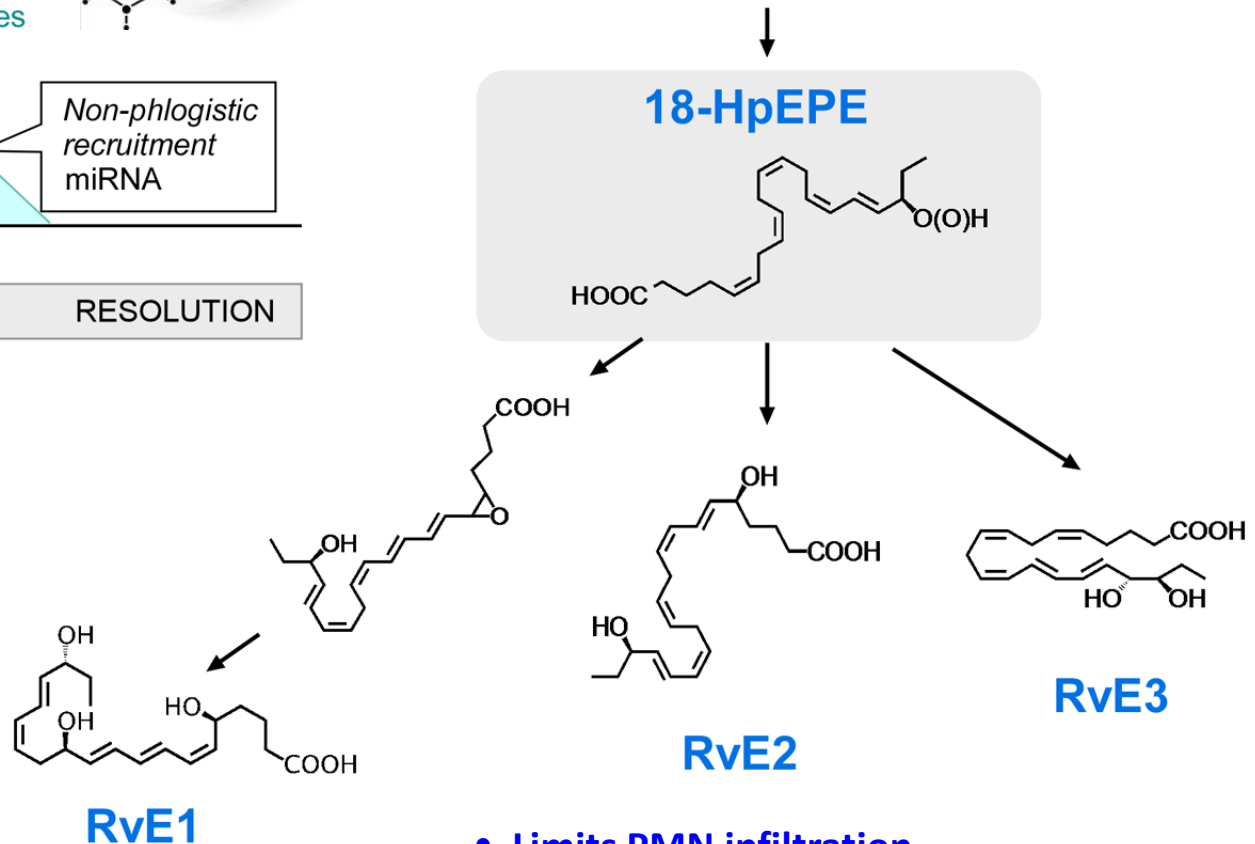EPA → 18-HpEPE → RvE1, RvE2, RvE3
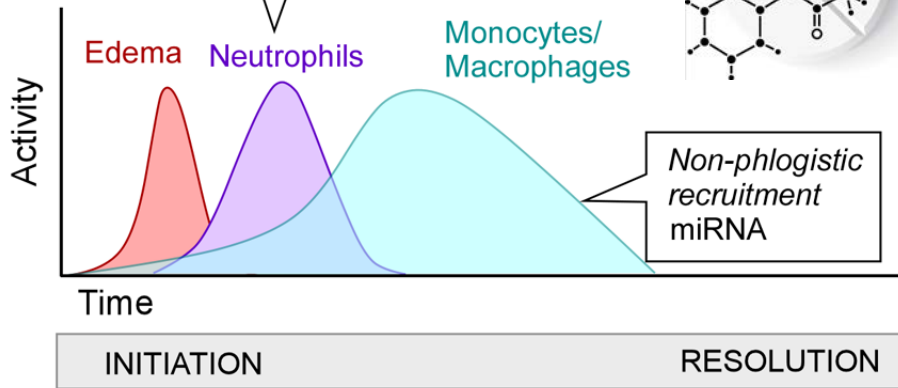
RvE1 >> Aspirin > Dex

- Regulates DC migration, IL-12 production, IL-10
- Blocks platelet aggregation

RvE2

RvE3
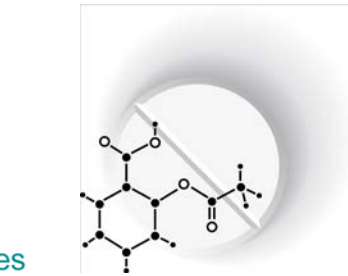
- Limits PMN infiltration
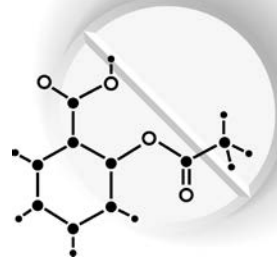- Stimulates Macrophage phagocytosis

Serhan CN. *Nature.* 2014;510:92-101.

Biosynthesis by Human Leukocytes : D- series Resolvins, Protectins & Maresins

Neutrophils in pus
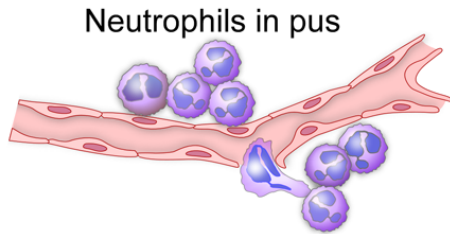
Resolution n-3 metabolome

Resolution microparticles

SPM

↑ **Efferocytosis**

Apoptotic PMN

Resolving macrophage (rMφ)

DHA

17S-H(p)DHA

17 position
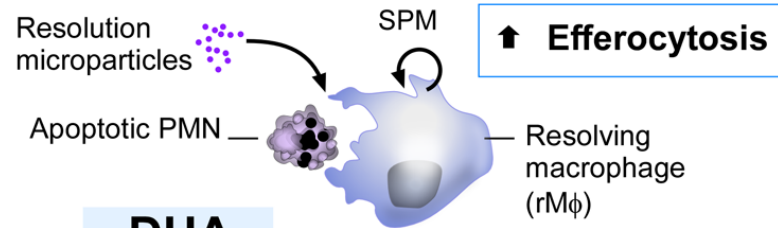
Complete Stereochemistry Assigned

Aspirin enhances 17R epimers

RvD1

RvD4

16S, 17S-epoxide-protectin

RvD2

RvD3

Protectin D1 (PD1) / Neuroprotectin D1 (NPD1)

D series Resolvins

Protectins

Serhan CN. *Nature.* 2014;510:92-101.
Buckley CD et al. *Immunity.* 2014;40:315-327.
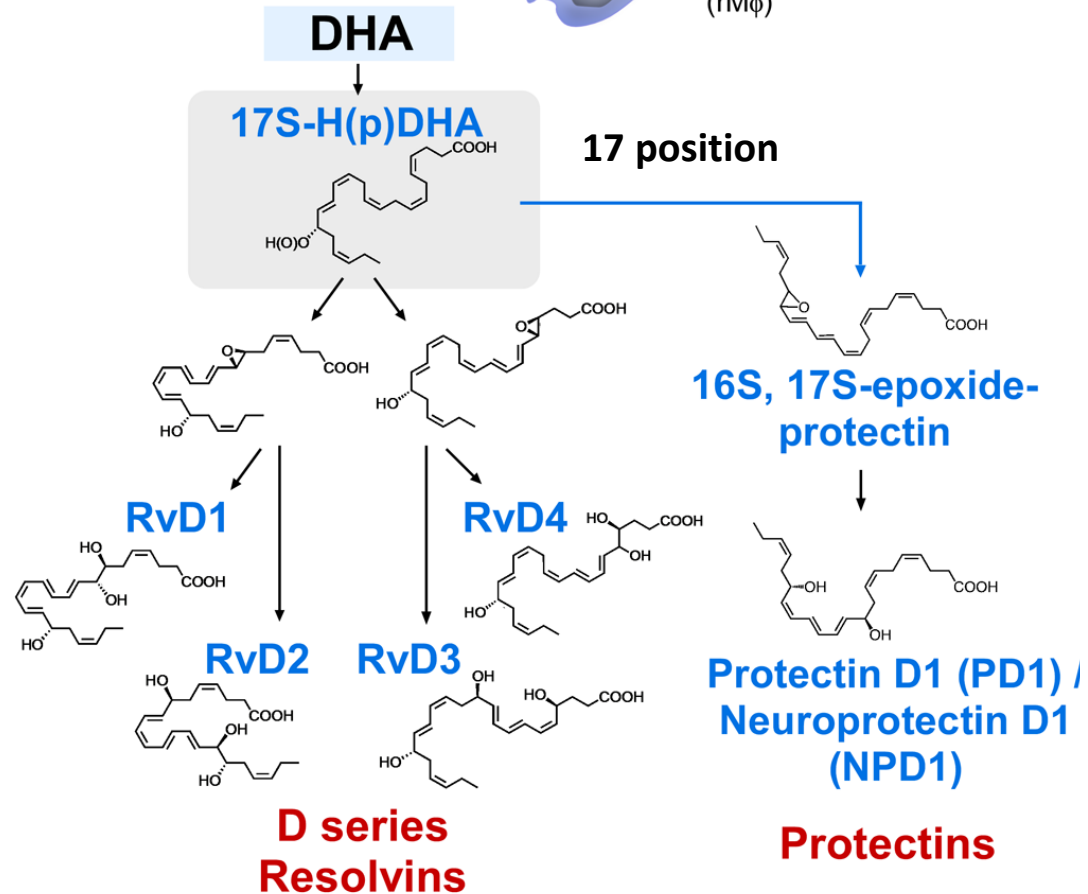
Key Functions Employed in Structural Elucidation of Novel Resolution Phase Mediators
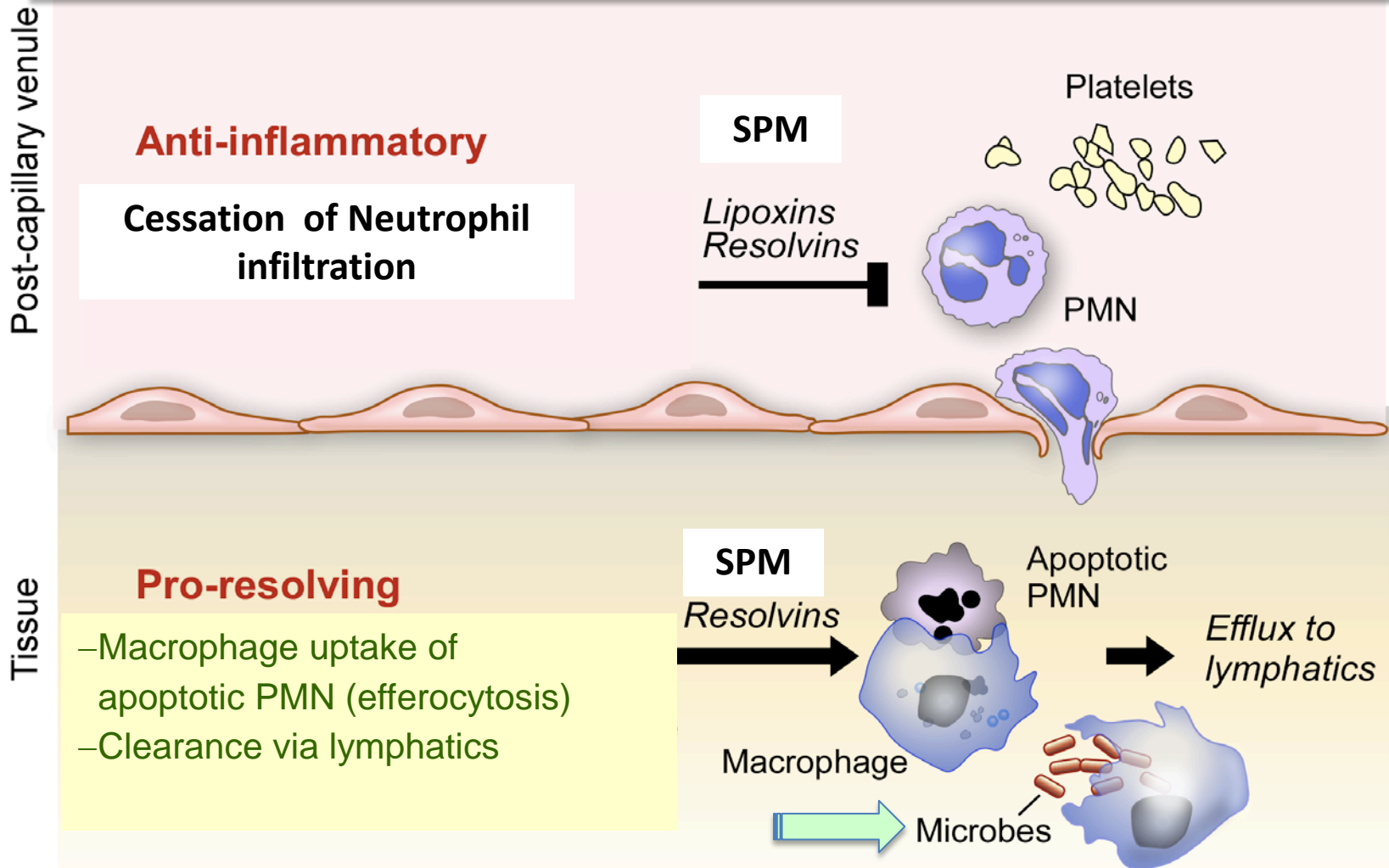
Main Cellular Responses in Resolution of Inflammation used for structural elucidation of novel mediators

Serhan CN. *Nature.* 2014;510:92-101.

Tissue Protective: Mouse 12/15-Lipoxygenase and SPM

Animal models	Alox15 gene modification	Actions/Phenotypes	Reference
Cornea thermal injury	Alox12/15 deficient mice	↑ Inflammation, corneal re-epithelialization ↓ Wound healing, **endogenous LXA₄ production** *LXA₄ rescues exacerbated inflammation and impaired wound healing in Alox15 deficient mice*	Gronert et al., 2005 Biteman et al., 2007
Suture-induced chronic cornea Injury	Alox12/15 deficient mice	↑ Inflammatory neovascularization ↑ VEGF-A and FLT4 expression *LXA₄ rescues 15-LOX knockout mice from exacerbated angiogenesis*	Leedom et al., 2010
Peritonitis	Alox12/15 deficient mice	Eosinophil depletion causes resolution deficit, *rescued by PD1* Alox12/15 deficient mice eosinophils did not rescue the resolution phenotype	Yamada et al., 2011
Dermal fibrosis	Alox12/15 deficient mice	↑ TGF-β stimulated MAPK pathway *LXA₄ counters TGF-β stimulated fibroblast activation*	Krönke et al., 2012
Endometriosis	Alox12/15 deficient mice	EPA decreases lesions in WT but not in Alox12/15 deficient mice. ↓ **RvE3** in Alox12/15 deficient mice compared to WT	Tomio et al., 2013
Airway inflammation	Alox12/15 deficient mice	↓ TLR7-mediated resolution of airway inflammation	Koltsida et al., 2013
Peritonitis	Alox12/15 deficient mice	Low dose inhaled CO reduces PMN infiltration in WT, but not in Alox12/15 deficient mice	Chiang et al., 2013

Change in Treatment for Inflammation Associated Diseases:
Agonist of Resolution   Immunoresolvents

Antagonists
Inhibitors
 i.e. NSAIDs
Resolution toxic

**Resolution
Agonists, SPM**
Traffic, Phenotype

Peak

Apoptosis/Phagocytosis
(TGFβ1)

Resolution

Resolving disease

Intensity

Onset

Lipoxins/Resolvins

PGD_2/J_2

**Failed Resolution
Disease Propagation**

Injury/
Infection

e.g. Histamine
5-HT
Complement

Time (days)

British Pharmacology Society Consensus Report: Serhan CN et al. *FASEB J.* 2006;21:325-332.

Chronic Inflammation is a Unifying Component of Many Diseases: Role for Pro-Resolving Mediators
Failed Resolution ?

Inflammatory Bowel Disease
Arita et al. (Serhan) *PNAS* 2005

Stem cells
Wada et al. (Serhan) *FASEB J* 2006

➡ **Sepsis**
Spite et al. (Serhan) *Nature* 2009

Obesity
Claria et al. (Serhan) *J Immunology* 2012

Tissue Regeneration
Serhan et al. *FASEB J* 2012

Asthma
Levy et al. (Serhan) *Nature Med* 2002

➡ **Infection**
Chiang, N. et al. (Serhan) *Nature* 2012

Stroke
Marcheselli et al. (Serhan and Bazan) *JBC* 2003

Atherosclerosis
Merched et al. (Serhan and Chan) *FASEB J.*

Retinal Angiogenesis
Connor et al. (Serhan and Smith) *Nature Med* 2007

Alzheimer Disease
Lukiw et al. (Serhan and Bazan) *JCI* 2005; **Wang et al 2014**

Periodontitis
Hasturk et al. (Serhan and Van Dyke,TE) *FASEB J* 2006

Pain
Xu, ZZ et al. (Serhan and Ji) *Nature Med* 2010

Planaria

RvE1, MaR1
↑ **Head regeneration**

Picogram to nanogram range potencies
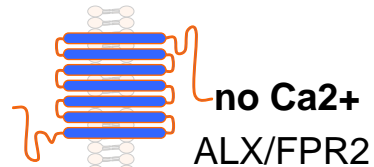
Pro-Resolving Mediators Activate GPCR

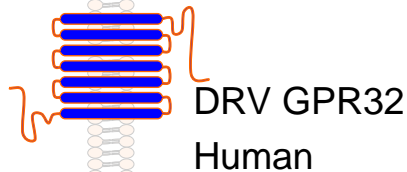*Ligand : Agonist*   *GPCR*   *TG mice*   *KO mice*

SPM

**LXA$_4$,
RvD1, 0.1nM**

no Ca2+
ALX/FPR2

**Accelerates
resolution with
LXA$_4$ and RvD1**

**RvD1 actions is
abolished**

**RvD1, RvD3, RvD5
nM**

DRV GPR32

Human

**RvE1, RvE2 (partial)
 nM**

ERV ChemR23
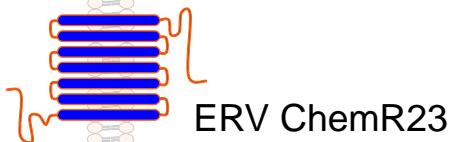
**Potentiates RvE1
action in limiting PMN**

 protecting bone loss

↓NFkB

 Agonist: **LTB$_4$**

Antagonist: *Partial Agonist*
  RvE1, RvE2

BLT1
PMN

↑**HO-1**

RvE1 regulate

PMN infiltration

& Apoptosis

Serhan CN, Chiang N. *Curr Opin Pharmacol.* 2013;13:632-640.
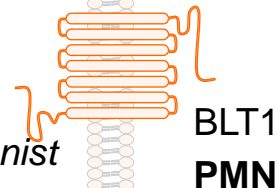
Infection regulates pro-resolving mediators that lower antibiotic requirements

Chiang N, Fredman G, Backhed F, Oh SF, Vickery T, Schmidt BA, Sherhan CN

Antibiotics

SPM Resolvins

Bacteria

Host response

Phagocytosis Containment, Killing Clearance

E. coli, S. aureus skin
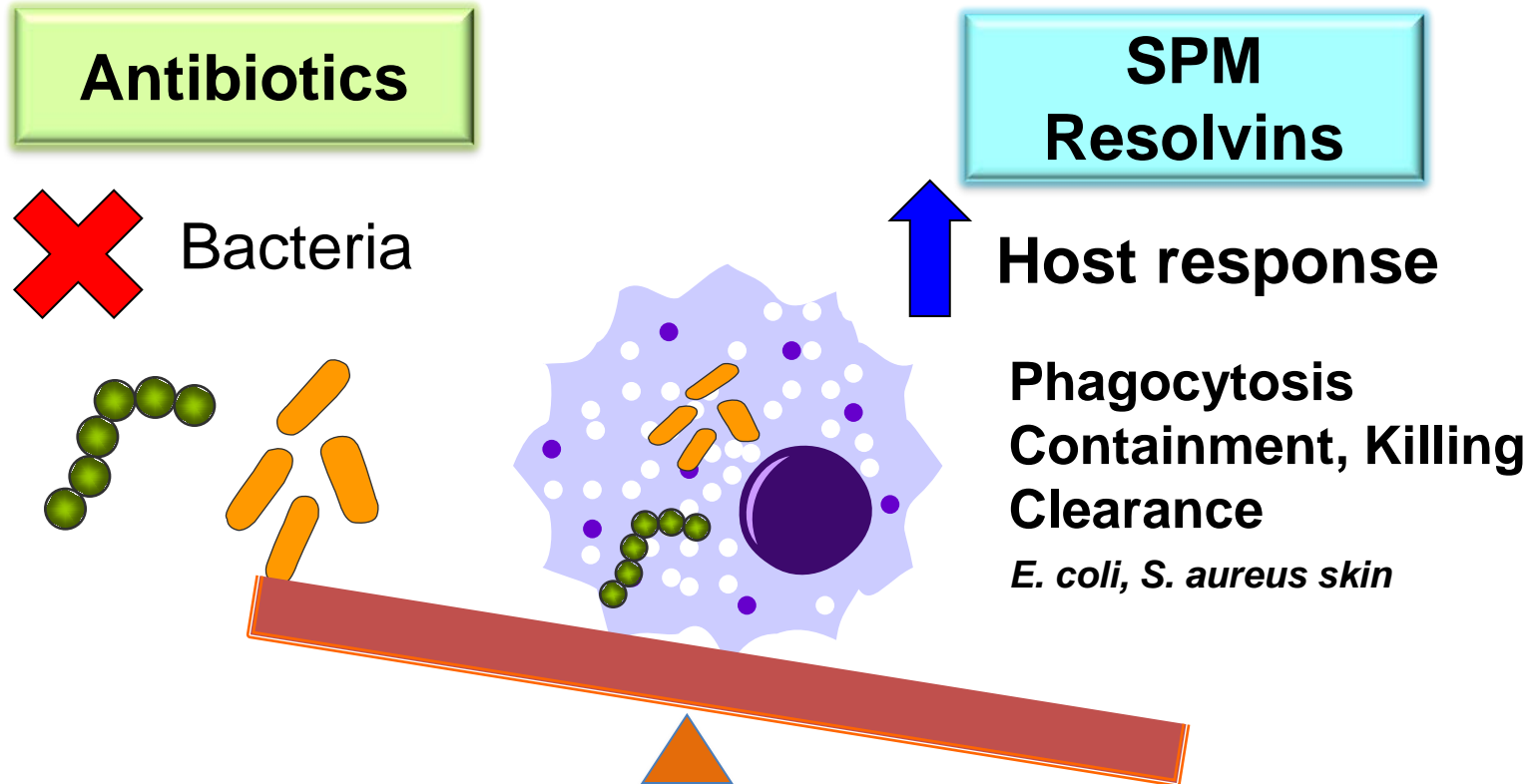
Resolvins Accelerate resolution of Infections
Enhance bacterial killing , reduce inflammation
Treating the Host SPM *Lowers* the required antibiotic doses

Chiang N et al. *Nature.* 2012;484:524-529.

Pro-Resolving Mediators: Towards Human Translation
Focus on Structure Function

Resolvins & Protectins

Structure elucidation of bioactive products
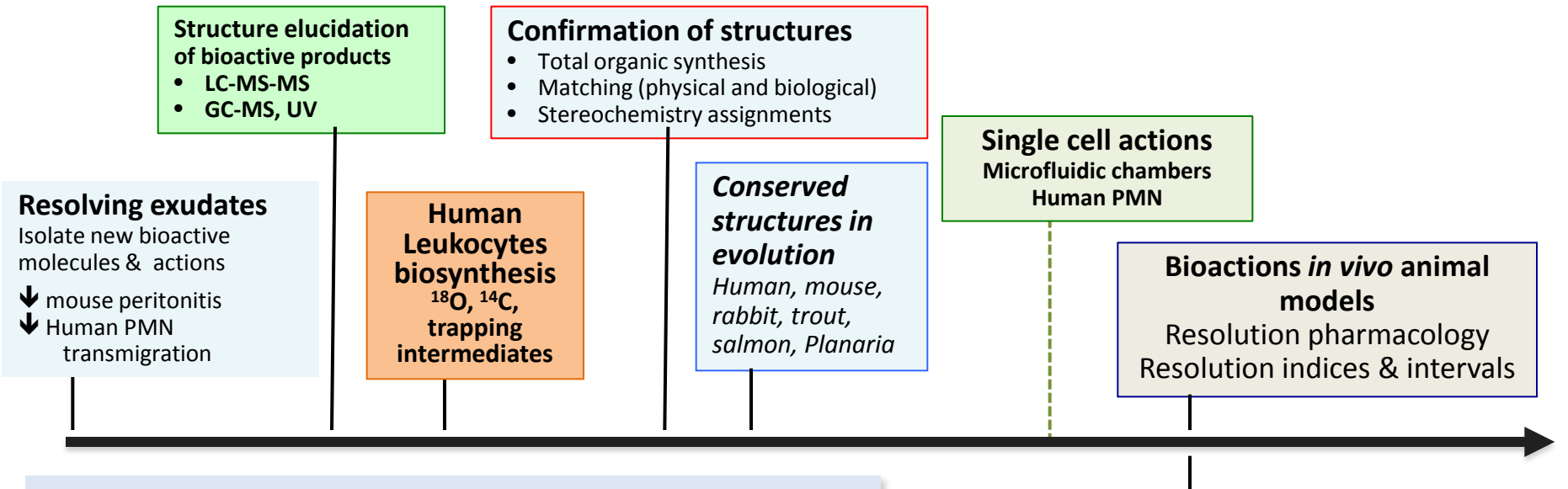- LC-MS-MS
- GC-MS, UV

Confirmation of structures
- Total organic synthesis
- Matching (physical and biological)
- Stereochemistry assignments

Single cell actions
Microfluidic chambers
Human PMN

Resolving exudates
Isolate new bioactive molecules & actions
↓ mouse peritonitis
↓ Human PMN transmigration

Human Leukocytes biosynthesis
^{18}O, ^{14}C, trapping intermediates

Conserved structures in evolution
Human, mouse, rabbit, trout, salmon, Planaria

Bioactions *in vivo* animal models
Resolution pharmacology
Resolution indices & intervals
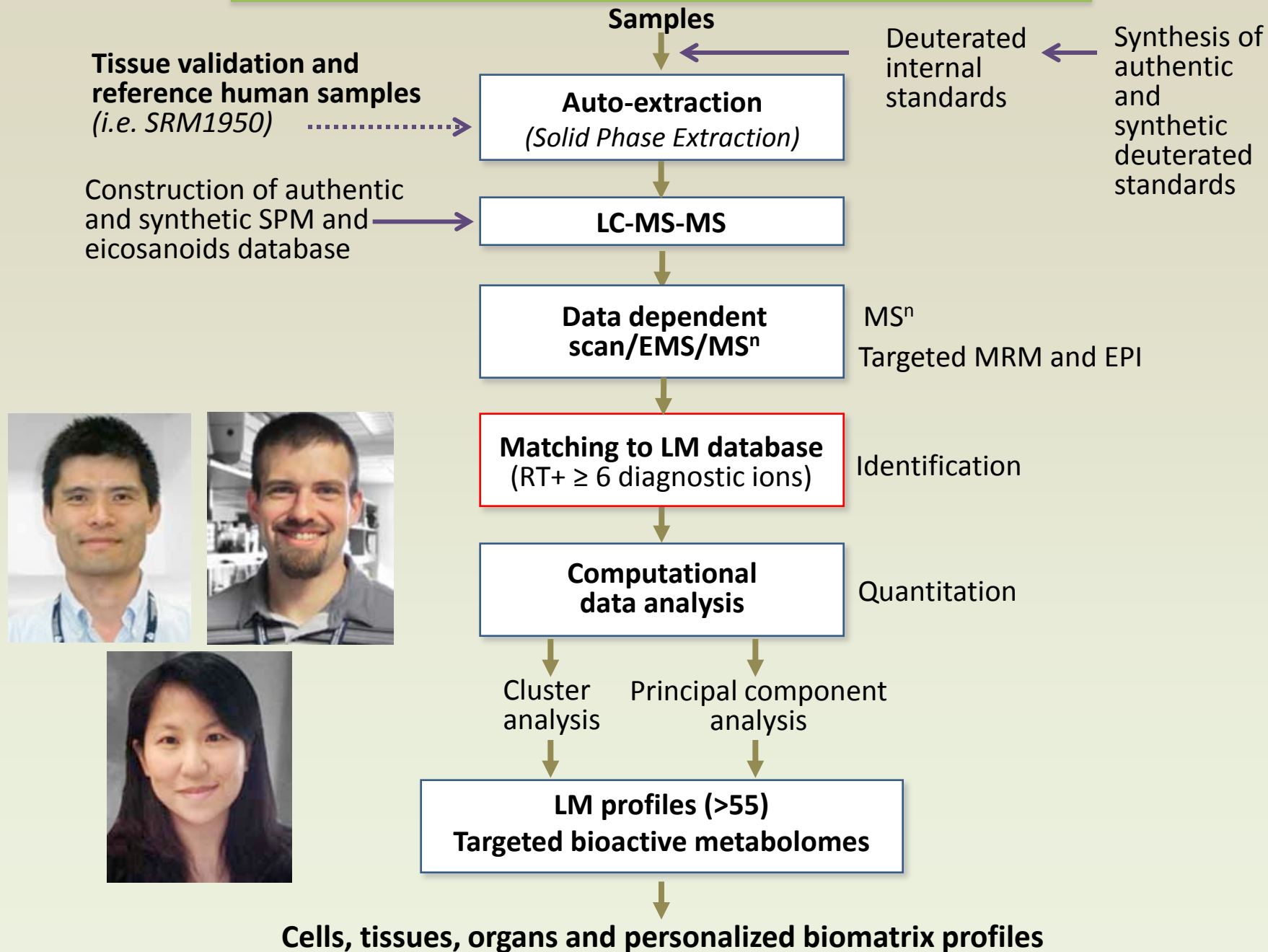
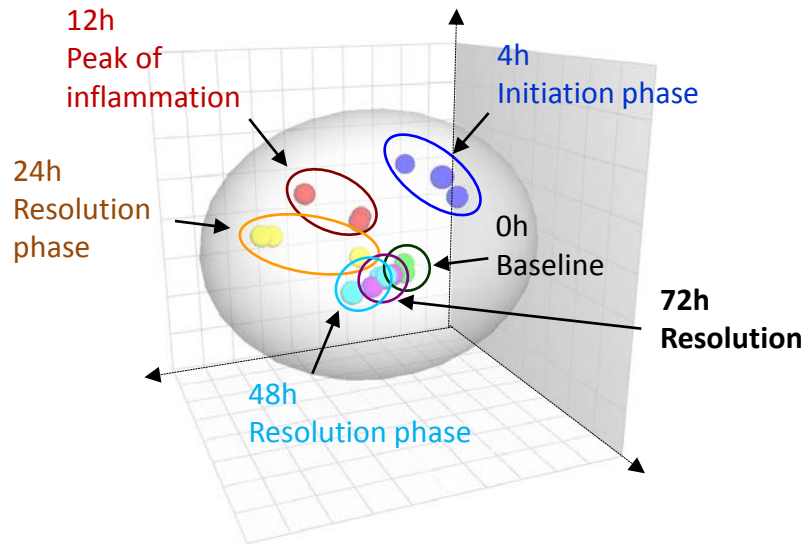## *Criteria for pro-resolving mediator functions:*

- *Produced in vivo at levels commensurate with actions*
- *Reduces PMN chemotaxis and infiltration in vivo*
- *Enhances macrophage phagocytosis & efferocytosis*
- *Accelerates resolution (shorten resolution interval)*
- *Reduces pro-inflammatory cytokines (TNFa, IL-1b) and lipid mediators (e.g. PAF, PGs, LTs)*
- *Increases anti-inflammatory mediators (e.g. IL-10 , LXA$_4$)*

Identification in Human Tissues
Plasma, synovial fluids, Asthmatic breath condensates

LC-MS-MS profiling

Serhan CN. *Nature.* 2014;510:92-101.

Operationalized Lipid Mediator Profiling Platform

Samples

Synthesis of authentic and synthetic deuterated standards

Deuterated internal standards

Tissue validation and reference human samples *(i.e. SRM1950)* ·····>

Auto-extraction *(Solid Phase Extraction)*

Construction of authentic and synthetic SPM and eicosanoids database →

LC-MS-MS

Data dependent scan/EMS/MSⁿ — MS^n / Targeted MRM and EPI

Matching to LM database (RT+ ≥ 6 diagnostic ions) — Identification

Computational data analysis — Quantitation

Cluster analysis

Principal component analysis

LM profiles (>55) Targeted bioactive metabolomes

Cells, tissues, organs and personalized biomatrix profiles

Time Dependent LM SPM Clusters in Self-Resolving Mouse Peritonitis

3D Score Plot

12h Peak of inflammation

4h Initiation phase

24h Resolution phase

0h Baseline

72h Resolution

48h Resolution phase

3D Loading Plot

PGE$_2$ PGD$_2$

RvD5

LTB$_4$

RvD1 RvD2

RvD3

2D Score Plot

24h Resolution phase

48h Resolution phase

12h Peak of inflammation

72h Resolution phase

0 h

4h Initiation phase
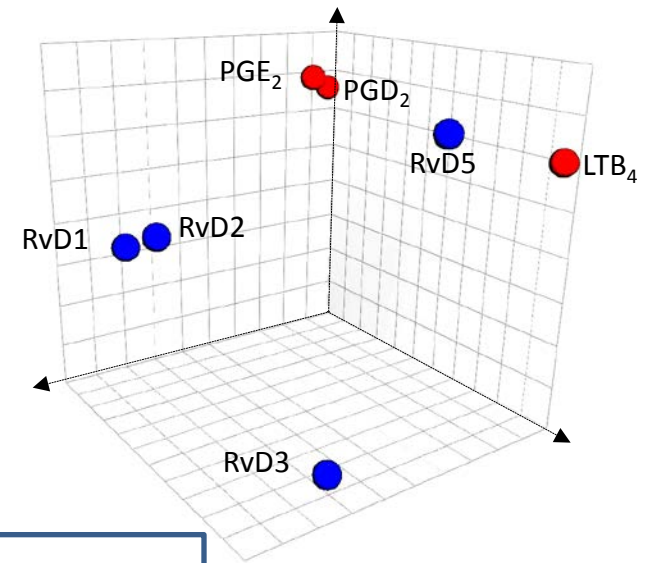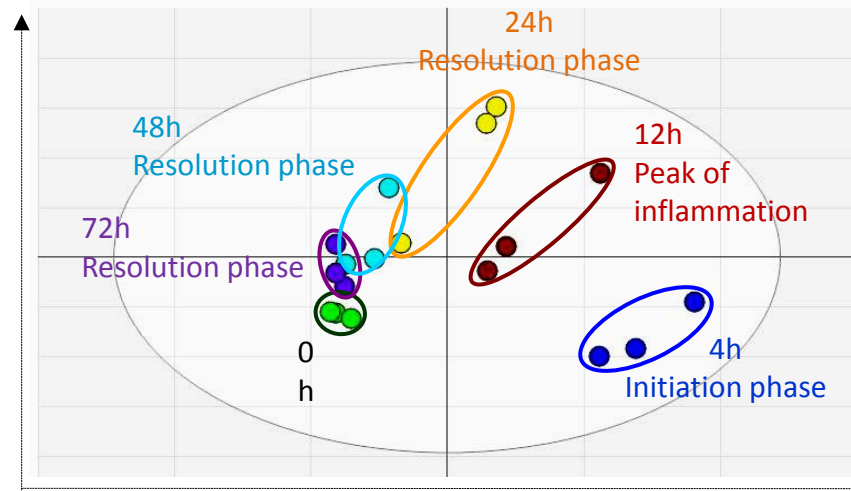
Zymosan mouse peritonitis: 1mg/ml i.p. n=3 in each cluster

Targeted LC-MS-MS lipidomics
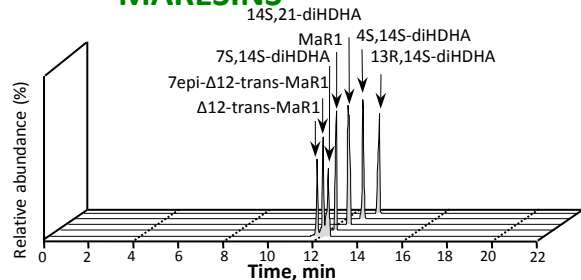
Colas and Shinohara *submitted* Am J Physiol Cell Physiol.

Endogenous LM-SPM in human peripheral blood : Serum

DHA bioactive metabolome

RESOLVINS
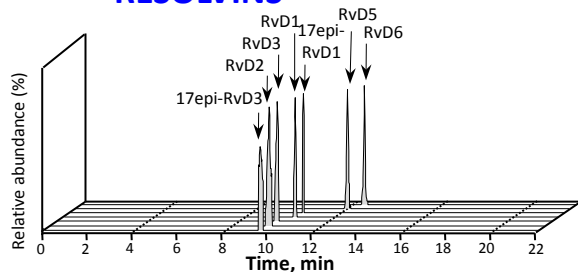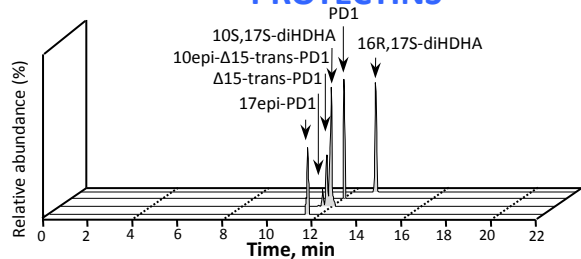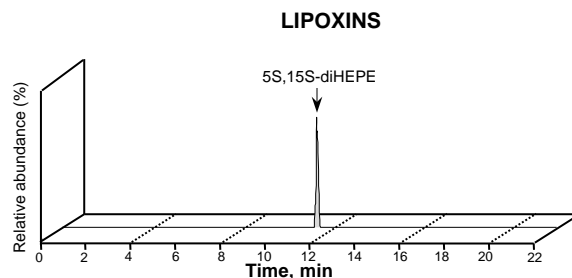
Labels: 17epi-RvD3, RvD2, RvD3, RvD1, 17epi-RvD1, RvD5, RvD6

MARESINS

Labels: 14S,21-diHDHA, 7S,14S-diHDHA, MaR1, 4S,14S-diHDHA, 13R,14S-diHDHA, 7epi-Δ12-trans-MaR1, Δ12-trans-MaR1

PROTECTINS

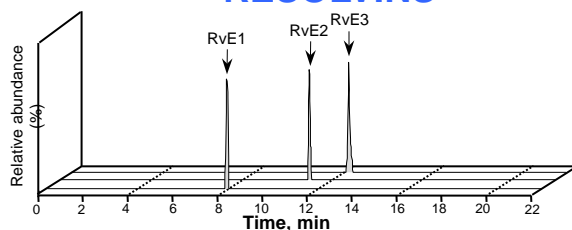Labels: 10S,17S-diHDHA, 10epi-Δ15-trans-PD1, Δ15-trans-PD1, 17epi-PD1, PD1, 16R,17S-diHDHA

EPA bioactive metabolome

LIPOXINS

Labels: 5S,15S-diHEPE

RESOLVINS

Labels: RvE1, RvE2, RvE3

>100 healthy donors

AA bioactive metabolome

LIPOXINS

Labels: 15epi-LXA$_4$, LXA$_4$, 15epi-LXB$_4$, LXB$_4$, 5S,15S-diHETE

LEUKOTRIENES

Labels: Δ6-trans-LTB$_4$, 20-OH-LTB$_4$, 12epi-Δ6-trans-LTB$_4$, 5S,12S-diHETE, 5S,6R-diHETE

Labels: TxB$_2$, PGD$_2$, PGE$_2$, PGF$_{2\alpha}$
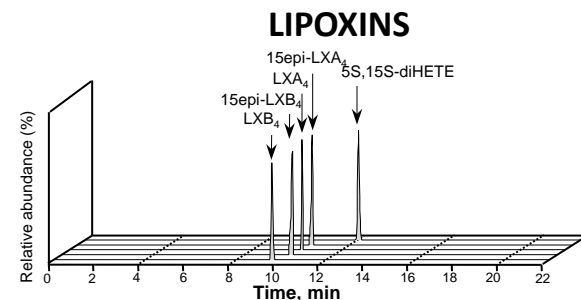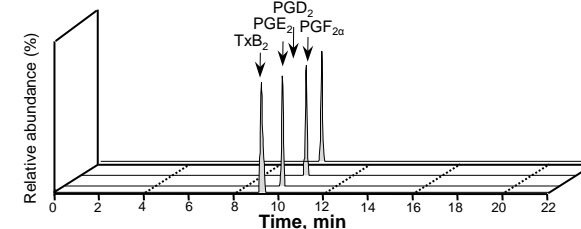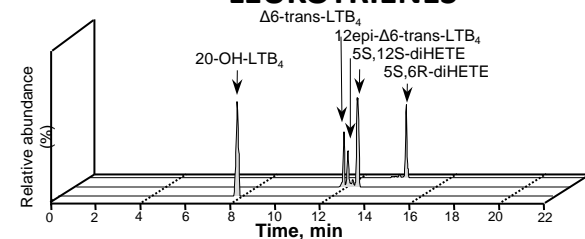
PROSTANOIDS

Multiple reaction monitoring (MRM) of signature ion pairse obtained using the precursor ion (Q1) and a characteristic product ion (Q3) for each lipid mediator (LM). Bioactive LM, isomers and pathway markers identified in human serum .

Representative of 3 different pooled sera, each from >100 individual healthy donor (USA demographics)

Human peripheral blood lipid mediators: NIST Plasma & Commercial Serum each 100 healthy Individuals pg/ml

AA bioactive metabolome	Commercial human serum		NIST human plasma reference material (SRM 1950)
LXA$_4$	115.6	± 45.5	*
15epi-LXA$_4$	59.2	± 49.6	*
LXB$_4$	48.7	± 25.2	*
15epi-LXB$_4$	106.6	± 67.0	*
5S,15S-diHETE	789.4	± 253.4	13.3 ± 1.1
LTB$_4$	-		3.4 ± 0.2
Δ6-trans-LTB$_4$	744.0	± 119.9	1.7 ± 0.1
12epi, Δ6-trans-LTB$_4$	662.5	± 193.3	3.8 ± 0.3
5S,12S-diHETE	2162.6	± 515.5	22.9 ± 0.7
20-OH-LTB$_4$	4.6	± 3.0	2.4 ± 0.4
20-COOH-LTB$_4$	-		-
PGD$_2$	271.0	± 57.7	7.0 ± 0.3
PGE$_2$	72.5	± 10.9	4.1 ± 0.2
PGF$_{2\alpha}$	73.9	± 23.2	4.8 ± 0.4
TxB$_2$	1061.0	± 1036.3	-

Bioactive Levels pg/ml (20-200pM)

Human pooled **serum (each pooled serum was a composite > 100 healthy individuals)** compared to human pooled plasma from NIST standard **reference (SRM 1950) (composite plasma 100 healthy individuals)**. Samples were extracted and lipid mediators (LM) investigated by LC-MS-MS-metabololipidomics. Results are expressed as pg/ml; mean ± SEM; n=3 of pooled commercial human serum , d=3 for SRM 1950; %RSD, relative standard deviation; %RSD = (SEM/mean) x100; *, below IS limits.

Human peripheral blood lipid mediators :
NIST Plasma & Commercial Serum each 100 healthy Individuals pg/ml

DHA bioactive metabolome	Commercial human serum	NIST human plasma reference SRM 1950
RvD1	30.9 ± 7.0	2.6 ± 0.1
17epi-RvD1	40.7 ± 13.9	-
RvD2	42.6 ± 13.9	-
RvD3	34.3 ± 9.4	-
17epi-RvD3	13.3 ± 4.6	-
RvD5	86.8 ± 42.2	1.2 ± 0.3
RvD6	687.0 ± 156.2	58.1 ± 5.2
PD1	5.6 ± 3.4	-
17epi-PD1	7.7 ± 1.4	-
Δ15-trans-PD1	207.9 ± 61.6	-
10epi-Δ15-trans-PD1	223.1 ± 33.1	-
10S,17S-diHDHA	227.4 ± 68.2	-
MaR1	21.2 ± 7.2	-
Δ12trans-MaR1	241.8 ± 64.6	-
7epi,Δ12-trans-MaR1	101.8 ± 42.7	-
7S,14S-diHDHA	131.1 ± 52.3	-
4S,14S-diHDHA	1579.7 ± 282.8	69.6 ± 6.5
14S,21-diHDHA	122.9 ± 2.7	-

EPA bioactive metabolome	Commercial human serum	NIST human plasma reference (SRM 1950)	
RvE1	12.5 ± 2.5	-	
RvE2	2212.6 ± 1587.6	130.6 ± 7.8	%
RvE3	361.8 ± 187.3	-	

Bioactive Levels (20-200pM)
Calibration between laboratories

These mediators have also been identified in:
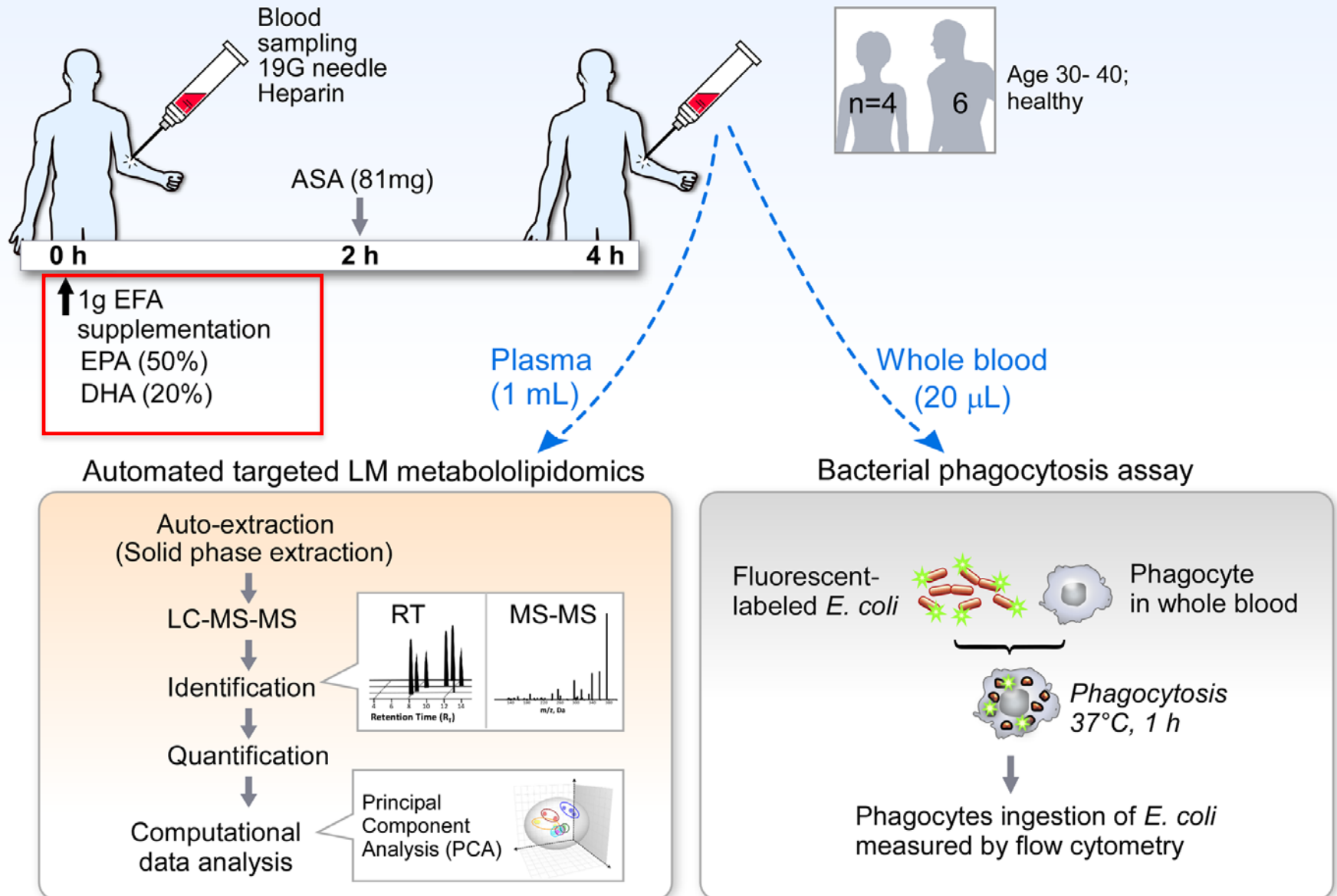**Human Milk** (Weiss et al. *Lipids Health and Dis* 2013.)
Urine (Sasaki et al. *Anal Bioanal Chem* 2015.)
Lymph nodes (Colas et al. *Am J Physiology* 2014.)
Adipose Tissues (Claria et al. *Am J Physiol Cell Physiol* 2013.)

Human pooled **serum (each pooled serum was a composite > 100 healthy individuals) and human pooled plasma from NIST standard reference (SRM1950) (composite plasma 100 healthy individuals)**. Samples were extracted and lipid mediators (LM) investigated. Results expressed as pg/ml; mean ± SEM; n=3 of pooled commercial human serum, d=3, **n> 330 for SRM 1950;** - below IS limits.

Demonstration: Human SPM Production & Assessment of Function

Blood sampling
19G needle
Heparin

ASA (81mg)

0 h   2 h   4 h

1g EFA supplementation
EPA (50%)
DHA (20%)

Age 30- 40; healthy
n=4   6

Plasma (1 mL)

Whole blood (20 μL)

Automated targeted LM metabololipidomics

Auto-extraction (Solid phase extraction)

LC-MS-MS

RT   MS-MS

Retention Time (R$_t$)   m/z, Da

Identification

Quantification

Computational data analysis

Principal Component Analysis (PCA)

Bacterial phagocytosis assay

Fluorescent-labeled E. coli   Phagocyte in whole blood

Phagocytosis 37°C, 1 h

Phagocytes ingestion of E. coli measured by flow cytometry

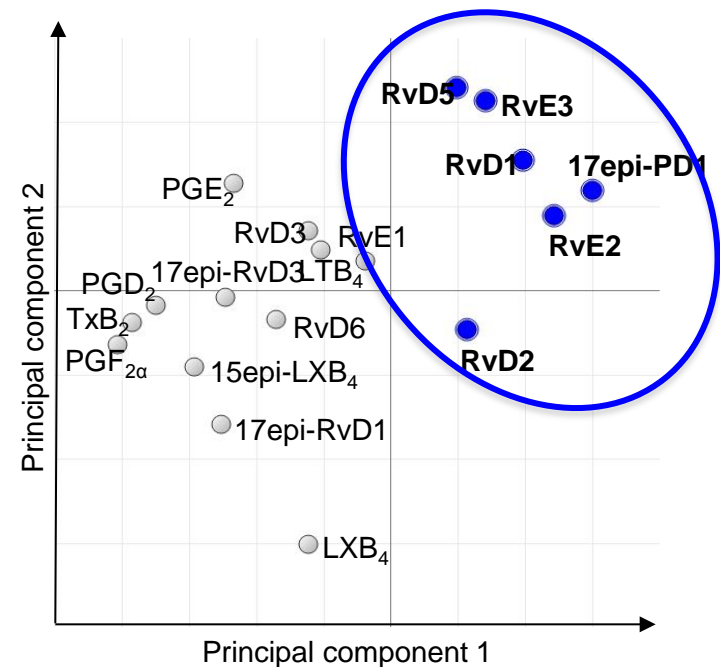Colas RA et al. *Am J Physiol Cell Physiol.* 2014;307:C39-C54.

Human Plasma LM-SPM signature profiles PCA
Partial Least Square-Discrimination Analysis: (PLS-DA)

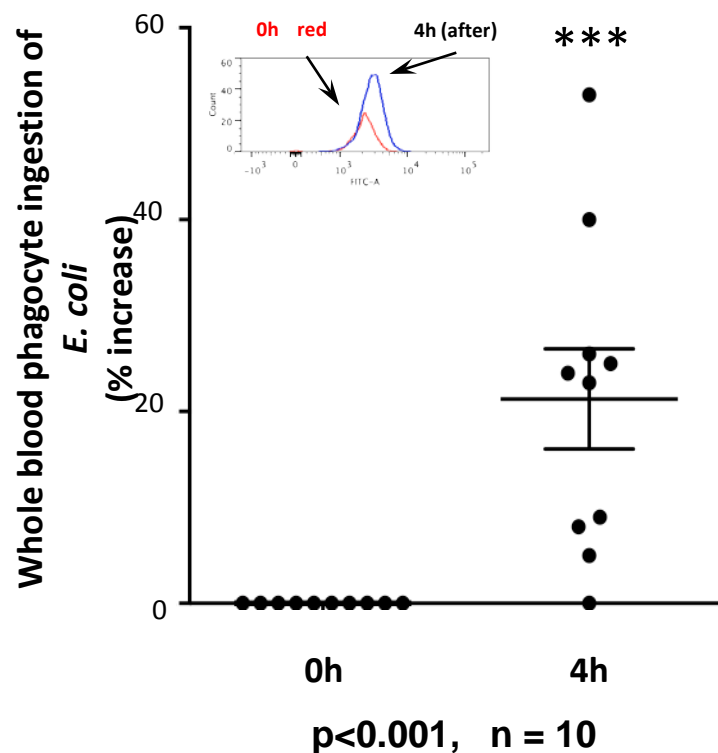Human plasma endogenous lipid mediators 2D Score Plot
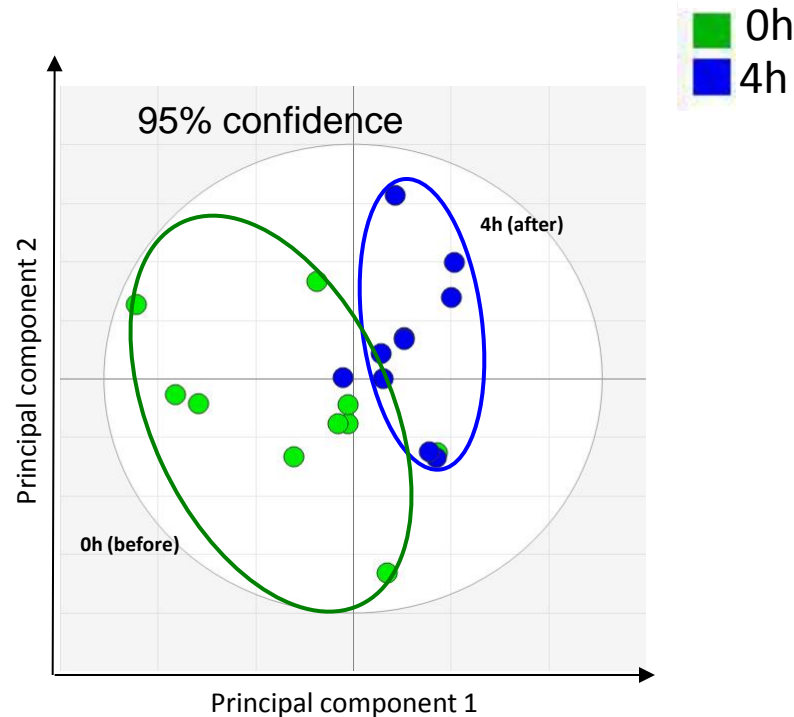
Human plasma endogenous lipid mediators 2D Loading Plot

2D loading plot. Gray ellipse in the score plots denotes 95% confidence regions; n = 10 healthy donors.

Colas RA et al. *Am J Physiol Cell Physiol.* 2014;307:C39-C54.

Human plasma LM-SPM signatures and increase in phagocytosis: PLS-DA

Summation of RvD1, RvD2, RvE2, RvE3 and 17epi-PD1 increases

$R^2=0.77$

p<0.001,   n = 10

Phagocytosis of live *E.coli* by phagocytes in whole blood increased at 4h
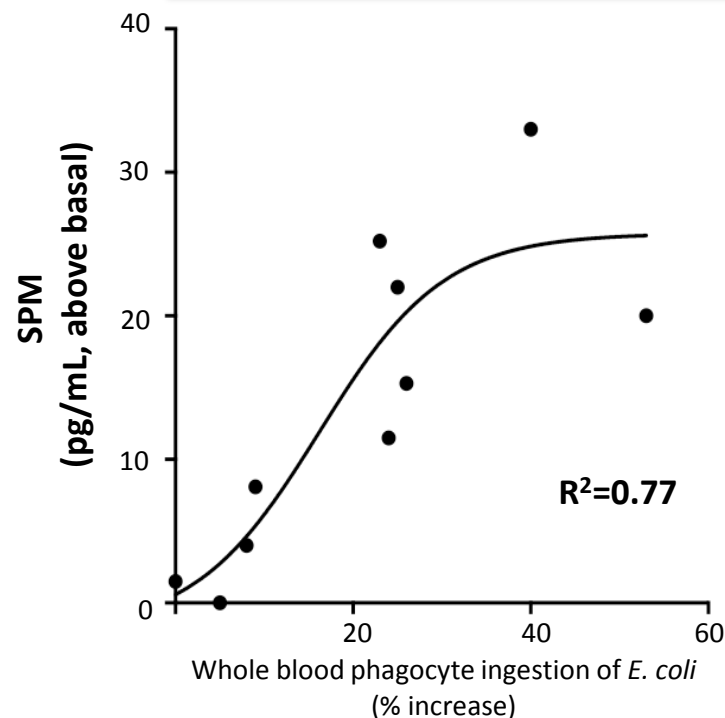
Increased phagocytosis positively correlated with the identified SPM increases

Colas RA et al. *Am J Physiol Cell Physiol.* 2014;307:C39-C54.

N-3 DPA Derived Pro-Resolving Mediators

Proposed Biosynthetic Pathways

n-3 Docosapentaenoic acid

17-hydroperoxy-DPA

16,17-epoxy-DPA

7,17-dihydro(peroxy)-DPA

14-hydroperoxy-DPA

13,14-epoxy-DPA

7,8-epoxy,17-hydroxy-DPA

PD1$_{n-3\ DPA}$

PD2$_{n-3\ DPA}$

RvD1$_{n-3\ DPA}$

RvD2$_{n-3\ DPA}$

RvD5$_{n-3\ DPA}$

MaR1$_{n-3\ DPA}$

MaR2$_{n-3\ DPA}$

MaR3$_{n-3\ DPA}$

Dalli J et al. *Sci Rep.* 2013;3:1940.
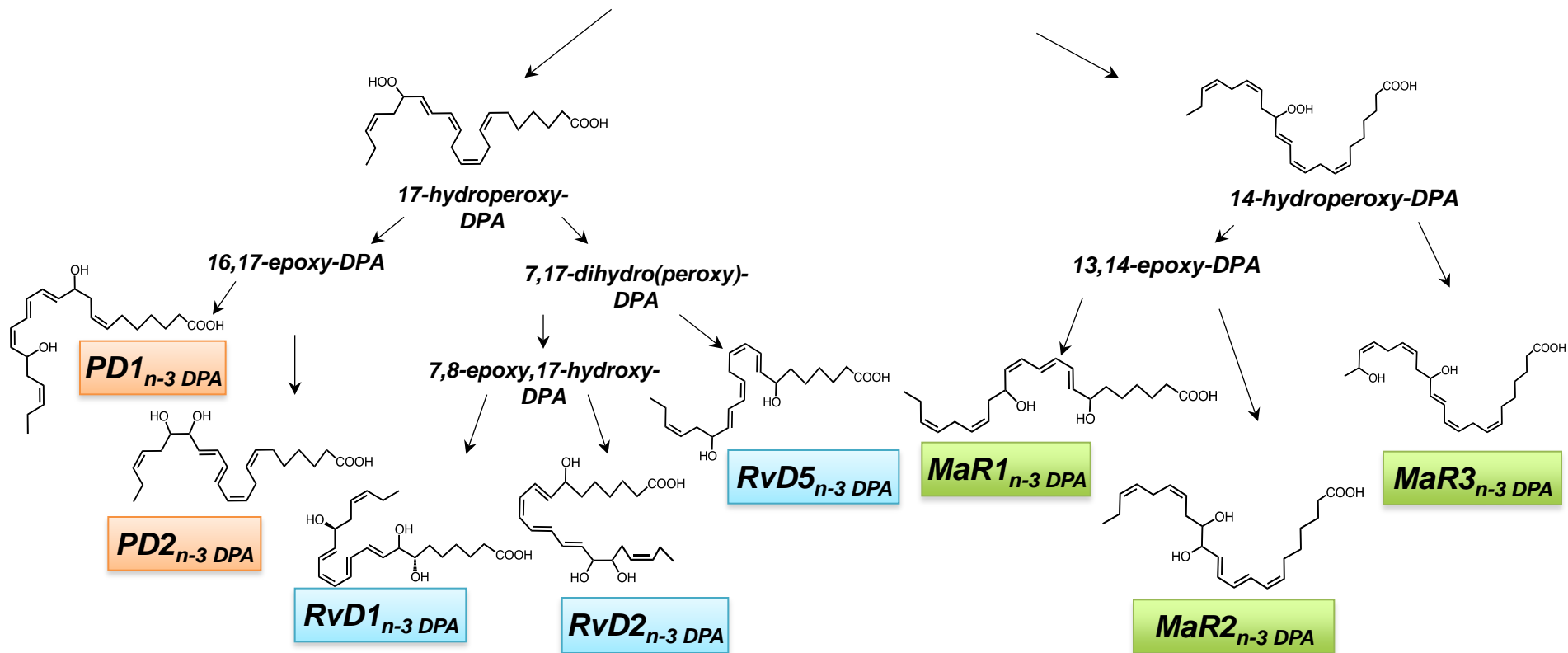
n-3 DPA derived Resolvins , Protectins & Maresins

Reduction in Human Neutrophil Adhesion (% of Veh Control)
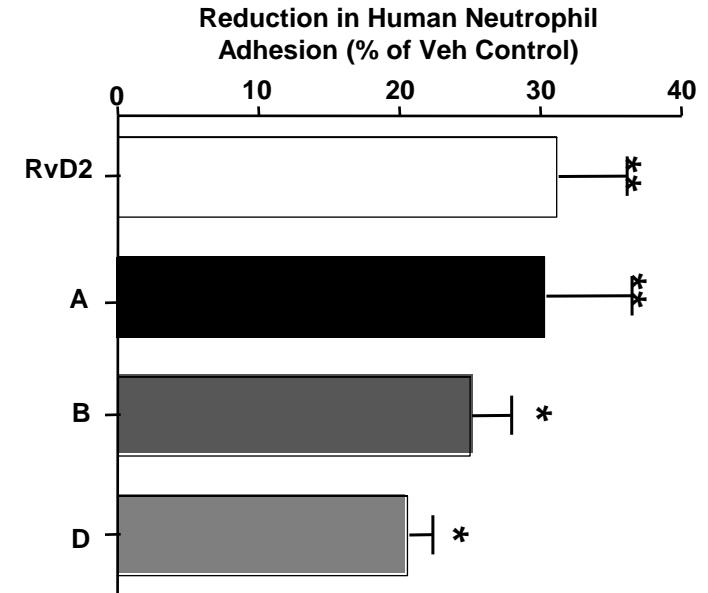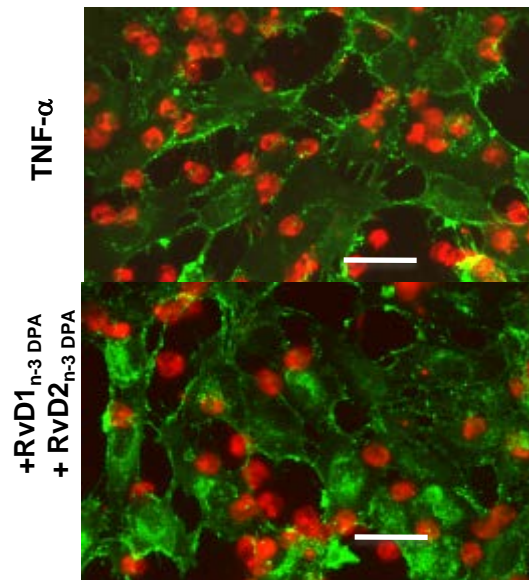
Novel n-3 Immunoresolvents: Structures and Actions
Dalli J, Colas RA, Serhan CN

- **Reduce I/R lung injury**
- **Stop PMN – Endothelial interactions**
- **Enhance Human Macrophage Phagocytosis**

Dalli J et al. *Sci Rep.* 2013;3:1940.

Key Points & Conclusions

- **Resolution is an active process with the biosynthesis of SPM**

- **Anti-inflammation is _not_ equivalent to Pro-Resolution**

- **Identified endogenous SPM bioactive metabolomes with human tissues**
  including **lipoxins , resolvins , protectins and maresins**
  at levels within their bioactive ranges (pg / ml in human plasma and serum) and
  lymphoid organs

- **Human Demonstration LM-SPM signatures : impact of omega-3 and aspirin**
  specific SPM increases correlated with enhanced phagocytosis of *E. coli* in human blood
  Functional SPM -Profliling

  - ✓ **Treatment of dry eye in humans:> 260 individuals RX-10045**
    Proof of concept for the broad clinical utility of resolvins
    as novel therapeutics

SPM and their receptors provide new opportunities for the control of unwanted inflammation & infection via Resolution Pharmacology
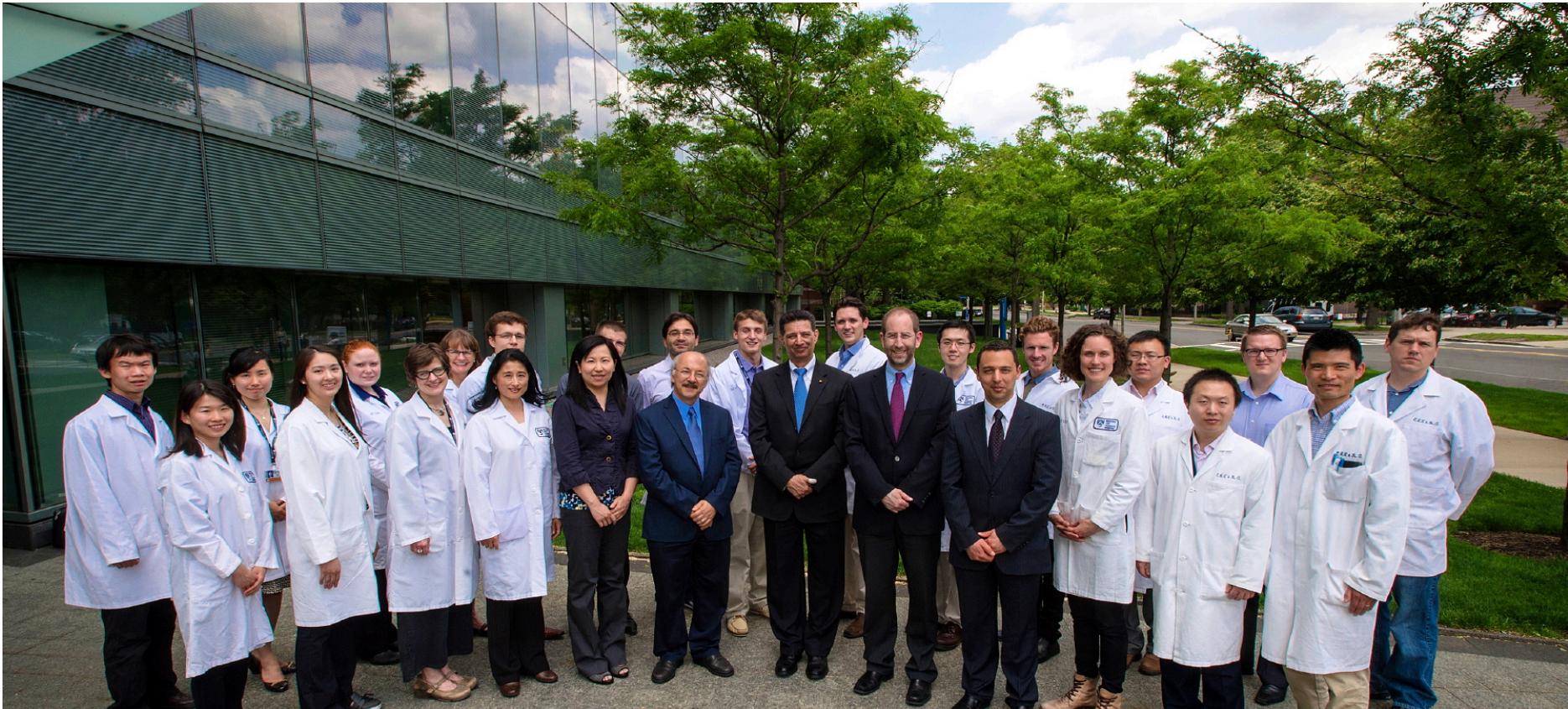
Specialized Center for Inflammation - Resolution