FUNCTIONAL MEDICINE
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Inflammation

When the Solution Becomes the Problem

Thomas G. Guilliams Ph.D.

Disclosures

- Dr. Guilliams is affiliated with Ortho Molecular Products. CEImpact has taken appropriate action for conflict resolution, including external peer review.

Objectives

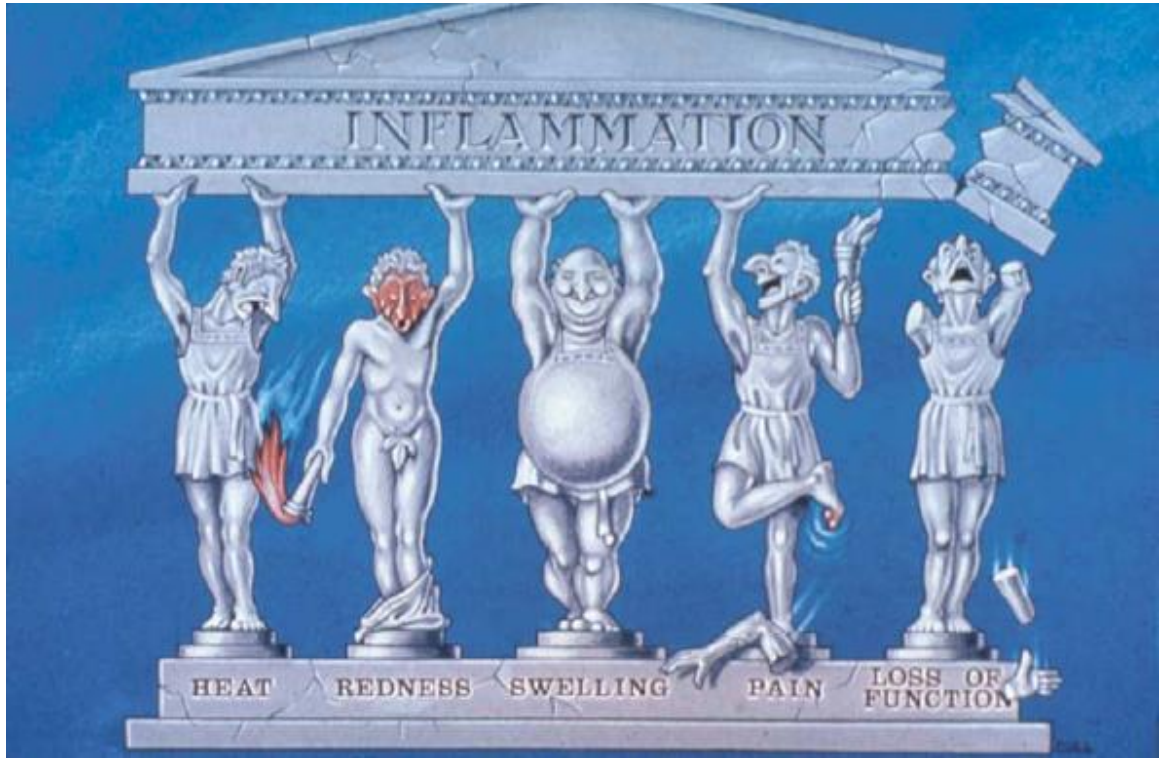
Upon successful completion of this knowledge-based course pharmacists should be able to:

- 1. Describe the pathophysiology of pain and inflammation.
- 2. Review inflammatory pathways and cascades.
- 3. Discuss natural product mechanisms of action for addressing pain and inflammation.

Inflammation is a response of distressed tissues

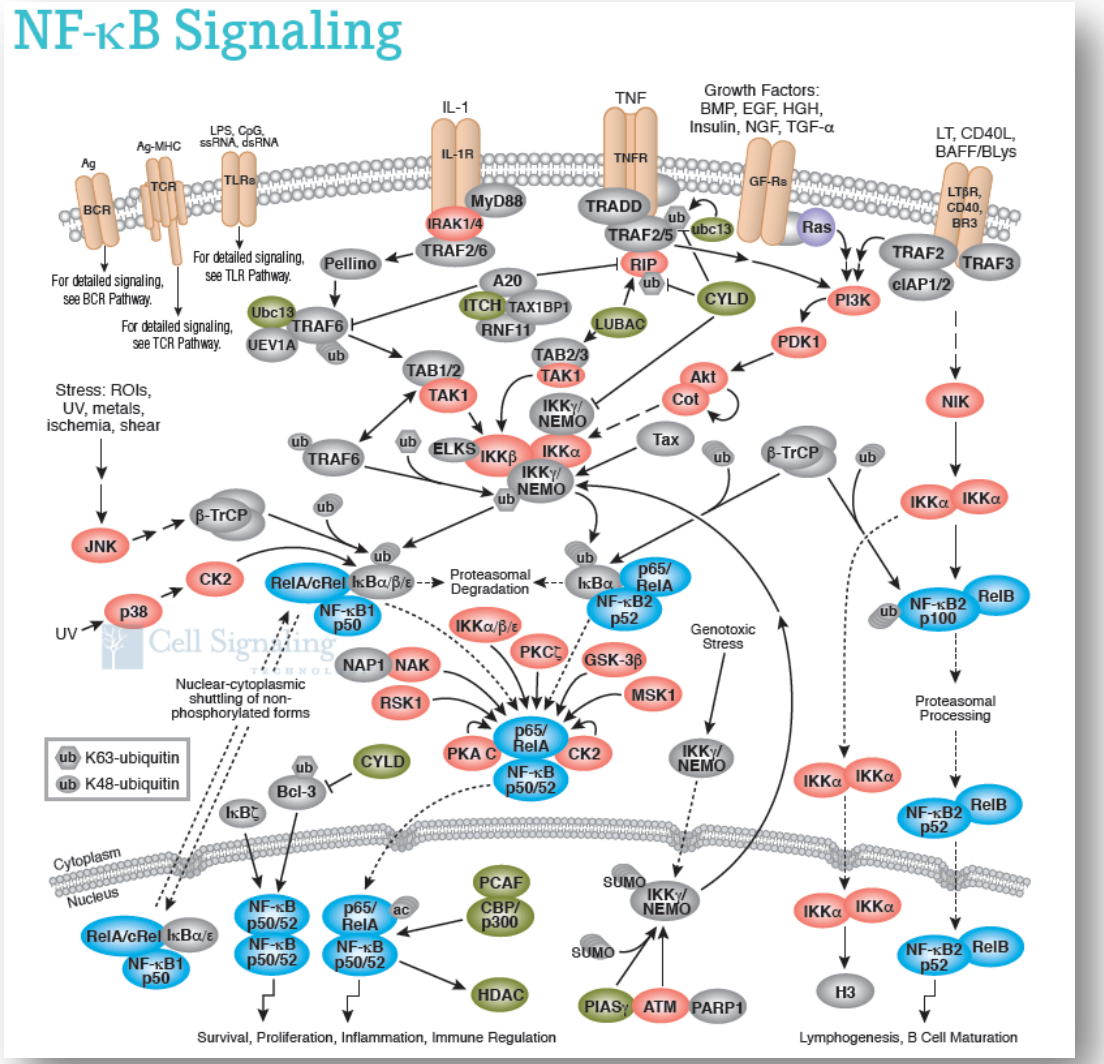
- Nearly every tissue has the ability to summon the immune system to begin a cascade of inflammatory signals
- Most of these signals should be turned off when the “stress” is resolved
- Unresolved stress on the tissues will result in continuous inflammation- damaging tissues- resulting in further inflammatory signals.

The Classical View of Inflammation



- “itis” – inflammatory condition
- appendicitis, bronchitis, prostatitis, arthritis, hepatitis, colitis, rhinitis….

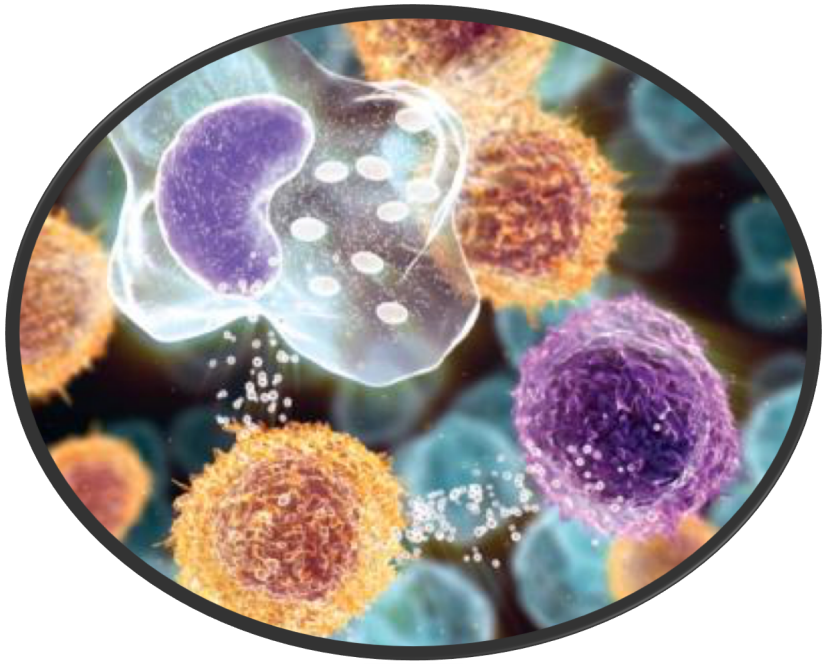
The “Modern” View of Inflammation

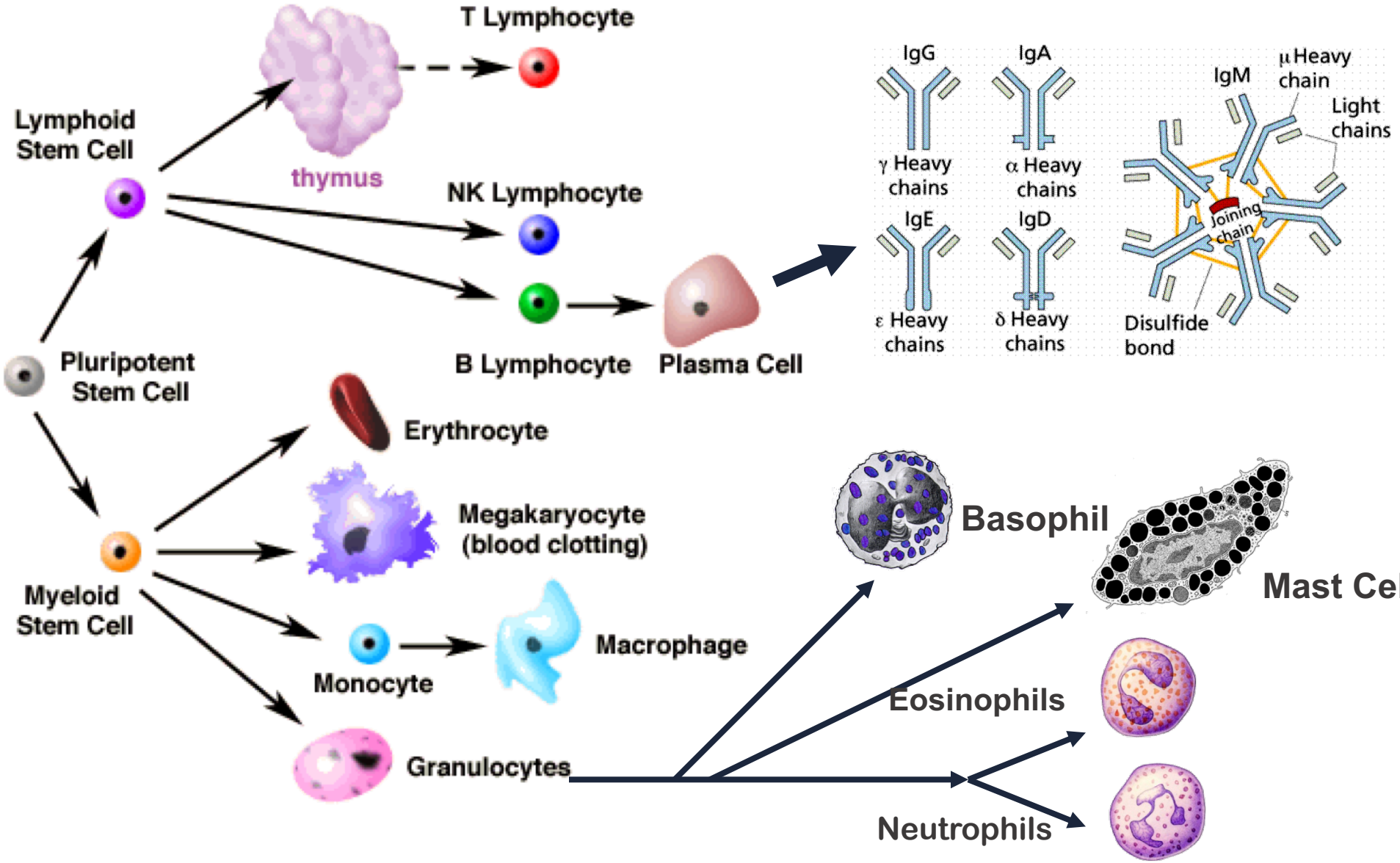


The Emerging Understanding

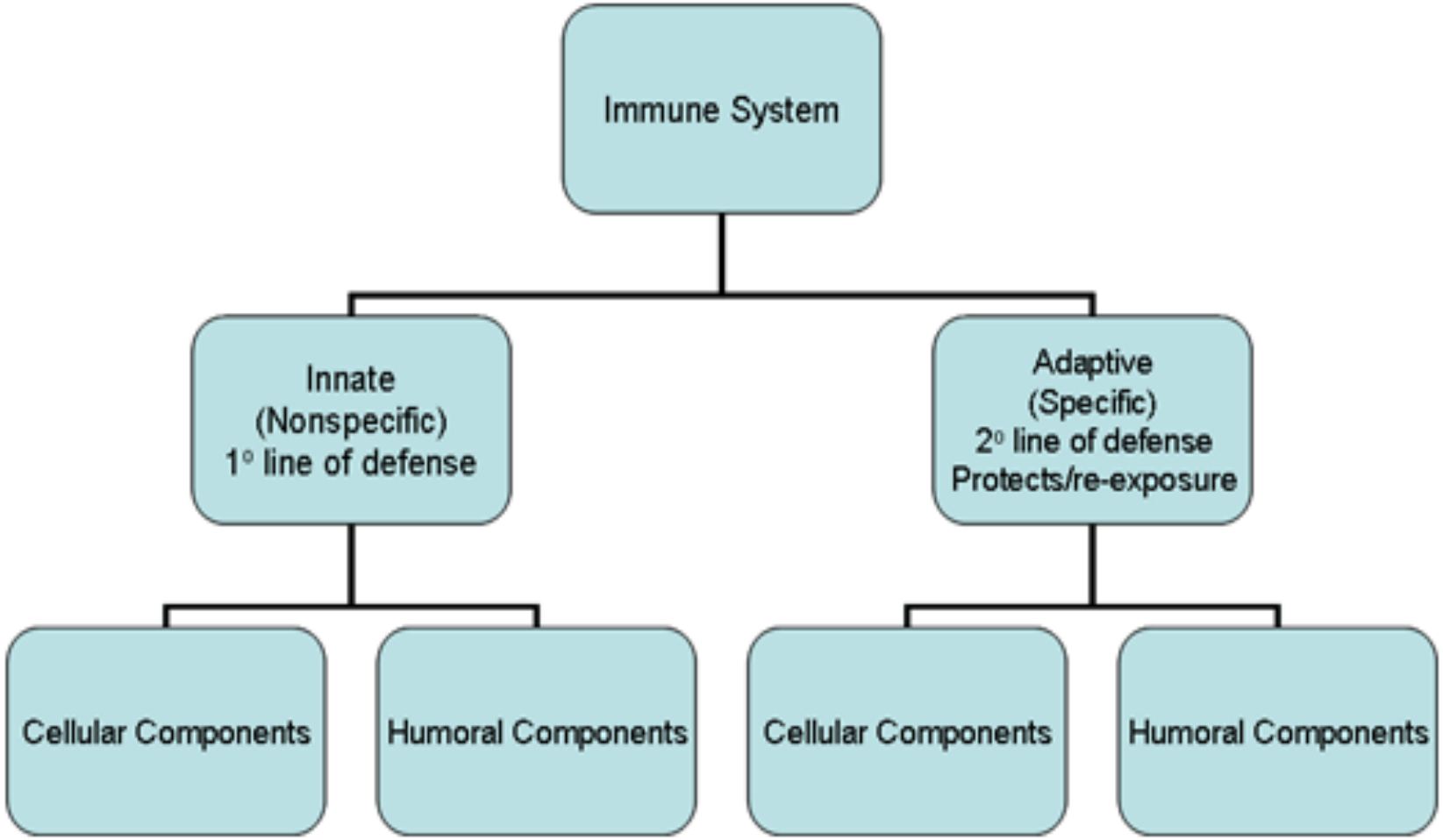
- Inflammation is the necessary and important non-specific alarm reaction of the innate immune system to nearly every potential cellular threat.
- There are many ways to trigger Inflammation
- Inflammatory Signals mediate most Acute and Chronic Disease pathways
- Inflammatory Signals initiate Tissue Repair
- There are Signals for Resolving Inflammation (not just preventing)

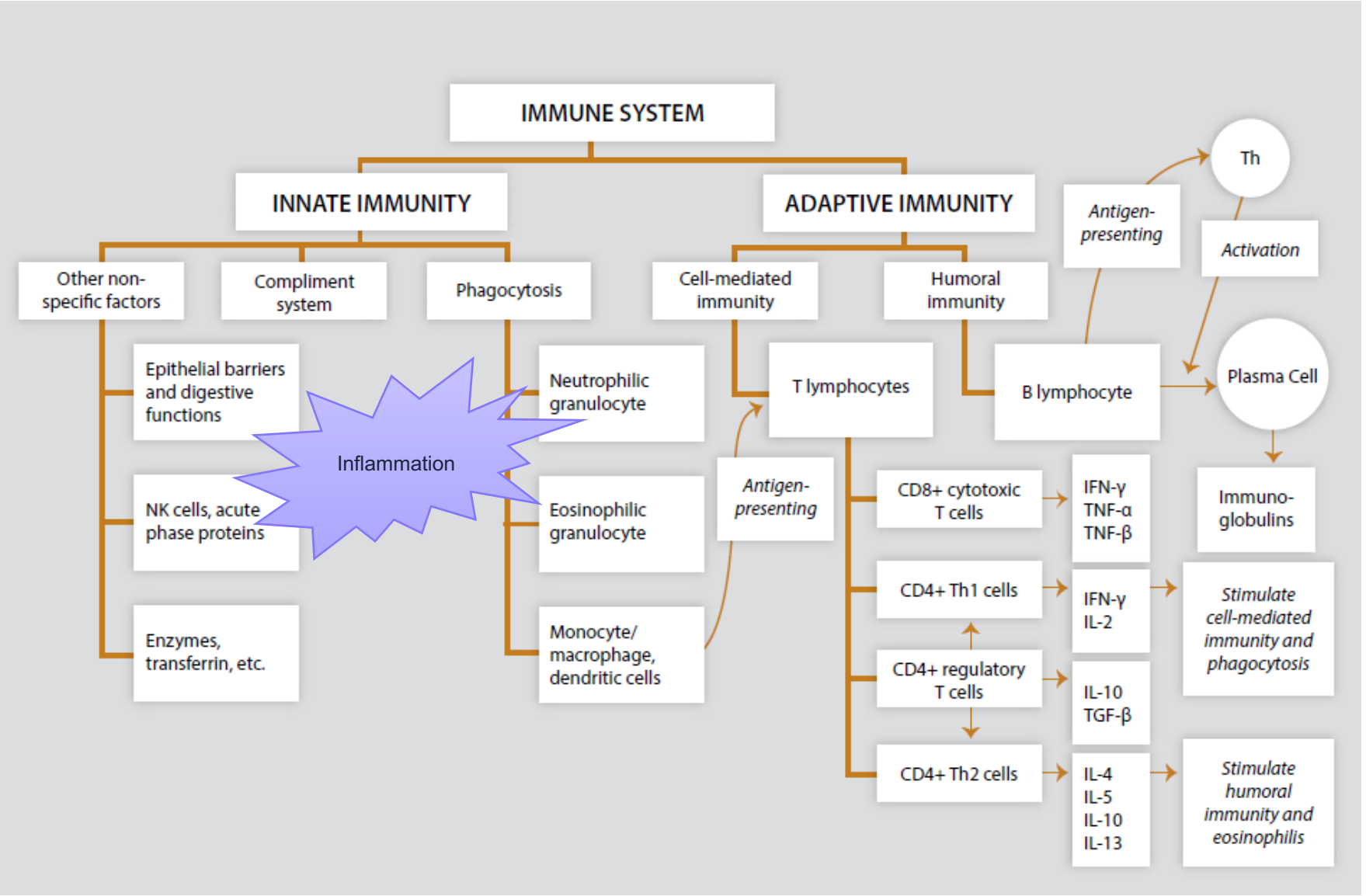
First: A reminder of the basics of the immune system and cells involved.





The Basic Divisions





Simple Dichotomy? The old view

TABLE 3-1 Innate and adaptive immunity

Attribute	Innate immunity	Adaptive immunity
Response time	Minutes/hours	Days
Specificity	Specific for molecules and molecular patterns associated with pathogens	Highly specific; discriminates even minor differences in molecular structure; details of microbial or nonmicrobial structure recognized with high specificity
Diversity	A limited number of germ line–encoded receptors	Highly diverse; a very large number of receptors arising from genetic recombination of receptor genes
Memory responses	None	Persistent memory, with faster response of greater magnitude on subsequent infection
Self/nonself discrimination	Perfect; no microbe-specific patterns in host	Very good; occasional failures of self/nonself discrimination result in autoimmune disease
Soluble components of blood or tissue fluids	Many antimicrobial peptides and proteins	Antibodies
Major cell types	Phagocytes (monocytes, macrophages, neutrophils), natural killer (NK) cells, dendritic cells	T cells, B cells, antigen-presenting cells

?

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Primary Defense: Non-specific

- Physical & Chemical Barriers
  - intact skin (secretions, pH, sweat)
  - mucus
  - stomach acid and enzymes
  - saliva
  - tears
  - nausea/vomiting
  - diarrhea

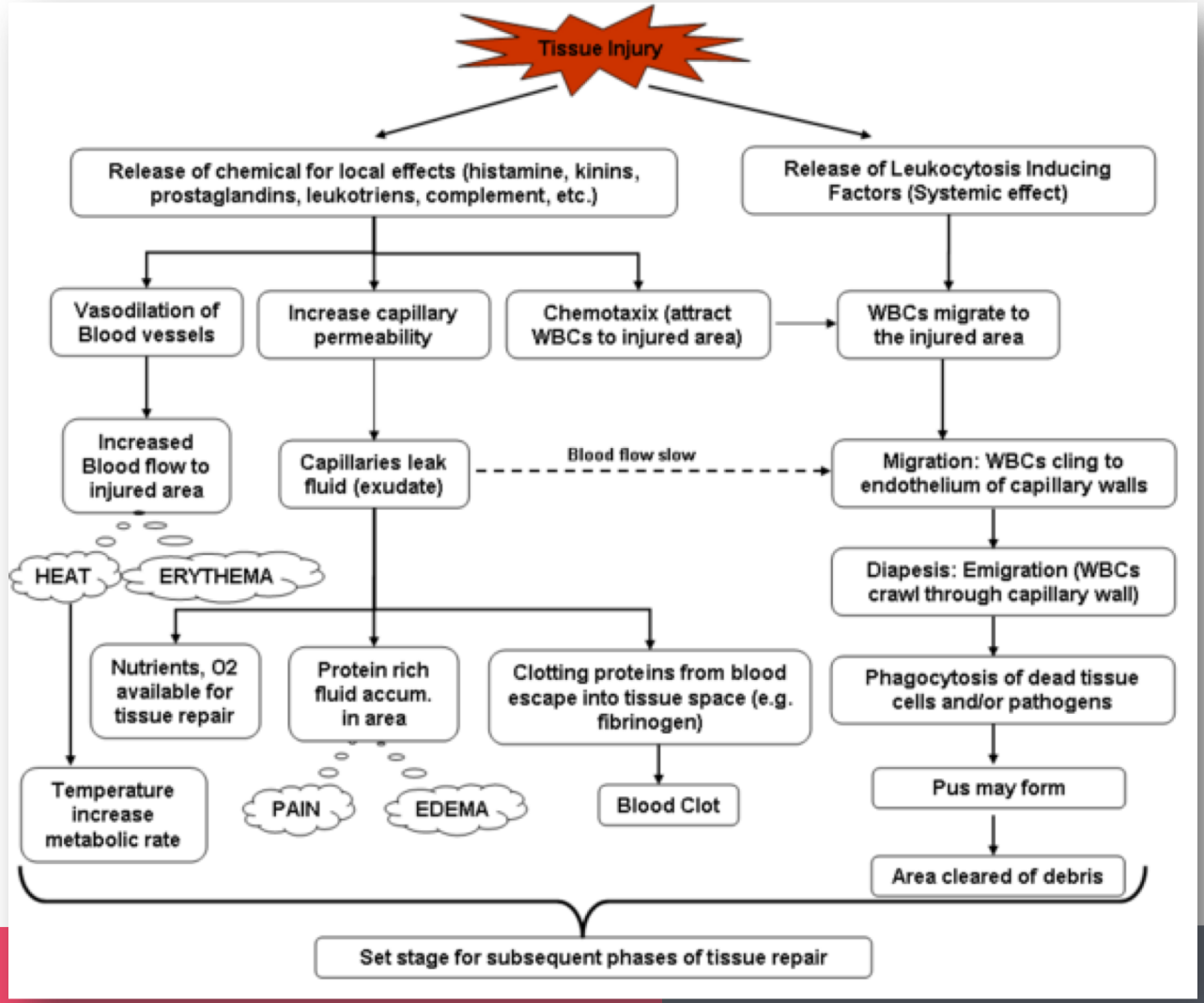
Primary Defense: Inflammation

- Non-specific response to burns, freezing, acid/base injury, impact injury, metabolic stress, environmental toxins, presence of foreign substances in tissue etc.
- The inflammatory response is (mostly) the same in each separate incident and has no (formal) “memory” of the incident for future responses.

Phases of the Injury Response

- Phases of typical Inflammatory Response
  - Isolation and removal of offense
  - Confinement (walling-off)
  - Signal the immune system (cells) for additional response- Inflammation
  - Trigger the healing response/Resolution

Rubor Tumor Calor Dolor



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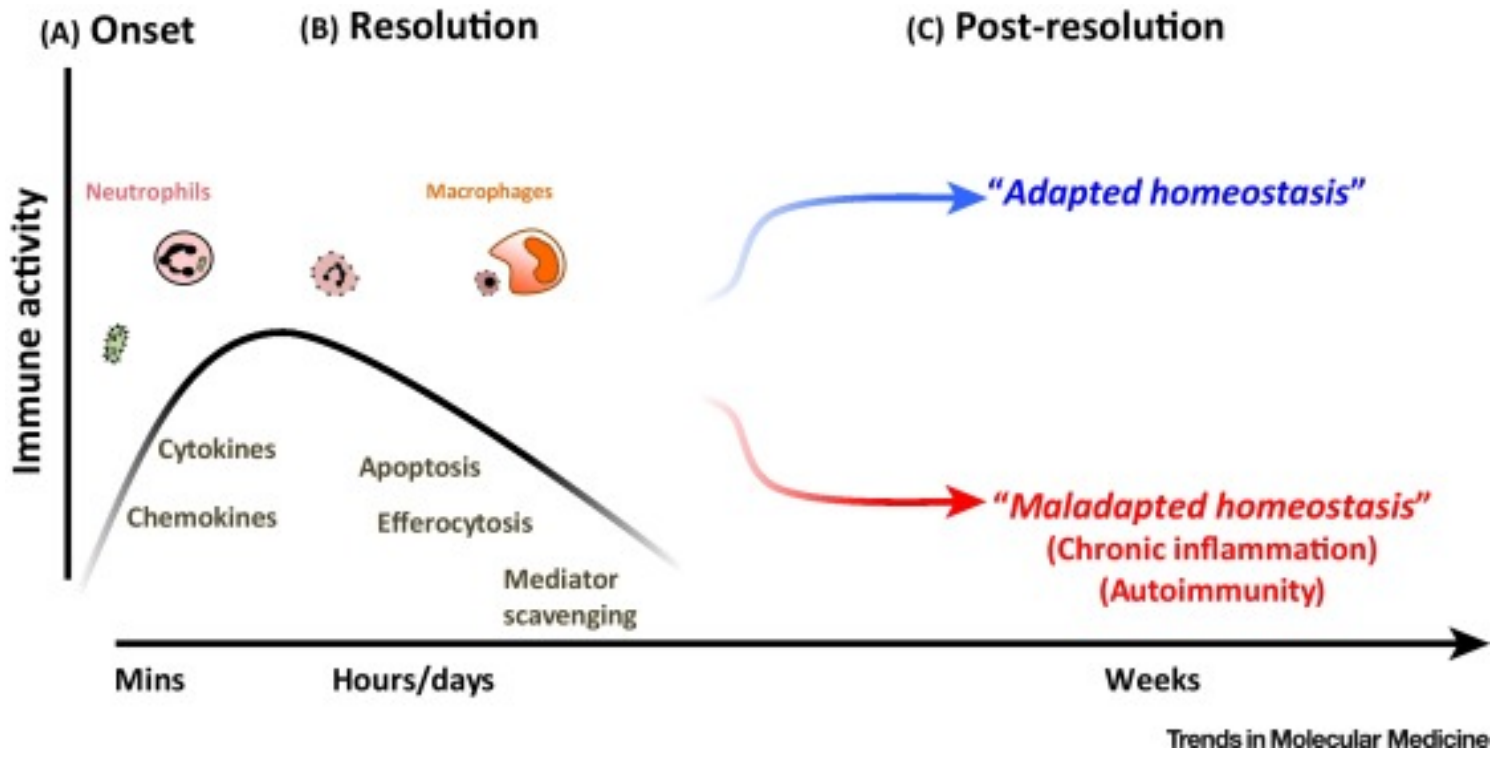
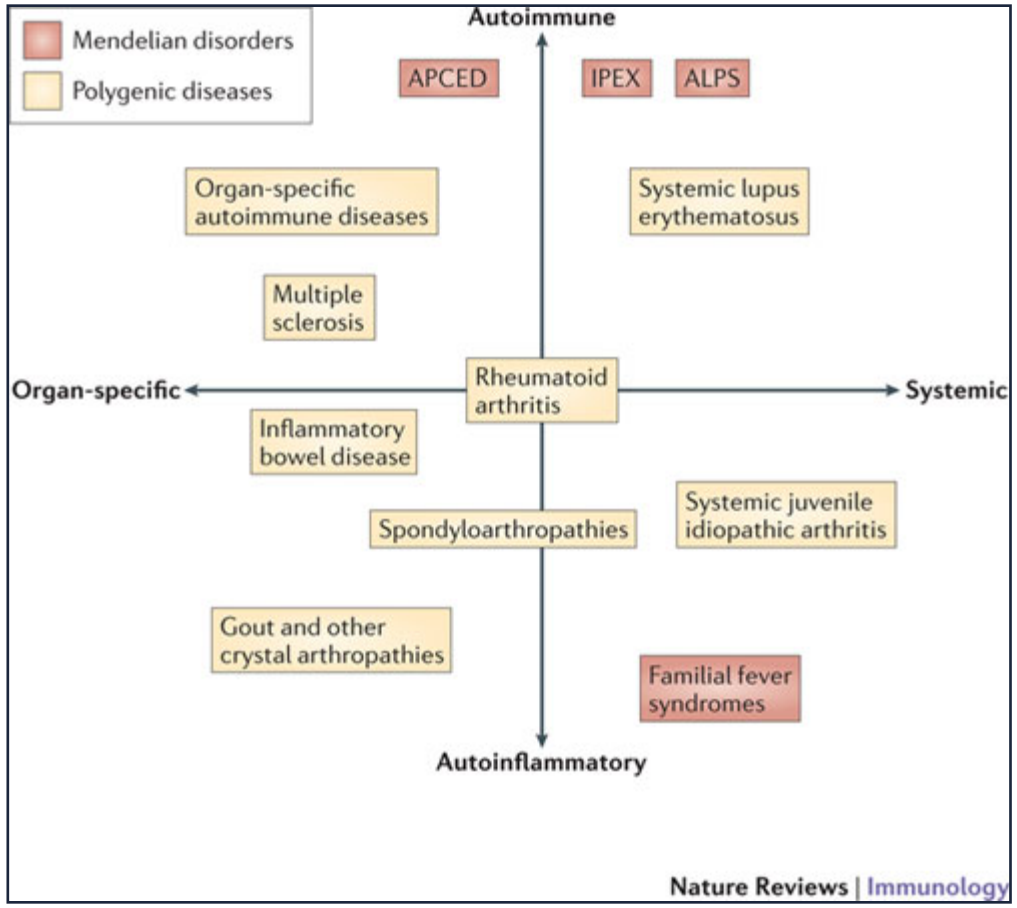
The Stages of Inflammation

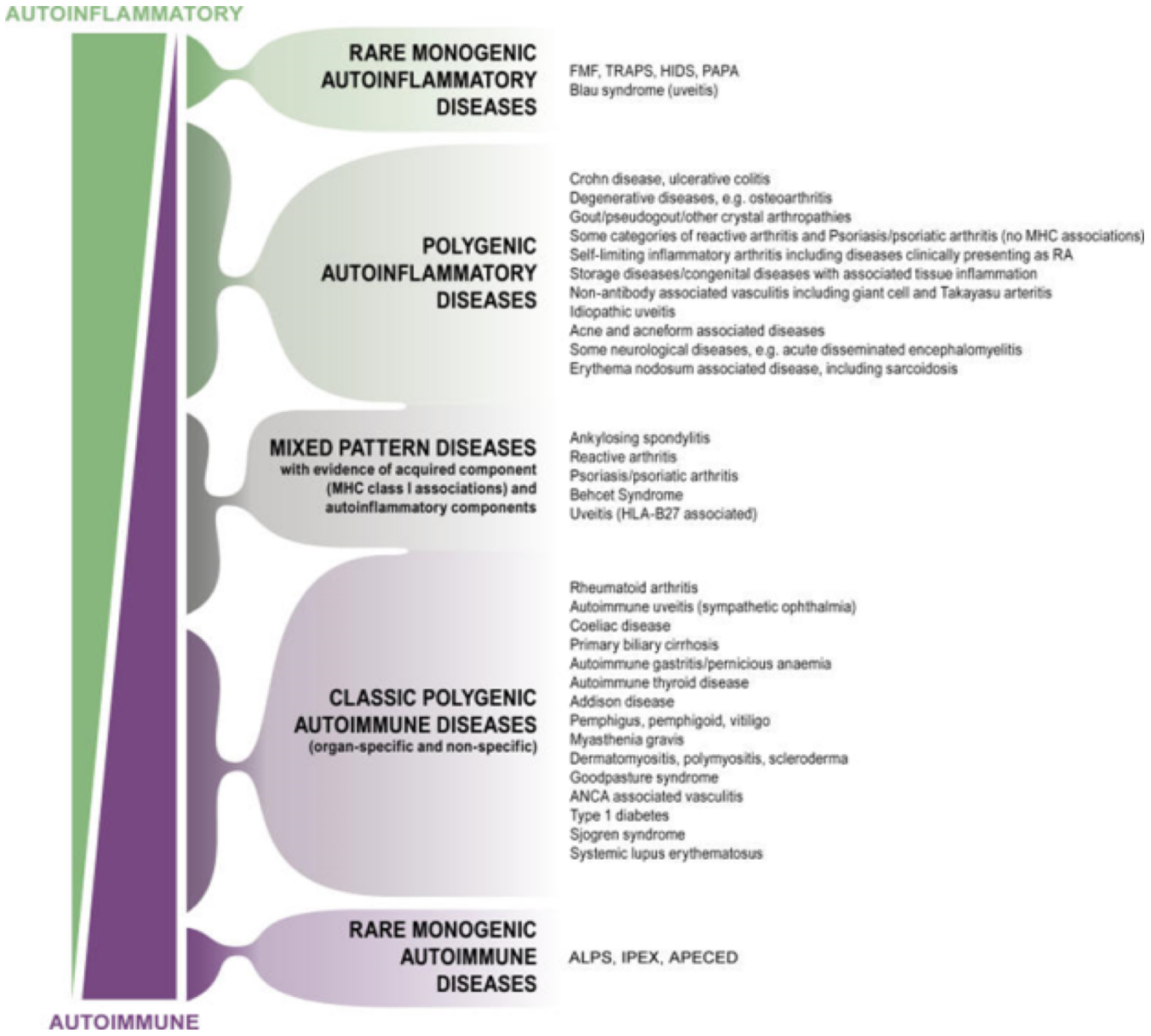


Trends Mol Med. 2019 Mar;25(3):198-214.

Autoimmunity vs. Auto-Inflammatory



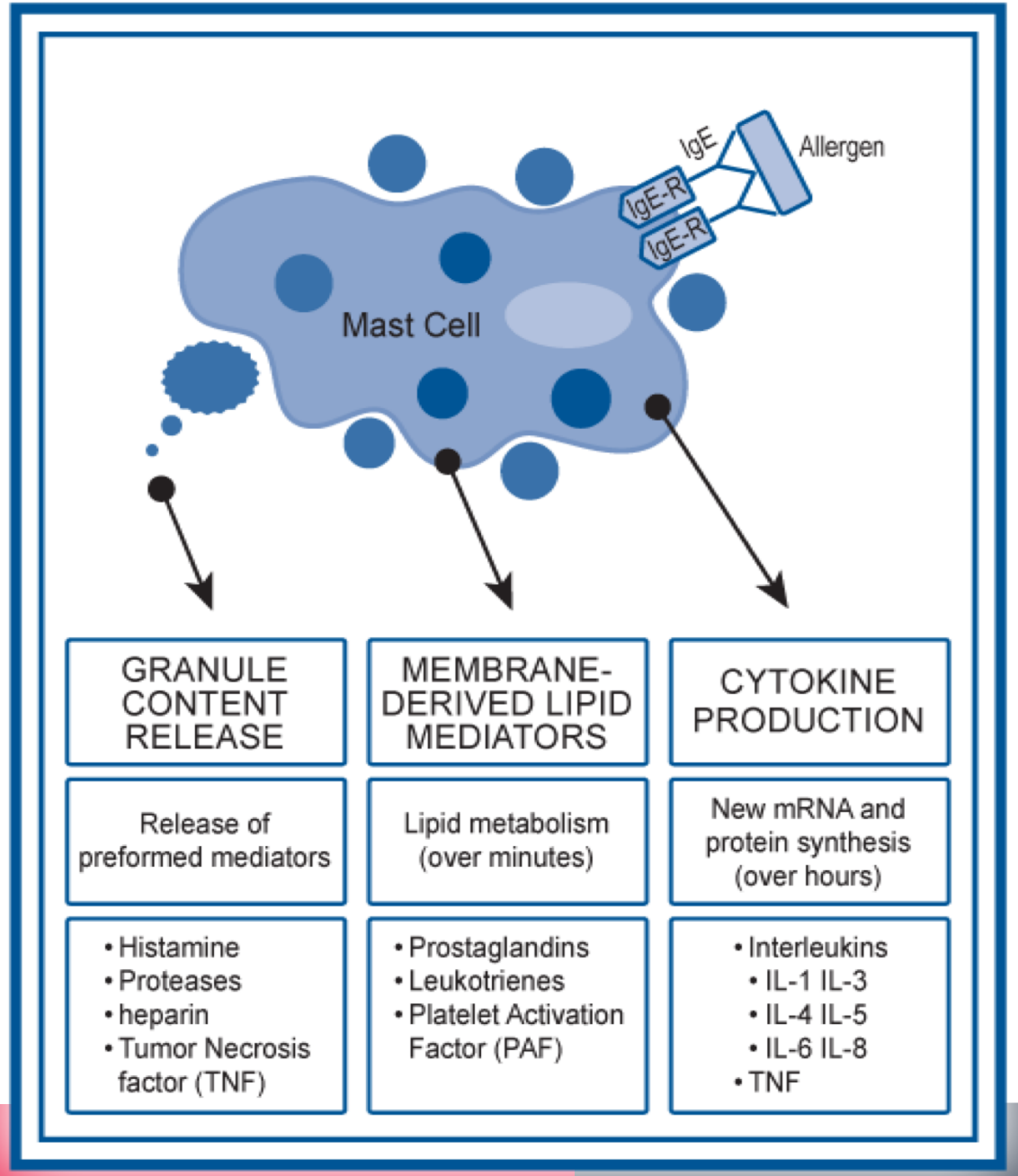
Nature Reviews Immunology 12, 570-580 (August 2012)



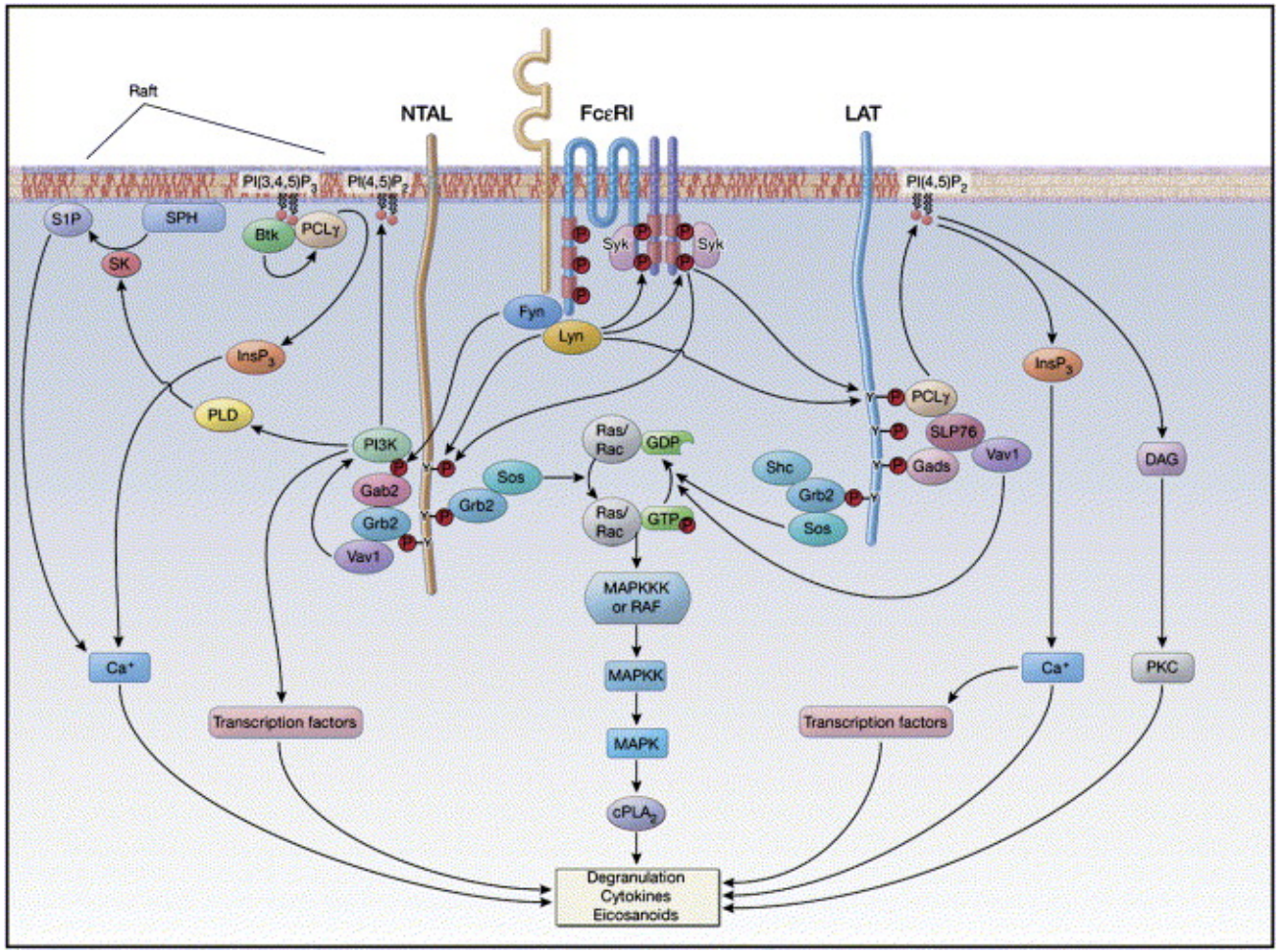
PLoS Med. 2006 August; 3(8): e297.

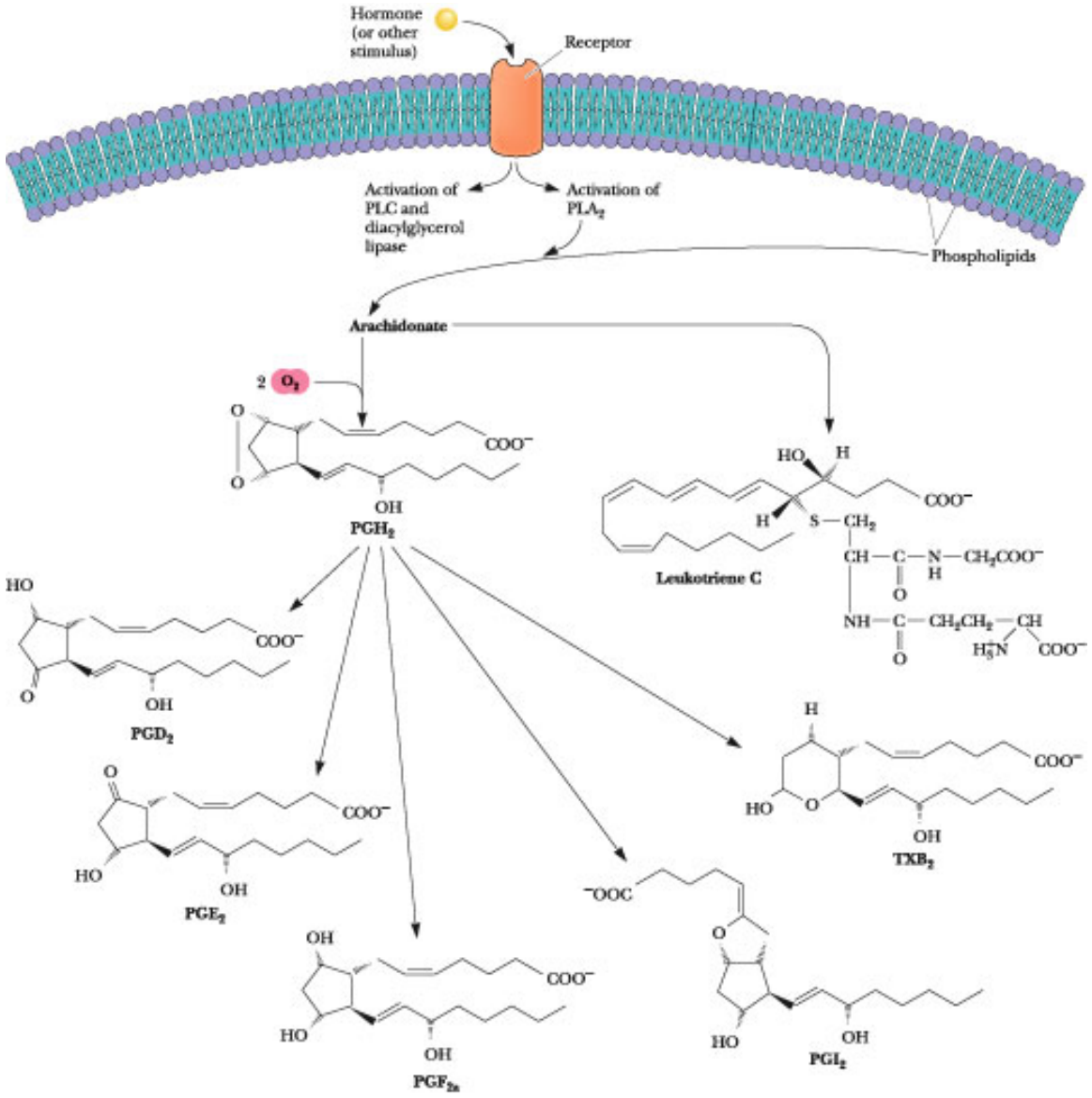
Inflammatory Mediators

- Cell Signaling molecules (cytokines)
- Functional Mediators
  - Preformed mediators (histamine)
  - Formed mediators (prostaglandins etc.)
- Adhesion molecules
- Intracellular signals- secondary messenger systems (NFkB)
- Body-wide signals/mediators (CRP)



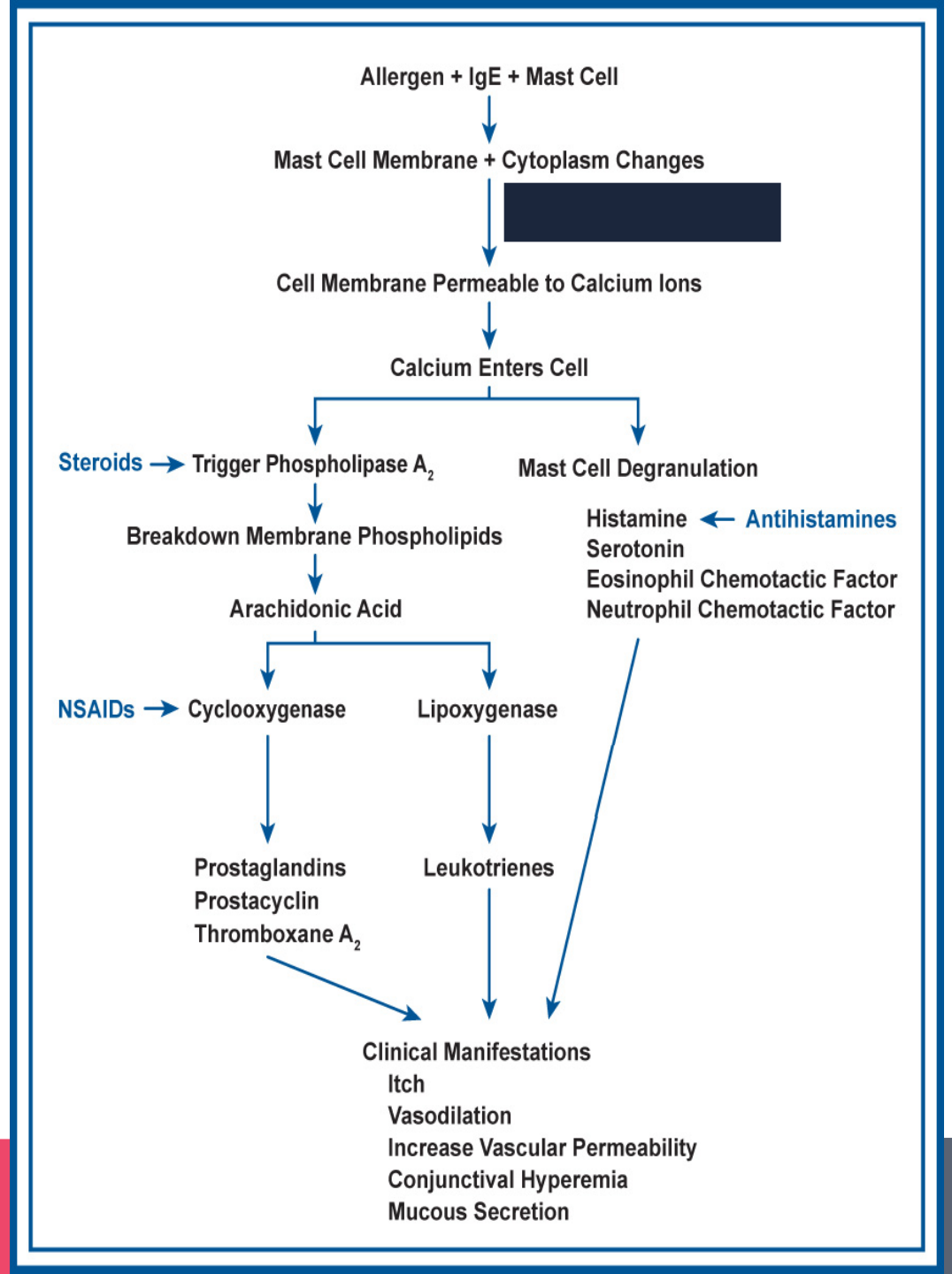
Lots more going on in the cell



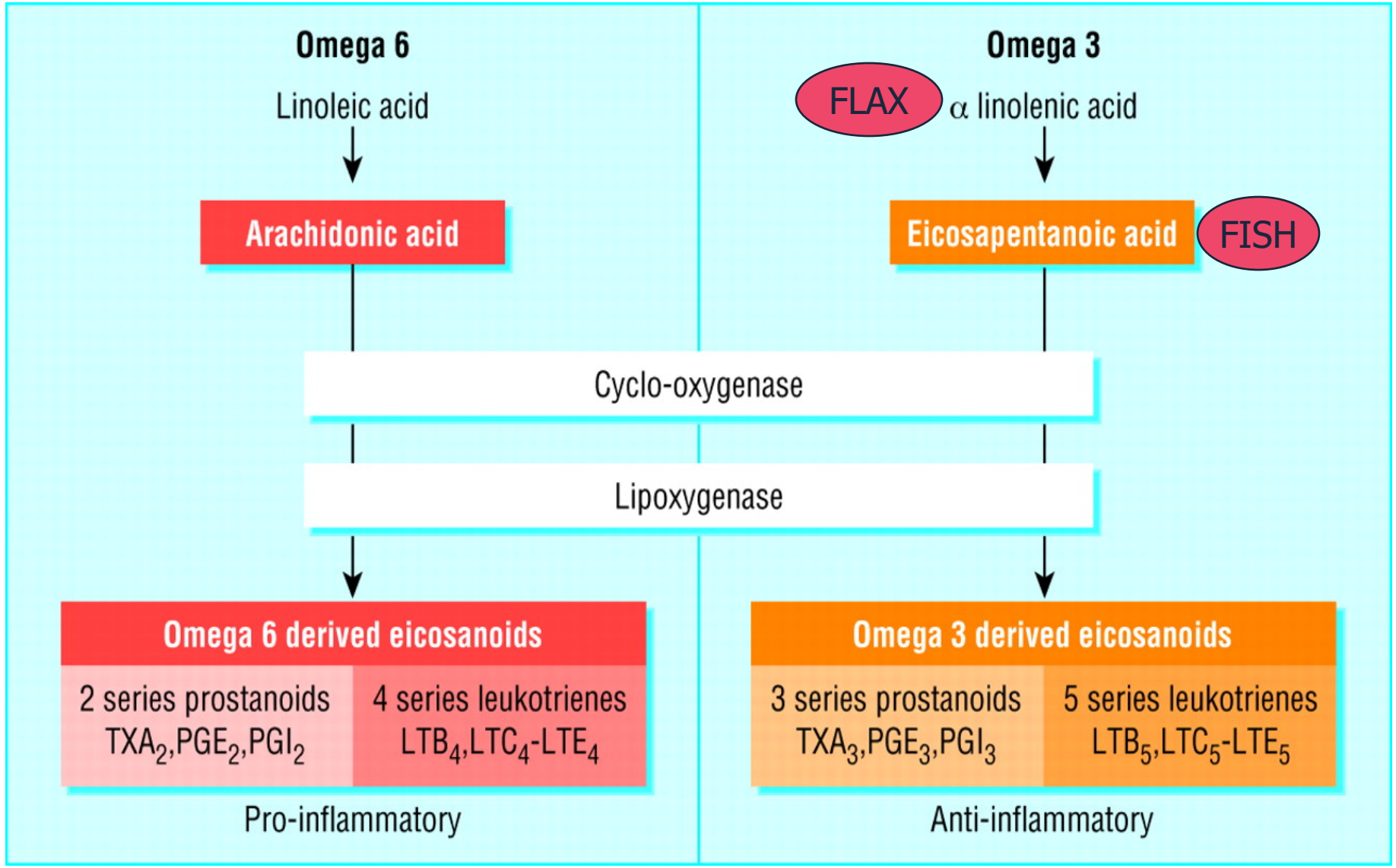


Arachidonic Acid Mediators

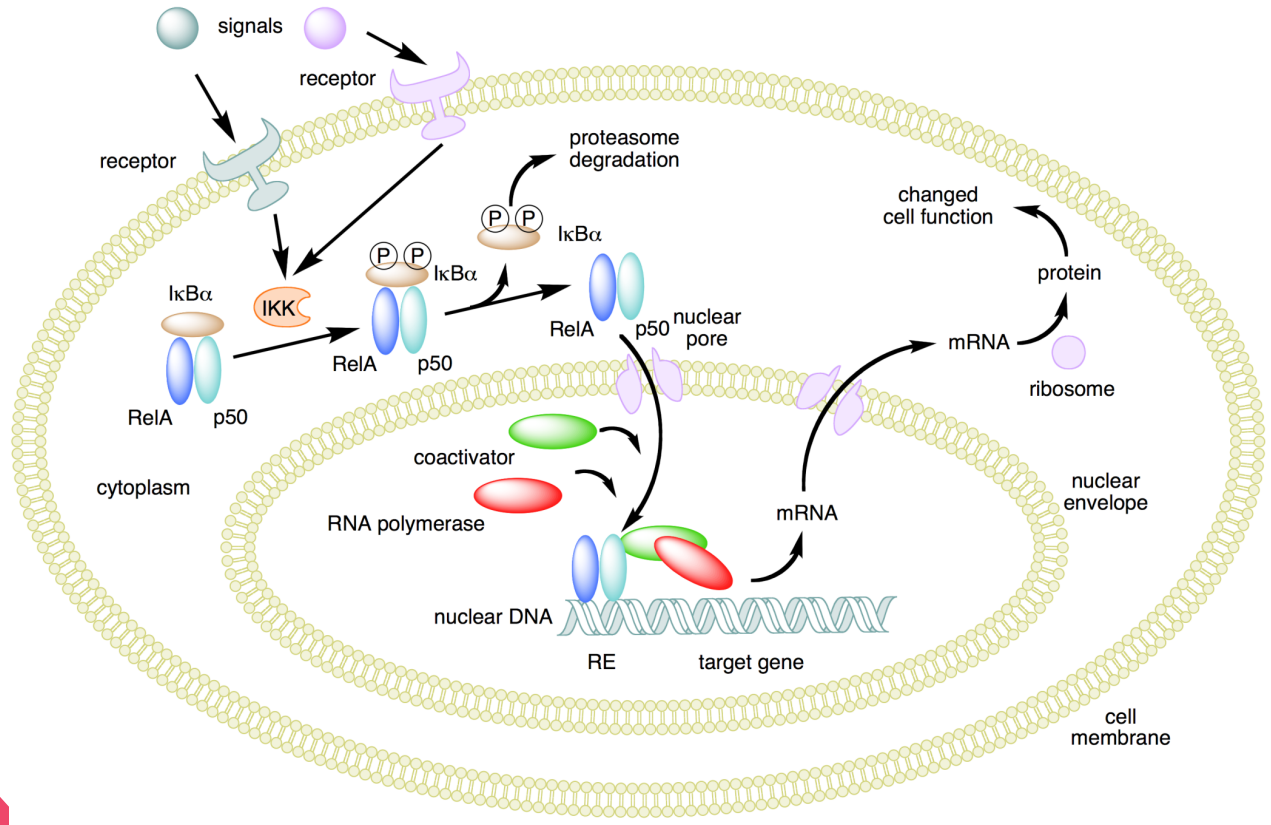
- Phospholipase A removes fatty acids (AA) from the cell membrane
- Cyclooxygenase (COX) converts to series 2 prostaglandins
- Lipoxygenase (LOX) converts to leukotrienes and thromboxanes



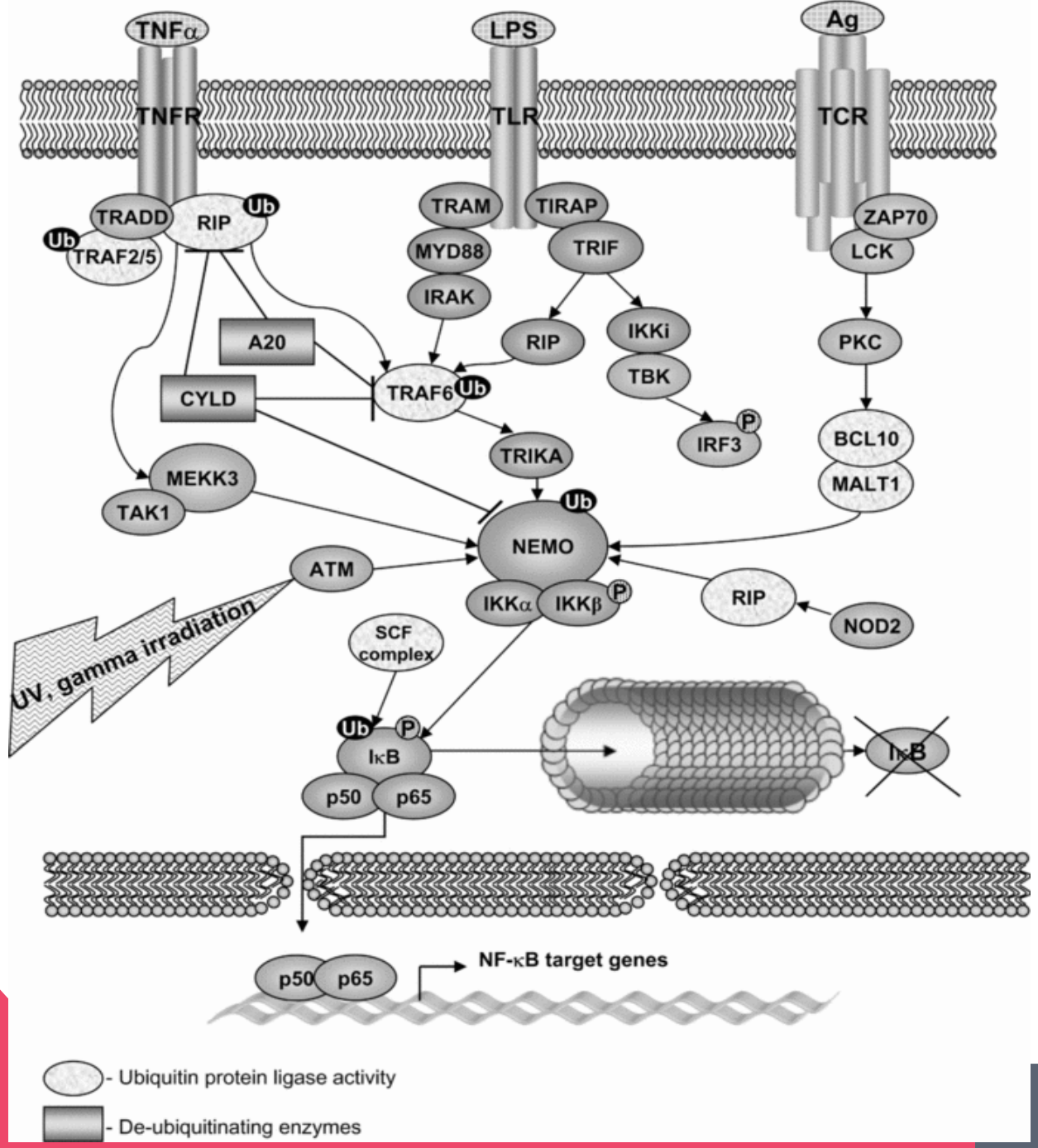
- Preventing Mast Cell Destabilization
  - Quercetin and related flavonoids have been shown to stabilize mast cells – preventing degranulation and other inflammatory mediators from forming



Nuclear Factor kappa B

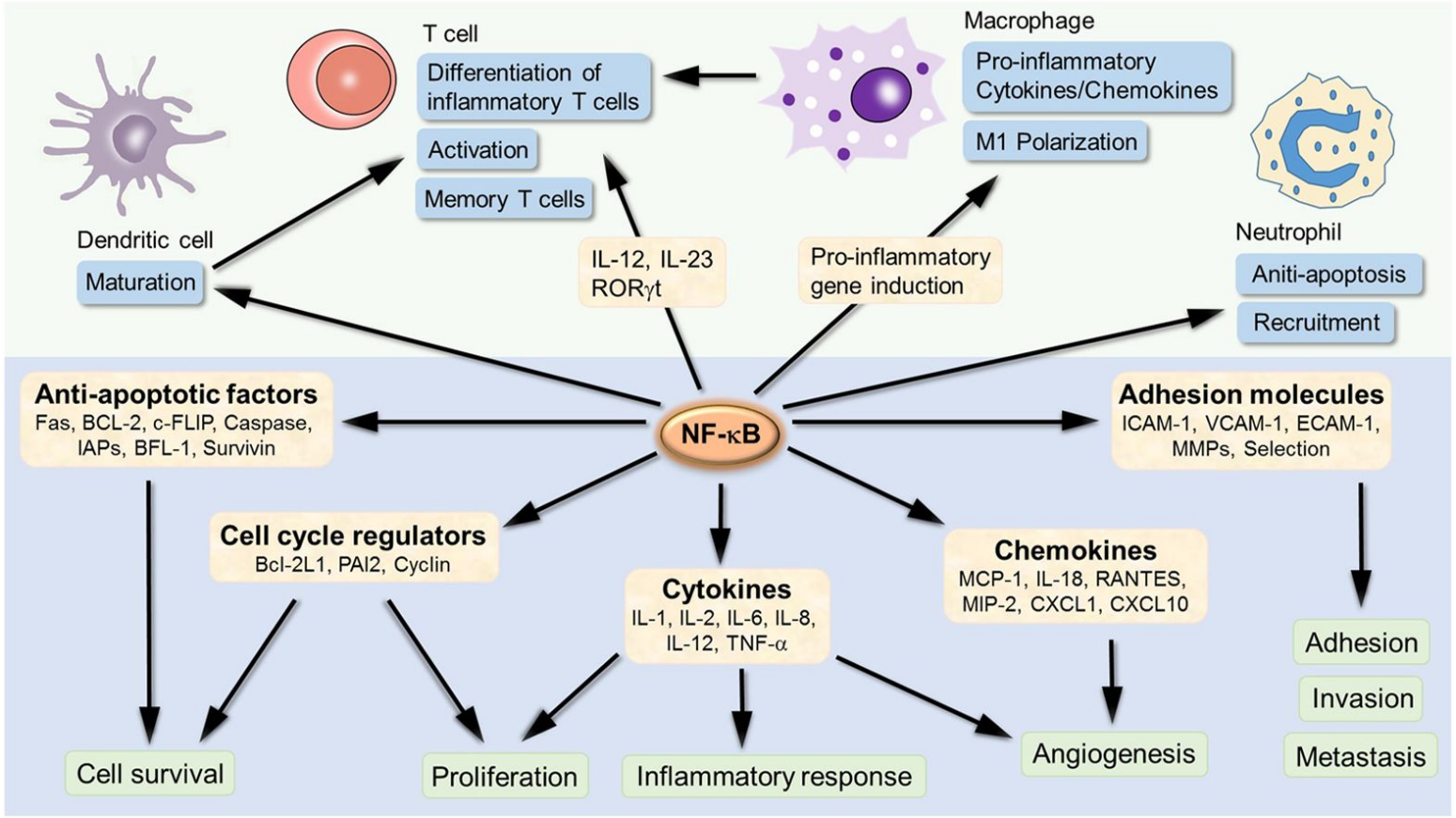


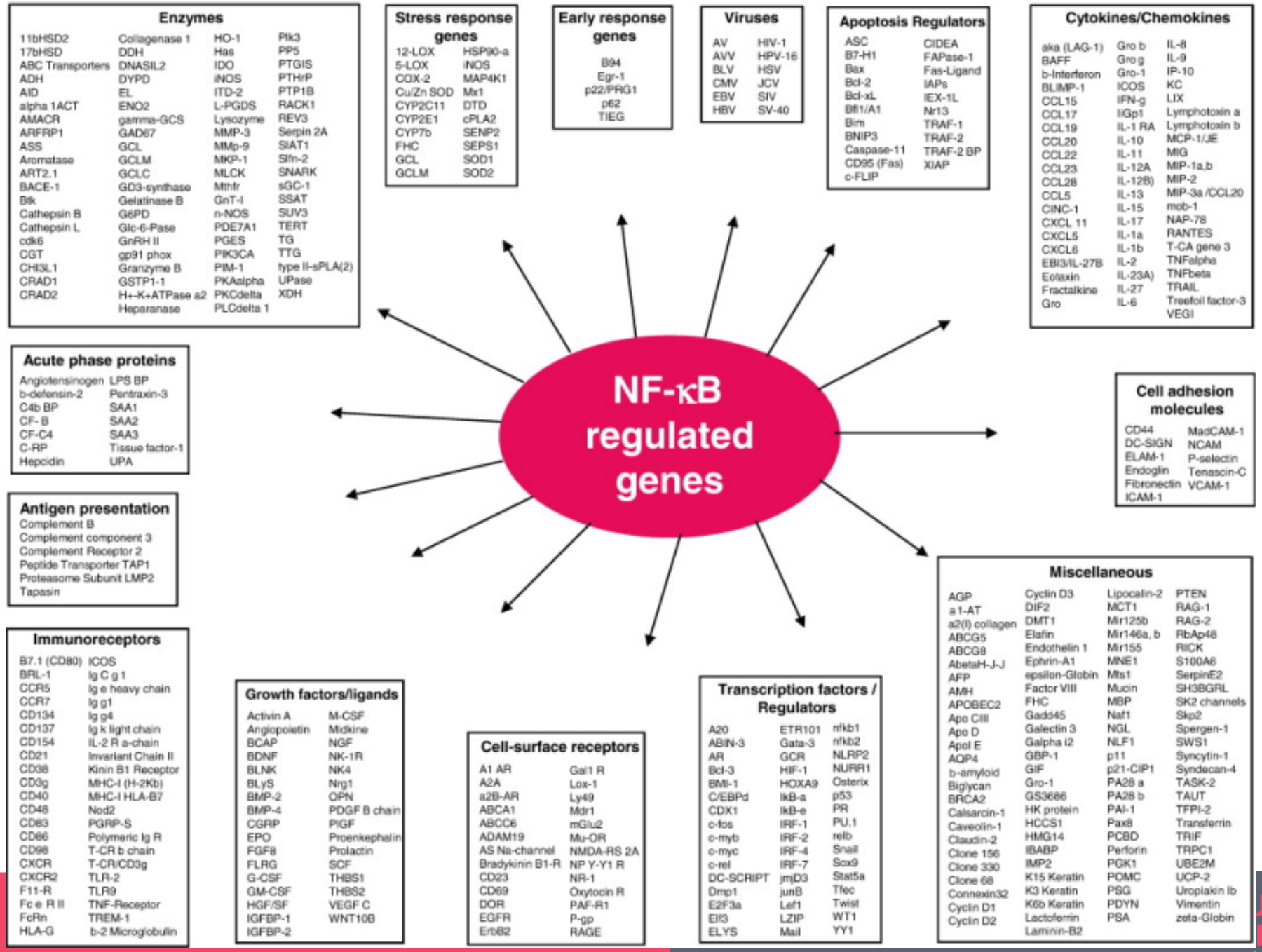
- Release of the potent pro-inflammatory transcription factor
- NFκB is a dimer (RelA, p50)
- Sequestered by IκB
- When IkB is phosphorylated by IKK, it releases NFkB to migrate into nucleus
- NFkB binds promoter regions and upregulates inflammation



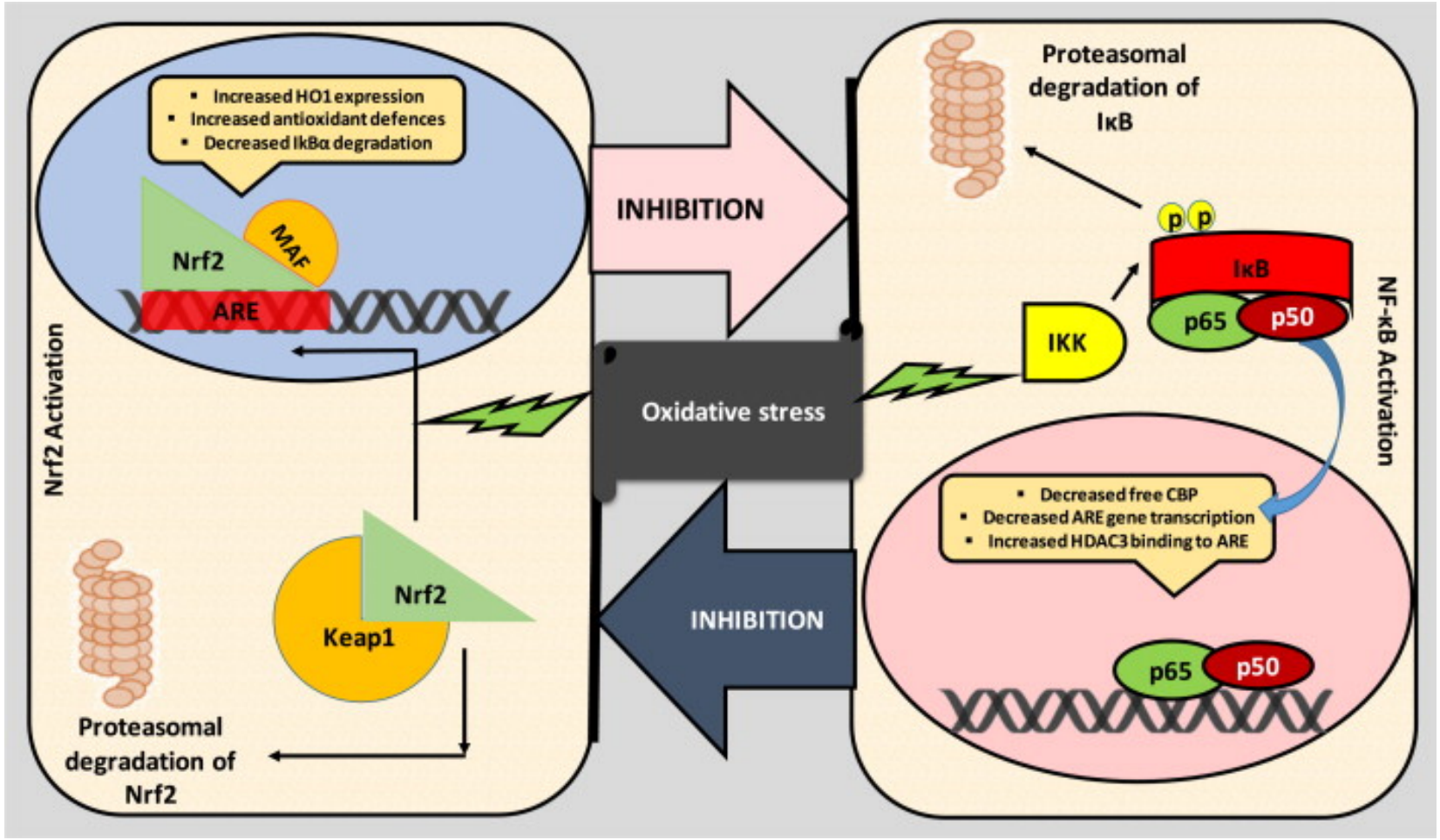
- Three of many different signaling cascades that can lead to the activation of NFκB.
- Shows the primary regulation of NFkB occurs upstream with NEMO (NF-κB essential modulator) and the release of IKKα

NFκB controls a range of immune responses




Enzymes
11bHSD2
17bHSD
ABC Transporters
ADH
AID
alpha 1ACT
AMACR
ARFRP1
ASS
Aromatase
ART2.1
BACE-1
Btk
Cathepsin B
Cathepsin L
cdk6
CGT
CHI3L1
CRAD1
CRAD2
Collagenase 1
DDH
DNASIL2
DYPD
EL
ENO2
gamma-GCS
GAD67
GCL
GCLM
GCLC
GD3-synthase
Gelatinase B
G6PD
Glc-6-Pase
GnRH II
gp91 phox
Granzyme B
GSTP1-1
H+-K+ATPase a2
Heparanase
HO-1
Has
IDO
iNOS
ITD-2
L-PGDS
Lysozyme
MMP-3
MMp-9
MKP-1
MLCK
Mthfr
GnT-I
n-NOS
PDE7A1
PGES
PIK3CA
PIM-1
PKAalpha
PKCdelta
PLCdelta 1
Plk3
PP5
PTGIS
PTHrP
PTP1B
RACK1
REV3
Serpin 2A
SIAT1
Sfn-2
SNARK
sGC-1
SSAT
SUV3
TERT
TG
TTG
type II-sPLA(2)
UPase
XDH
Stress response genes
12-LOX
5-LOX
COX-2
Cu/Zn SOD
CYP2C11
CYP2E1
CYP7b
FHC
GCL
GCLM
HSP90-a
iNOS
MAP4K1
Mx1
DTD
cPLA2
SENP2
SEPS1
SOD1
SOD2
Early response genes
B94
Egr-1
p22/PRG1
p62
TIEG
Viruses
AV
AVV
BLV
CMV
EBV
HBV
HIV-1
HPV-16
HSV
JCV
SIV
SV-40
Apoptosis Regulators
ASC
B7-H1
Bax
Bcl-2
Bcl-xL
Bfl1/A1
Bim
BNIP3
Caspase-11
CD95 (Fas)
c-FLIP
CIDEA
FAPase-1
Fas-Ligand
IAPs
IEX-1L
Nr13
TRAF-1
TRAF-2
TRAF-2 BP
XIAP
Cytokines/Chemokines
aka (LAG-1)
BAFF
b-Interferon
BLIMP-1
CCL15
CCL17
CCL19
CCL20
CCL22
CCL23
CCL28
CCL5
CINC-1
CXCL 11
CXCL5
CXCL6
EBI3/IL-27B
Eotaxin
Fractalkine
Gro
Gro b
Gro g
Gro-1
ICOS
IFN-g
IGp1
IL-1 RA
IL-10
IL-11
IL-12A
IL-12B)
IL-13
IL-15
IL-17
IL-1a
IL-1b
IL-2
IL-23A)
IL-27
IL-6
IL-8
IL-9
IP-10
KC
LIX
Lymphotoxin a
Lymphotoxin b
MCP-1/JE
MIG
MIP-1a,b
MIP-2
MIP-3a /CCL20
mob-1
NAP-78
RANTES
T-CA gene 3
TNFalpha
TNFbeta
TRAIL
Treefoil factor-3
VEGI
NF-κB regulated genes
Acute phase proteins
Angiotensinogen
b-defensin-2
C4b BP
CF- B
CF-C4
C-RP
Hepcidin
LPS BP
Pentraxin-3
SAA1
SAA2
SAA3
Tissue factor-1
UPA
Cell adhesion molecules
CD44
DC-SIGN
ELAM-1
Endoglin
Fibronectin
ICAM-1
MadCAM-1
NCAM
P-selectin
Tenascin-C
VCAM-1
Antigen presentation
Complement B
Complement component 3
Complement Receptor 2
Peptide Transporter TAP1
Proteasome Subunit LMP2
Tapasin
Miscellaneous
AGP
a1-AT
a2(I) collagen
ABCG5
ABCG8
AbetaH-J-J
AFP
AMH
APOBEC2
Apo CIII
Apo D
Apol E
AQP4
b-amyloid
Biglycan
BRCA2
Calsarcin-1
Caveolin-1
Claudin-2
Clone 156
Clone 330
Clone 68
Connexin32
Cyclin D1
Cyclin D2
Cyclin D3
DIF2
DMT1
Elafin
Endothelin 1
Ephrin-A1
epsilon-Globin
Factor VIII
FHC
Gadd45
Galectin 3
Galpha i2
GBP-1
GIF
Gro-1
GS3686
HK protein
HCCS1
HMG14
IBABP
IMP2
K15 Keratin
K3 Keratin
K6b Keratin
Lactoferrin
Laminin-B2
Lipocalin-2
MCT1
Mir125b
Mir146a, b
Mir155
MNE1
Mts1
Mucin
MBP
Naf1
NGL
NLF1
p11
p21-CIP1
PA28 a
PA28 b
PAI-1
Pax8
PCBD
Perforin
PGK1
POMC
PSG
PDYN
PSA
PTEN
RAG-1
RAG-2
RbAp48
RICK
S100A6
SerpinE2
SH3BGRL
SK2 channels
Skp2
Spergen-1
SWS1
Syncytin-1
Syndecan-4
TASK-2
TAUT
TFPI-2
Transferrin
TRIF
TRPC1
UBE2M
UCP-2
Uroplakin Ib
Vimentin
zeta-Globin
Immunoreceptors
B7.1 (CD80)
BRL-1
CCR5
CCR7
CD134
CD137
CD154
CD21
CD38
CD3g
CD40
CD48
CD83
CD86
CD98
CXCR
CXCR2
F11-R
Fc e R II
FcRn
HLA-G
ICOS
Ig C g 1
Ig e heavy chain
Ig g1
Ig g4
Ig k light chain
IL-2 R a-chain
Invariant Chain II
Kinin B1 Receptor
MHC-I (H-2Kb)
MHC-I HLA-B7
Nod2
PGRP-S
Polymeric Ig R
T-CR b chain
T-CR/CD3g
TLR-2
TLR9
TNF-Receptor
TREM-1
b-2 Microglobulin
Growth factors/ligands
Activin A
Angiopoietin
BCAP
BDNF
BLNK
BLyS
BMP-2
BMP-4
CGRP
EPO
FGF8
FLRG
G-CSF
GM-CSF
HGF/SF
IGFBP-1
IGFBP-2
M-CSF
Midkine
NGF
NK-1R
NK4
Nrg1
OPN
PDGF B chain
PlGF
Proenkephalin
Prolactin
SCF
THBS1
THBS2
VEGF C
WNT10B
Cell-surface receptors
A1 AR
A2A
a2B-AR
ABCA1
ABCC6
ADAM19
AS Na-channel
Bradykinin B1-R
CD23
CD69
DOR
EGFR
ErbB2
Gal1 R
Lox-1
Ly49
Mdr1
mGlu2
Mu-OR
NMDA-RS 2A
NP Y-Y1 R
NR-1
Oxytocin R
PAF-R1
P-gp
RAGE
Transcription factors / Regulators
A20
ABIN-3
AR
Bcl-3
BMI-1
C/EBPd
CDX1
c-fos
c-myb
c-myc
c-rel
DC-SCRIPT
Dmp1
E2F3a
Elf3
ELYS
ETR101
Gata-3
GCR
HIF-1
HOXA9
IkB-a
IkB-e
IRF-1
IRF-2
IRF-4
IRF-7
jmjD3
junB
Lef1
LZIP
Mail
nfkb1
nfkb2
NLRP2
NURR1
Osterix
p53
PR
PU.1
relb
Snail
Sox9
Stat5a
Tfec
Twist
WT1
YY1

Cross-Talk Between Nrf2 and NFκB



Redox Biol. 2013 Aug 1;1:394-7.

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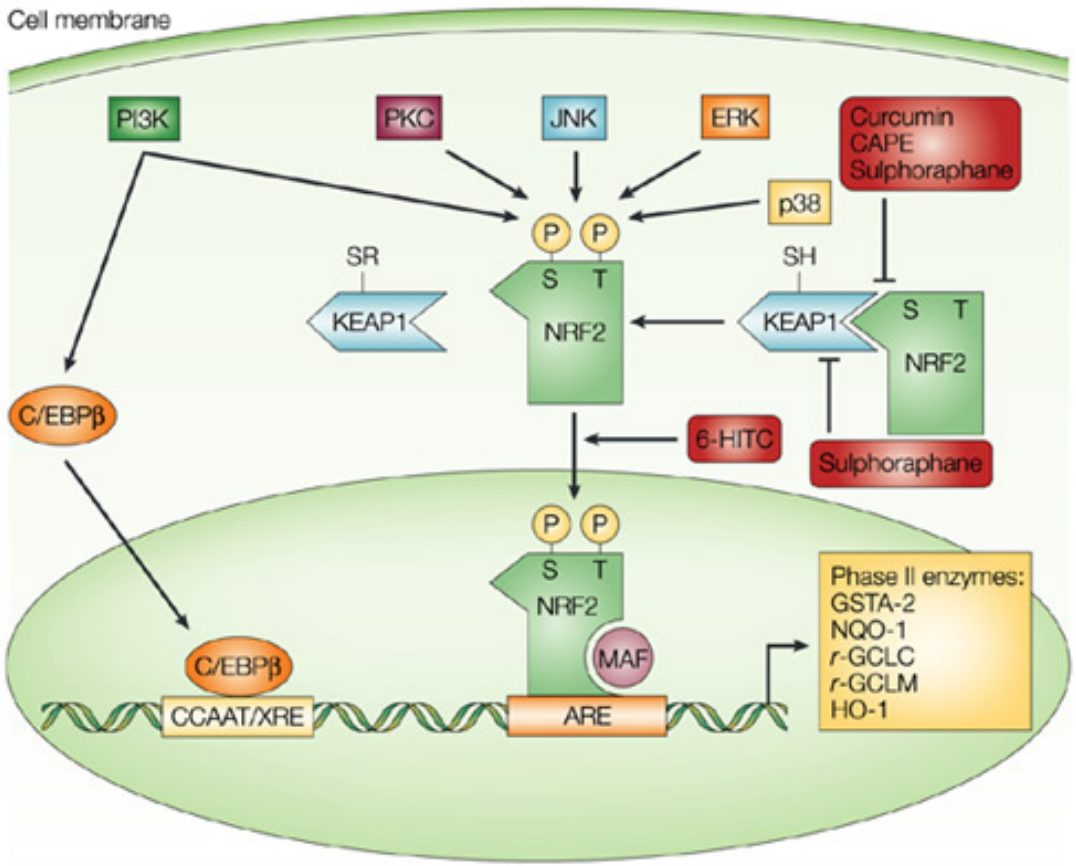
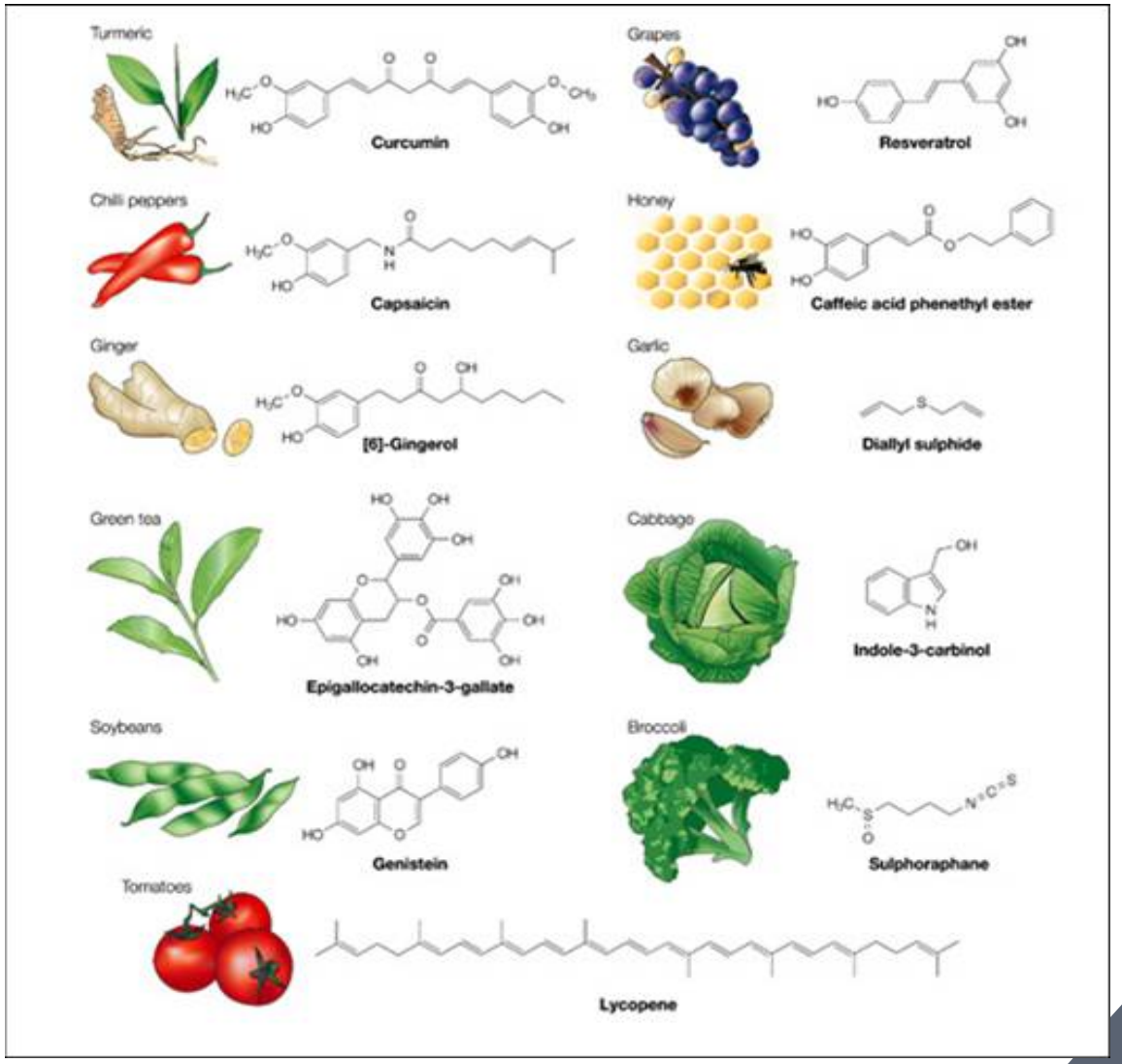
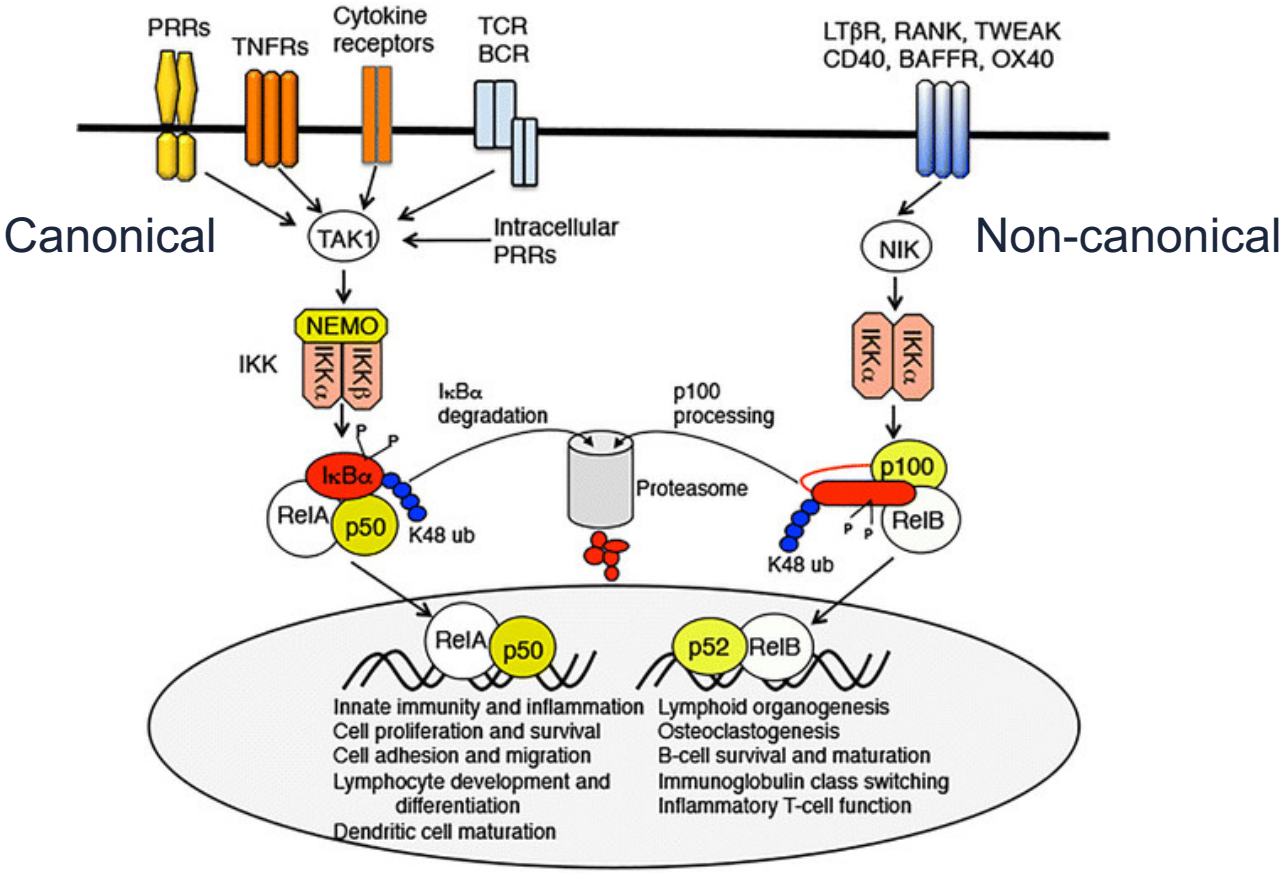


Nature Reviews | Cancer

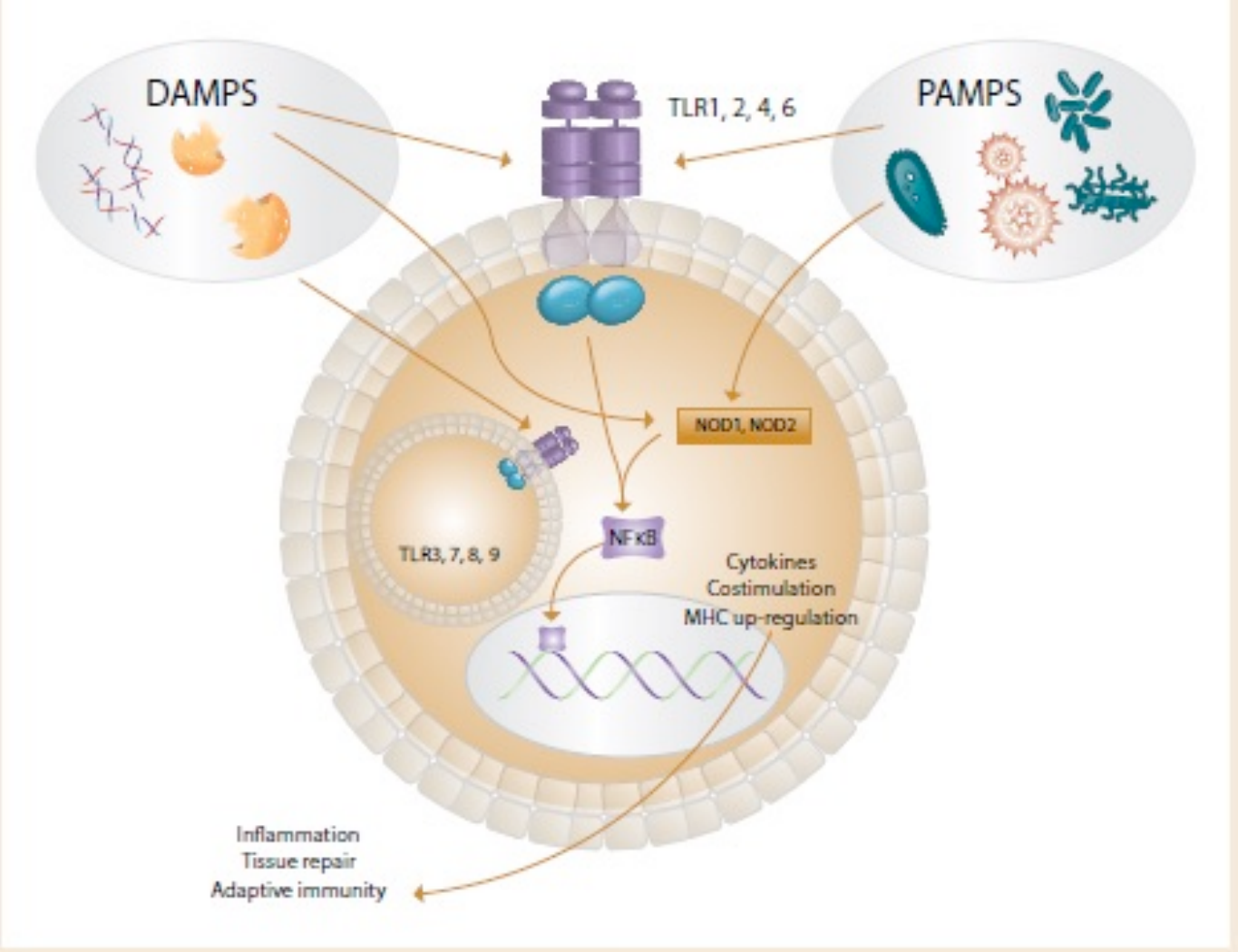


What Signals Turn on Inflammation?



- Pattern Recognition Receptors (PRRs)
- TNF-a
- Cytokines (IL-1b, IL-6, etc.)
- Signals from antibody or TCR binding
- Non-canonical signaling

Pattern Recognition Receptors



Toll-like receptor family (TLRs)

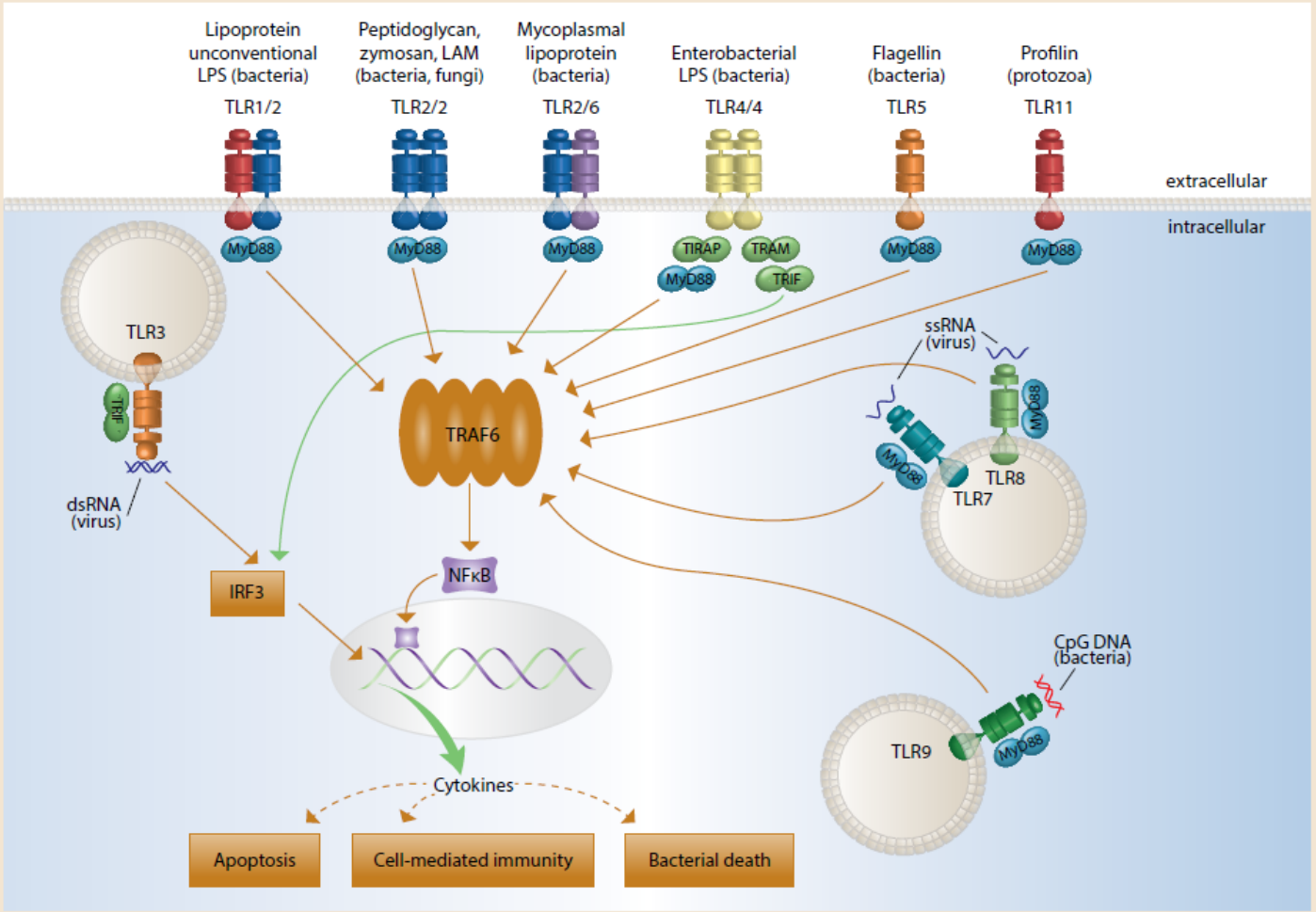
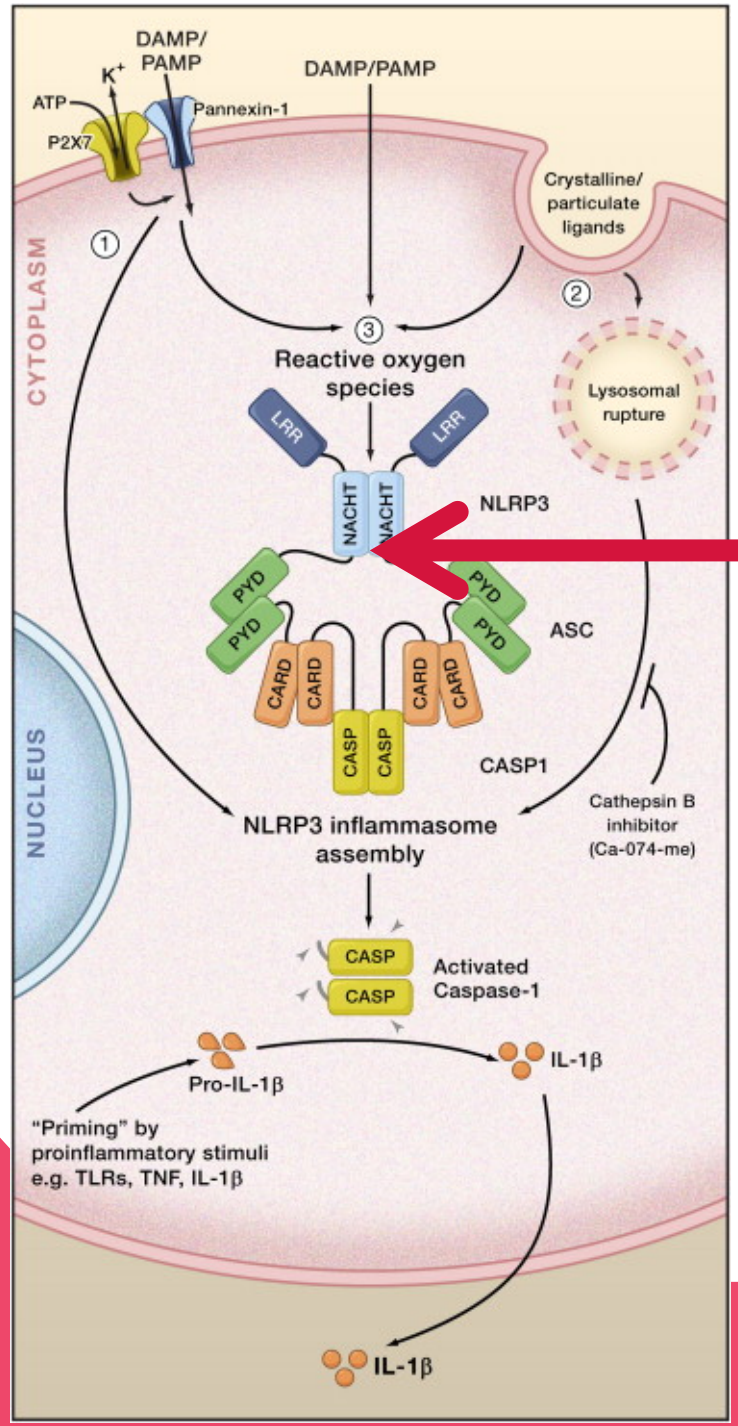
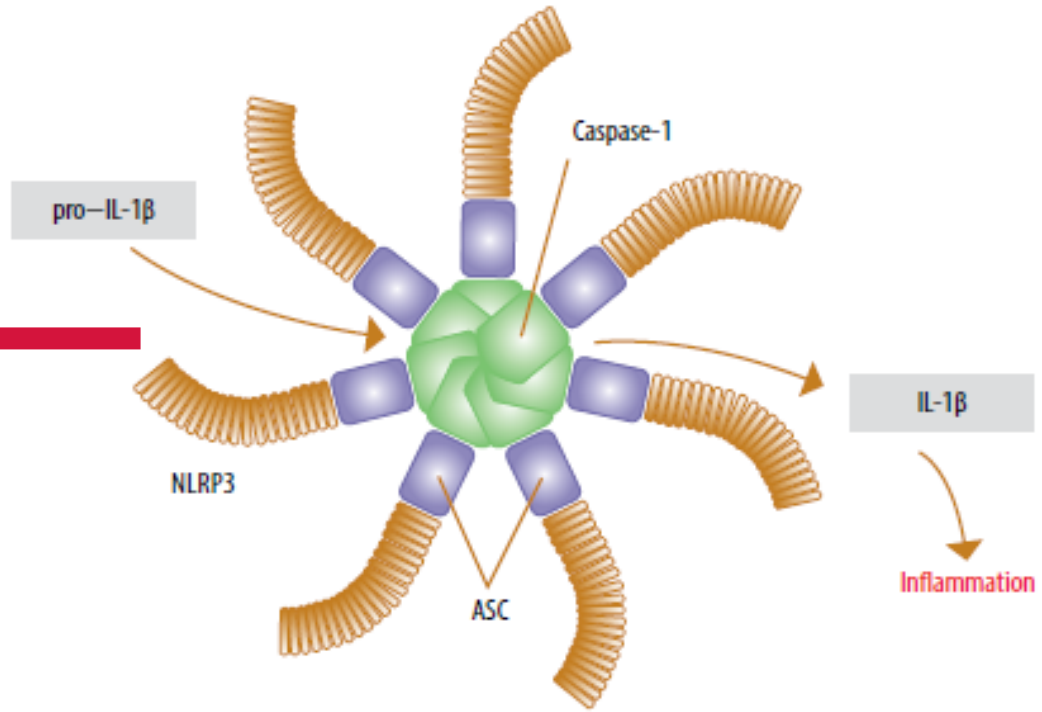


Figure: Toll-like receptor (TLR) signaling. This diagram shows the different types of TLRs, their locations and the patterns they recognize. See text for more details about the signaling pathways. Image adapted from Minireview: Toll-like Receptors (TLR)-www.abdserotec.com.

Inflammasome (NLRP3-type)

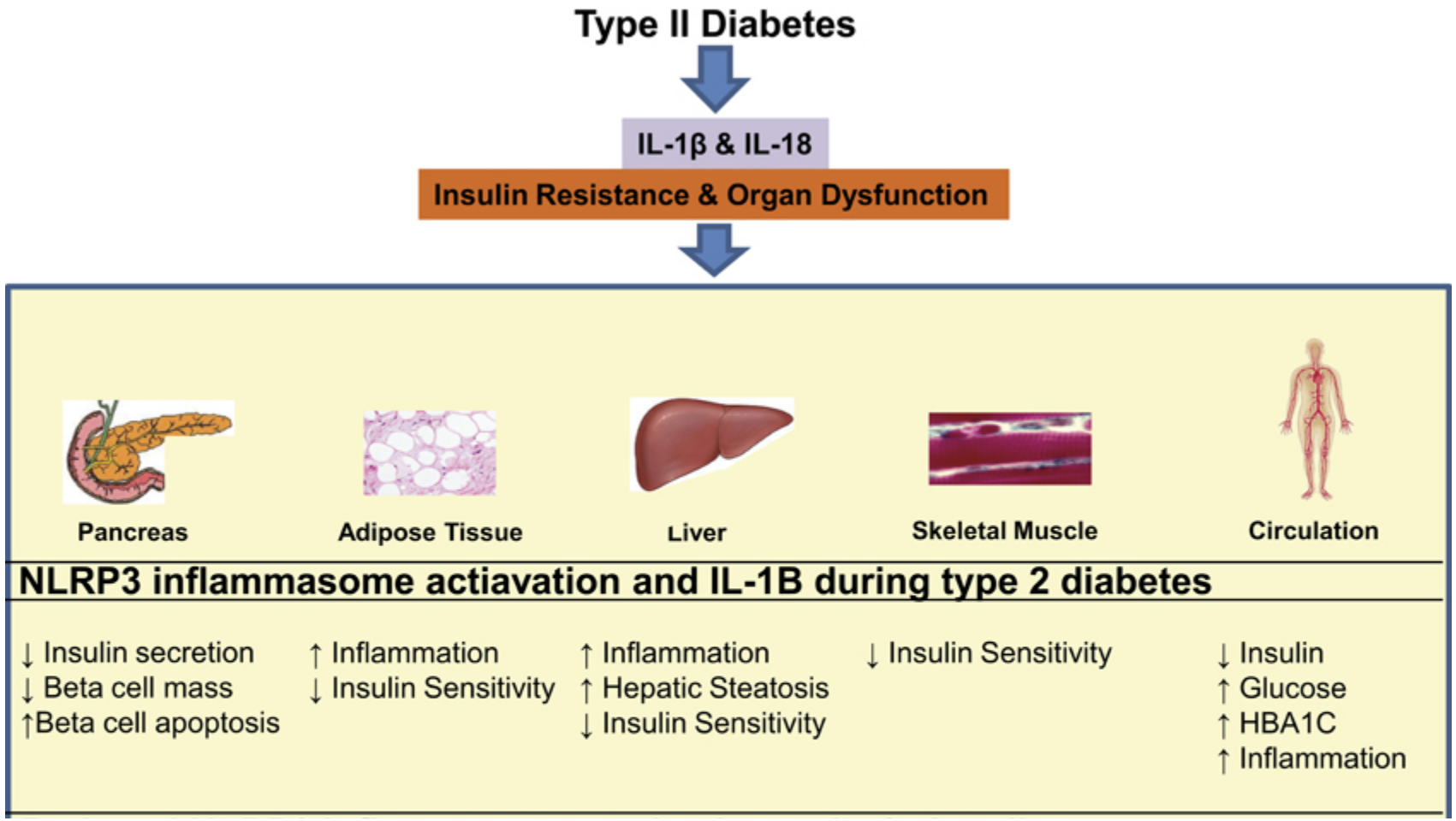




- Heptamer complex between caspase and NRLP3
- Caspase activates the release of IL-1β, furthering inflammatory cascade
- 3 potential triggers

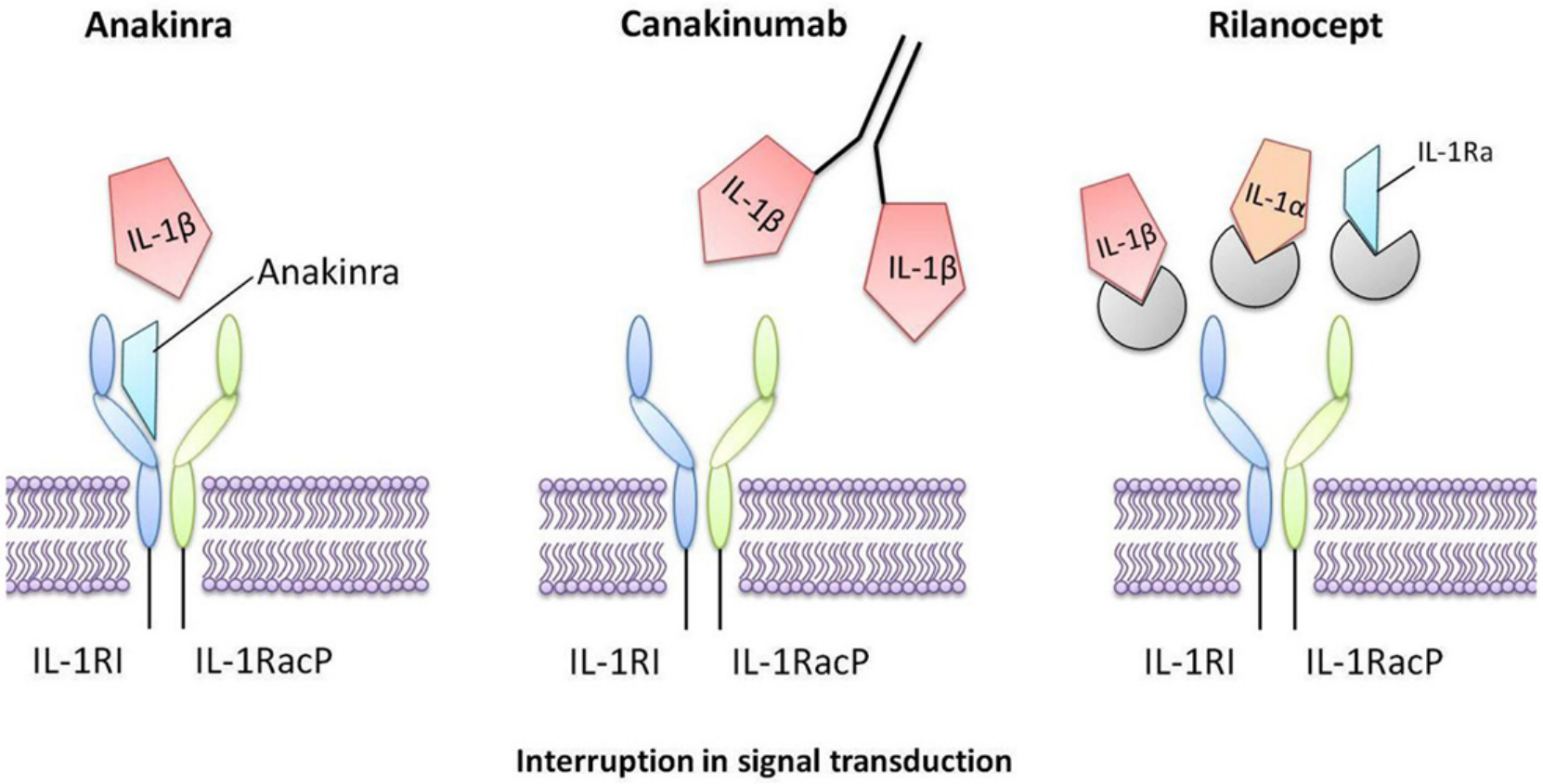
Cell: Volume 140, Issue 6, 19 March 2010, Pages 821–832

Inflammasome and chronic disease



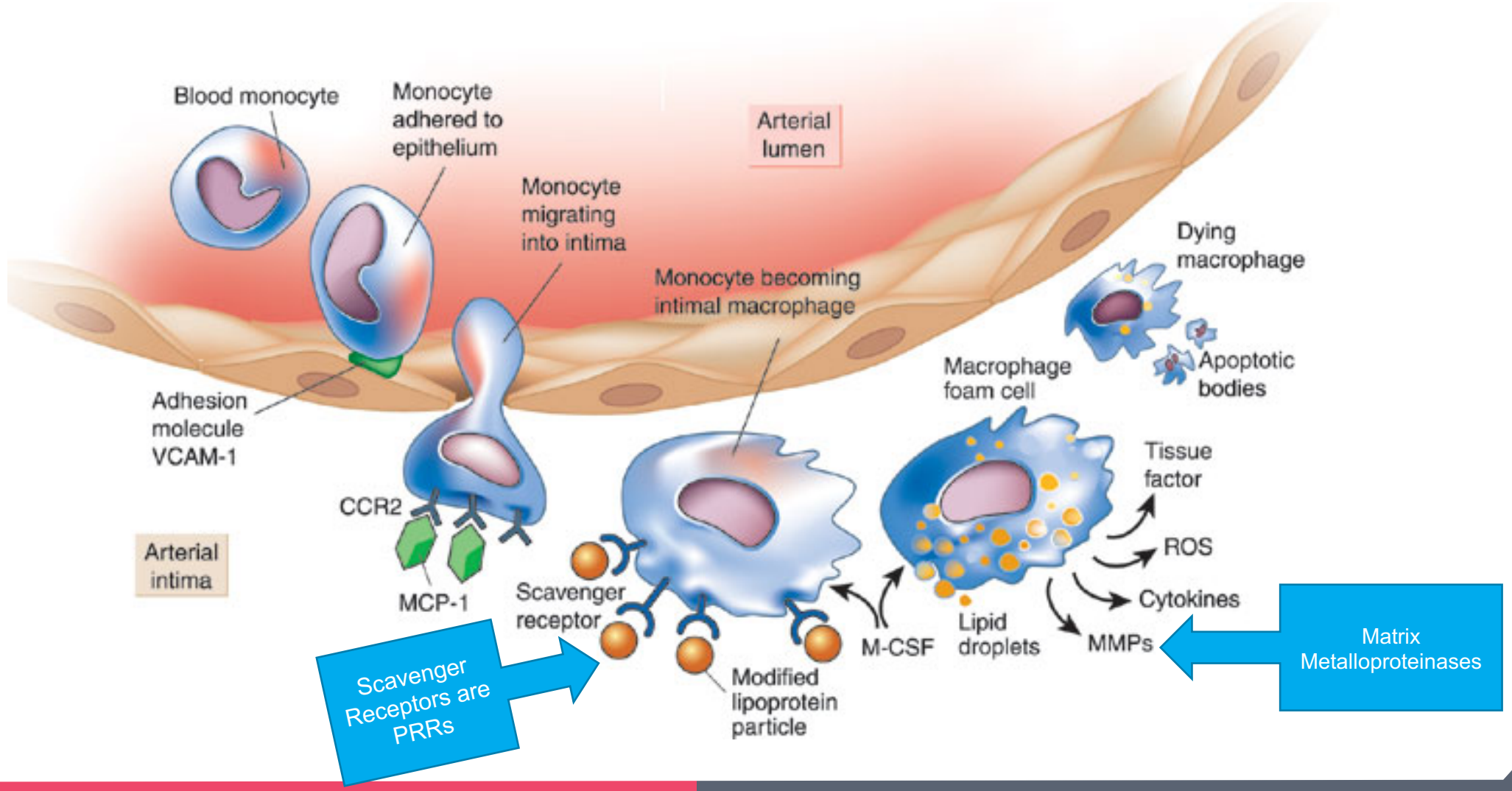
Front. Immunol., 08 March 2013

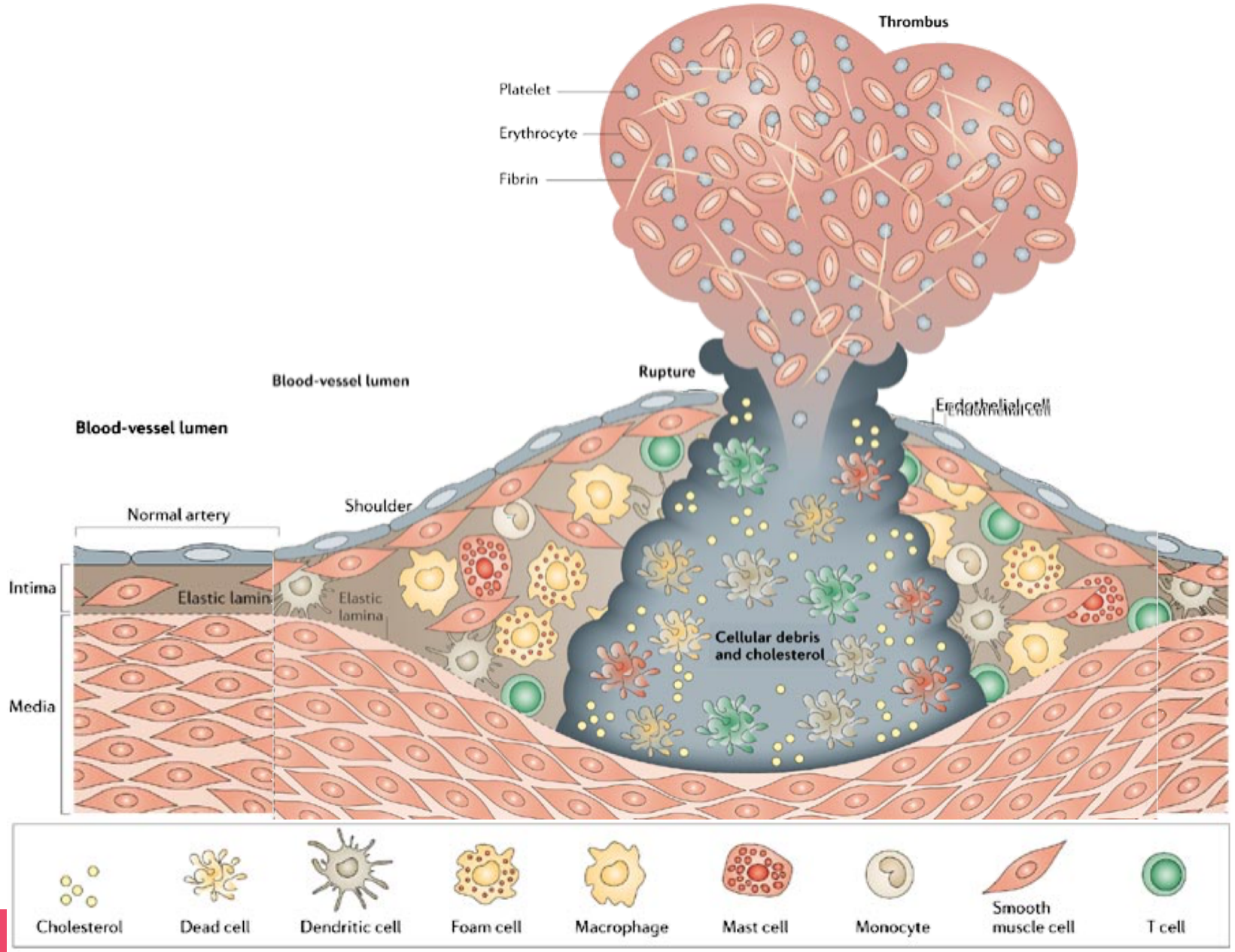
Significant Investment in Biologics to Block IL-1β



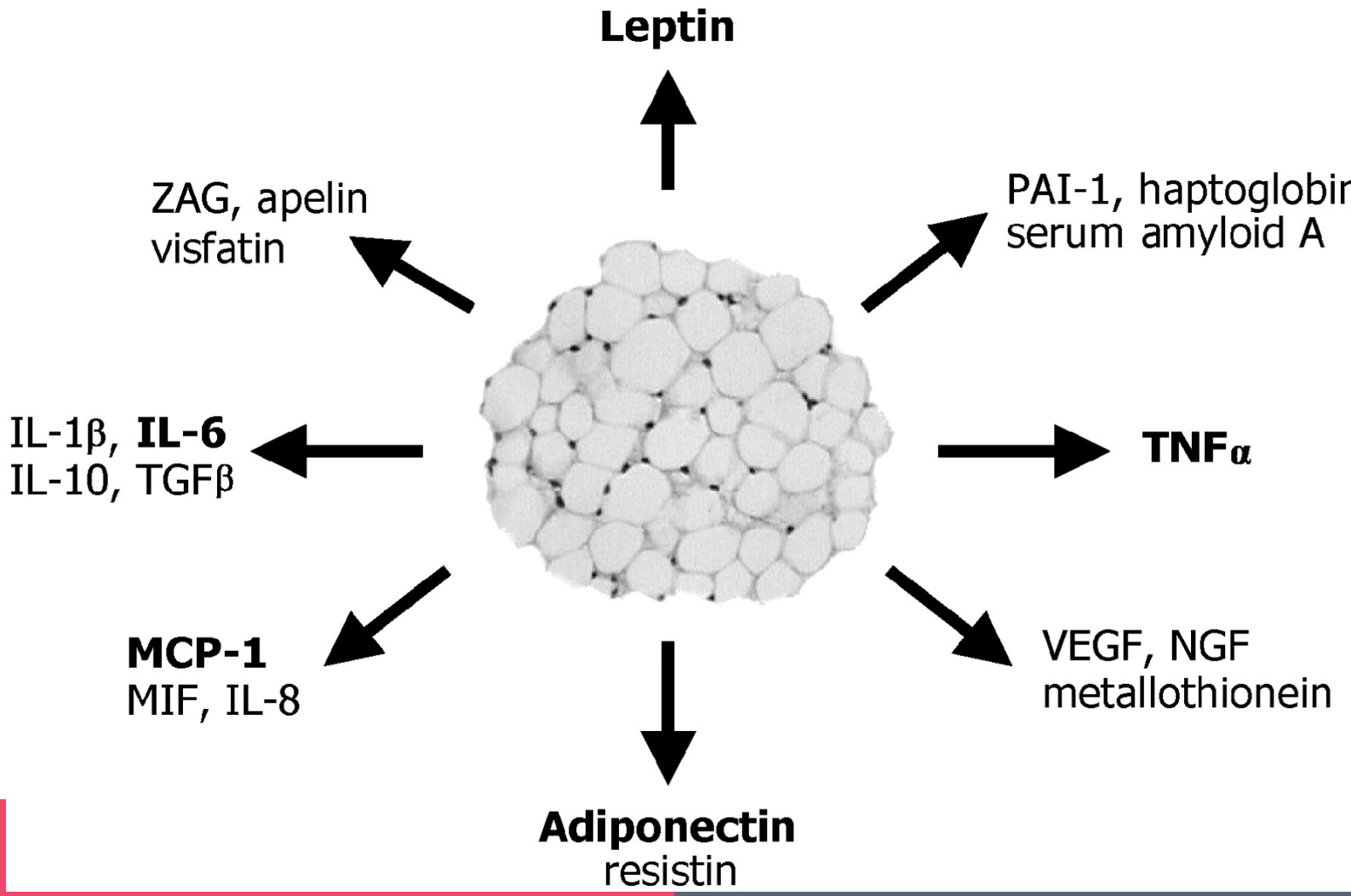
Front. Cell. Neurosci., 06 February 2015

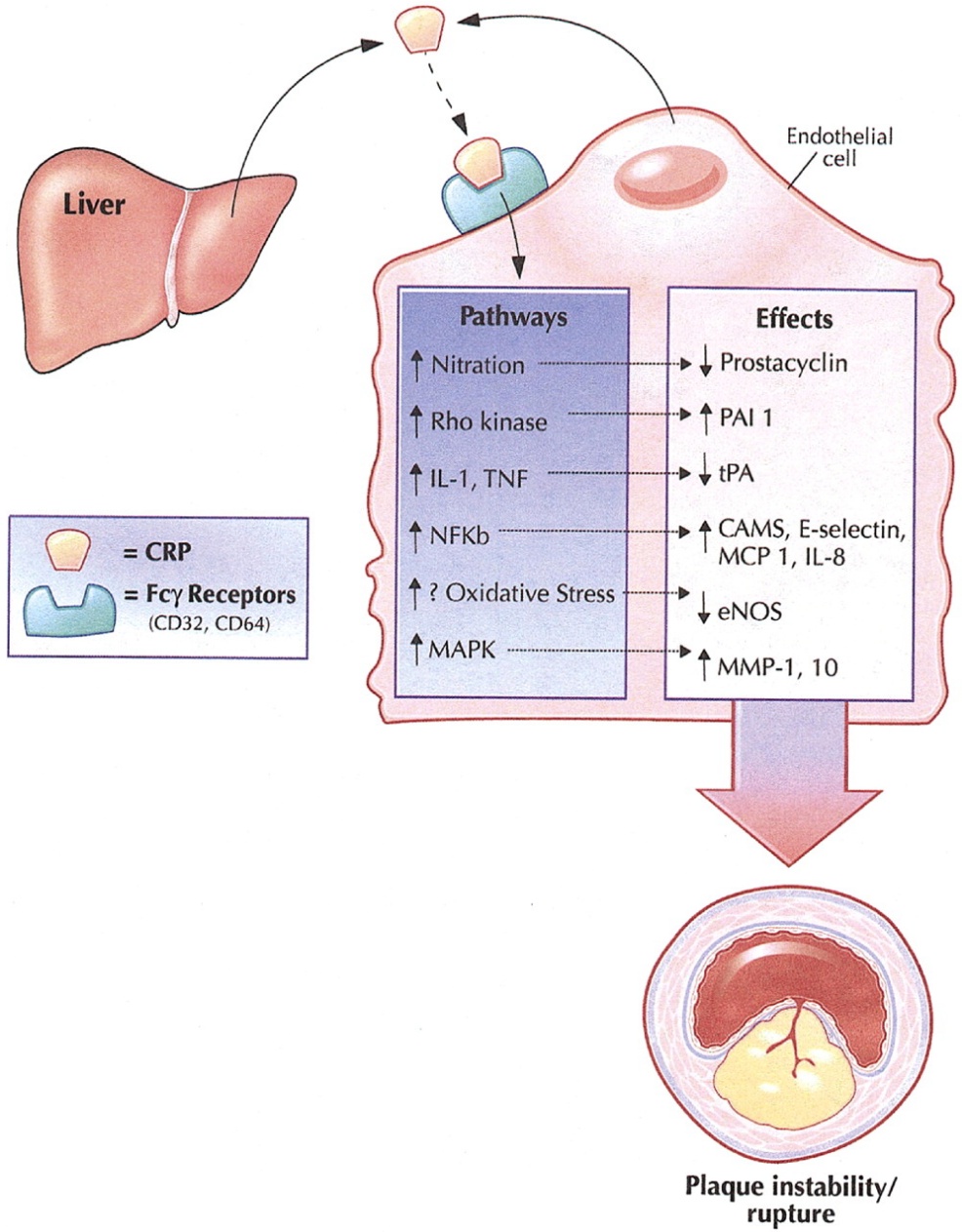
Inflammatory Cascade & CAD





Fat cells (white adipose tissue) are cytokine factories!





The Role of CRP

Marker or Mediator?

- PAI-1: plasminogen activator inhibitor 1
- tPA: tissue plasminogen activator
- MCP1: monocyte chemoattractant protein 1
- eNOS- endothelial nitric oxide synthase
- MMP- matrix metaloproteases

Clinical Research

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Fish Oil Supplementation Lowers C-Reactive Protein Levels Independent of Triglyceride Reduction in Patients With End-Stage Renal Disease

Rodney G. Bowden, PhD[1]; Ronald L. Wilson, MD[2]; Erika Deike, MS[1]; and Mindy Gentile, RD, LD, MS[3]

Financial disclosure: Fish oil and placebo supplements were provided by Royal Numico, Wageningen, The Netherlands.

Background: Inflammation has been identified as a marker for cardiovascular disease. The purpose of this study is to examine the effects of fish oil fatty acid supplementation on C-reactive protein (CRP) levels. *Methods:* The study uses a double-blind, permuted-randomized, and placebo-controlled experimental protocol. Patients are randomly placed into a fish oil group or a control group. Thirty-three patients in the experimental and control groups ingest 2 soft-gel pills (1 g each) of fish oil supplements containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) or placebo at each meal. Patients follow the supplementation protocol for 6 months. Analysis of variance (ANOVA) is used to measure pretest and posttest differences in the variable of interest. A Kolmogorov-Smirnov test for normality is used to test whether CRP levels are normally distributed. *Results:* The Kolmogorov-Smirnov test for CRP finds a P value of .273 (KS = .997), revealing that the distribution is normal. ANOVA reveals no statistically significant difference between groups at baseline for CRP ($F = 4.118$, $P = .053$). ANOVA reveals a significant main effect ($F = 4.29$, $P = .048$) for CRP, with the EPA/DHA group having a significant change in values from pretest (16 mg/dL, standard deviation [SD] = 13.80) to posttest (10.22 mg/dL, SD = 7.87). The placebo group's CRP levels do not change significantly from pretest (13.37, standard deviation [SD] = 7.94) to posttest (13.67, SD = 7.07). An observed power calculation using Cohen's D with a computed α of .05 is .588. *Conclusions:* The study demonstrates that consuming 960 mg/d of EPA and 600 mg/d of DHA can lower CRP. *(Nutr Clin Pract. XXXX;xx:xx-xx)*

Keywords: C-reactive protein; fish oils; docosahexaenoic acids; ω-3 fatty acids

Cardiovascular disease (CVD) is a comorbid condition among patients with end-stage renal disease (ESRD) and is frequently associated with mortality in this patient population. The risk for CVD in patients undergoing chronic hemodialysis can be ascribed to traditional measures of CVD risk such as elevated serum lipid levels and blood pressure, but additional factors such as acute phase reactants may lead to a risk profile that is 10-20 times higher than that of an apparently healthy population[1] and that is responsible for 50% annual mortality in ESRD patients.[2] These additional risk factors may include one such acute phase reactant called C-reactive protein (CRP) and other inflammatory markers[3,4] that are significantly elevated in patients with ESRD.

Low-grade inflammation, measured by circulating levels of CRP, has been included as a risk factor for CVD in previous studies.[5-10] The role of CRP as a marker of disease has been primarily studied in healthy women, healthy men, elderly people, postmenopausal women,[11] premenopausal women,[12] arthritis patients,[13] and healthy older adults,[14] with few studies recruiting ESRD patients.

The use of ω-3 polyunsaturated fatty acids (fish oil) has been proposed as a means to control endothelial dysfunction and thereby control CRP levels. Fish oil has anti-inflammatory, anti-arrhythmic, and antithrombotic properties and has been reported to improve insulin sensitivity,[15] yet research regarding fish oil and CRP is poorly understood.[11,1,6-18] Additionally, ESRD patients normally do not consume enough ω-3 fatty acids in the diet.[19] Studies[16-18] have demonstrated the relationship between fish oil and CVD for a number of years, but studies linking fish oil and circulating markers of inflammation in ESRD patients have been less apparent.[5,20,21] Only 5 studies were discovered that measured the effects of fish oil supplementation on CRP levels in ESRD patients undergoing chronic hemodialysis.[1,2,10,15,16] Most of the fish oil studies that were included in this article focused specifically on docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

From [1]Baylor University, Wako, Texas; [2]Central Texas Nephrology Associates, Wako, Texas; and [3]NutrePletion Resources, Bensalem, Pennsylvania.

Address correspondence to: Rodney G. Bowden, School of Education, Baylor University, Waco, TX, 76798-7304; e-mail: Rodney_Bowden@baylor.edu.

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THE JOURNAL OF Nutritional Biochemistry

Dietary fish oil decreases C-reactive protein, interleukin-6, and triacylglycerol to HDL-cholesterol ratio in postmenopausal women on HRT

Irina Ciubotaru, Ye-Sun Lee, Rosemary C. Wander*

Department of Nutrition, The University of North Carolina at Greensboro, Greensboro, NC 27402, USA

Received 20 February 2003; received in revised form 20 May 2003; accepted 15 June 2003

Abstract

Background: Atherogenesis is a complex process involving both a low-grade inflammation and a disturbed lipid profile. Although dietary fish and fish oil improve the latter of these two risk factors, their impact on the former is less clear.

Objective: This study addressed the effect of supplementation with fish oil in doses achievable with diet on serum C-reactive protein (CRP), interleukin-6 (IL-6), and the lipid profile.

Methods and results: Thirty healthy subjects taking HRT were randomly divided into three groups and supplemented for five weeks with 14g/day safflower oil (SO), 7g/day of both safflower oil and fish oil (LFO), or 14g/day fish oil (HFO). Measurements included serum high-sensitivity CRP, IL-6 in plasma and in cell culture supernatant collected from 24-hr lipopolysaccharide (LPS)-stimulated whole blood, and lipid profile markers. CRP and IL-6 were adjusted for body mass index (BMI). Fish oil supplementation significantly decreased CRP and IL-6 compared to SO, with a greater effect in the LFO than HFO groups. Plasma triacylglycerol (TG) and the TG/HDL-C ratio were significantly lower in the HFO compared to the SO group.

Conclusions: These results suggest that dietary fish oil may decrease the risk for cardiovascular disease through the modulation of both plasma lipids and inflammatory markers in healthy postmenopausal women.

Keywords: C-reactive protein; Interleukin-6; Triacylglycerol/HDL-cholesterol; Fish oil; HRT

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States. The main factor leading to CVD is atherosclerosis manifested at the coronary, cerebral, and peripheral level of the arterial system. The mechanism is a generalized cellular and humoral inflammatory response that leads to the formation of the atheromatous plaque [1]. Key participants in this process are the proinflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), IL-6, and acute phase reactants (APR) such as CRP. The potential role of CRP as a marker of subclinical atherosclerosis in identifying at-risk individuals has been studied in healthy women, healthy men, and elderly [2-5]. Postmenopausal women are considered at-risk individuals because of the imbalanced production of estrogens that renders them at risk for CVD [6]. HRT has been shown to increase CRP concentrations compared to non-users, and this effect is irrespective of the hormonal preparation [7]. Additional factors that may up-regulate CRP levels in healthy individuals are age [8], body mass index (BMI) [9], level of physical activity [10], smoking [11], alcohol consumption [12], and the polymorphism of genes associated with CRP production [13-15]. In addition to inflammation, serum lipids have been shown to play an important role in atherogenesis. After menopause changes in the lipid profile consist in increased triacylglycerol (TG), low density lipoprotein-cholesterol (LDL-C), and decreased high-density lipoprotein-cholesterol (HDL-C) concentrations, and constitute independent risk factors for CVD [6]. In postmenopausal women, HDL-C/TG ratio has emerged as a better predictor of myocardial infarction than the routinely used TC/HDL-C and LDL-C/HDL-C ratios [16].

Coldwater fish are rich in long chain polyunsaturated

* Corresponding author. Tel.: +1-336-256-0426; fax: +1-336-334-3009.
E-mail address: rcwander@uncg.edu (R.C. Wander).

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Inverse association of erythrocyte n-3 fatty acid levels with inflammatory biomarkers in patients with stable coronary artery disease: The Heart and Soul Study

Ramin Farzaneh-Far[a,*], William S. Harris[b], Sachin Garg[a], Beeya Na[c], Mary A. Whooley[a,c]

[a] Department of Medicine, University of California San Francisco, United States
[b] Sanford School of Medicine and Research, University of South Dakota, United States
[c] Veterans Affairs Medical Center, San Francisco, CA, United States

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ABSTRACT

Objective: Dietary intake of polyunsaturated n-3 fatty acids has been associated with a reduced incidence of adverse cardiovascular events. The protective mechanisms involved are not fully understood, but may include anti-inflammatory factors. We sought to investigate the relationship between n-3 fatty acid levels in erythrocyte membranes and markers of systemic inflammation in 992 individuals with stable coronary artery disease.

Methods: Cross-sectional associations of C-reactive protein (CRP) and Interleukin-6 (Il-6) with docosahexaenoic acid (DHA) and eicosapentaenoic acid (EHA) were evaluated in multivariable linear regression models adjusted for demographics, cardiovascular risk factors, medication use, exercise capacity, body-mass index, and waist-to-hip ratio.

Results: After multivariable adjustment, n-3 fatty acid levels (DHA + EPA) were inversely associated with CRP and IL-6. The inverse association of n-3 fatty acids with CRP and IL-6 was not modified by demographics, body-mass index, smoking, LDL-cholesterol, or statin use (p values for interaction> 0.1).

Conclusions: In patients with stable coronary artery disease, an independent and inverse association exists between n-3 fatty acid levels and inflammatory biomarkers. These findings suggest that inhibition of systemic inflammation may be a mechanism by which n-3 fatty acids prevent recurrent cardiovascular events.

1. Introduction

Epidemiologic studies have demonstrated a protective effect of dietary n-3 fatty acids on adverse cardiovascular events, particularly sudden cardiac death [1–4]. The mechanisms underlying this protective effect are not well understood, and may include anti-inflammatory factors [5]. Systemic inflammation is a key component in the development and progression of atherosclerosis, and the circulating inflammatory biomarkers C-reactive protein (CRP) and interleukin-6 (IL-6) are independent risk factors for cardiovascular disease [6,7]. The n-3 fatty acids exhibit anti-inflammatory properties which have proven useful in the treatment of systemic inflammatory diseases such as rheumatoid arthritis and Crohn's disease [8].The relationship between blood levels of n-3 fatty acids and inflammatory biomarkers in persons with stable coronary artery disease has not previously been reported. The primary aim of this study was to examine the relationship between blood levels of two n-3 fatty acids (docosahexaenoic acid, eicosapentaenoic acid)[1] and two inflammatory biomarkers (CRP, IL-6). A secondary aim was to determine whether the relationship between n-3 fatty acids and inflammatory biomarkers was modified by demographics, body-mass index, smoking, LDL-cholesterol, or statin use.

2. Methods

2.1. Participants

The Heart and Soul Study is a prospective cohort study investigating the influence of psychosocial factors on cardiovascular events in outpatients with stable coronary artery disease. The enrollment process for the Heart and Soul Study has been pre-

* Corresponding author at: Department of Medicine, Box 0124, University of California San Francisco, CA-94143-0124, United States. Tel.: +1 415 742 0814; fax: +1 415 376 1175.
E-mail address: rfarzanehfar@medicine.ucsf.edu (R. Farzaneh-Far).

[1] The term "n-3 fatty acids" in this paper refers to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Alpha-linoleic acid, a vegetable oil-derived n-3 fatty acid was not studied.

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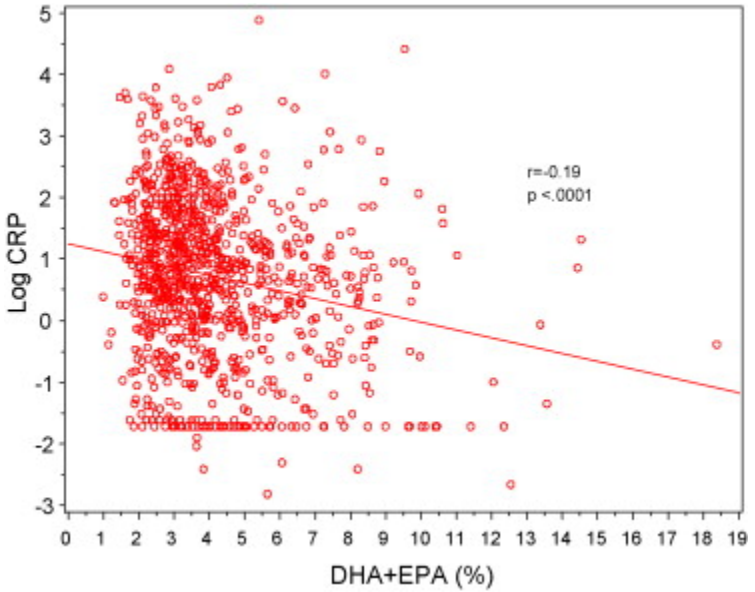
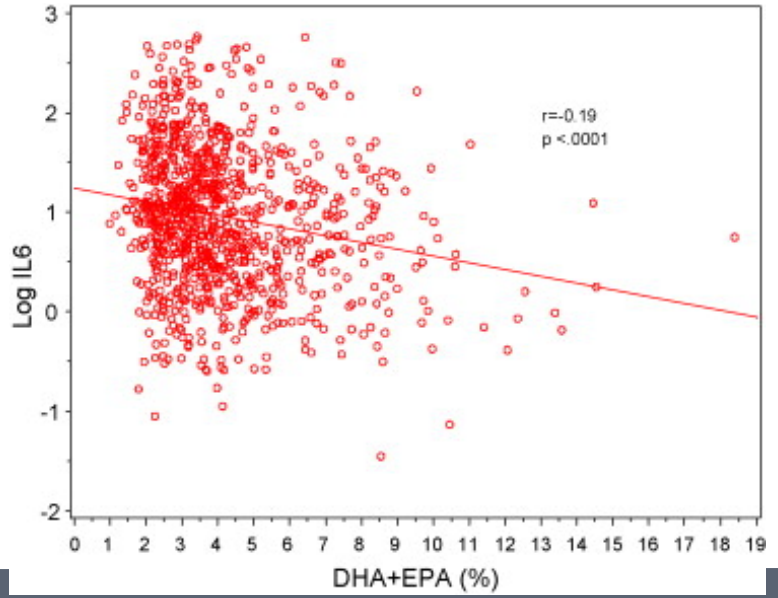




SPM defining actions

Specialized Pro-Resolving Mediators (SPMs)

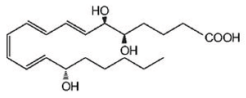
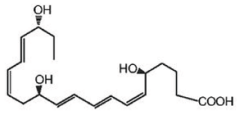
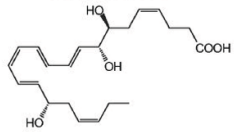
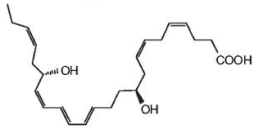
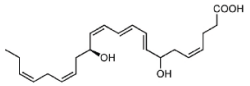
SPMs shorten resolution interval

Neutrophils

- Stop PMN transmigration and chemotaxis, brake eosinophils
- Block prostaglandins and leukotrienes
- Reduce cytokine release and TNF-α release actions

Monocytes Macrophages

- Non-phlogistic monocyte recruitment
- Uptake and removal of apoptotic PMN and microbial particles by macrophages
- Enhance anti-microbial defense mechanisms and clearance at mucosal surfaces

Precursors	Arachidonic acid (AA)	Eicosapentaenoic acid (EPA)		Docoshexaenoic acid (DHA)	
Families	Lipoxins	Resolvins E-series	Resolvins D-series	Protectins (Neuroprotectin D1)	Maresins
Some target cells	Neutrophils, Monocytes Macrophages	Mucosal epithelial cells, Neutrophils	Neutrophils Macrophages	Endothelial cells	T cells, Microglial cells	Monocytes Macrophages, Neutrophils
SPM specific actions by potencies and stereoselectivity	**LXA_4** ⇩ PMN-mediated tissue damage ⇩ Pain signals ⇩ Angiogenesis and cell proliferation ⇩ Adhesion PMN ⇧ Phagocytosis and IL-10 production	**RvE1** ⇩ DC IL-12 production and migration ⇩ Phosphorylation signals Inhibit NF-κB reporter gene activation ⇩ Reduce organ fibrosis ⇧ Regulates PMN apoptosis ⇧ Mucosal clearance of PMN by CD55 ⇧ LXA_4 production ⇧ Microbial killing	**RvD1** ⇩ Adhesion receptors ⇩ ROS genera tion & pro-inflammatory cytokines (TNF-α, IL-8) ⇩ PMN transmigration ⇩ Reduce organ fibrosis ⇧ Phagocytosis and IL-10 production ⇧ Organ production (Lung)	**RvD2** ⇩ PMN adhesion to endothelial cells ⇧ Nitric oxide and prostacyclin in endothelial cells ⇧ Microbial killing and clearance	**PD1-NPD1** ⇩ NF-κB and COX-2 expression ⇩ Renal fibrosis ⇩ T-cell migration ⇩ TNF and IFN-γ release ⇩ Microglial cell cytokines ⇧ Protection of retinal pigmented epithelial cells ⇧ Neuroprotective actions ⇧ CCR5 expression on T-cells	**MaR1** ⇧ Tissue regeneration ⇩ Reduces pain ⇧ Macrophage phenotype switch from M1 to M2
Molecule images	 Lipoxin A4	 Resolvin E1	 Resolvin D1	 Protectin D1	 Maresin 1

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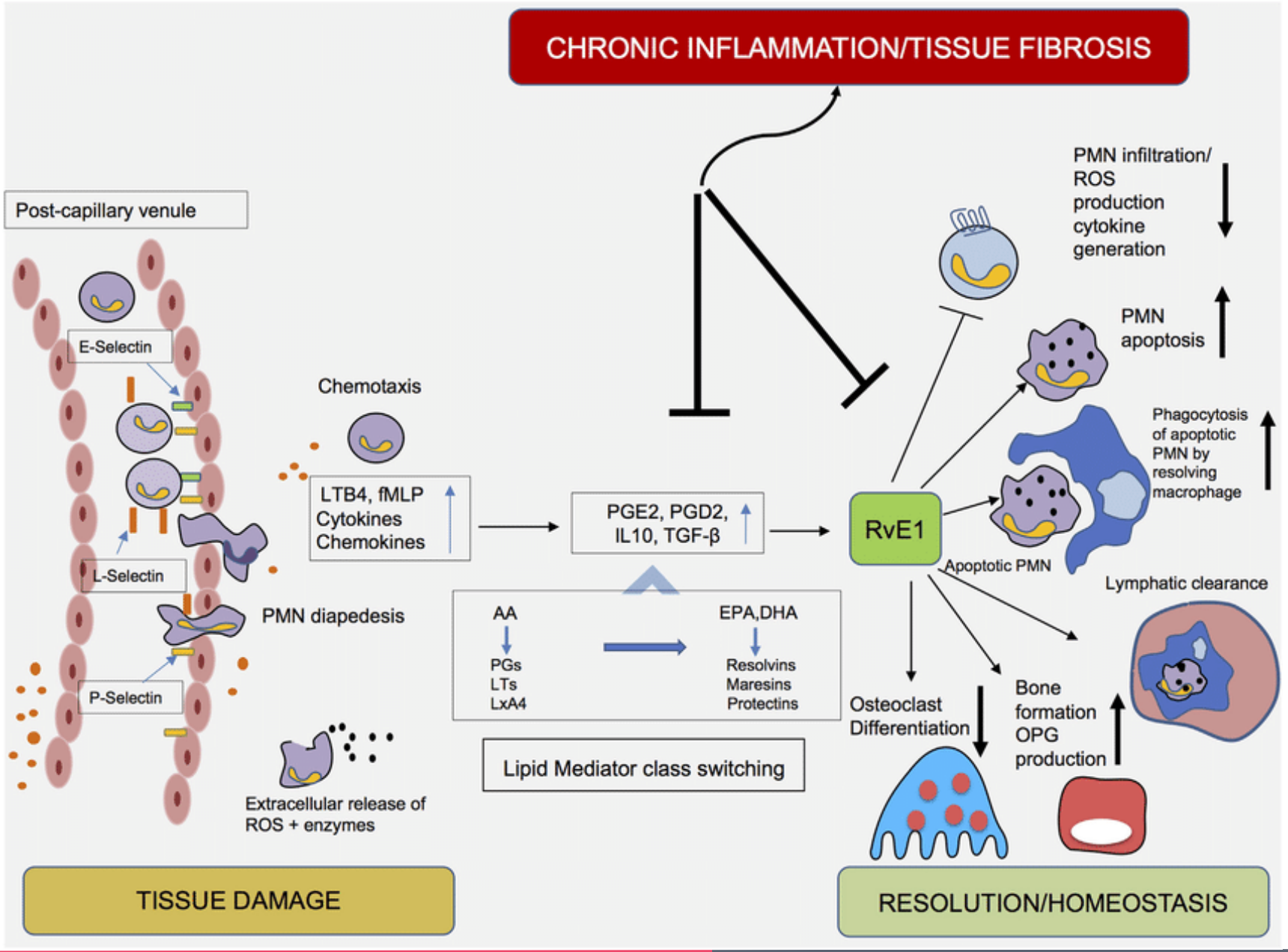

Front Immunol. 2017 Dec 14;8:1682.

The Journal of Nutrition
Nutrition and Disease

Serum C-Reactive Protein Concentrations Are Inversely Associated with Dietary Flavonoid Intake in U.S. Adults[1]

Ock Kyoung Chun,[2,4] Sang-Jin Chung,[3] Kate J. Claycombe,[2] and Won O. Song[2]*

[2]Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824 and [3]Department of Foods and Nutrition, Kookmin University, Seoul, Korea 136-702

Abstract

Serum C-reactive protein (CRP) is a biomarker for chronic inflammation and a sensitive risk factor for cardiovascular diseases. Though CRP has been reported to be related to food intake, there is no documentation of a direct association with flavonoid intake. We aimed to test the associations between dietary flavonoid intake and serum CRP concentrations among U.S. adults after adjusting for dietary, sociodemographic, and lifestyle factors. Data from the NHANES 1999–2002 were used for this cross-sectional study. Subjects were ≥19-y-old adults ($n = 8335$), and did not include pregnant and/or lactating women. Flavonoid intake of U.S. adults was estimated by the USDA flavonoid databases matched with a 24-h dietary recall in NHANES 1999–2002. The serum CRP concentration was higher in women, older adults, blacks, and smokers, and in those with high BMI or low exercise level, and in those taking NSAID, than in their counterparts ($P < 0.01$). Intakes of apples and vegetables were inversely associated with serum CRP concentrations after adjusting for covariates ($P < 0.05$). Total flavonoid and also individual flavonol, anthocyanidin, and isoflavone intakes were inversely associated with serum CRP concentration after adjusting for the covariates ($P < 0.05$). Among the flavonoid compounds investigated, quercetin, kaempferol, malvidin, peonidin, daidzein, and genistein had inverse associations with serum CRP concentration ($P < 0.05$). These associations did not change even after the additional adjustment for fruit and vegetable consumption. Our findings demonstrate that intake of dietary flavonoids is inversely associated with serum CRP concentrations in U.S. adults. Intake of flavonoid-rich foods may thus reduce inflammation-mediated chronic diseases. J. Nutr. 138: 753–760, 2008.

TABLE 5 Serum CRP concentration by individual flavonoid intake of U.S. adults[1–3]

	Intake (tertiles)				*P*-value for
Flavonoid	Nonconsumers	T1	T2	T3	linear trend
Quercetin					
n	254	2850	2857	2848	
Range	0	0–2.9	2.9–9.0	>9.0	
CRP, mg/L	2.56 ± 0.12	1.97 ± 0.04	1.86 ± 0.02	1.77 ± 0.03	<0.01
Kaempferol					
n	827	2700	2527	2655	
Range	0	0–0.1	0.1–0.5	>0.5	
CRP, mg/L	2.20 ± 0.05	1.90 ± 0.04	1.84 ± 0.03	1.80 ± 0.04	<0.01

Introduction

Cardiovascular diseases (CVD)[5] are the leading causes of death worldwide (1). A growing body of evidence suggests that systemic inflammation plays a key role in the pathogenesis of CVD (2–5). C-reactive protein (CRP) is 1 of the major acute-phase reactants secreted by the liver in response to increased levels of inflammatory cytokines such as interleukin-6 and interleukin-1β (6). High concentrations of CRP have consistently been linked to an increased risk for CVD and thus have been used as a sensitive predictor of acute cardiovascular events compared with other widely used biomarkers such as total and LDL cholesterol (7,8).

Even though several population-based observational or randomized clinical trial studies (9–13) have reported that serum CRP concentrations are inversely associated with dietary intake of fruits, vegetables, and tea, which are rich in polyphenolic antioxidants such as flavonoids (14,15), data directly delineating the protective mechanisms are scant. One proposed mechanism for the benefit of dietary flavonoids is the antioxidant properties (16–23). These polyphenols are effective scavengers of reactive oxygen species (24) and can inhibit lipid peroxidation through chelation of transition metal ions (25,26) or their chain breaking antioxidants (27). These properties suggest that flavonoids might prevent LDL oxidation, an early key inflammatory event in the development of atherosclerosis (28). However, studies on the antiinflammatory effects of specific flavonoid compounds or flavonoid-rich foods are still controversial (29–31). We speculate that, in addition to being confounded by high baseline antioxidant vitamin intakes and sociodemographic status of the participants, inaccurate estimates of dietary intake of flavonoids due to incomplete flavonoid food composition data in those studies have contributed to the inconsistent findings (11,30,32,33).

Recently, we reported estimates of total dietary flavonoid intake of U.S. population based on the recently released USDA flavonoid databases matched with food consumption data from the NHANES 1999–2002 (14). Building upon our previous research scheme to estimate flavonoid intake, we aimed to test the associations between dietary flavonoid intake and serum CRP concentrations among U.S. adults after adjusting for dietary,

[1] Author disclosures: O. K. Chun, S.-J. Chung, K. J. Claycombe, and W. O. Song, no conflicts of interest.
[4] Present address: Department of Nutrition and Dietetics, East Carolina University, Greenville, NC 27858.
[5] Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; DR, dietary recall; MET, metabolic equivalent; NF, nuclear factor; NSAID, nonsteroidal antiinflammatory drug; WHS, Women's Health Study.
* To whom correspondence should be addressed. E-mail: song@msu.edu.

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Journal of Thrombosis and Haemostasis, **5**: 1309–1317

ORIGINAL ARTICLE

Effect of wine phenolics on cytokine-induced C-reactive protein expression

G. KAUR,* L. V. M. RAO,* A. AGRAWAL† and U. R. PENDURTHI*

**Center for Biomedical Research, The University of Texas Health Center at Tyler, Tyler, TX; and†Department of Pharmacology, East Tennessee State University, Johnson City, TN, USA*

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Summary. *Background:* Elevation of C-reactive protein (CRP) levels in blood was recognized as one of the cardiac disease risk factors. Consumption of wine is shown to reduce the risk from heart disease and improve longevity. *Objectives:* In the present study, we evaluated the effect of various wine polyphenolic compounds and several active synthetic derivatives of resveratrol on the inflammatory cytokines (IL-1ß + IL-6)-induced CRP expression in Hep3B cells. *Results:* Among the wine phenolics tested, quercetin and resveratrol, in a dose-dependent manner, suppressed cytokine-induced CRP expression. Two of the synthetic derivatives of resveratrol, R3 and 7b, elicited a fiftyfold higher suppressive effect compared with resveratrol. The inhibitory effects of resveratrol and its derivatives on CRP expression were at the level of mRNA production. Investigation of signaling pathways showed that the cytokines induced the phosphorylation of p38 and p44/42 MAP kinases. Inhibitors of p38 and p44/42 mitogen-activated protein kinase (MAPK) activation inhibited CRP expression, implicating the involvement of both pathways in cytokine-induced CRP expression. These data revealed a previously unrecognized role of the p44/42 MAPK signaling pathway in CRP expression. Wine polyphenolics or the synthetic compounds of resveratrol did not affect cytokine-activated phosphorylation of these MAPKs. *Conclusions:* Wine phenolics inhibit CRP expression; however, to do so, they do not utilize the MAPK pathways.

Keywords: atherosclerosis, C-reactive protein, inflammation, quercetin, resveratrol.

Correspondence: Usha R. Pendurthi, Center for Biomedical Research, The University of Texas Health Center at Tyler, 11937 US Highway 271, Tyler, TX 75703, USA.
Tel.: + 1 903 877 7342; fax: + 1 903 877 7426; e-mail: usha.pendurthi@uthct.edu

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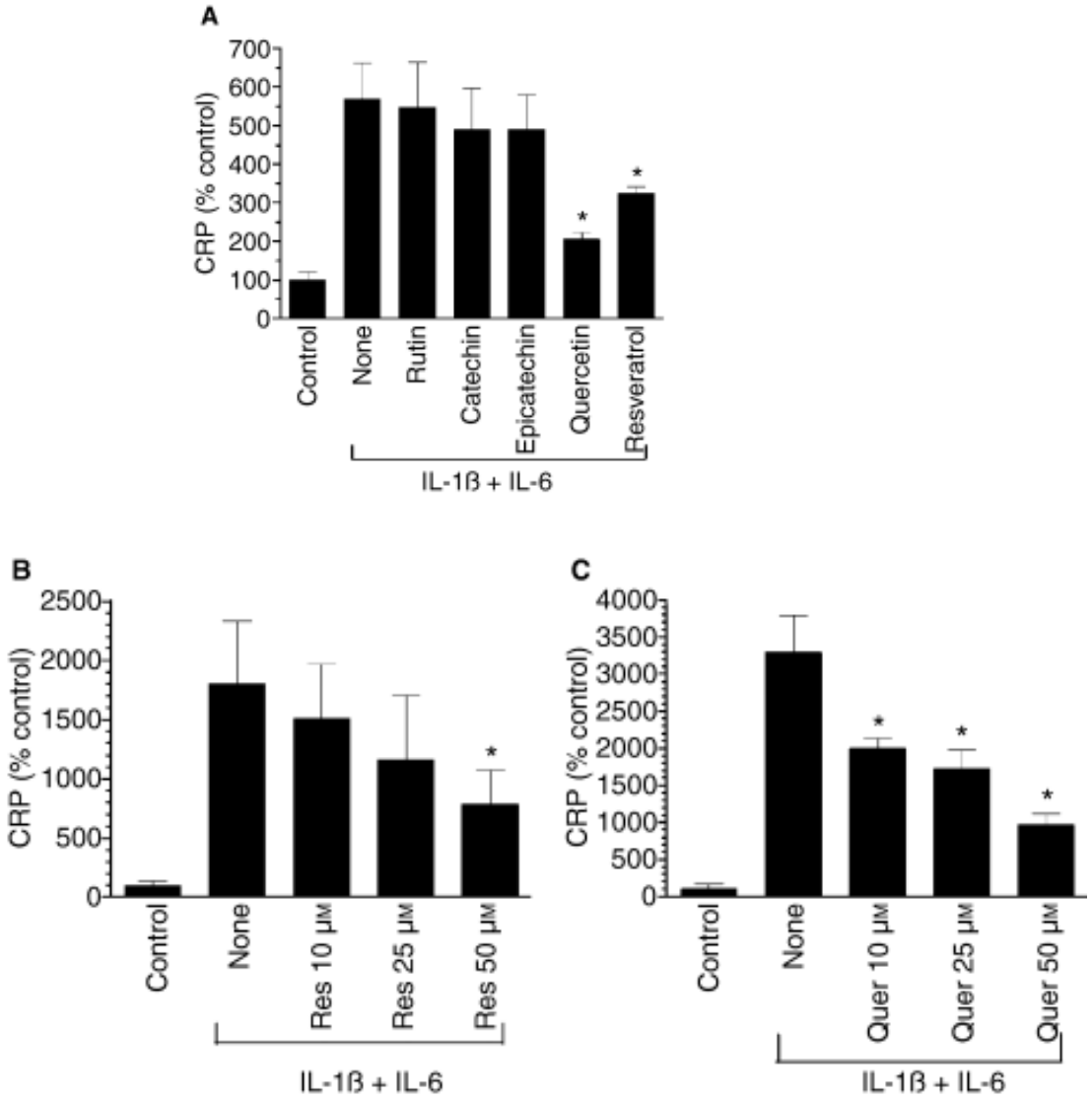
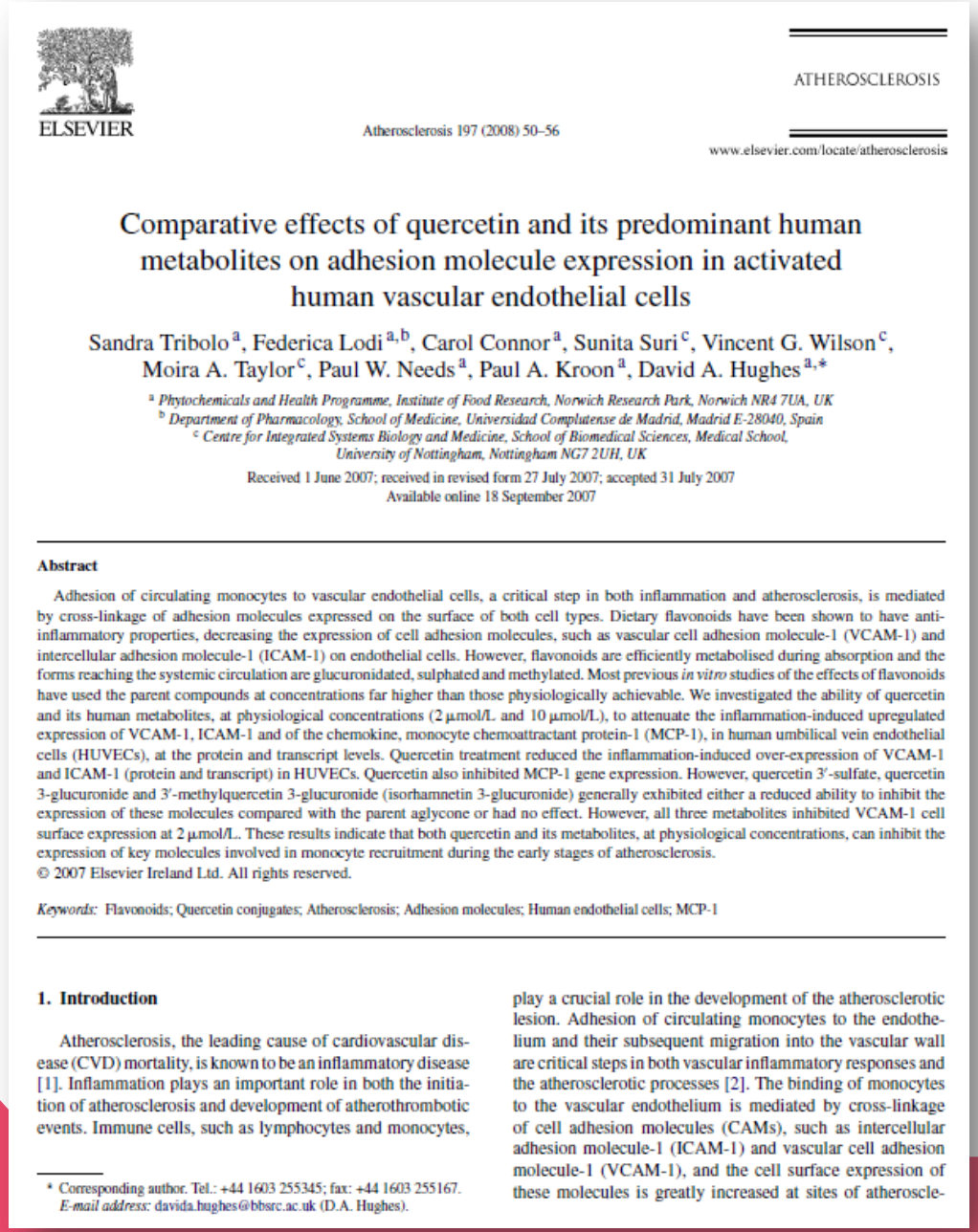
Introduction

C-reactive protein (CRP) is an acute phase protein whose concentration also increases under chronic inflammatory conditions [1]. Epidemiological studies suggest that CRP is both a marker of and a causal agent in the development of atherosclerosis [2–5]. Although it is generally accepted that CRP is a well-proven clinical marker of increased cardiovascular risk, still it is debated whether CRP is a causal agent in atherogenesis [6,7]. Nonetheless, an accumulating evidence from *in vitro* and *in vivo* studies strongly suggest that CRP acts as a proatherogenic factor and promotes atherothrombosis [8,9]. CRP is shown to promote endothelial cell activation and dysfunction [10,11], affect vascular smooth muscle cell migration and proliferation [7,12,13], induce changes in matrix biology [14], and promote coagulation [15]. If CRP plays a role in pathogenesis of atherosclerosis, then the blockade of CRP synthesis or its actions would be beneficial in inhibiting the development of atherosclerosis.

Overwhelming epidemiological evidence suggests that moderate consumption of alcoholic beverages, particularly red wine, lowers mortality rates from coronary heart diseases [16–21]. Cardiovascular benefits associated with moderate wine consumption have been thought to stem, at least partly, from antioxidant [22–24], anti-inflammatory [25–27], antiplatelet [28–30] and anticoagulant [31,32] activities of wine phenolics, particularly resveratrol. Resveratrol is shown to mimic calorie restriction by stimulating Sir2 (sirtuin 2, a histonedeacetylase), increasing DNA stability and extending lifespan of yeast by 70% [33]. Recent studies showed that resveratrol improves health and survival of mice in a high calorie diet by producing changes associated with longer lifespan, such as increased insulin sensitivity, reduced insulin-like growth factor-1 levels, and increased mitochondrial numbers [34].

Recent epidemiological studies found an inverse/U-shaped relation between alcoholic beverage consumption and plasma concentration of CRP expression [35]. In age-adjusted analyses, wine consumption appears to be more effective in reducing CRP levels compared with other alcoholic beverages. However, this difference disappeared when BMI was taken into account.





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Comparative effects of quercetin and its predominant human metabolites on adhesion molecule expression in activated human vascular endothelial cells

Sandra Tribolo [a], Federica Lodi [a,b], Carol Connor [a], Sunita Suri [c], Vincent G. Wilson [c], Moira A. Taylor [c], Paul W. Needs [a], Paul A. Kroon [a], David A. Hughes [a,*]

[a] *Phytochemicals and Health Programme, Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, UK*
[b] *Department of Pharmacology, School of Medicine, Universidad Complutense de Madrid, Madrid E-28040, Spain*
[c] *Centre for Integrated Systems Biology and Medicine, School of Biomedical Sciences, Medical School, University of Nottingham, Nottingham NG7 2UH, UK*

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Abstract

Adhesion of circulating monocytes to vascular endothelial cells, a critical step in both inflammation and atherosclerosis, is mediated by cross-linkage of adhesion molecules expressed on the surface of both cell types. Dietary flavonoids have been shown to have anti-inflammatory properties, decreasing the expression of cell adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) on endothelial cells. However, flavonoids are efficiently metabolised during absorption and the forms reaching the systemic circulation are glucuronidated, sulphated and methylated. Most previous *in vitro* studies of the effects of flavonoids have used the parent compounds at concentrations far higher than those physiologically achievable. We investigated the ability of quercetin and its human metabolites, at physiological concentrations (2 μmol/L and 10 μmol/L), to attenuate the inflammation-induced upregulated expression of VCAM-1, ICAM-1 and of the chemokine, monocyte chemoattractant protein-1 (MCP-1), in human umbilical vein endothelial cells (HUVECs), at the protein and transcript levels. Quercetin treatment reduced the inflammation-induced over-expression of VCAM-1 and ICAM-1 (protein and transcript) in HUVECs. Quercetin also inhibited MCP-1 gene expression. However, quercetin 3′-sulfate, quercetin 3-glucuronide and 3′-methylquercetin 3-glucuronide (isorhamnetin 3-glucuronide) generally exhibited either a reduced ability to inhibit the expression of these molecules compared with the parent aglycone or had no effect. However, all three metabolites inhibited VCAM-1 cell surface expression at 2 μmol/L. These results indicate that both quercetin and its metabolites, at physiological concentrations, can inhibit the expression of key molecules involved in monocyte recruitment during the early stages of atherosclerosis.

Keywords: Flavonoids; Quercetin conjugates; Atherosclerosis; Adhesion molecules; Human endothelial cells; MCP-1

1. Introduction

Atherosclerosis, the leading cause of cardiovascular disease (CVD) mortality, is known to be an inflammatory disease [1]. Inflammation plays an important role in both the initiation of atherosclerosis and development of atherothrombotic events. Immune cells, such as lymphocytes and monocytes, play a crucial role in the development of the atherosclerotic lesion. Adhesion of circulating monocytes to the endothelium and their subsequent migration into the vascular wall are critical steps in both vascular inflammatory responses and the atherosclerotic processes [2]. The binding of monocytes to the vascular endothelium is mediated by cross-linkage of cell adhesion molecules (CAMs), such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and the cell surface expression of these molecules is greatly increased at sites of atheroscle-

* Corresponding author. Tel.: +44 1603 255345; fax: +44 1603 255167.
E-mail address: davida.hughes@bbsrc.ac.uk (D.A. Hughes).

- Physiologically relevent concentrations of Quercetin and Q-metabolites reduce:
  - VCAM-1 expression
  - ICAM-1 expression
  - MCP-1 expression

  3 key components to the initiation and progression of atherosclerosis.

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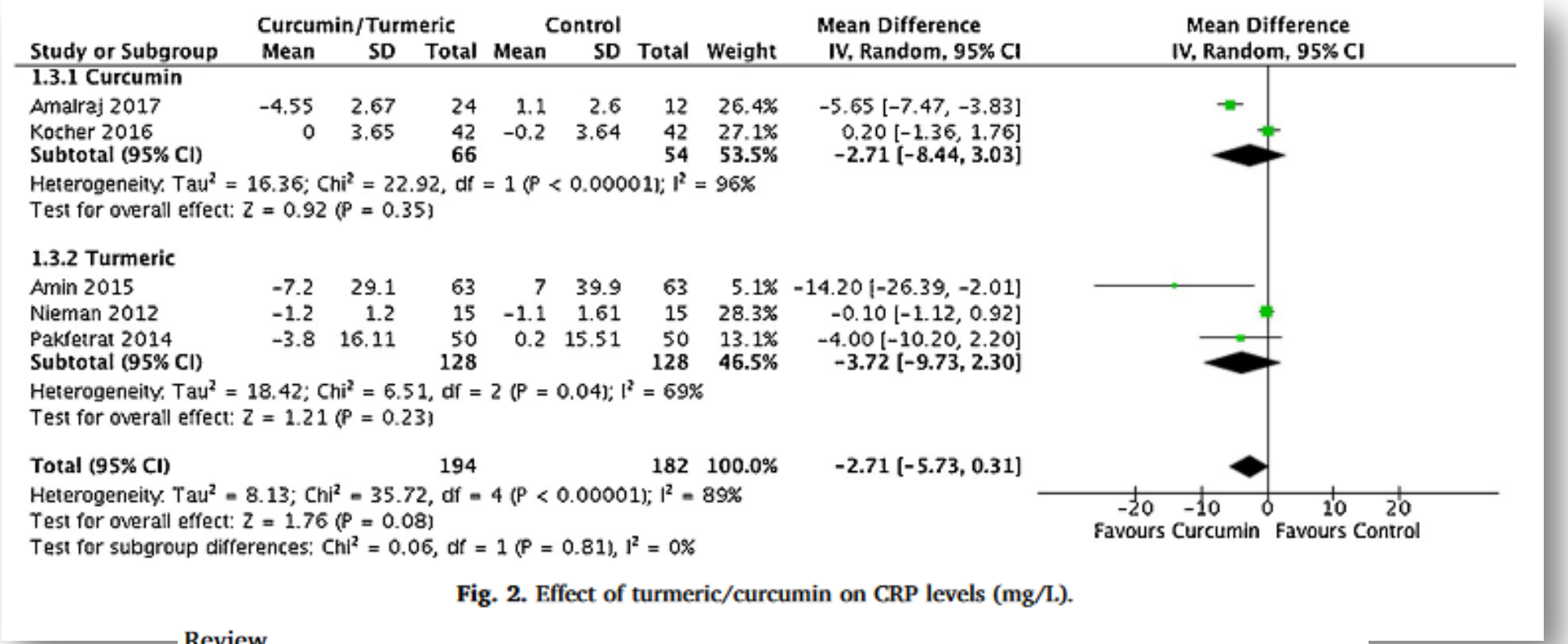
Turmeric (*Curcuma longa*)

- One of the most potent natural anti-inflammatory agents (NFκB-inhibition)
- Decreases prostaglandin production by inhibiting both COX and LOX enzymes
- Strong antioxidant
- Boswellia and Ginger work in similar fashion

Turmeric and Curcumin and CRP levels



Study or Subgroup	Curcumin/Turmeric Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
1.3.1 Curcumin								
Amalraj 2017	-4.55	2.67	24	1.1	2.6	12	26.4%	-5.65 [-7.47, -3.83]
Kocher 2016	0	3.65	42	-0.2	3.64	42	27.1%	0.20 [-1.36, 1.76]
Subtotal (95% CI)			**66**			**54**	**53.5%**	**-2.71 [-8.44, 3.03]**
Heterogeneity: Tau^2 = 16.36; Chi^2 = 22.92, df = 1 (P < 0.00001); I^2 = 96%								
Test for overall effect: Z = 0.92 (P = 0.35)								
1.3.2 Turmeric								
Amin 2015	-7.2	29.1	63	7	39.9	63	5.1%	-14.20 [-26.39, -2.01]
Nieman 2012	-1.2	1.2	15	-1.1	1.61	15	28.3%	-0.10 [-1.12, 0.92]
Pakfetrat 2014	-3.8	16.11	50	0.2	15.51	50	13.1%	-4.00 [-10.20, 2.20]
Subtotal (95% CI)			**128**			**128**	**46.5%**	**-3.72 [-9.73, 2.30]**
Heterogeneity: Tau^2 = 18.42; Chi^2 = 6.51, df = 2 (P = 0.04); I^2 = 69%								
Test for overall effect: Z = 1.21 (P = 0.23)								
Total (95% CI)			**194**			**182**	**100.0%**	**-2.71 [-5.73, 0.31]**
Heterogeneity: Tau^2 = 8.13; Chi^2 = 35.72, df = 4 (P < 0.00001); I^2 = 89%								
Test for overall effect: Z = 1.76 (P = 0.08)								
Test for subgroup differences: Chi^2 = 0.06, df = 1 (P = 0.81), I^2 = 0%								

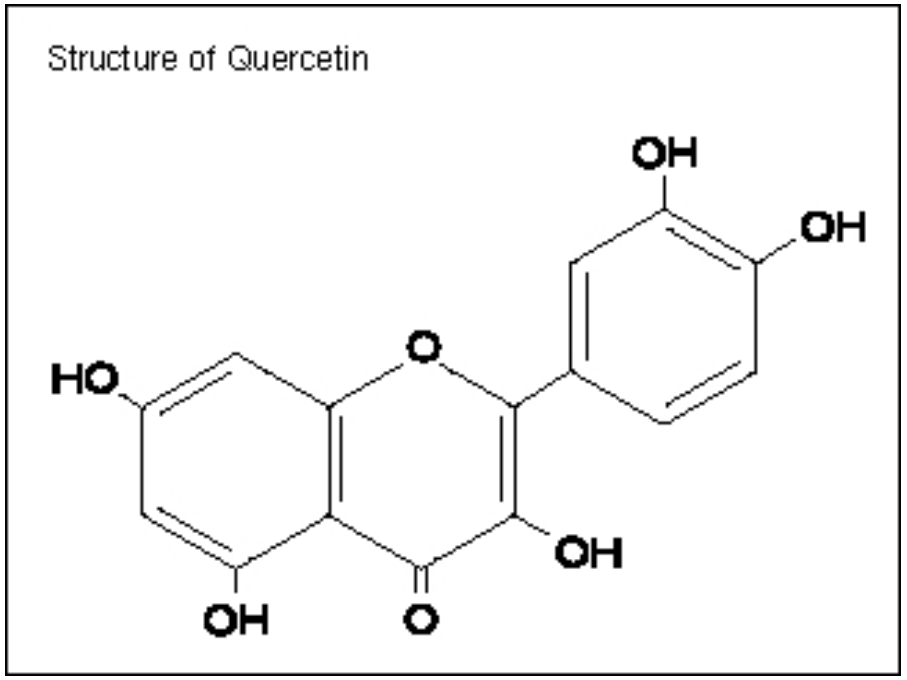
Fig. 2. Effect of turmeric/curcumin on CRP levels (mg/L).

Review

Oral turmeric/curcumin effects on inflammatory markers in chronic inflammatory diseases: A systematic review and meta-analysis of randomized controlled trials

Pharmacol Res. 2019 Aug;146:104280.

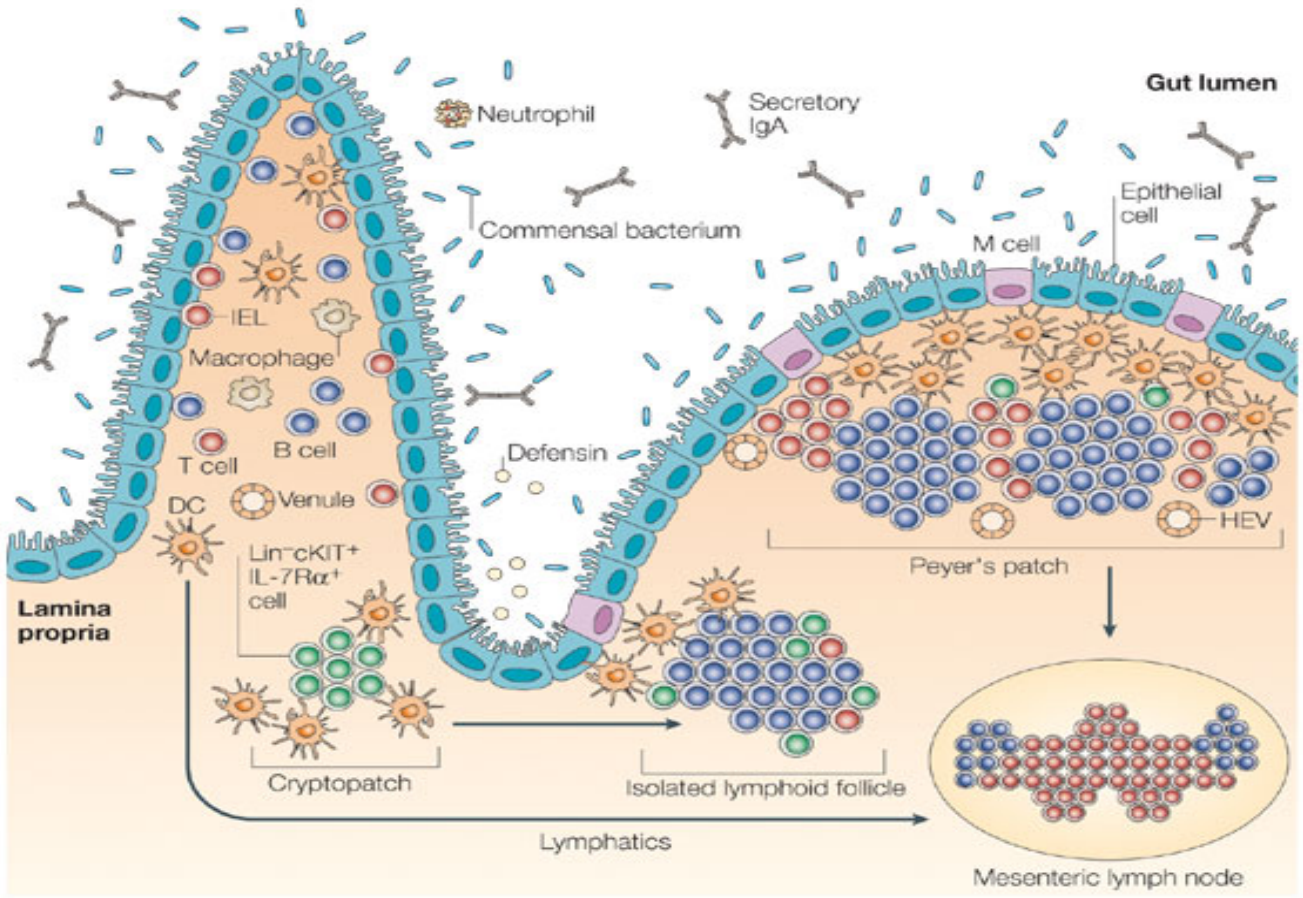
Quercetin



The aglycone of Rutin, commercially prepared from Rutin

- Mast cell stabilizer preventing degranulation
- Inhibits COX and LOX enzymes decreasing inflammatory mediators
- Strong flavonoid Anti-oxidant
- Numerous other biological activities

The Gut as a modulator of Immune Function



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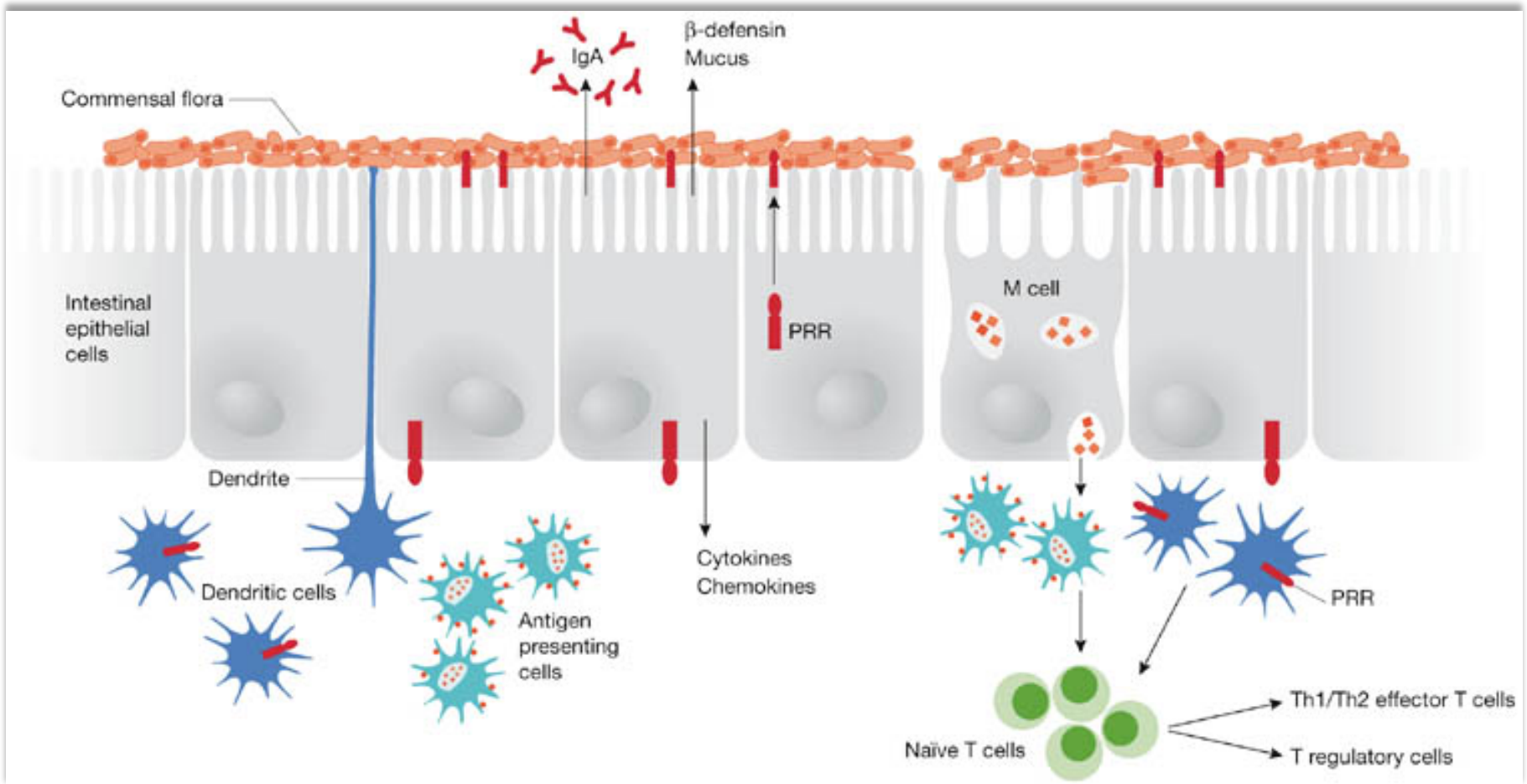
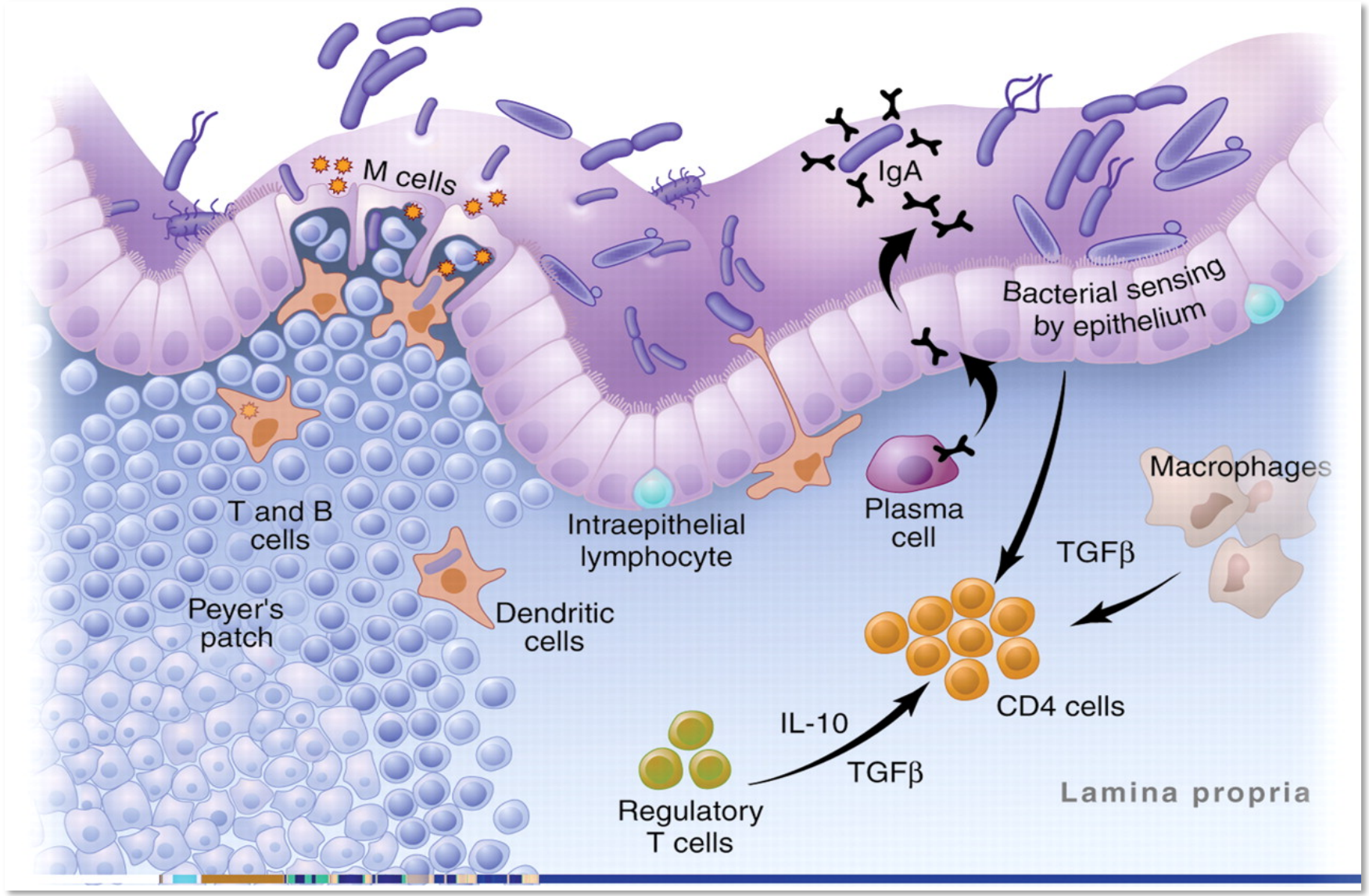
Commensal Flora and Immune Control





Keys to reducing inflammatory signaling

- Reduce pro-inflammatory inputs
  - Foods, UV radiation, stress, toxins
- Target NFkB pathways (reduce)
- Target Nrf-2 pathways (stimulate)
- Modulate COX/LOX precursors and activities
- Modulate immune responses to decrease inflammation (influence on TH cell differentiation, RAGE (glycation), PRR interactions)

Summary

- The inflammatory process is a vital first-line of defense against pathogens and other tissue damage.
- Inflammation is non-specific and can be triggered by any tissue under stress (metabolic stress, oxidative stress etc.)
- Inflammation is mediated by several layers of mediators within and between cells
- Uncontrolled inflammation is a key mediator of most chronic diseases.
- Chronic disease management must include therapies which breaks the cycle of chronic inflammatory signaling and promote resolution signaling.