We are Still Immunologists

Week 3

Ed Roy, Marie Roy, Sue Ingels, Mary Kuetemeyer, Class Moderator

Learning Objectives for Week 3

- Learn about Tolerance, controlling immune responses (Finish Innate responses by discussing resolution of inflammation in innate immunity)
- Start on adaptive immunity: structure of antibodies and T cell receptors, encoded by modifications of DNA sequences
- Learn about translation and transcription, how DNA codes for mRNA which codes for proteins

To be effective the immune system must:

1. Recognize and destroy a diversity of pathogens
   (bacteria, fungi, viruses, protozoans, helminths)

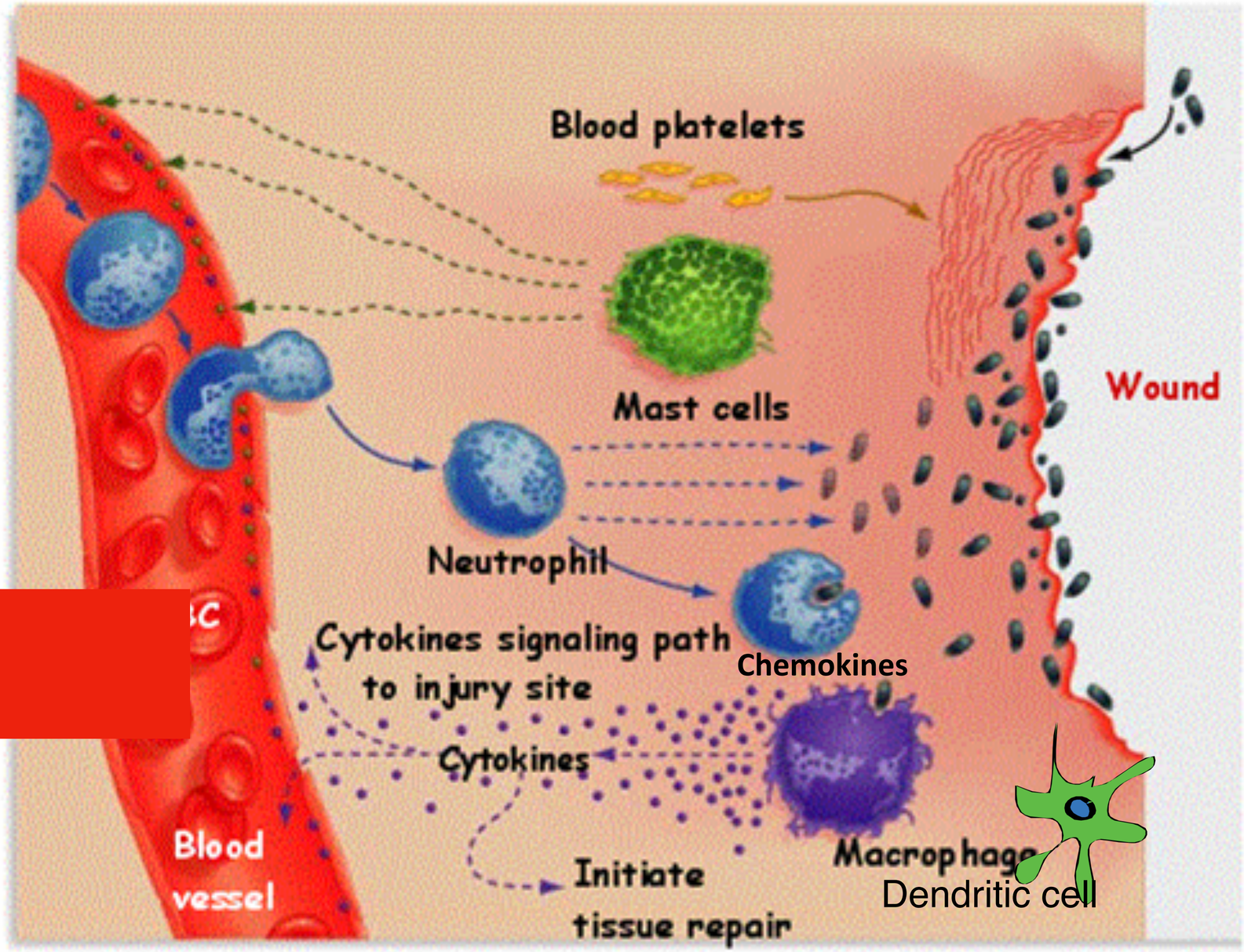
2. Recognize and destroy altered or damaged self
   (apoptotic, necrotic, effete and cancerous cells)

3. Minimize collateral damage to self
   (avoid chronic inflammation, autoimmunity)

- Innate System has mechanisms to resolve inflammation
- Adaptive System has specific “Tolerance” mechanisms to avoid attacking self antigens
- Both types act as “negative feedback” systems, initiated at the same time as activating signals

- Tolerance is achieved by intrinsic mechanisms of immune regulation and systemic mechanisms mediated by the nervous and endocrine systems

Tissue resident Macrophages and Dendritic Cells become activated by tissue damage and by microbes to release proinflammatory molecules that cause increased vasodilation and vascular leakage which enables circulating immune components to access the site of infection.



Self-Amplifying

Inflammation Resolution

It is a popular misconception that once the inflammatory response has neutralized an injurious stimulus, inflammation somehow fizzles out, possibly from proinflammatory mediator catabolism.On the contrary, the resolution of acute inflammation is a highly coordinated and active process that is controlled by endogenous ‘pro-resolving’mediators. These factors switch off leukocyte trafficking to the inflamed site, reverse vasodilation and vascular permeability, and bring about the safe disposal of inflammatory leukocytes, exudate and fibrin, thereby leading to the restoration of the inflamed tissue to its prior physiological function. Importantly, successful resolution will limit excessive tissue injury and give little opportunity for the development of chronic, immune-mediated inflammation. However, if the host is unable to neutralize the injurious agent and/or there is a failure of endogenous pro-resolving mediators to invoke resolution, then acute inflammation might perpetuate, resulting in varying degrees of tissue injury.

Gilmore 04

defervescence

Resolution of Inflammation

Gilmore ’04:

Misconception that once the inflammatory response has neutralized an injurious stimulus, inflammation somehow fizzles out

The resolution of acute inflammation is a highly coordinated and active process that is controlled by endogenous ‘pro-resolving’mediators.’

These factors switch off leukocyte trafficking to the inflamed site, reverse vasodilation and vascular permeability, and bring about the safe disposal of inflammatory leukocytes.

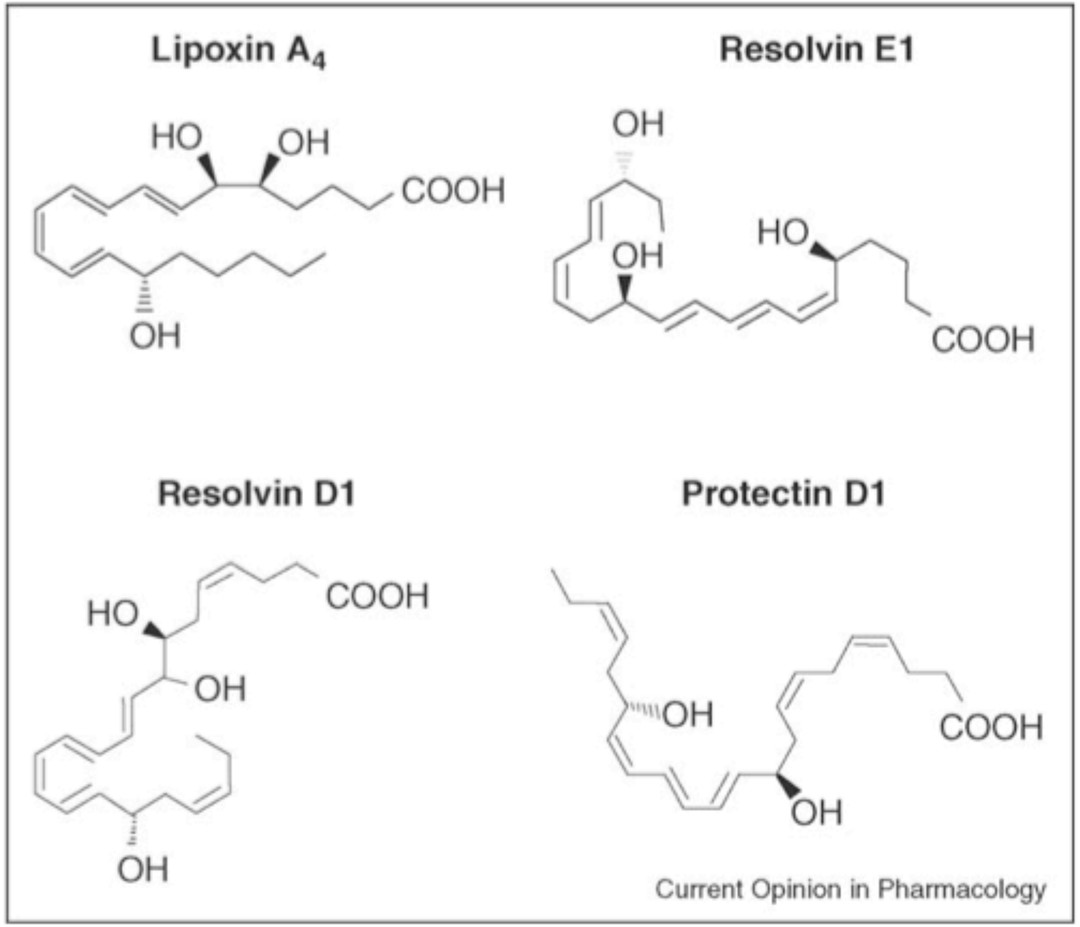
Restoration of normal physiology of tissue.

Successful resolution will limit excessive tissue injury and give little opportunity for the development of chronic, immune-mediated inflammation.

Another name for the process is “defervescence.”

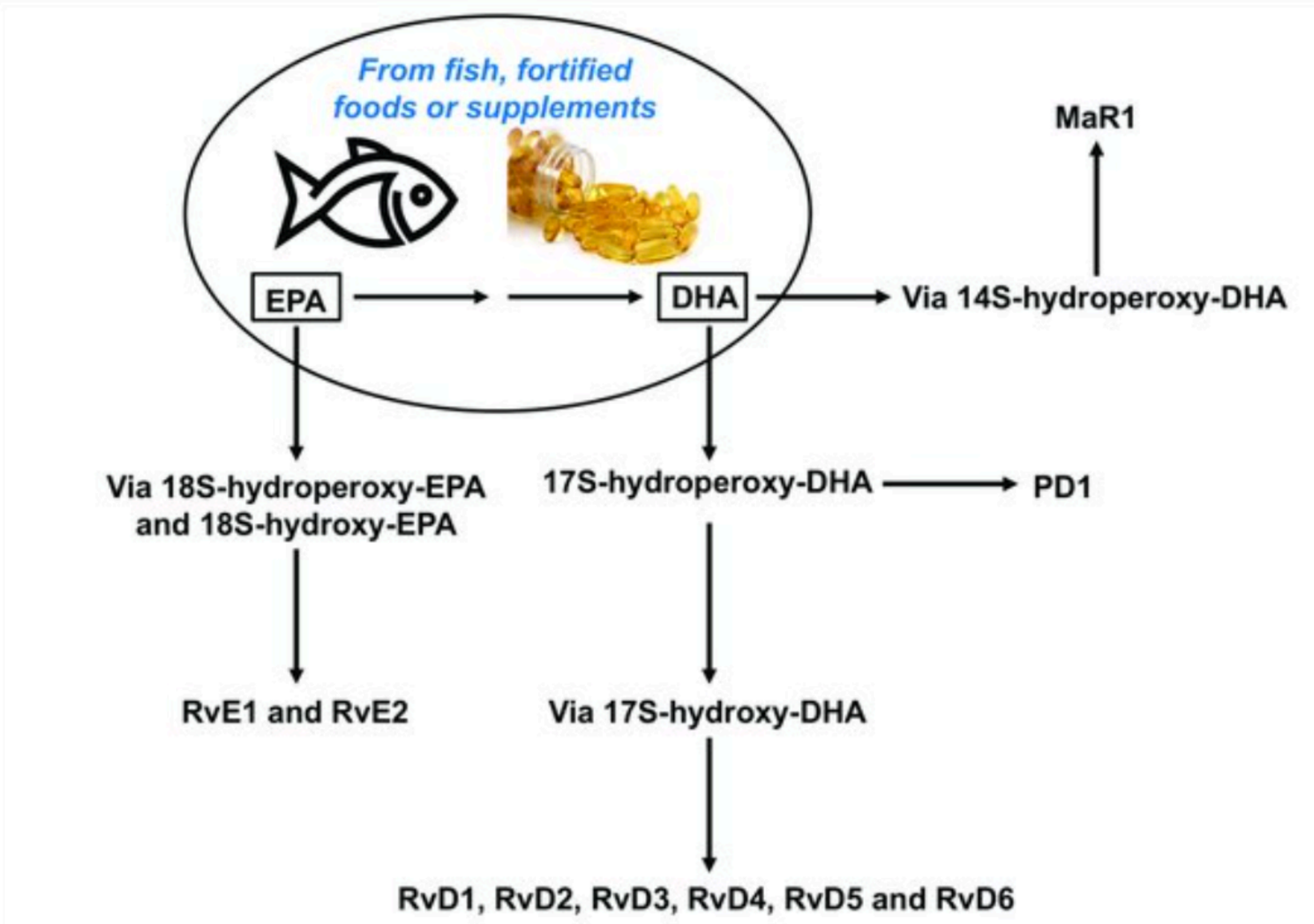
Inflammation Resolution

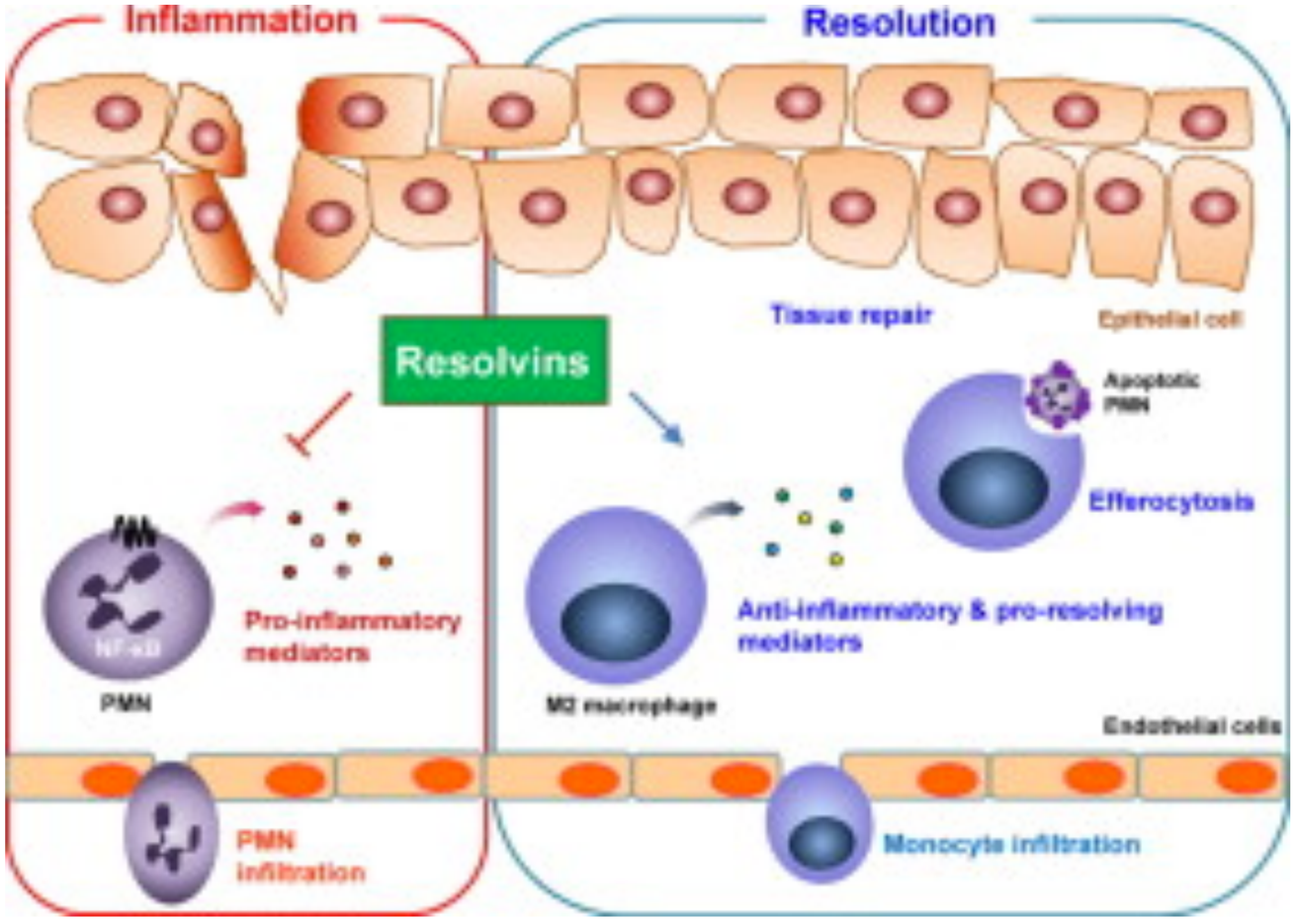
- Intracellular: signaling pathway changes
- Intercellular:Lipid mediators and lipid binding proteins
- Systemic: glucocorticoids and parasympathetic influences



Founding members in resolution. LXs were the first family of endogenous lipid mediators deriving from essential PUFAs that serve as endogenous stop signals in abrogating the inflammation phase (by preventing inflammatory cell recruitment and stopping 'cell entry') and promoting resolution (by removing inflammatory cells from the lesion site through phagocytosis of PMNs and promotion of 'cell exit'), exemplified by LXA_4. The essential ω-3 PUFAs, EPA and DHA, are converted to three novel families of lipid mediators that play pivotal roles in promoting resolution: RvE series generated from EPA, such as RvE1; RvD series generated from DHA, such as RvD1; and PDs, also generated from DHA, such as PD1 (or NPD1) that are enriched in neural systems [9–11].

Schwab 06


From fish, fortified foods or supplements
EPA
DHA
MaR1
Via 14S-hydroperoxy-DHA
Via 18S-hydroperoxy-EPA and 18S-hydroxy-EPA
17S-hydroperoxy-DHA
PD1
RvE1 and RvE2
Via 17S-hydroxy-DHA
RvD1, RvD2, RvD3, RvD4, RvD5 and RvD6


Inflammation
Resolution
Tissue repair
Epithelial cell
Resolvins
Apoptotic PMN
Efferocytosis
Pro-inflammatory mediators
PMN
Anti-inflammatory & pro-resolving mediators
M2 macrophage
Endothelial cells
PMN infiltration
Monocyte infiltration

Resolvins

- Omega-3 polyunsaturated fatty acids are precursors for resolvins
- Omega-3 polyunsaturated fatty acids being tested for COVID-19

Inflammation contributes to pathogenicity of SARS

The Role of Innate Immunity and Bioactive Lipid Mediators in COVID-19 and Influenza

Sabina Sahanic[1], Judith Löffler-Ragg[1], Piotr Tymoszuk[1], Richard Hilbe[1], Egon Demetz[1], Rebecca K Masanetz[1], Markus Theurl[2], Johannes Holfeld[3], Can Gollmann-Tepeköylü[3], Alexandar Tzankov[4], Guenter Weiss[1], Martin Giera[5] and Ivan Tancevski[1]**

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In this review, we discuss spatiotemporal kinetics and inflammatory signatures of innate immune cells specifically found in response to SARS-CoV-2 compared to influenza virus infection. Importantly, we cover the current understanding on the mechanisms by which SARS-CoV-2 may fail to engage a coordinated type I response and instead may lead to exaggerated inflammation and death. This knowledge is central for the understanding of available data on specialized pro-resolving lipid mediators in severe SARS-CoV-2 infection pointing toward inhibited E-series resolvin synthesis in severe cases. By investigating a publicly available RNA-seq database of bronchoalveolar lavage cells from patients affected by COVID-19, we moreover offer insights into the regulation of key enzymes involved in lipid mediator synthesis, critically complementing the current knowledge about the mediator lipidome in severely affected patients. This review finally discusses different potential approaches to sustain the synthesis of 3-PUFA-derived pro-resolving lipid mediators, including resolvins and lipoxins, which may critically aid in the prevention of acute lung injury and death from COVID-19.

Keywords: innate immunity, COVID-19, lipid mediator, metabololipidomics, influenza virus, macrophages and neutrophils

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The Role of Innate Immunity and Bioactive Lipid Mediators in COVID-19 and Influenza

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dicated that supplementation with omega-3 PUFA in patients
n the PaO2/FiO2 ratio, with a shorter ICU stay, a shorter
ward reduced mortality (Dushianthan et al., 2019; Langlois

Of particular interest was the observation that specific pro-resolving mediators, including RvE3 and RvD4, were increased in serum from patients with moderate COVID-19 compared to subjects with severe disease.

Omega-3 Fatty acids are precursors for anti-inflammatory mediators

Omega-3 fatty acids are high in fish oil, walnuts, flax seeds, Brussels sprouts

- Production of metabolites such as resolvins depends on precursor concentrations and enzyme concentrations

- The immune system does not operate in isolation
- It interacts with and is regulated by other physiological systems, especially the nervous system and endocrine system

Bidirectional Interactions

- Nervous system influences immune system
- Immune system influences nervous system

- Pro-inflammatory and anti-inflammatory

Ways Brain Influences Immune Responses

- Hormonal:
  - Hypothalamo-Pituitary-adrenal
    - HPA (adrenal)
      - Corticosteroids (cortisol/corticosterone)
    - Macrophage Migration Inhibitory Factor (MIF)
    - Growth hormone, opiates, prolactin
- Neuro-Hormonal (sympathetic/adrenal)
- Direct Neural (autonomic and somatic)

Evidence for neural influence

- Immune responses can be classically conditioned
- Hypothalamic lesions influence immune function
- Innervation of bone marrow, thymus, spleen and lymph nodes
- Receptors for hormones and neurotransmitters on lymphocytes

Organization of Nervous System

- Central Nervous System
  - Brain and spinal cord
- Peripheral Nervous system
  - Autonomic (afferent and efferent)
    - Enteric
    - Sympathetic
    - Parasympathetic
  - Somatic Nervous System
    - Sensory and motor

Systemic Negative Feedback (Neural and Hormonal)

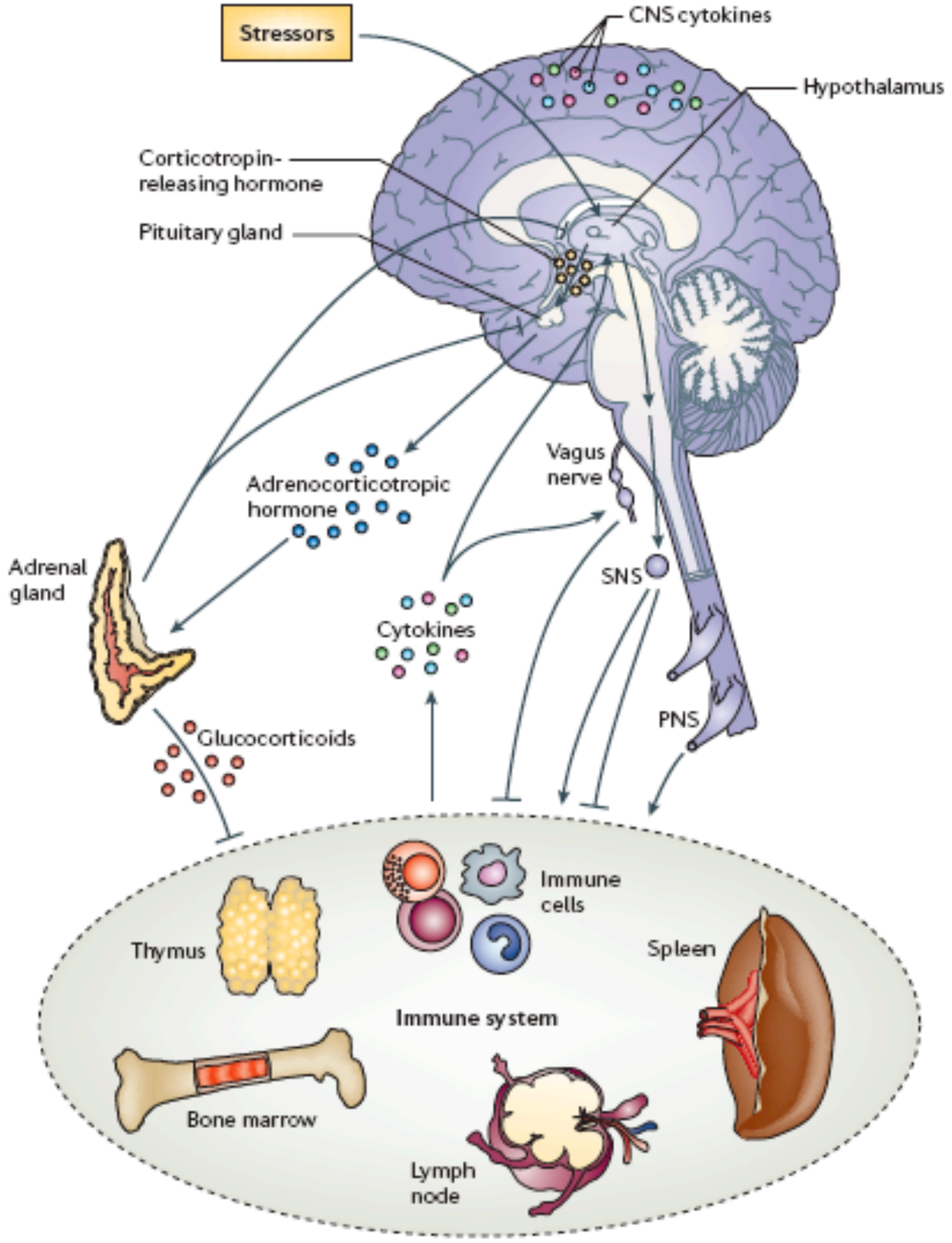
- Corticosteroids (Glucocorticoids)
  - GR on leukocytes and non-transcription-activating inhibition of NFkB
- Autonomic Nervous System
  - Sympathetic Nervous system
  - Parasympathetic nervous system

Neuroendocrine Systems: HPA

- Hypothalamus Corticotropin Releasing Hormone (CRH)
- Pituitary Adrenocorticotropic Hormone (ACTH)
- Adrenal Cortex cortisol (or corticosterone in rats)

Immune response elicits self-limiting immunosuppession mediated by hormones

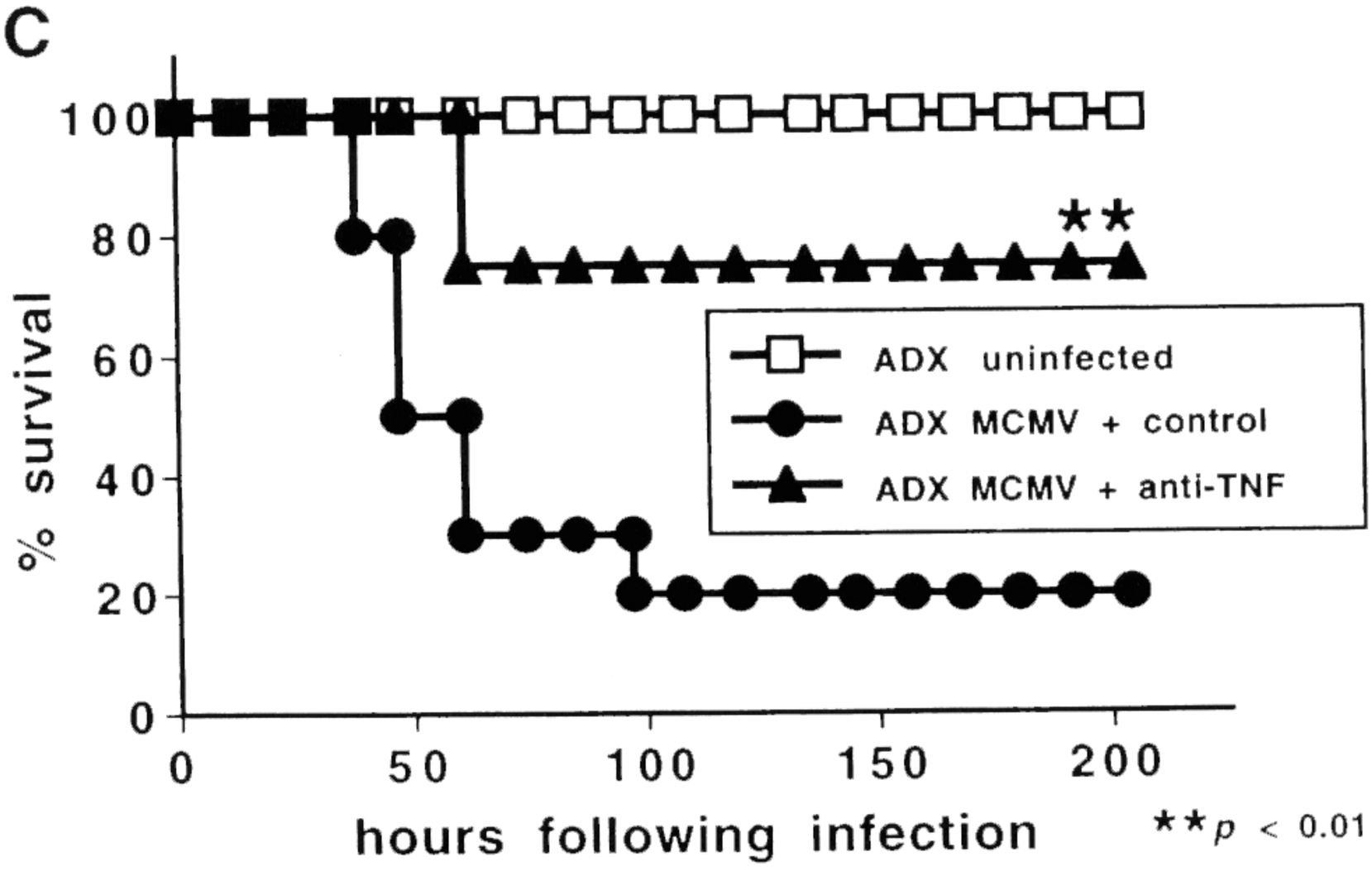
- Signals brain, which starts stress response
- Stress response modulates immune response



Sternberg, 2006

Stress Hormones provide negative feedback on immune response

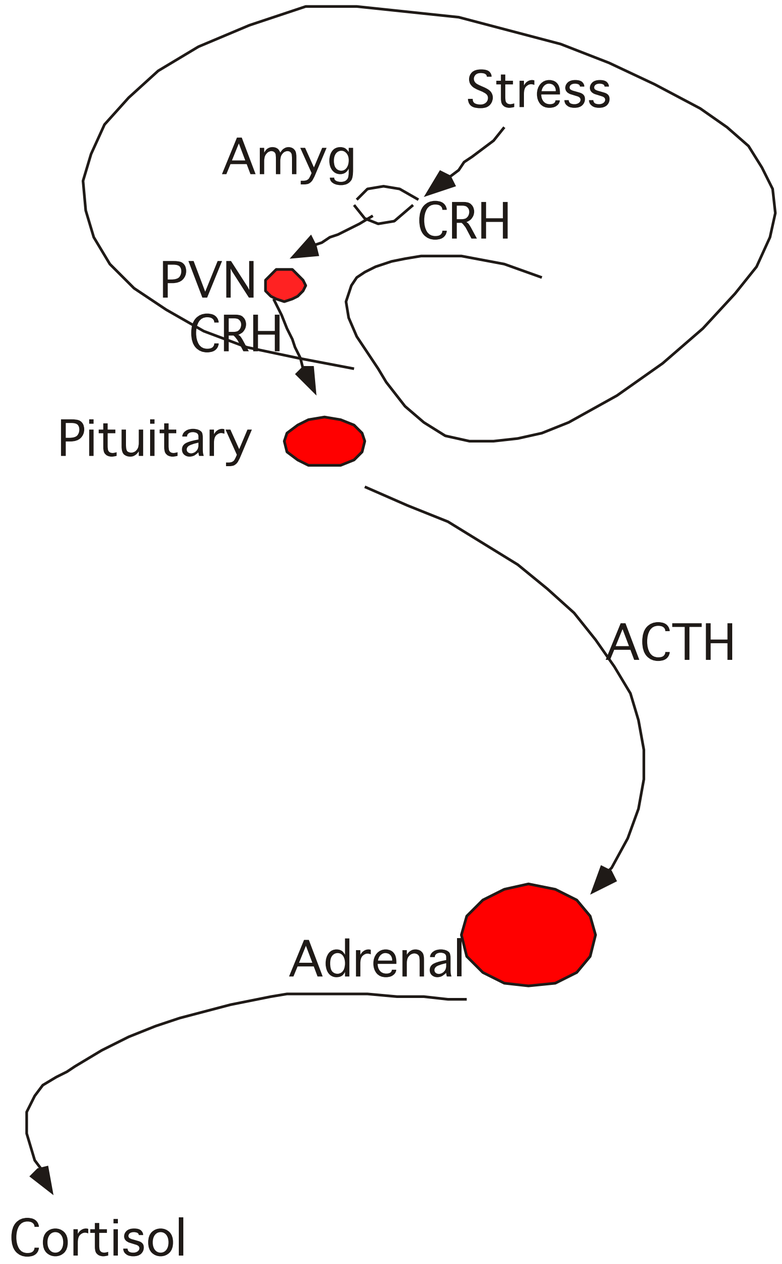
- Adrenalectomized rats die from viral infection due to overactive cytokines, not viral load (cort replacement protects)


C
100
80
60
40
20
0
% survival
0
50
100
150
200
hours following infection
**
ADX uninfected
ADX MCMV + control
ADX MCMV + anti-TNF
**p < 0.01

Does stress immunosuppress via glucocorticoids?

- Responses to stress are complex with multiple factors directing in opposite ways
- Acute vs chronic stress
- Most attempts to demonstrate adverse effects of stress in real-life immune mediated health effects have been unimpressive

Psychological stress can tap into the same system



Types of stress examined in humans

- Life events
- Marital discord
- Final Exams
- Bereavement
- Clinical Depression
- Alzheimer’s Caregivers
- Intervention studies (therapy, support)

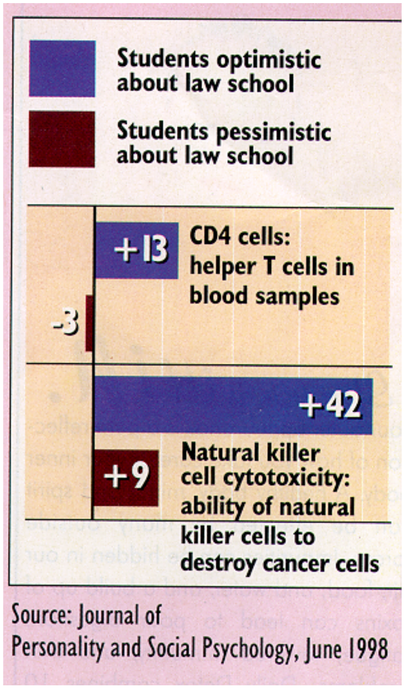
Immune Measures -- Final Exams for med students

- decreased proliferative responses
- decreased NK cell activity
- decreased cytokine production
- Depends on student’s level of loneliness

Optimism correlated with stronger immune response to acute stress

- Law school students during exams
- (Segerstrom 98)

- However, when stress persisted at high levels, optimists showed more subsequent immune decrements than pessimists (Cohen 99)



- Positive Role for Humor?
  - No methodologically sound data yet

Acute stress enhances immune responses
(chronic stress impairs)

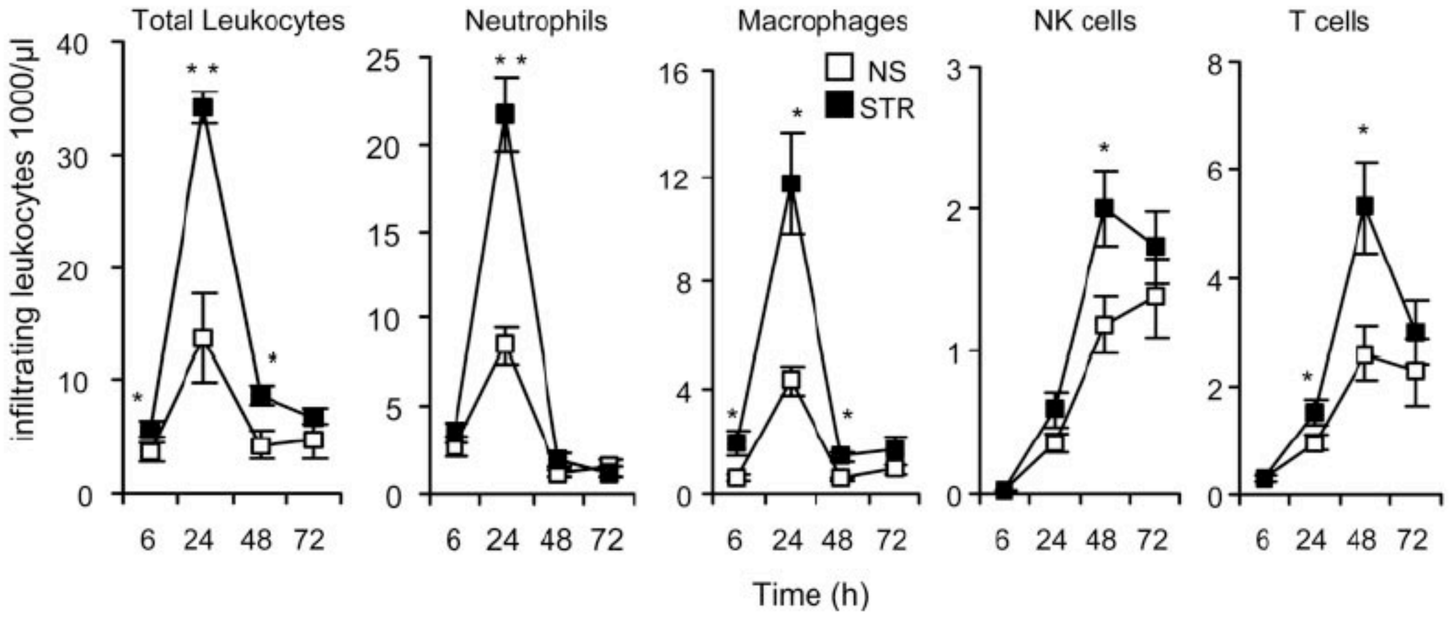


Fig. 1. Acute stress enhances leukocyte trafficking into an implanted surgical sponge. Saline-treated gelatin sponges were s.c.-implanted in NS or STR mice. Sponges were retrieved at 6, 24, 48, and 72 h after implantation. Compared with NS animals, sponges from STR animals had significantly higher total leukocyte numbers at 6, 24, and 48 h after implantation. STR animals had ≈200% higher macrophage numbers at 6 h, 300% higher neutrophil and macrophage numbers at 24 h, and 200% higher NK cell and T cell numbers at 48 h after implantation. Sponges from NS and STR animals had comparable and lower neutrophil, macrophage, NK cell, and T cell numbers at 72 h, indicating resolution of inflammation in both groups. Data are expressed as means ± SEM. Statistically significant differences between means are indicated (*, $P < 0.05$; **, $P < 0.01$, Student's t test).

Viswanathan 05

Human Studies

General Immune Measures -- Yes

Wound healing -- Yes

Chronic viral infections (Herpes) -- Yes

AIDS Dementia -- Mostly No

Respiratory Infections (colds) -- Yes, interactions complex

Autoimmune Disorders -- Probably yes

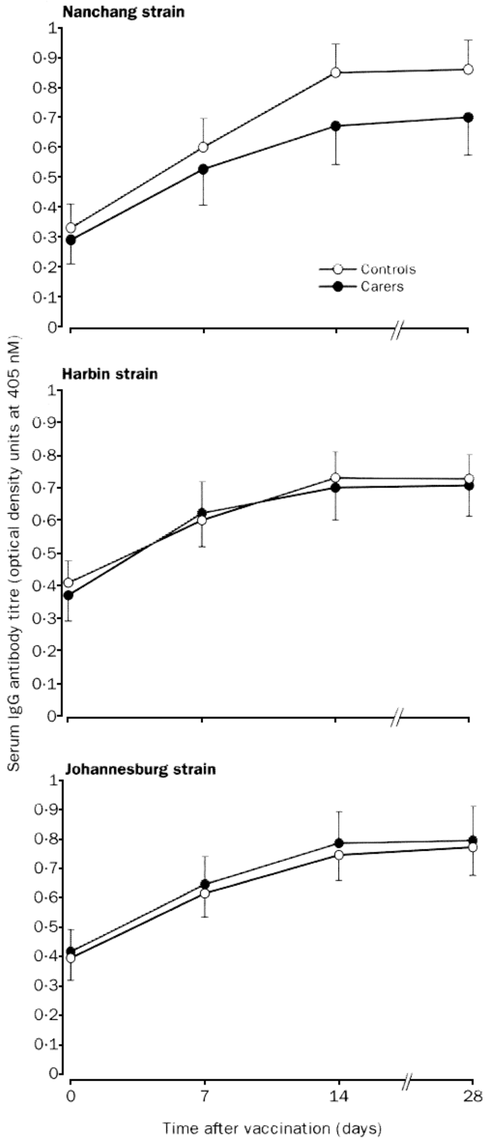
Cancer -- Uncertain, probably not

Antibody Formation and stress

Kiecolt-Glaser 96

- Care-givers for Alzheimer's patients
- Functional measure: antibody response to flu vaccine
- Higher percentage responded to vaccine in controls
- Only significant for subjects >70 yrs old

Antibody formation in response to flu vaccine in Alzheimer caregivers (stressed) and controls



What other factors are activated by stressors?

- Sympathetic nervous system
- Macrophage Migration inhibitory factor (MIF)

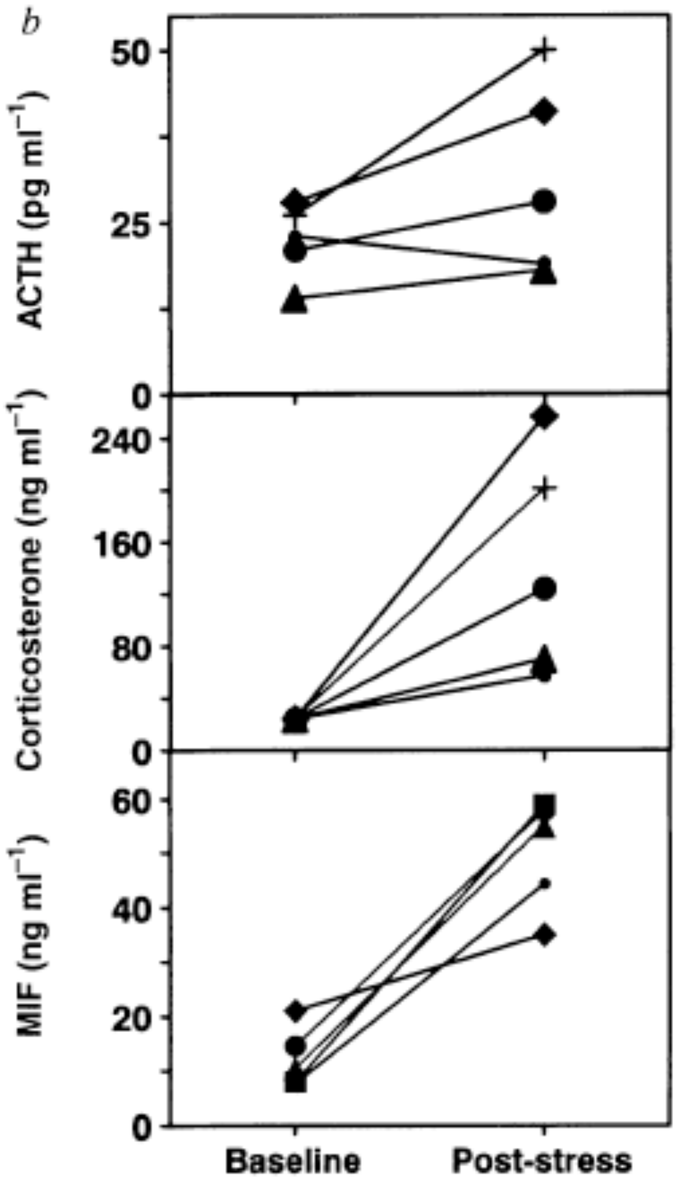
MIF

- Macrophage Migration Inhibitory Factor
- First cytokine, isolated in 1966
- Secreted by T cells, anterior pituitary, skin, macrophages in liver
- Localizes macrophages at sites of inflammation and activates them
- MIF and TNFa enhance each other
- Counters cortisol’s inhibitory effects

Pituitary release of MIF controlled by CRH

- MIF released from pituitary
- Cortisol causes release of MIF from macrophages
- MIF inhibits suppression by cortisol of inflammatory cytokines

Psychological stress (handling) induces both corticosterone and MIF secretion



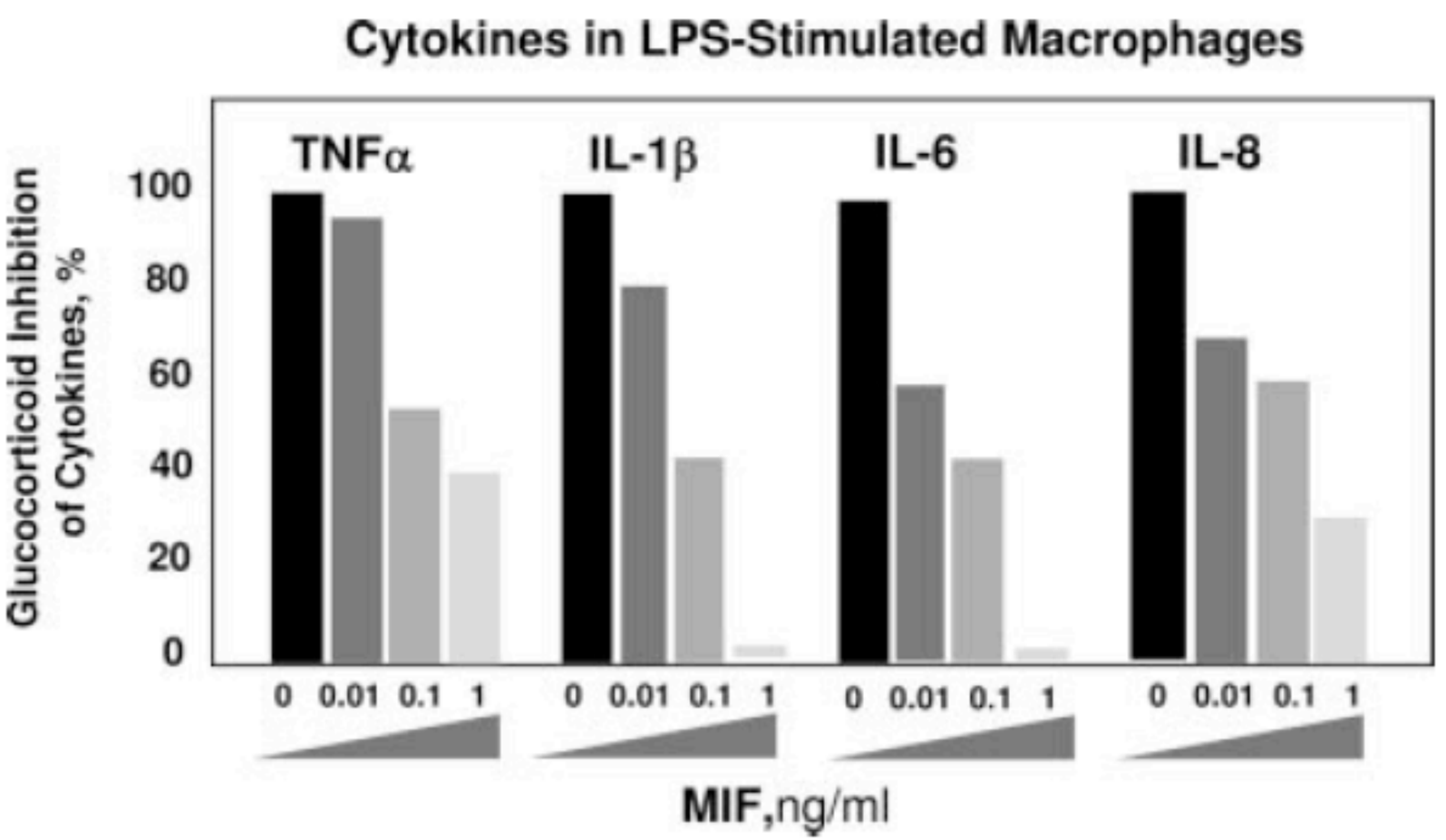


Fig. 2. MIF Overrides Glucocorticoid-Mediated Suppression of Cytokine Production by Peripheral Blood Mononuclear Cells

Cells were preincubated with dexamethasone or with dexamethasone and MIF before the addition of lipopolysaccharide (LPS) as a stimulus for cell activation. [Reproduced with permission from T. Calandra *et al*.: *Nature* 377:68–71,

- Polymorphisms in regulatory elements for MIF secretion correlated with autoimmune diseases

Human pathologies associated with increased MIF

- Sepsis, septic shock, graft rejection
- Asthma
- Rheumatoid arthritis
- Crohn’s disease
- Atopic dermatitis, psoriasis
- Multiple sclerosis
- Uveitis
- Atherosclerosis

Calandra, 2003

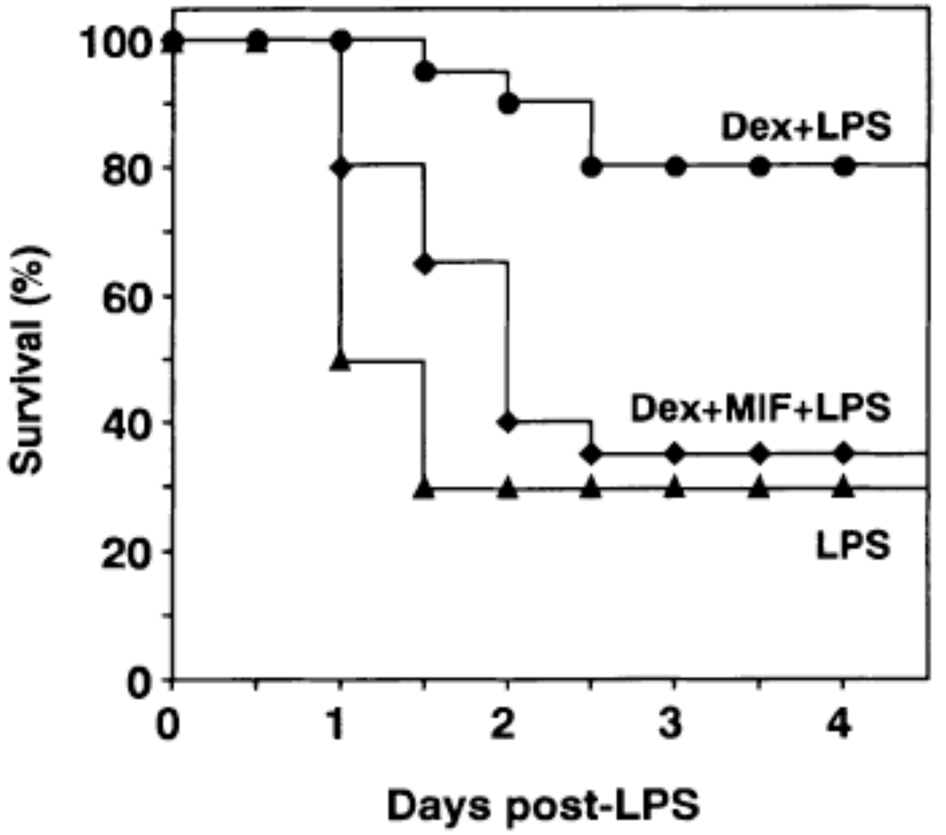
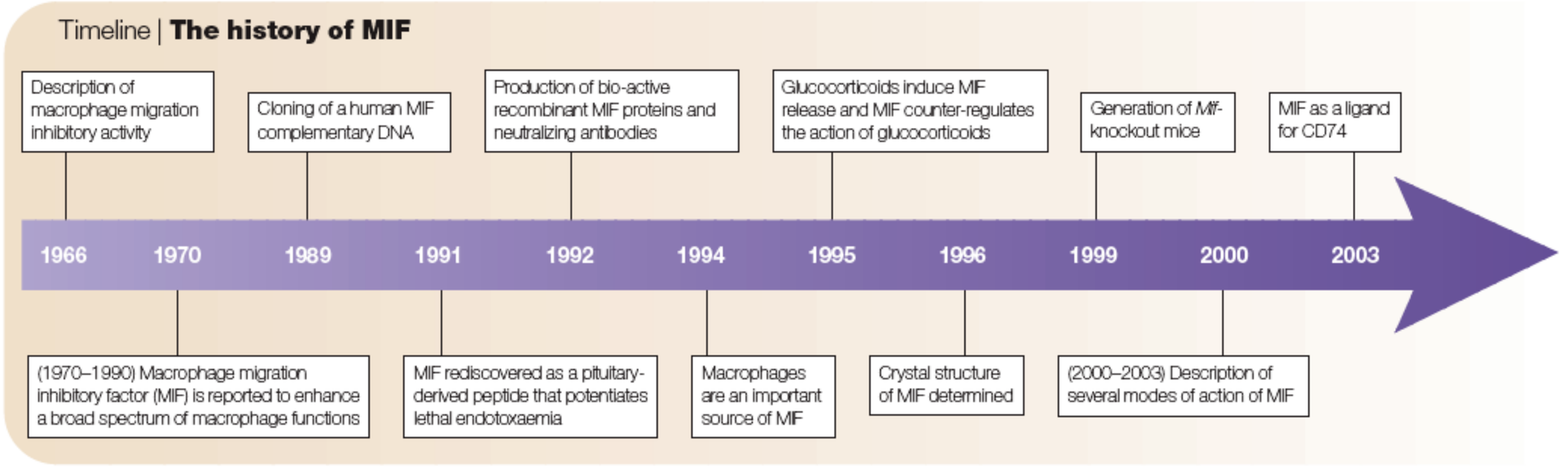


FIG. 4 MIF overrides glucocorticoid inhibition of LPS lethality. Ten-week-old BALB/c mice (19–20 g) were injected intraperitoneally with dexamethasone (Dex) (1.25 mg per kg, that is 25 μg or 67 nmol) with or without murine rMIF (0.6 mg per kg, that is 12 μg or 1 nmol) or saline.

Calandra 95


Timeline | The history of MIF
Description of macrophage migration inhibitory activity
Cloning of a human MIF complementary DNA
Production of bio-active recombinant MIF proteins and neutralizing antibodies
Glucocorticoids induce MIF release and MIF counter-regulates the action of glucocorticoids
Generation of Mif-knockout mice
MIF as a ligand for CD74
1966
1970
1989
1991
1992
1994
1995
1996
1999
2000
2003
(1970–1990) Macrophage migration inhibitory factor (MIF) is reported to enhance a broad spectrum of macrophage functions
MIF rediscovered as a pituitary-derived peptide that potentiates lethal endotoxaemia
Macrophages are an important source of MIF
Crystal structure of MIF determined
(2000–2003) Description of several modes of action of MIF

Peripheral Nervous System

- Somatic Sensory, Sympathetic, Parasympathetic and Enteric all influence immune system by direct connections
- Both pro-inflammatory and anti-inflammatory

Somatic Neural response to tissue damage also contributes directly to inflammation

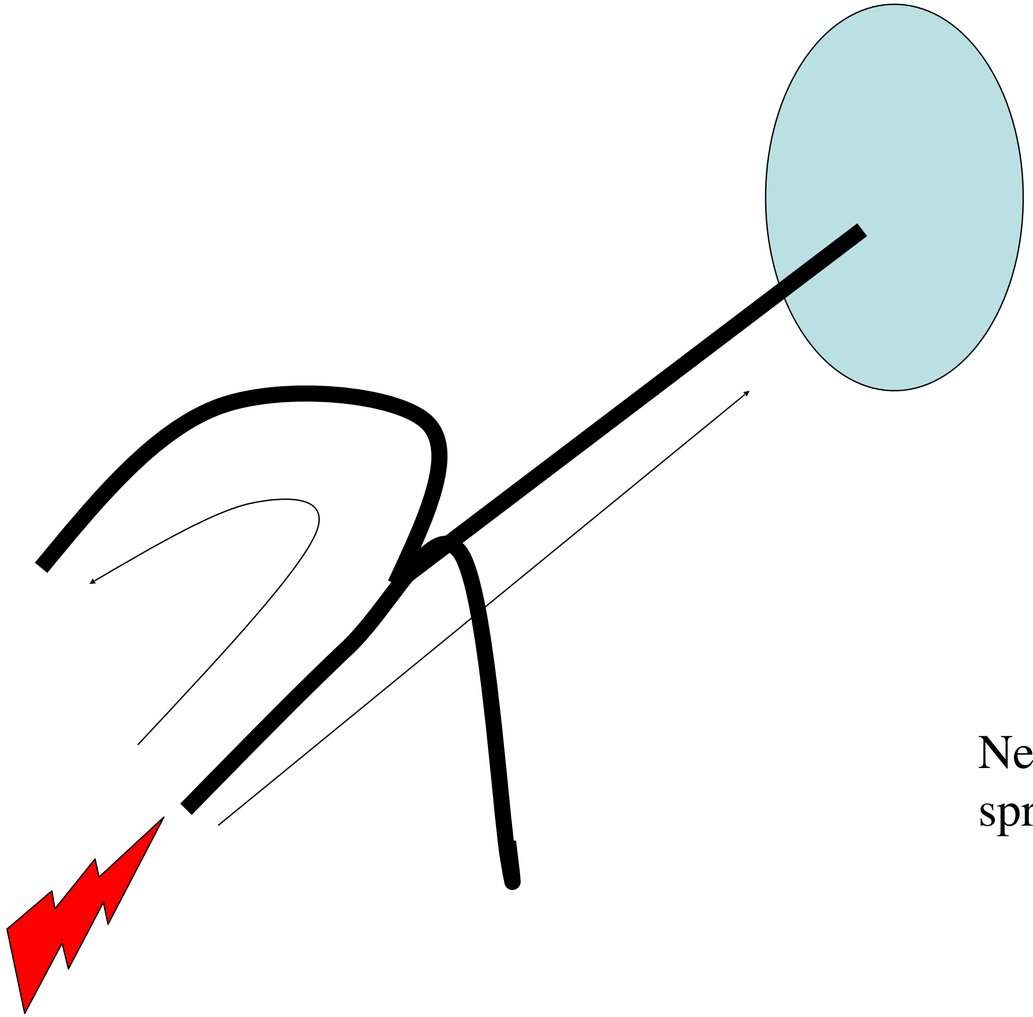
- Pain neurons are mediators of inflammation by mechanical damage
- Neurogenic inflammation also interacts with other stimuli for inflammation

Tissue Damage Can Start Inflammation

- E.g., bradykinin is produced by damaged blood vessels
  - Bradykinin stimulates pain nerve terminals
  - Other neurotransmitters then released
- Mechanical and osmotic stimulation releases endocannabinoids that act on VR1 receptors on pain afferent neurons (same as capsaicin receptors)

Neurotransmitters released from sensory *afferents*

- Corticotropin Releasing Hormone
- Glutamate
- Neurotensin
- Substance P
- Calcitonin gene-related peptide
- Anandamide



Neighboring terminal branches
spread inflammation

Sensory fiber responses to trauma

- Locally released neurotransmitters cause vasodilation and vascular leakage and stimulate immune inflammatory cells
- Prompt mast cell degranulation
  - Increased platelet aggregation
  - Vascular permeability
  - Attraction of neutrophils and then macrophages

Can be centrally induced

- Pain fibers are afferent
- CNS circuits and efferent sympathetic innervation of mast cells can lead to neurogenic inflammation
  - Contributes to arthritis, colitis, asthma

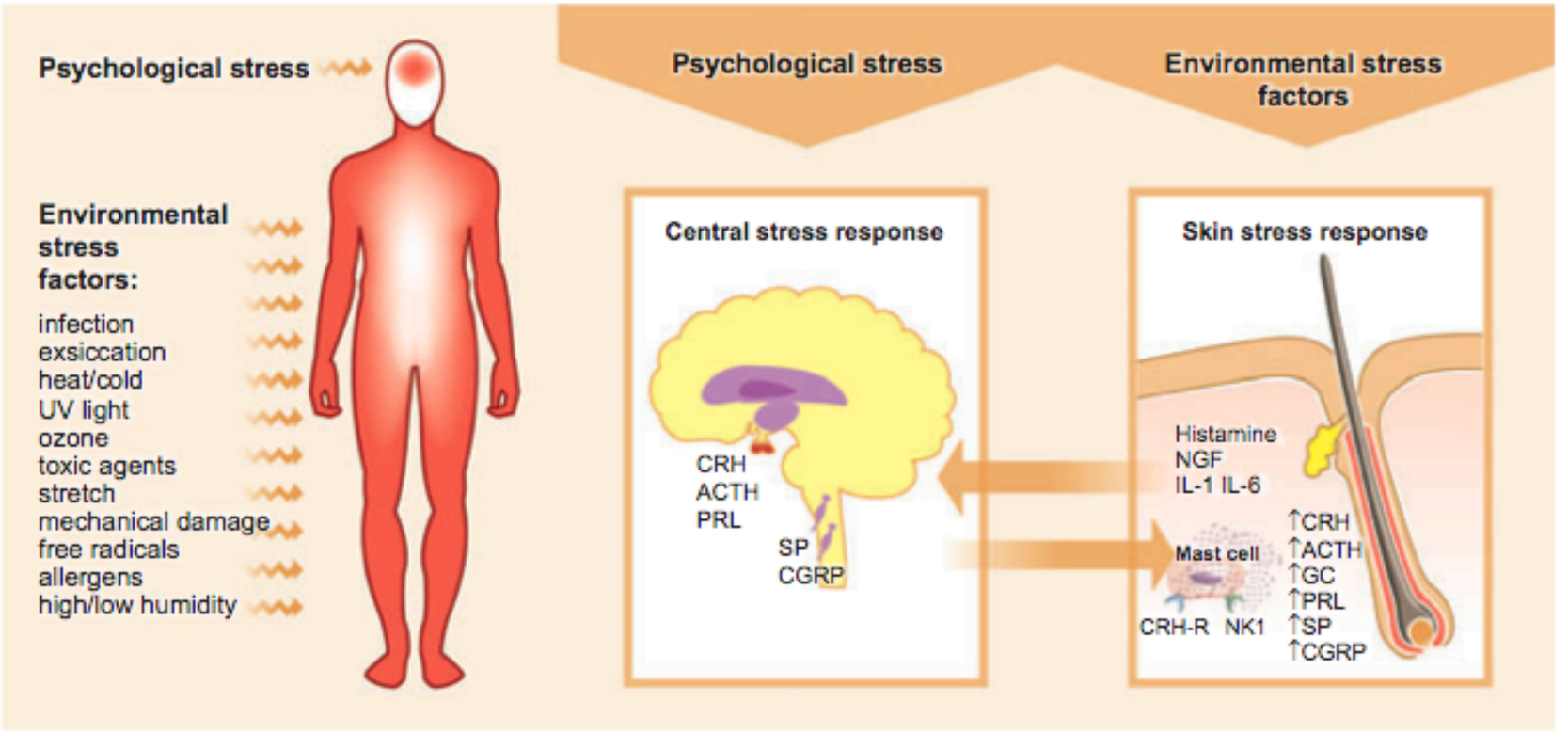


Figure 1. Brain–skin cross-talk upon exposure to psychological stress or environmental stress factors. Upon perception of psychological stress, the central stress response leads to the activation of the hypothalamic–pituitary–adrenal axis, which causes the release of corticotropin-releasing hormone (CRH), ACTH, and prolactin (PRL). Further, an upregulation of substance P (SP) and calcitonin gene-related peptide (CGRP) can be observed in the dorsal root ganglia. Such stress response patterns may be translated into a skin stress response, including the local production of CRH, ACTH, and glucocorticoids (GCs), the release of inflammatory cytokines, and the sprouting of SP^+ nerve fibers. In the skin response to stress, mast cells occupy a central switchboard position, as they are targets for stress-triggered factors as well as effector cells that contribute, for example, to neurogenic inflammation in the skin. Environmental factors are also capable of inducing a skin stress response, and this may be signaled to the brain, where it affects behavior and leads to an increased vulnerability to additional stress perception.

Neural Anti-Inflammatory Reflexes

- Afferents:
  - Cytokines induce stress responses via afferent vagus
  - Pain-induced anti-inflammatory responses
- Parasympathetic anti-inflammatory
- Sympathetic anti-inflammatory (beta-adrenergic)
- Neuroendocrine anti-inflammatory responses (cort)

(Somatic nerves release proinflammatory peptides)

Parasympathetic Nervous System

- Vagus nerve major pathway (afferent and efferent)
- Acetylcholine is postganglionic neurotransmitter
- Recent work showing the vagal ACh is anti-inflammatory
- Part of “anti-inflammatory reflex”