Oestrogens & Immune Function

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Overview

- **Understand estrogen’s influence on immune function and the impact of menopause**
- **Obtain a foundational understanding of the innate and adaptive immune systems**
- **Recognize the critically important role played by microbiomes, and in particular the gut microbiome, in the maintenance of immune balance and its involvement in the development of autoimmunity**
- **Acknowledge the contributions of the Circadian Rhythm to gut and immune health**
- **Learn how optimizing the gut microbiome and the circadian rhythm, along with key nutritional modalities, can make a profound difference in immune homeostasis and reduce the incidence of autoimmunity**

Fundamental Premise:

Optimal Hormones are Needed for Optimal Immune Function

Reminder: PRIME DIRECTIVE OF LIFE

- REPRODUCTION & SURVIVAL OF PRODIGY -
- TO GROW TO SEXUAL MATURITY

Then to repeat the process……

TO THAT END:

- Females have a more responsive & robust immune system compared to males

BUT WHEN THINGS GO WRONG:

- Females respond more aggressively to self-antigens
- Females are more susceptible to autoimmune diseases
- Estrogen acts on all cellular subsets of immune system through estrogen receptor - dependent & independent mechanisms

Women and men have different gut microbiomes

Women and men have different immune systems

Males & Females have Different Immune Systems

Sex-based immunological differences historically overlooked – but research & awareness growing!

Differences driven by:

- **X chromosomes**
- **Epigenetics**
- **Hormones**
- **Microbiome**
- **Circadian Rhythm**
- **Age and Reproductive status**

Sex-based immunological differences not unique to humans - insects, lizards, birds & other mammals also demonstrate immunological differences between sexes

Khan et al. Front Immunol.2015;6:635
Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38.

Men and Women have Different Genes

- **X chromosome contains approximately 1100 genes – many involved in immune functions**
- **One X chromosome is randomly silenced during X chromosome inactivation but up to 15% of genes escape silencing in humans may have large impact**

Alebert et al. Nat Rev Immunol. 2010;10(8):594-604
Carrel et al. Nature.2005;434(7031):400-4

Men & Women have Different Genes

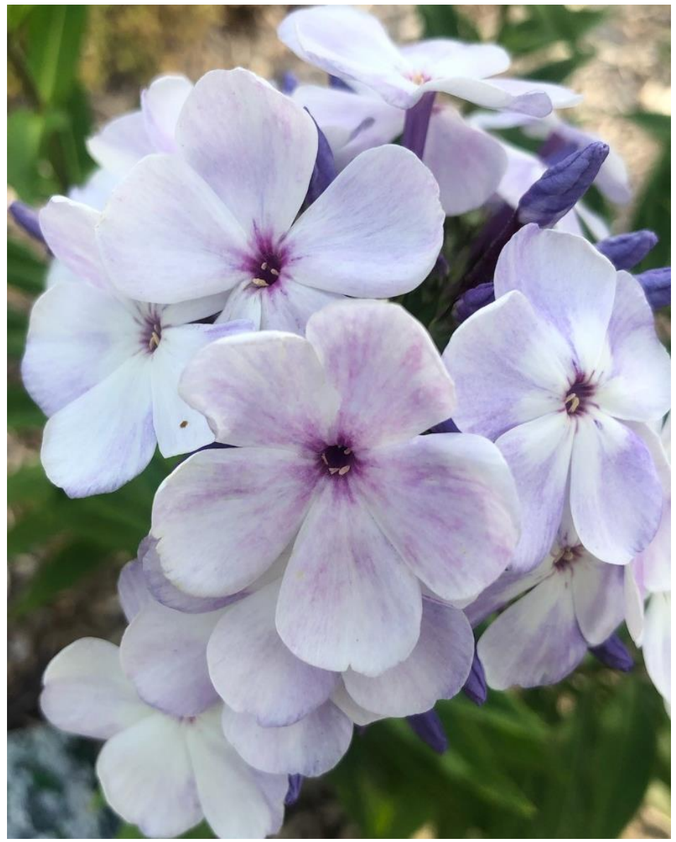
Immunological genes - located on sex chromosomes.
For example:

- **Pattern recognition receptors genes**
- **Cytokine receptor genes**
- **Transcriptional factor genes**
- **Non-coding DNA regions**
- **Immune cells**

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38..

Differences in CD4 T Cells

- **Adult females produce more T helper 1 (Th 1) cytokines (IFN)**
- **Females have greater antibody responses than males**
- **Higher basal immunoglobulin levels**
- **Higher B cell numbers**



Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38.

Men & Women - Different Hormonal Effects

Estradiol acts on all cellular subsets of immune system – ER dependent & independent mechanisms

Modulates Immune System Directly and Can:

- **Increase TLR4, TLR7 and TLR9**
- **Modulate NF-κB activity**
- **Modulate dendritic cell activation**
- **Increase neutrophil numbers and degranulation**
- **Increase IFNγ production by NK cells**
- **Increase in B cells and production of antibodies**

Modulate Immune System Indirectly: Microbiome & Circadian Rhythm

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38..

Differences in Adaptive Immunity

- **Following invitro stimulation of peripheral monocytes women have higher numbers of activated CD4 and CD8 T cells**
- **Analysis shows greater cytotoxic T cell activity in adult females**
- **Stimulation leads to higher upregulation of antiviral genes and pro-inflammatory genes compared to male T cells**
- **Half of activated genes in female T cells have Estrogen Response Elements (EREs) in their promoters**

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38

There are Trade-offs for Each Gender

- **MALES: Testosterone Reduces Immune Response – to support energy utilization priorities – energy to mount immune response could instead be used for growth, maintenance of secondary sex characteristics, sperm production**
  Theory: Higher pathogen load & reduced immune function - but more successful reproduction & overall survival of the species

- **FEMALES: Increased vaccine efficacy, increased survival due to infections and trauma – but increased susceptibility to autoimmune diseases**

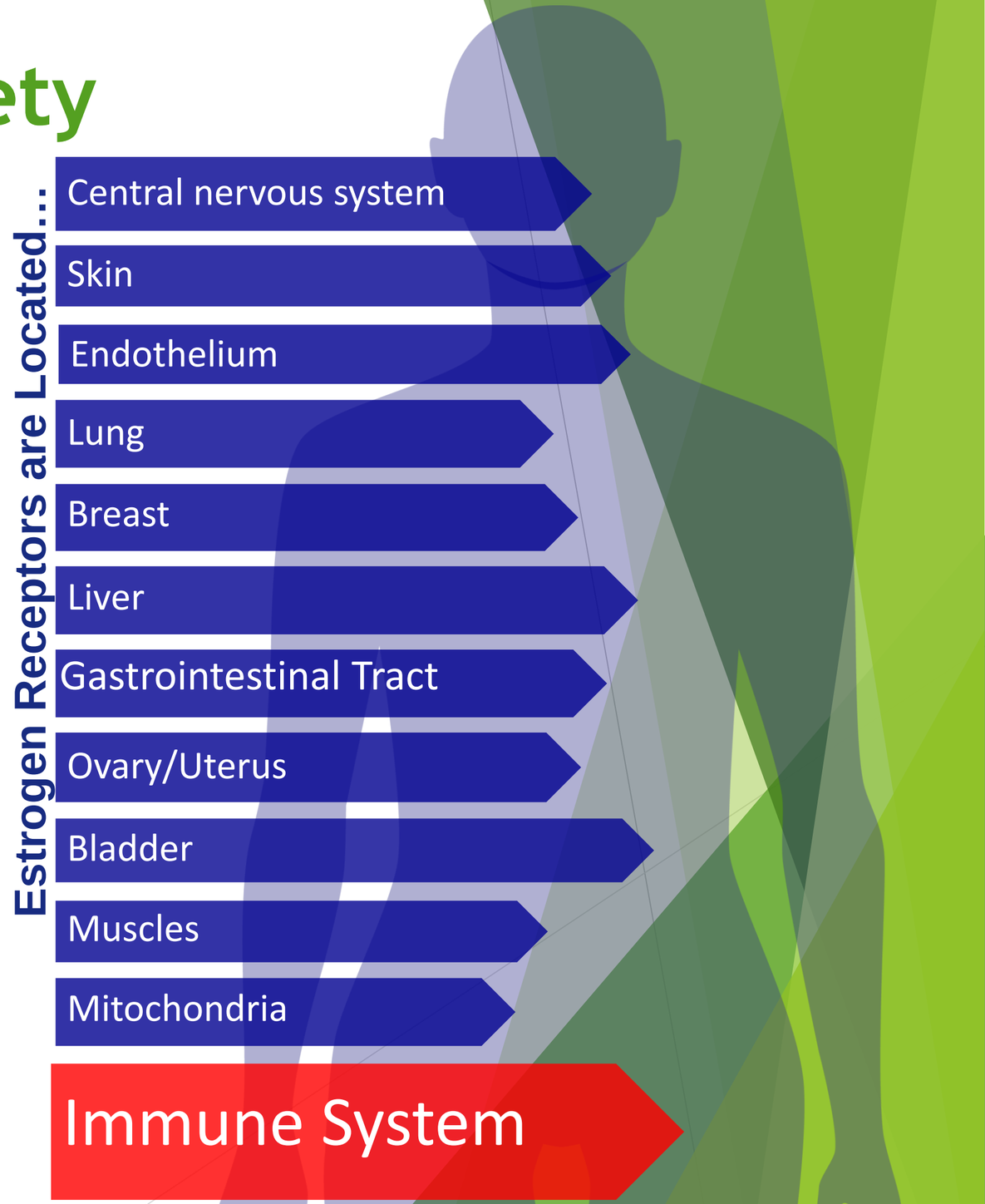
Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38.

Estrogen is the “MOTHER HORMONE”

The master of immune function

Estradiol supports wide variety of physiological functions

- **Estradiol influences number, activity, & function of immune cells**
- **Estradiol supports gene activation in immune cells**
- **Estradiol mediated effects seen in all major innate & adaptive immune cells**

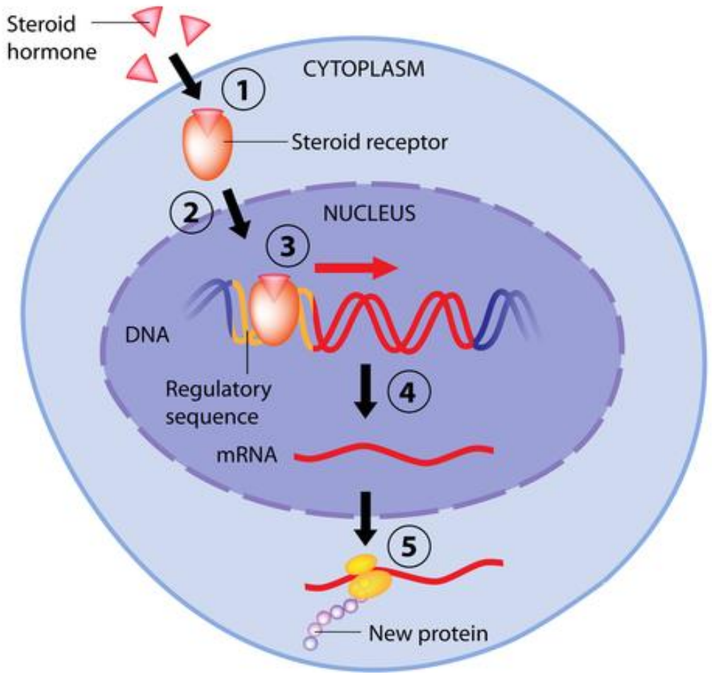


Estrogen Basics:
Estrogens are a Family of Hormones

Review of the Estrogens

	Forms of Estrogen		
	Estrone (E1)	**Estradiol (E2)**	**Estriol (E3)**
Features	Not beneficial to the brain – dominant in menopause	Dominant estrogen of reproductive aged women	Hormone of Pregnancy
Source	Ovaries, adipose and other hormones	Ovaries	Other estrogens and the placenta
Receptor	**ER alpha**	**ER alpha** **ER beta** **GPERS**	**ER beta**

Estrogen Basics: Receptors
High ER beta down-regulates ER alpha



ER alpha → **Regulates genes & membranes**

Expressed in reproductive organs: uterus, ovary, prostate, testes, and breast, hypothalamus of brain, innate immune cells, T cells, bone, muscle, mitochondria

ER beta → **Regulates genes & membranes**

Expressed in GI tract, colon, bone marrow, vascular endothelium, lung, bladder, B cells, mitochondria, brain

Membrane-associated ER →

No effect on genes, rapid effects on cellular signaling

Mendelsohn ME, and Karas RH. *N Engl J Med*. 1999: 340; 1801-1811
Dahlman-Wright et al. *Aspet Pharmacological Reviews*. 2006: 58

Modulated by Estradiol:

Immune activity
- Vascular-cell adhesion molecule
- Cytokines (IL1, IL6, TNFα)
- Cytokine receptors
- Superoxide Dismutase

Coagulation
- Fibrinogen
- Coagulation factors
- Protein S

Angiogenesis
- Matrix metalloproteinase
- Vascular endothelial growth factor

Non-Genomic Effects
- Fast-acting actions such as NO facilitated vasodilation

Vasodilation and vasoconstriction
- Endothelial NO synthase
- Prostacyclin cyclooxygenase
- Prostacyclin synthase
- Renin and angiotensin
- Endothelin-1

Lipid Metabolism
- Lipoprotein lipase
- Apolipoproteins
- Leptin
- PON 1
- LDL receptors
- HMG-CoAR activity

Saltiki, K and Alevizaki M.. *Hormones*. 2007; 6(1): 9-24

Innate and Adaptive Immune System Genes Regulated by Estrogen

Table 1

List of key selected genes that are regulated by estrogen in cells of innate and adaptive immune system.

Immune cell	List of genes	Reference
Neutrophil	CINC-1, CINC-2β, CINC-3, TNFα, IL-6, IL-1β	(8–10)
Macrophage	iNOS, NO, IL-6, TNFα	(12–16)
Dendritic cells	IL-6, IL-10, CXCL8, CCL2, TGFβ, IL-23, IL-12	(17, 18, 22)
Th1	IFNγ	(23–25)
Th2	IL-4	(26)
Tregs	FoxP3, PD-1, CTLA-4	(27–30)
B cells	Immunoglobulin, CD22, SHP-1, Bcl-2, VCAM-1	(31)

JVI
Journals.ASM.org

17β-Estradiol Protects Females against Influenza by Recruiting Neutrophils and Increasing Virus-Specific CD8 T Cell Responses in the Lungs

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ABSTRACT

17β-Estradiol (E2) treatment limits the pathology associated with pulmonary diseases caused by pathogens, allergens, and asthma, partly by reducing the production of proinflammatory cytokines and chemokines. To test the hypothesis that E2 protects against influenza A virus (IAV) infection by altering the recruitment and activity of innate immune cells and T cells, chemokine concentrations were measured and innate and adaptive immune cells were enumerated from the lungs of E2- and placebo-treated ovariectomized female C57BL/6 mice following infection. Females treated with E2 experienced less morbidity but had similar lung virus titers to placebo-treated females. Females treated with E2 had lower induction of CCL2 but higher CCL3 and CXCL1 responses in their lungs than placebo-treated females. Pulmonary recruitment of neutrophils, NK cells, macrophages, and dendritic cells was increased following infection, but only neutrophil numbers were greater in E2-treated than placebo-treated females. Neutrophils enhance the responses of influenza virus-specific CD8 T cells to promote virus clearance and improve the outcome of infection. Total numbers of virus-specific CD8 T cells were not altered by treatment with E2, but the proportion of gamma interferon (IFN-γ)- and tumor necrosis factor alpha (TNF-α)-producing, virus-specific CD8 T cells was increased. Neutrophil depletion in E2-treated females increased morbidity, reduced pulmonary production of chemoattractants for neutrophils, and reduced IFN-γ production by virus-specific CD8 T cells. Neutrophils mediate both inflammation and tissue repair during IAV infection and are regulated by E2 to improve the outcome of influenza in females.

IMPORTANCE

Severe influenza is associated with excessive inflammation that leads to tissue damage. We demonstrate that estradiol (E2) is a potent anti-inflammatory hormone that reduces the severity of influenza A virus infection in females. Treatment of female C57BL/6 mice with E2 does not affect virus replication but rather alters the production of chemokines, pulmonary recruitment of neutrophils, and the cytokine responses of virus-specific CD8 T cells to protect females against severe influenza.

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PLoS PATHOGENS

Elevated 17β-Estradiol Protects Females from Influenza A Virus Pathogenesis by Suppressing Inflammatory Responses

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Estrogen receptors regulate innate immune cells and signaling pathways

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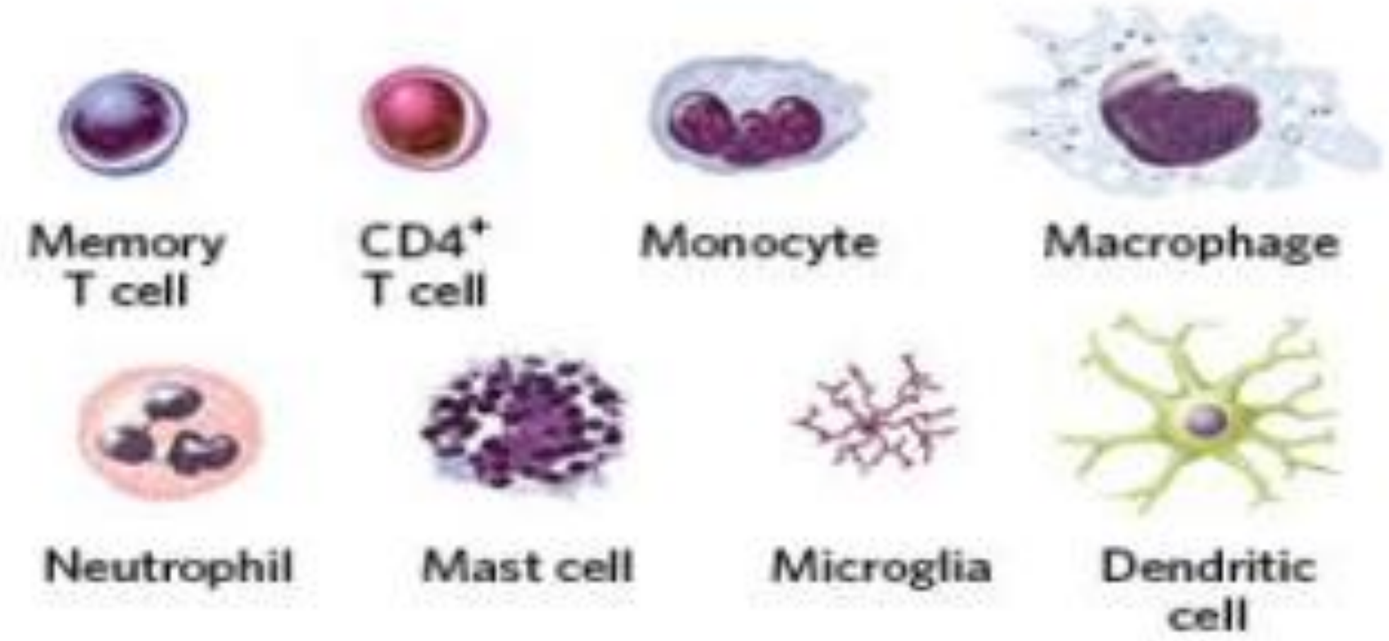
Abstract

Humans show strong sex differences in immunity to infection and autoimmunity, suggesting sex hormones modulate immune responses. Indeed, receptors for estrogens (ER) regulate cells and pathways in the innate and adaptive immune system, as well as immune cell development. ERs are ligand-dependent transcription factors that mediate long-range chromatin interactions and form complexes at gene regulatory elements, thus promoting epigenetic changes and transcription. ERs also participate in membrane-initiated steroid signaling to generate rapid responses. Estradiol and ER activity show profound dose- and context-dependent effects on innate immune signaling pathways and myeloid cell development. While estradiol most often promotes the production of type I interferon, innate pathways leading to pro-inflammatory cytokine production may be enhanced or dampened by ER activity. Regulation of innate immune cells and signaling by ERs may contribute to the reported sex differences in innate immune pathways. Here we review the recent literature and highlight several molecular mechanisms by which ERs regulate the development or functional responses of innate immune cells.

Beginnings: What is the Immune System & Why Do We Have It?

- **Essential for survival - network of cells, tissues, organs – work together to protect the body**
- **Keeps us healthy as we drift through a sea of pathogens (the PAMPS)**
- **Distinguishes self from non-self**
- **Recognizes and clears away dead, damaged and faulty cells (the DAMPS)**
- **Divisions: Innate, Adaptive, Passive**

Cells of the Innate and Adaptive Immune System



Innate & Adaptive Immune System: Regulated by Estradiol

Estradiol modulates proinflammatory cytokine production:

- TNF-alpha
- IL-1 beta
- IL-6
- Receptor activator of NF-kappa B ligand
- Regulates IFN-gamma, iNOS, immunoglobulins, chemokines

Shu et al. J Demt Res2008; 87(2):142-147

What is the Role of Inflammation?

- **Biological defense mechanism induced by innate immune system against microbial infections**
- **Macrophage Toll-like receptors recognize conserved structures on pathogens - bacteria, parasites, fungi & viruses**
- **TLR4 signaling pathway tightly controlled by *circadian clock* - Prepares immune cells for integrated response at time of greatest risk**

White Blood Cells -
Circulate in Blood & Lymphatics

Innate Immune System:

- First Line Responders Interface with Acquired
- Immune System to Perform Phagocytosis

The Players:

- Mast cells
- Neutrophils
- Monocytes
- Macrophages

Estradiol & the Innate Immune System

Estradiol impacts:
Neutrophils, macrophages/monocytes, natural killer cells, dendritic cells

- **Neutrophils:**
- **Regulate numbers and functions: chemotaxis, infiltration, production of superoxide anion and myeloperoxidase**
- **Induction of chemokines**
- **Induction of cytokines (TNF alpha, IL-6, IL-1beta)**
- **Regulate genes**

Cutolo et al. Lupus.2004;13(9):635

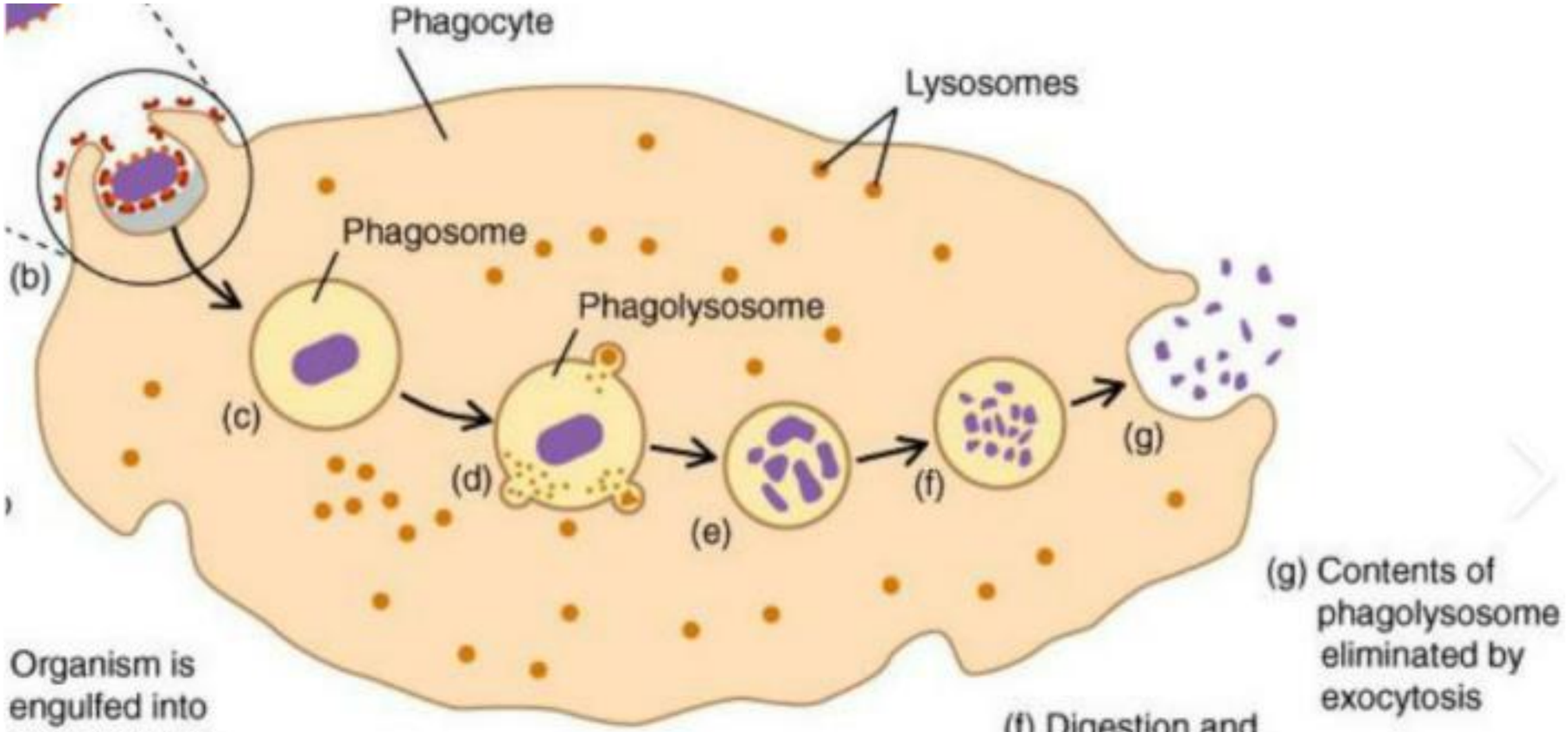
Estradiol:
Innate Immune System

Macrophages:

- **Regulate chemotaxis**
- **Phagocytic activity**
- **Induction of cytokines, iNOS, and Nitric Oxide**
- **E2 regulates macrophages in brain and gut - microglia (involvement in brain health and dementia, gut health)**

Karpuzoglu et al. Endocrinology.2009;150(3):1310

Phagocytosis



http://www.cellsalive.com/mac.htm

Estradiol: Innate Immune Cell Signaling

Dendritic Cells:

Messengers between innate & adaptive immune systems

- **Present antigen material to T cells**
- **Enhance differentiation of immature DC's into mature functional DCs**
- **Regulate expression of cytokines and chemokines (IL-6, IL-10, CXCL8, CCL2**

Lelu et al. J Immunol.2011;187(5):2386

Mast Cells

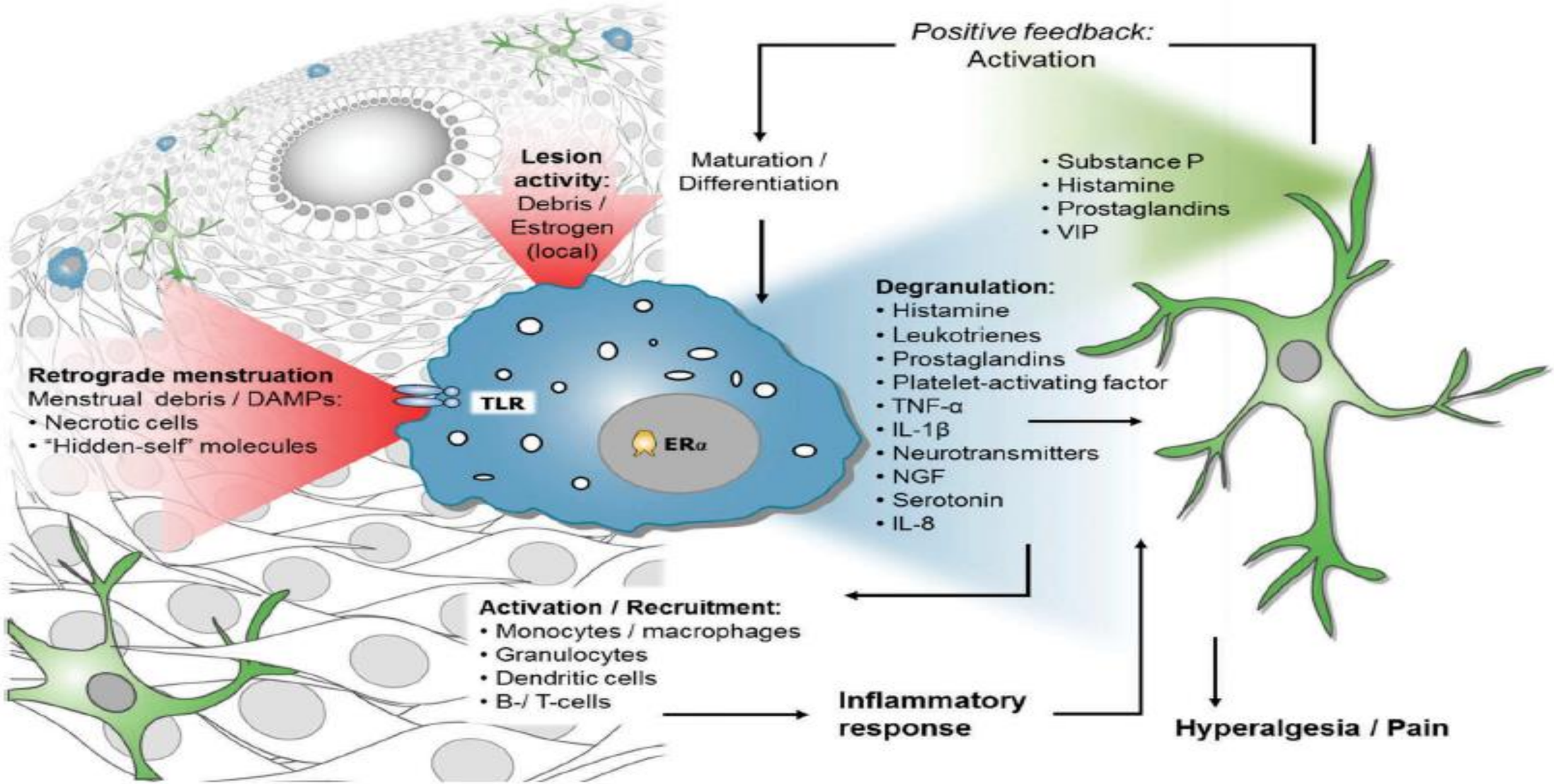
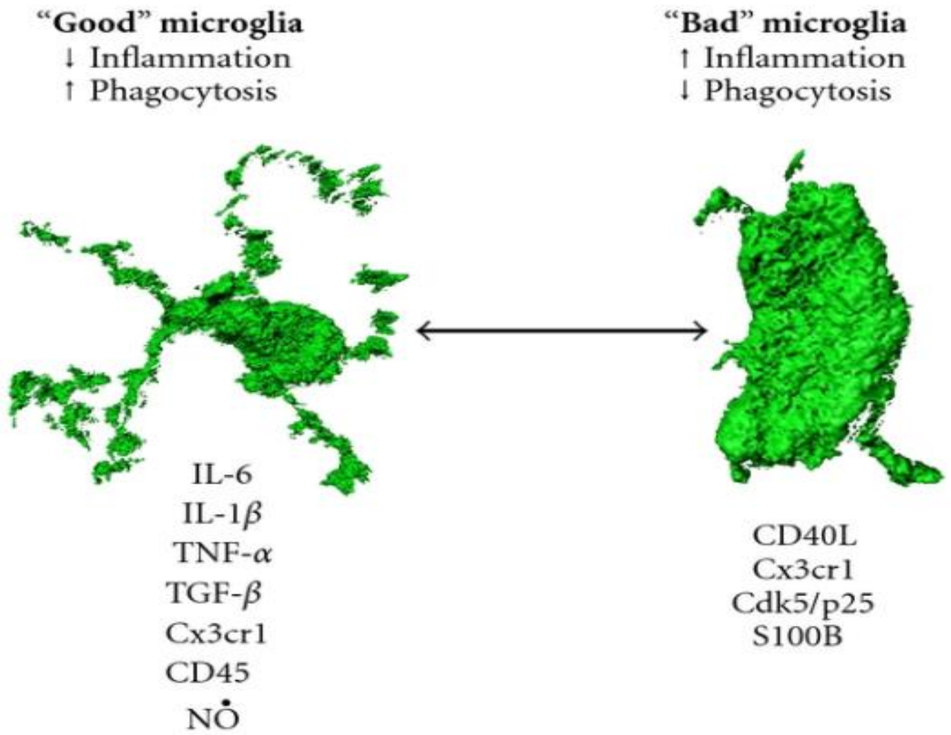


Figure 1. **Mast cells as key players in the pathology of endometriosis.** Kirchhoff D et al. [31] are acknowledged for kindly providing.

Estradiol Controls Neuroinflammation

- Effects on T cell activation:
  vary based on different hormone concentrations -
  can decrease MMP-9 expression
- Inhibits microglia activation by LPS, controls astrocytes



Neumann et al. Brain (2008) 132 (2): 288-295
Drew et al. J. Neuroimmunol. 2000;111: 77-85
Kato et al.Science. 1995;270(524):1491-1494

Miraculous Estradiol: Suppression of Local Brain Inflammation

- **ER beta: dampens LPS stim of NO production in microglia**
- **ER alpha: attenuates oxidative damage in hippocampus after hypoxia, inhibits chemokines**
- **Decreases inflammatory responses (IL 6 and NF kappa B) in neurodegenerative conditions, reduces expression of cyclooxygenase-2**
- **Antagonize pro-inflammatory glial cytokine responses**
- **Pro-survival factors (glutamate metabolism and growth factor supply)**

Habib et al. J Steroid Biochem and Mol Bio 2014;146:3-14

Estradiol modulates Adaptive Immune System

T Cells:

- **Modulates subsets of T cells - includes CD+4 (Th1, Th2, Th17, & Tregs)**
- **Promotes expansion and frequency of T reg cells – critical role in downregulating immune responses via ER alpha mediated signaling**

Lelu et al. J Immunol.2011;187(5):2386

Estradiol & Adaptive Immune Cells

T Lymphocytes

- **CD4+ cells have more ER alpha compared to ER beta**

- **Modulates IFN gamma secreting Th1 cells - E2 driven Th1 cell responsiveness dependent on ER alpha mediated signaling**

Khan et al. Front Immunol.2015;6:635.
Lelu et al. J Immunol.2011;187(5):2386

Estradiol Modulates Adaptive Immune System

B Cells:

- **E2 affects B cell differentiation, activity, function, and survival by increasing expression of genes**
- **Increases plasma cell and autoantibody producing cell numbers**
- **B Cells – have more ER beta than ER alpha**
- **Both ER alpha and beta shown to alter B cell maturation, but ER alpha engagement critical for autoimmunity**

Think about these issues – oral contraceptives, endocrine disruptors, menopausal choice of hormone therapy

Lelu et al. J Immunol.2011;187(5):2386

Lymphocytes:
Adaptive (Acquired) Immune System

Bone marrow:

- **B lymphocytes:**
- **Produce antibodies and help alert T lymphocytes**

Thymus:

- **T lymphocytes:**
- **Destroy compromised cells in body and help alert other leukocytes**
- **T Helper cells, Killer T cells**

Antibodies lock onto antigen but do not kill it - just mark it for death. Killing is job of phagocytes!

Amount of Estradiol Matters

Concentration of estradiol influences impact on immune system

“E2 has bi-potential effect on monocytes & macrophages – low doses enhance production of pro-inflammatory cytokines & high doses reduce production of these cytokines”

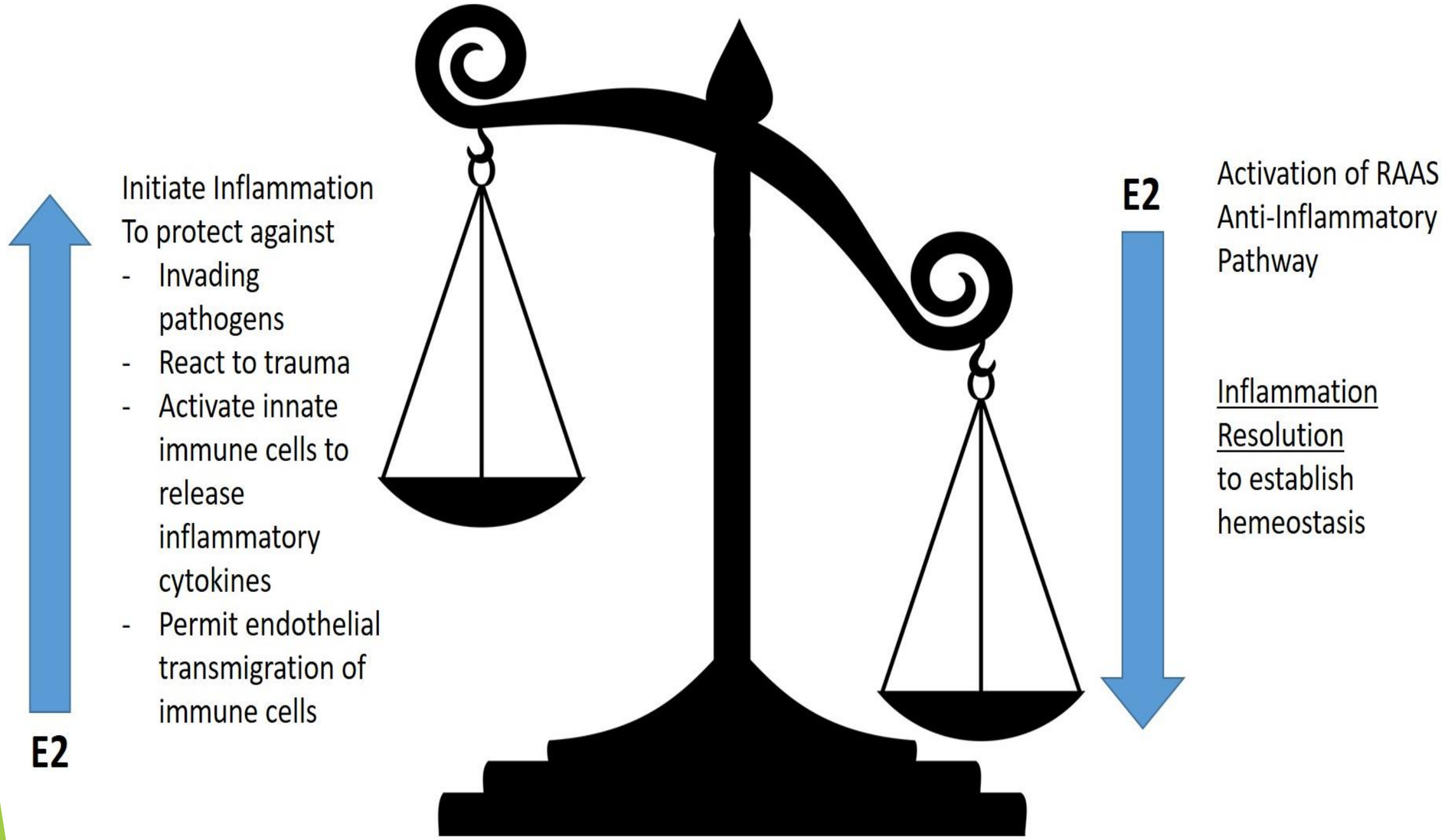
Controlling inflammation:

a key function of ESTROGEN!

Lateef A and Petri M. J of Autoimm. 2012; 38: J170-J176.

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38..

Estradiol:
Master Immunomodulator & Regulator of the RAAS



Review: Estradiol Effects

- **Low E2 promotes Th1 type responses and cell mediated immunity**
- **High E2 augments Th2 type responses and humoral immunity**
- **Low dose E2 stimulates processes that increases production of IFN gamma by T cell and can upregulate pro-inflammatory responses mediated by NF Kappa B**
- **Exogenous E2 enhances expansion of T reg cell populations in mice and healthy women**
- **High doses E2 lowers IL 17 production by Th 17 cells**
- **Ovariectomy of mice increases Th17 cells and IL 17 production**
- **E2 at physiological doses stimulates humoral responses to infection**

Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38

80% of Autoimmune Diseases in Females

Most pronounced for:

- **Sjögren syndrome**
- **Systemic lupus erythematosus**
- **Thyroid diseases**
- **Scleroderma**
- **Myasthenia gravis**

Why?

Khan et al. Front Immunol.2015;6:635
Klein S and Flanagan K. Nat Rev Immunol. 2016 Oct;16(10):626-38

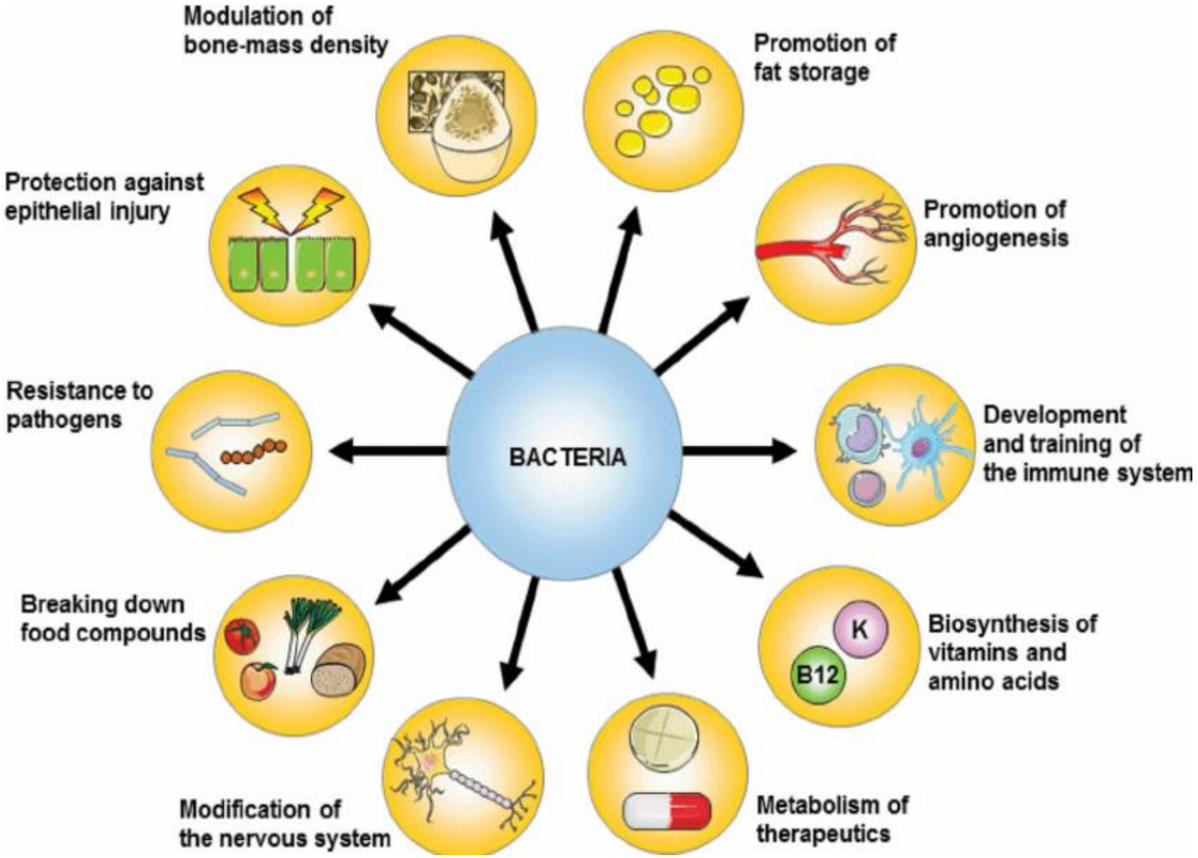
Pregnancy & Menopause

- **Pregnancy:**
- **Immune system skews from Th1 (IFN gamma) to Th2 (IL-4)**
- **More complex and varies with trimester**
- **Pregnancy often associated with improved symptoms of autoimmune disease – down-regulation of ER alpha by ER beta**
- **Menopause:**
- **Associated with increased Th17 – increase in inflammation!!!**

Protective effects of estrogen in autoimmune conditions such as MS and RA are believed to be related to estrogen-mediated T reg expansion and activation

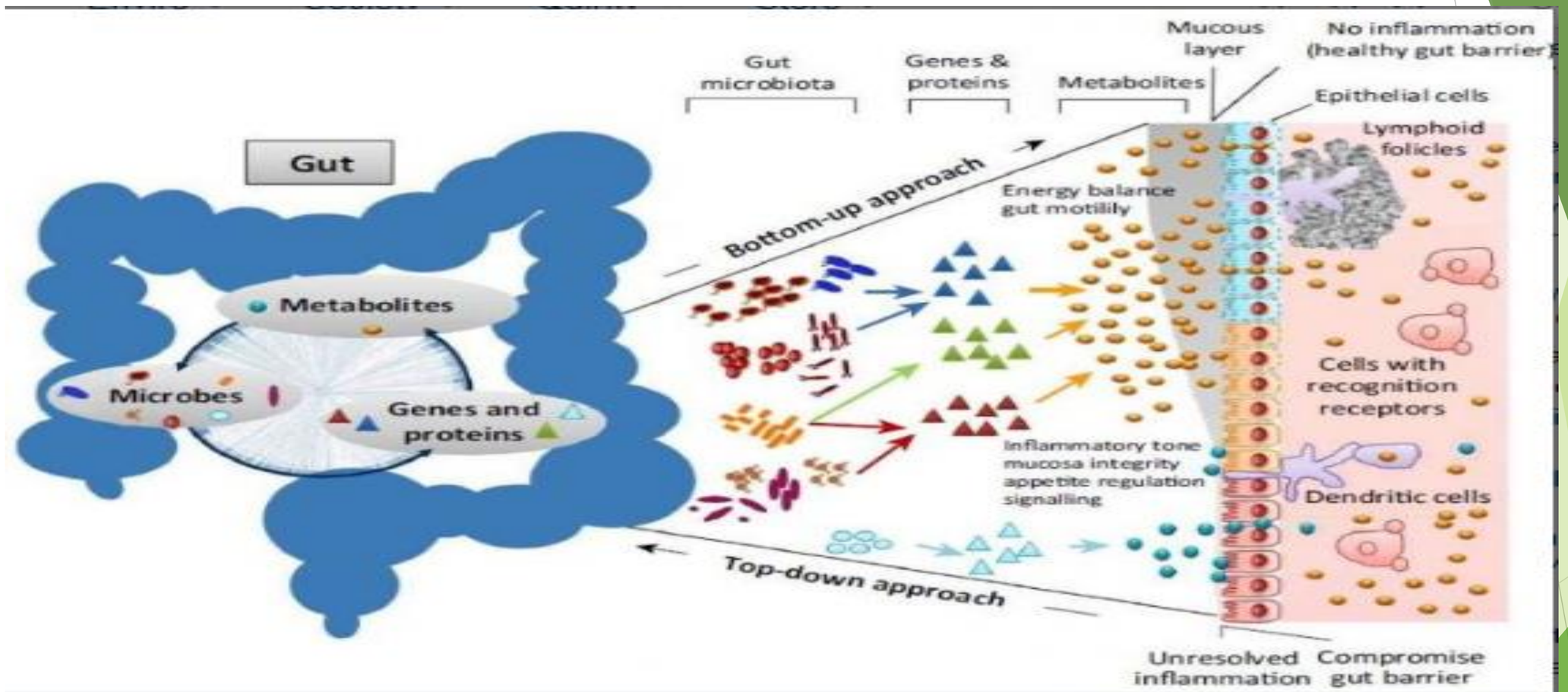
Estradiol & Gut Immune System

- **E2 has important role in immune system involving gut – maintains healthy host – microbiota interactions**
- **Peyer's Patches, as part of GALT, acts as inductive sites of intestinal immune responses - generating immune tolerance and preventing systemic inflammations**



Aug 2015 · FEMS microbiology reviews
Bird et al. Cell Immunol. 2008;252(1-2):57-67

The Complex World of the Gut

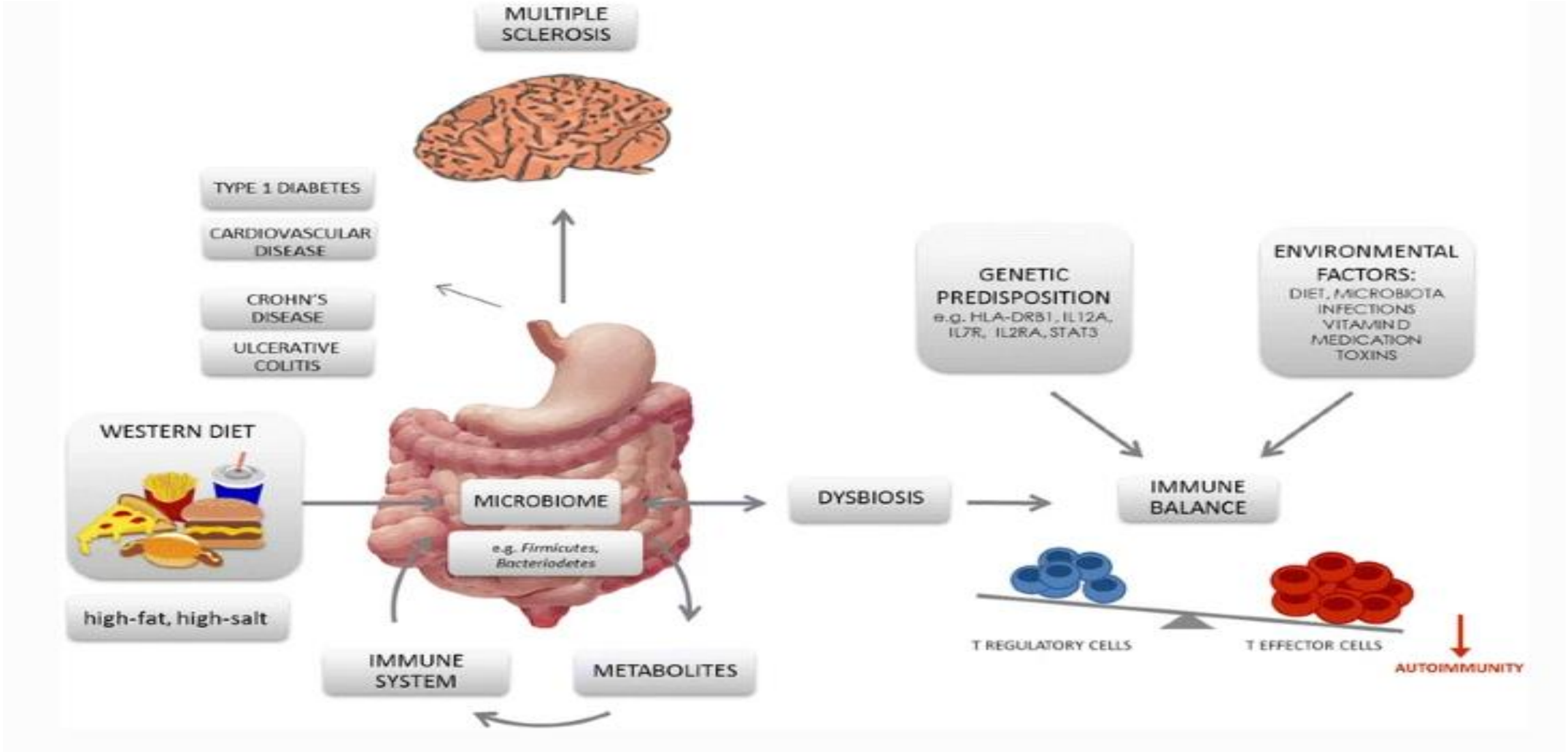


This figure shows how microbiota species are interchangeable in terms of functions by means of the metabolites produced by the action of gene products contained in the gut bacteria.

Credit: Moya and Ferrer/Trends in Microbiology 2016

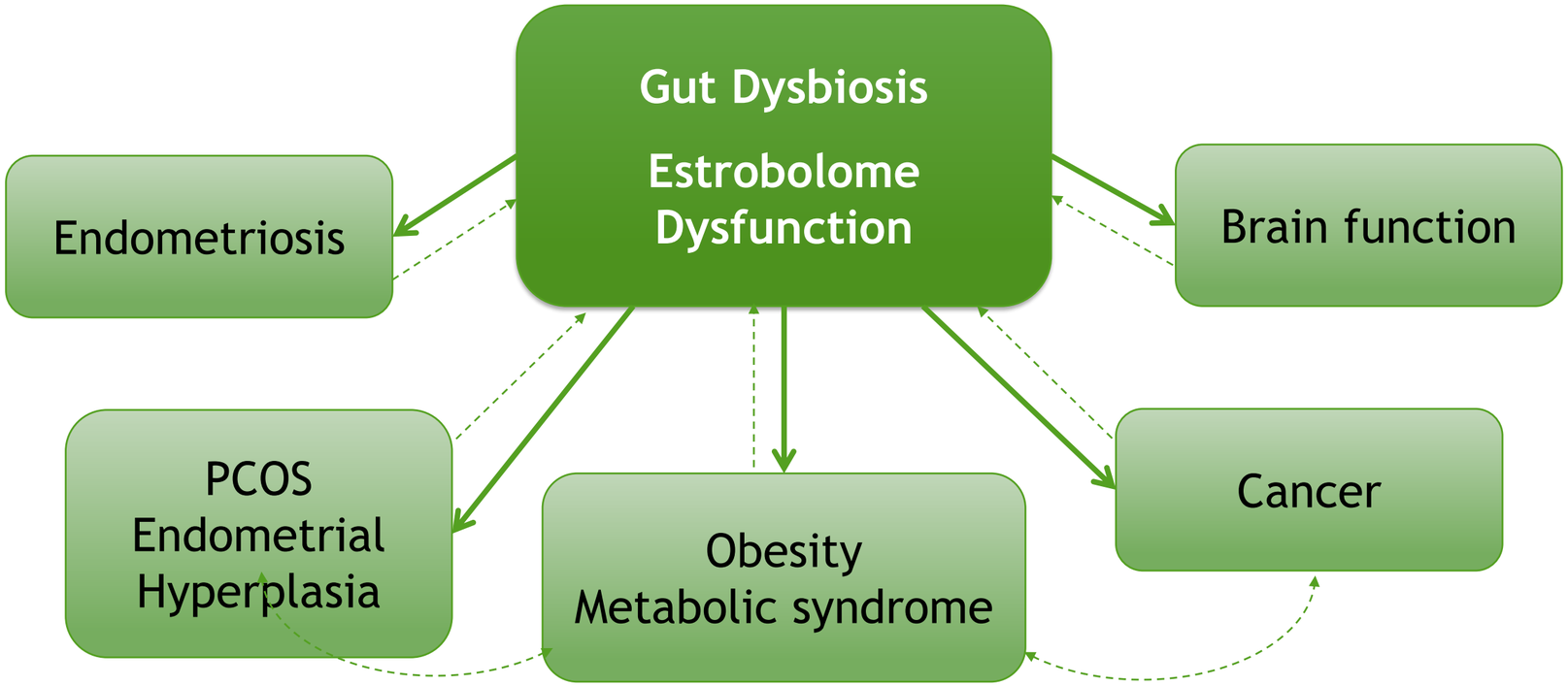
Close

Dysbiotic Microbiome -
Treg Deficiency & Activation of Proinflammatory Th17 cells



Jorg et al. Cell Mol Life Sci. 2017;73(24):4611-4622

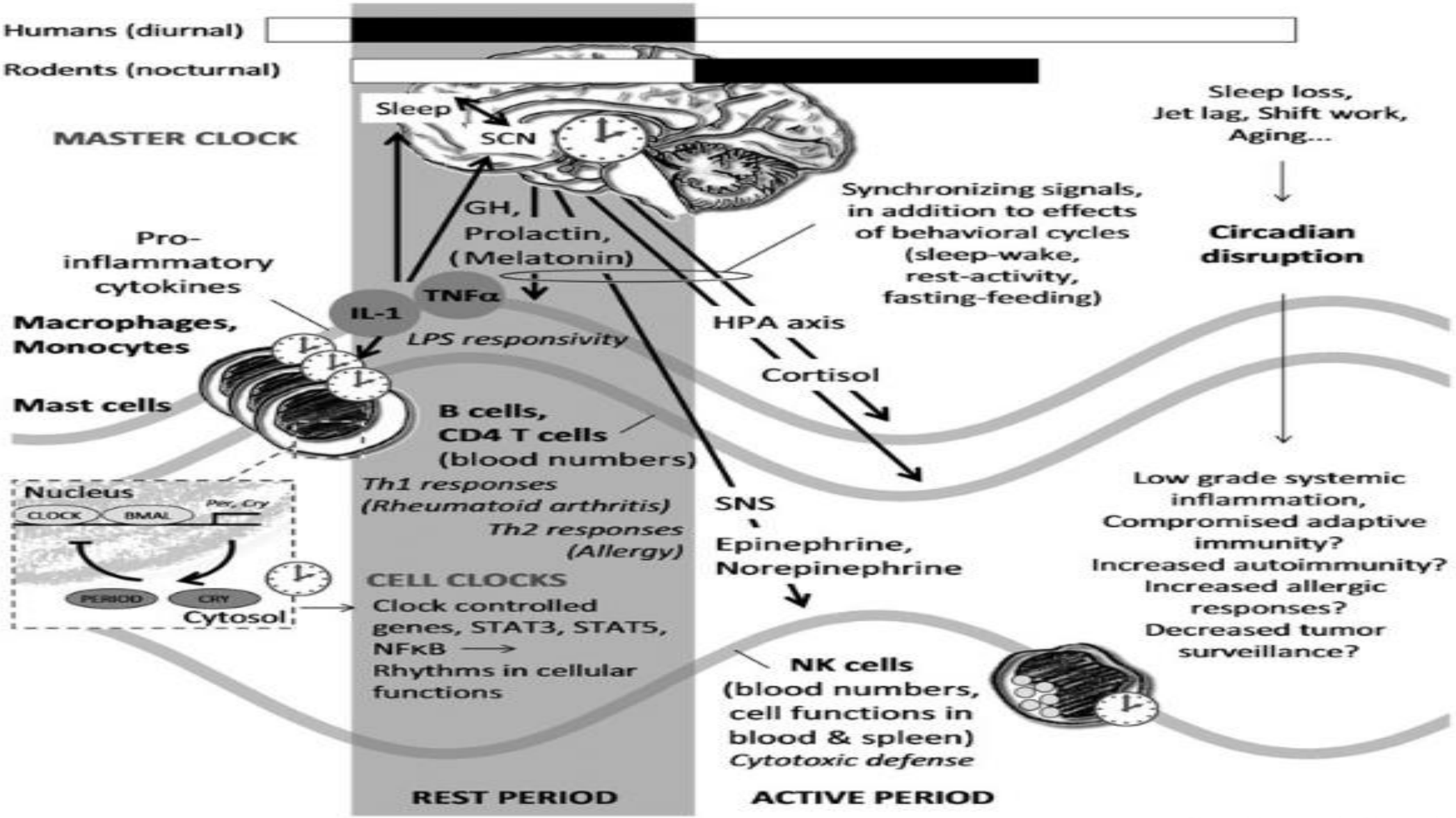
Impact of Estrobolome with Dysbiosis



- **Estrobolome: Unique portion of gut microbiome - metabolizes & modulates estrogen**
- **Dysbiosis impacts estrobolome & wide range of diseases**

Plottel CS et al. *Cell Host Microbe*. 2011;10(4):324-335.
Baker JM et al. *Maturitas*. 2017;103;45-53.

Immune System + Circadian Rhythm

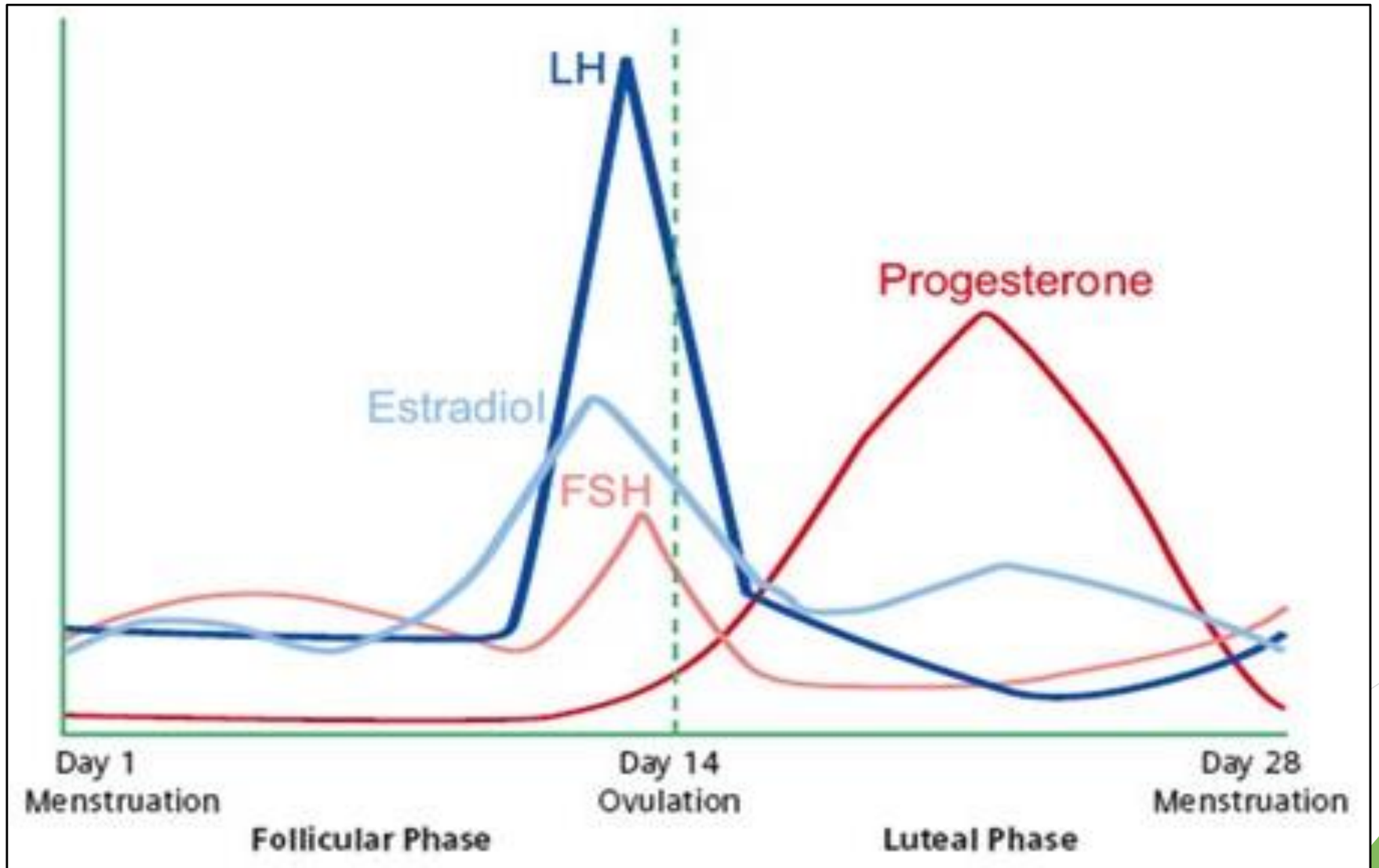


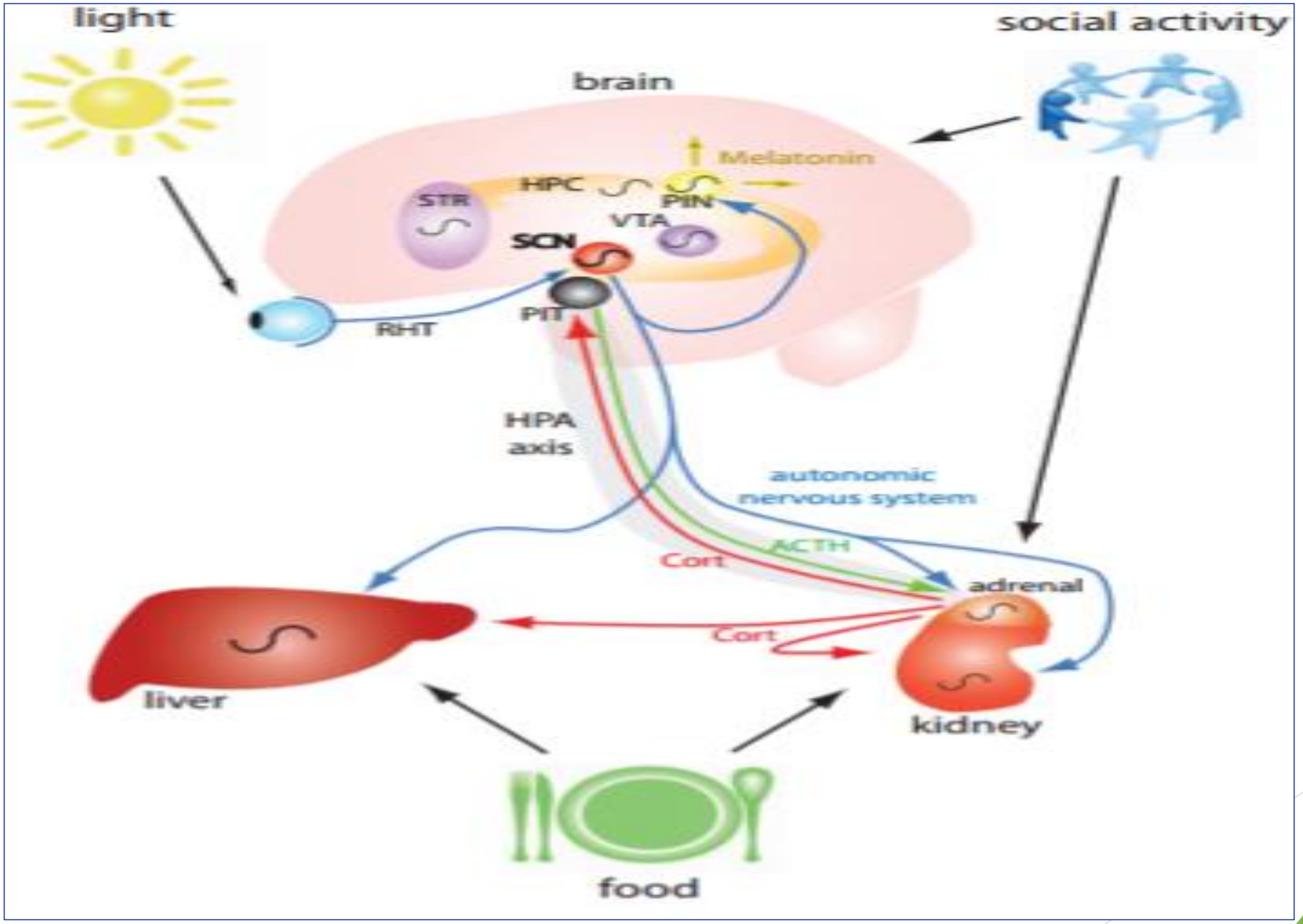
Cirmakian et al. Chronobio Intern.2013;1-19

Supplements for Immune Health

- Omega 3
- Vitamin D
- Quercetin
- Astralagus
- Astrographis
- Reishi Mushrooms
- NAC and Glutathione
- Zinc
- Probiotics and Prebiotics
- Multi-vitamin

Menstrual Cycle and Health





Behav Neurosci 128(3) pg 326-43

THANKS SO MUCH!

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