Innate Immunity, Inflammation and Toll-like Receptors (TLRs)

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Overview

I. Inflammation and the Immune Response

II. Positive and Negative Outcomes of and Immune Response

III. Toll-like Receptor (TLR) Biology

IV. Innate Immunity in the CNS

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Immune System

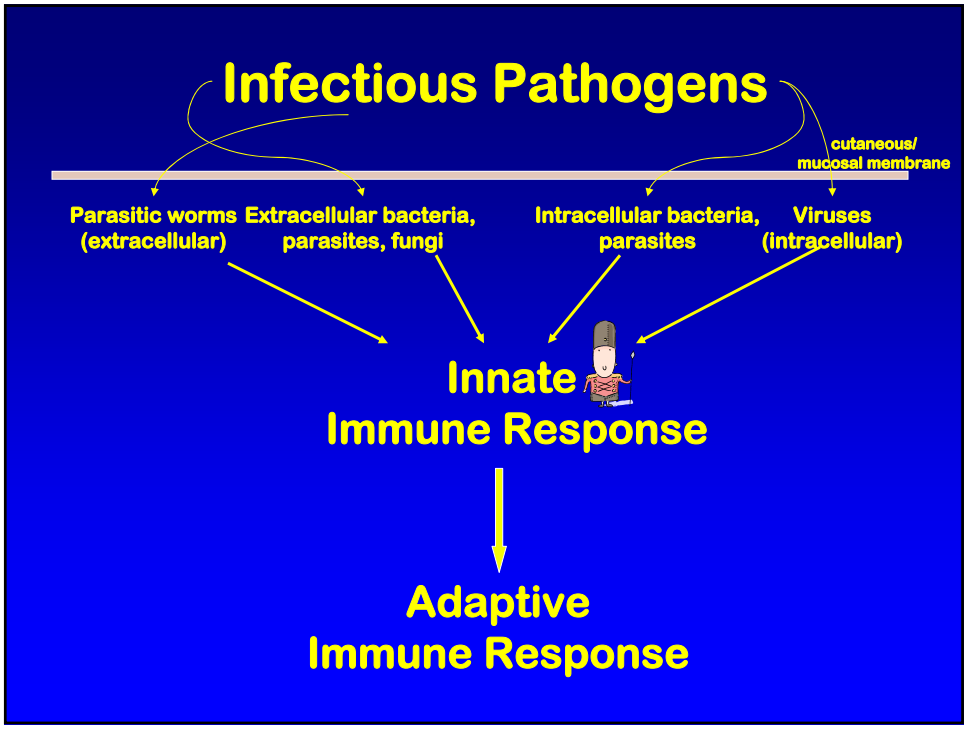
A system of defenses
by which the body
(host)
recognizes self from non-self
(foreign material)

The immune system
destroys or neutralizes
foreign matter, both living and nonliving.

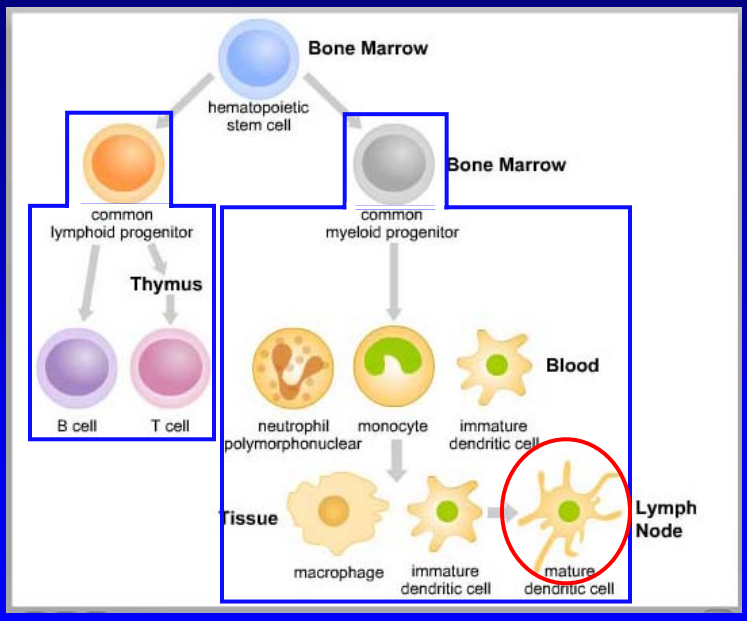
White Blood Cells are Mediators of the Immune Response

White Blood Cell Lineages

The Immune Response to Infectious Pathogens



Both Innate and Adaptive Immunity Depend on the Activities of White Blood Cells



Innate Immune Response

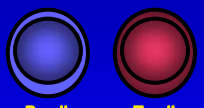
neutrophils

macrophages

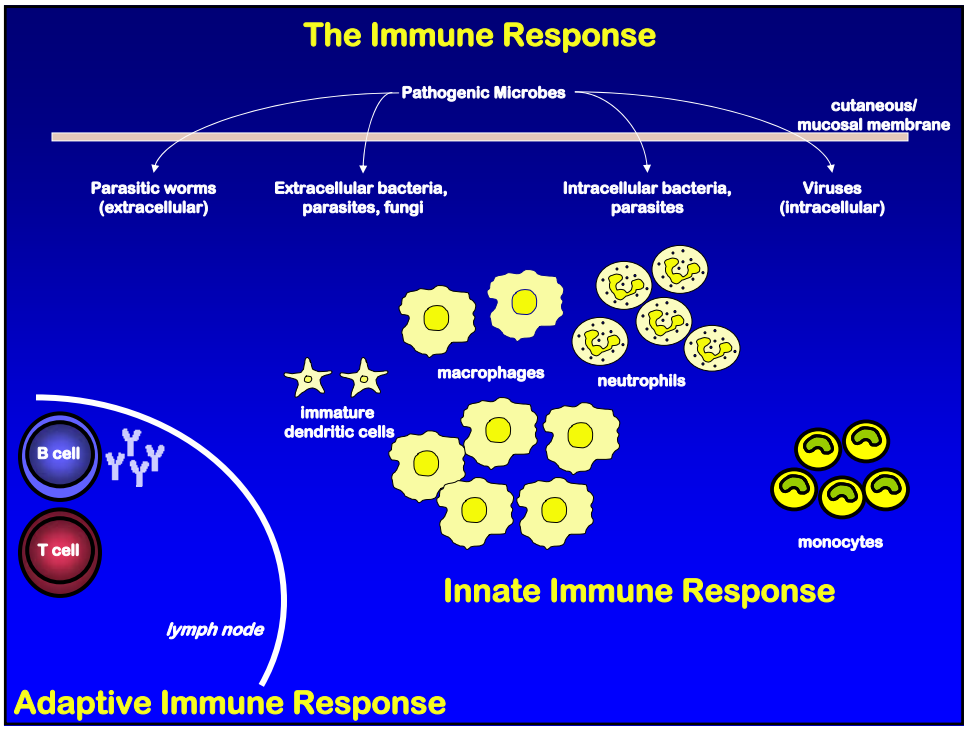
Adaptive Immune Response

dendritic cells "DC"

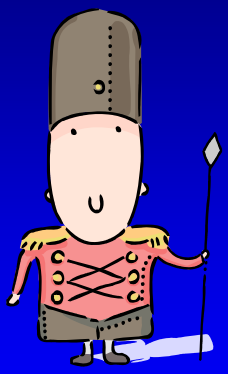
lymphocytes



Innate Immune Response	Adaptive Immune Response
- Immediate response 0-96 hours	- Gradual response > 96 hours
-Targets groups of pathogens	-Targets specific pathogens
- No Memory	- Memory

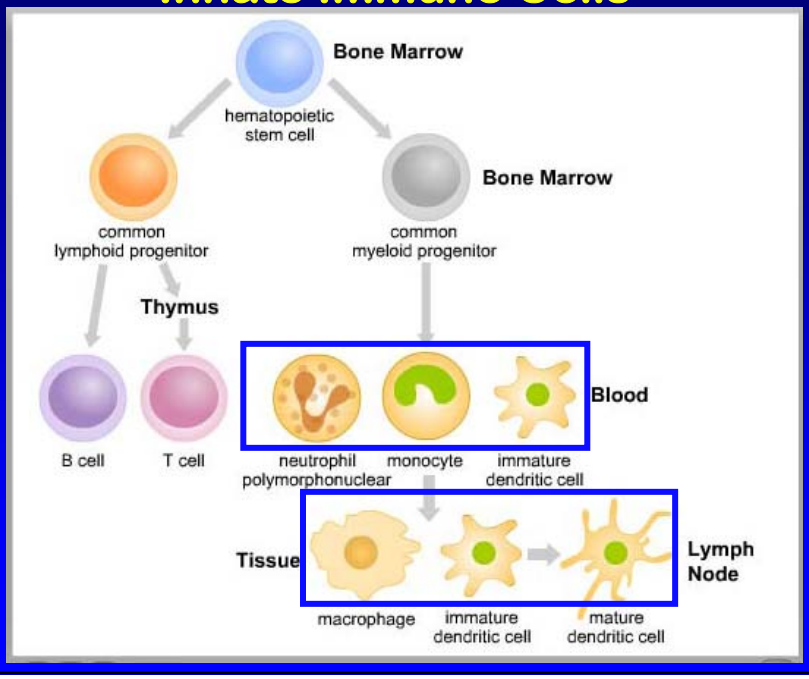


Innate Immunity



The First Line of Defense

Innate Immune Cells



Recognition of Pathogens by Innate Immune Cells

initiated when innate immune cell

Pattern Recognition Receptors

including *Toll-like receptors (TLRs), Nod-like receptors (NLRs)and RIG-like receptors (RLRs)*

are triggered by microbe-specific motifs,

Pathogen-Associated Molecular Patterns (PAMPs)

Events Elicited by Triggering of Macrophage and Neutrophil TLRs

- phagocytosis
- secretion of inflammatory cytokines
- secretion of chemokines (chemoattractants); recruitment of additional innate immune cells

Ilya Ilich (a.k.a. Elie) Mechnikov

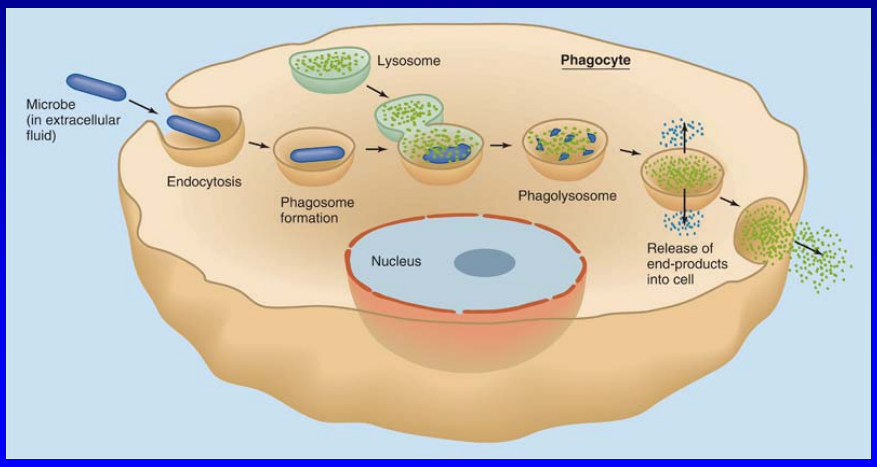
First Observed Phagocytosis by Phagocytes

a fundamental process of the innate immune response

http://www.pasteur.fr/pasteur/musees

Phagocytosis

Microbe or other foreign material taken up by endocytosis and isolated and destroyed within a phagolysosome



Vander's Human Physiology. The McGraw-Hill Companies, Inc. , Editors

Agents produced or released by *phagocytes* on ingestion of microorganisms

Acidification	pH 3.5-4.0 bacteriostatic bacteriocidal
Toxic O_2-derived products	superoxide O_2^- H_2O_2 hydroxyl radical OH^+
Toxic nitrogen oxides	nitric oxide NO
Antimicrobial peptides	defensins cationic proteins
Enzymes	lysozyme acid hydrolases
Competitors	lactoferrin (binds Fe) vitamin B_{12}-binding protein

Secretion of Inflammatory Cytokines and Chemokines

Cytokines

- secreted in response to an activating stimulus
- stimulate cellular effector functions
  (eg. bacteriocidal activity of macrophages)
- induce responses by binding to specific receptors
  autocrine acting
  paracrine acting
  endocrine acting

Chemokines

- class of cytokines
- *chemoattractant properties*
- induce cells with appropriate chemokine receptors to migrate toward the chemokine source

Acute Inflammatory Events During Innate Immune Response to Infection

1. ***Vasodilation*** of the microcirculation leading to increased blood flow to the infected area

2. ***Increased permeability*** of capillaries and venules with diffusion of blood proteins and filtration of fluid into the interstitial spaces

Above events occur within seconds to minutes of infection
Subsequently.....

3. ***Chemotaxis*** with movement of leukocytes from venules into the interstitium of the infected area

4. ***Destruction*** of pathogens in the tissues by phagocytosis and other mechanisms

Acute Inflammation

classic signs and symptoms

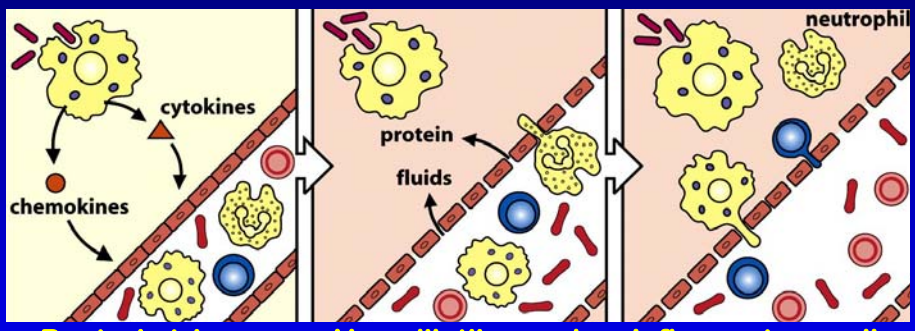
Redness

Heat

Swelling

Pain

Inflammation



Bacteria trigger macrophages to release cytokines & chemokines

Vasodilatiion and increased vascular permeability cause redness, heat & swelling

Inflammatory cells migrate into tissue, releasing inflammatory mediators causing pain

Adapted from: Immunobiology . Janeway, Charles A.; Travers, Paul; Walport, Mark; Shlomchik, Mark New York and London: Garland Science; c2001

Important Cytokines Secreted by *Pathogen Activated Macrophages*

interleukin-1 (IL-1)

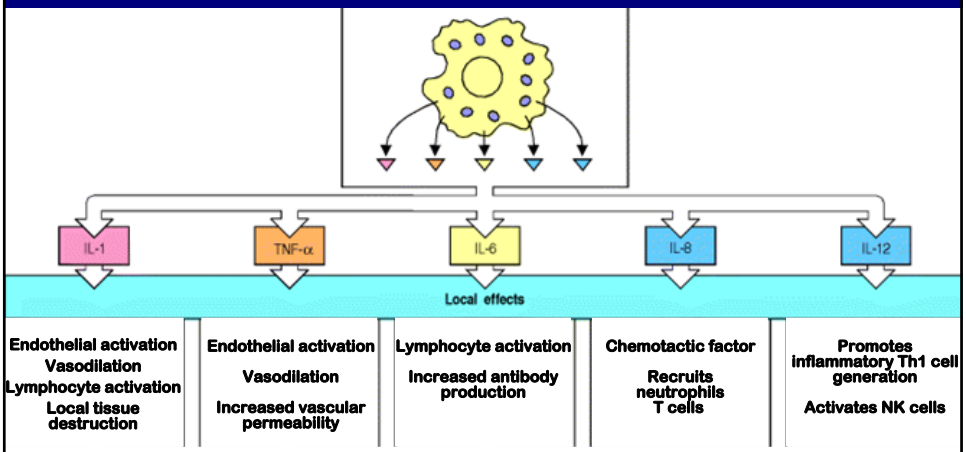
interleukin-6 (IL-6)

TNF-α

interleukin-12 (IL-12)

interleukin-8 (IL-8)

Cytokine Secretion by Activated Macrophages



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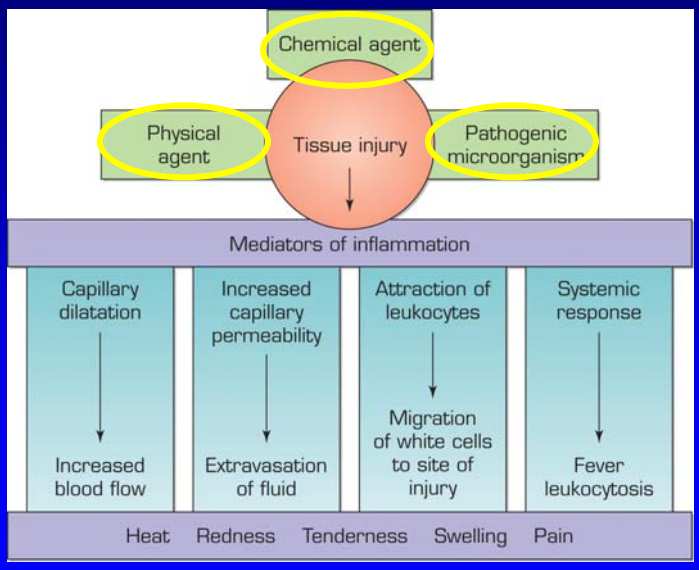
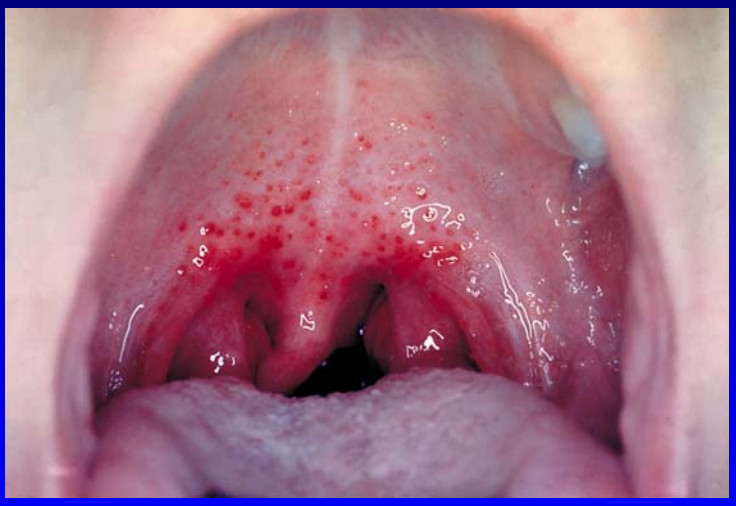
Inflammation



Vander's Human Physiology. The McGraw-Hill Companies, Inc. , Editors

Inflammation Associated with Strep Throat



Note the inflammation of the oropharynx and small red areas of hemorrhage (petechiae).
Strep throat is caused by group A *Streptococcus* bacteria which can spread through direct contact with persons who are infected.

Summary of the Innate Response to an Invading Pathogenic Microbe

macrophage

- TLRs and other pattern recognition receptors bind pathogenic microbe motifs trigger macrophage to *phagocytize* and *destroy* infecting microbe
- activated macrophages secrete *chemokines* that attract additional innate immune cells neutrophils & monocytes

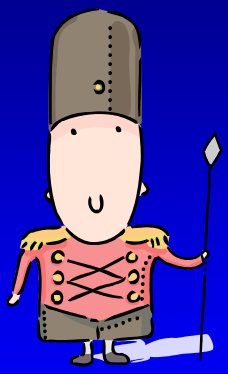
neutrophil

- primary cell seen early in response to pathogens
- phagocytize and destroy invading microbes

monocytes

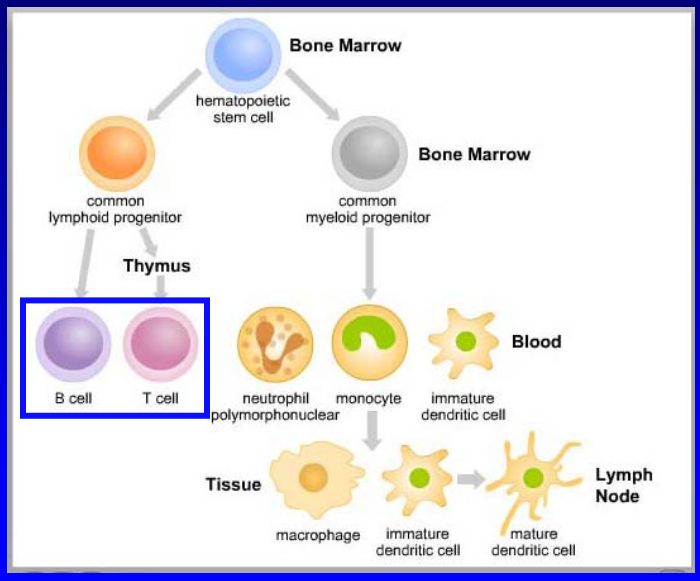
- rapidly differentiate into macrophages adding to the defenses

Adaptive Immunity



The Backup Line of Defense

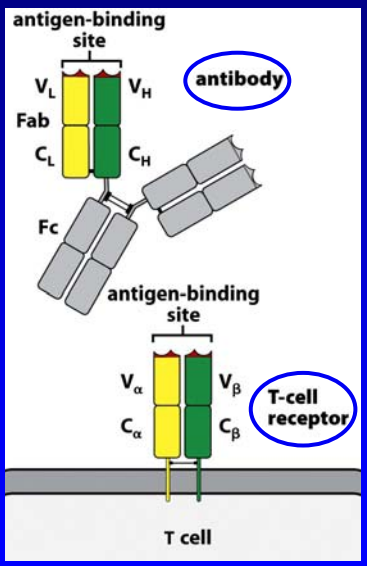
Adaptive Immune Cells



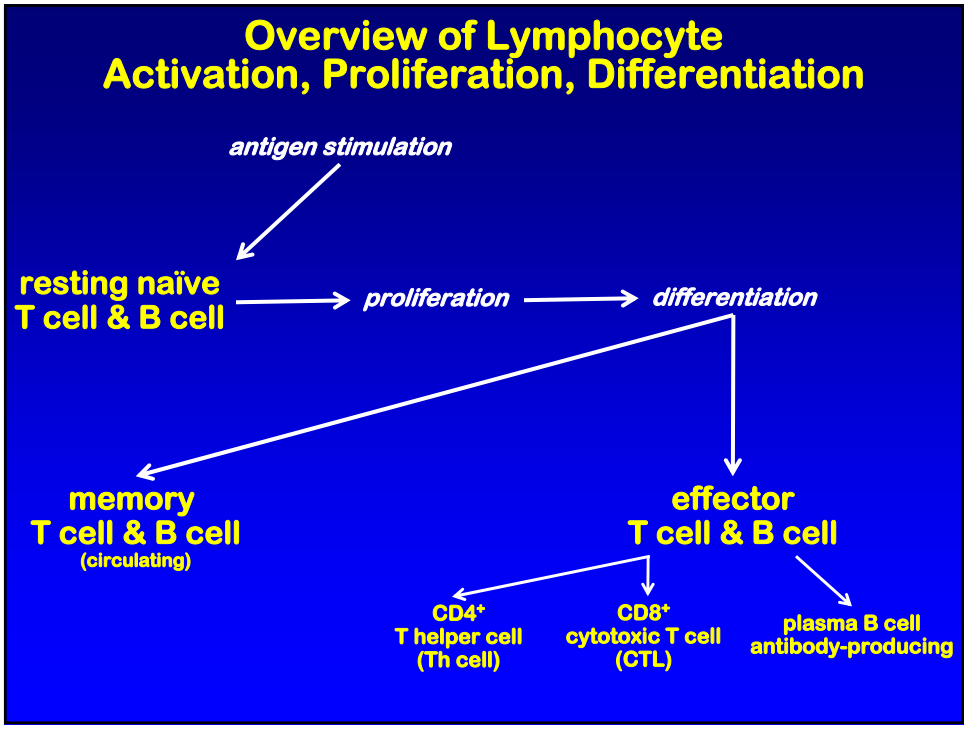
Antigen

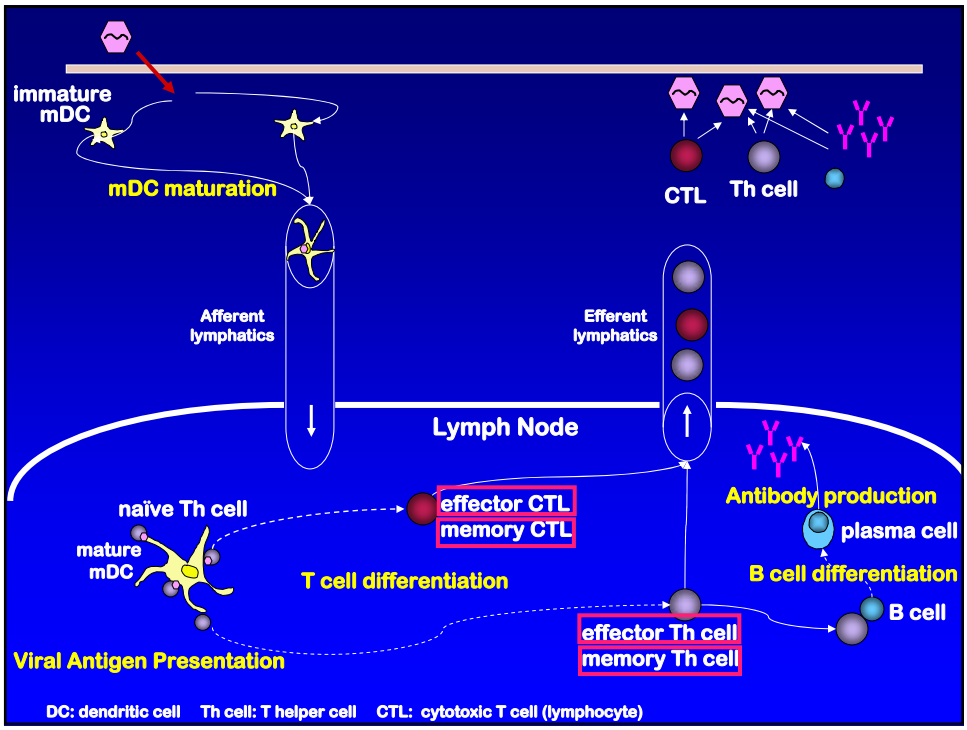
that which is recognized by the adaptive immune system

Antigen Binding Site of Antibody and T-cell Receptor Molecules

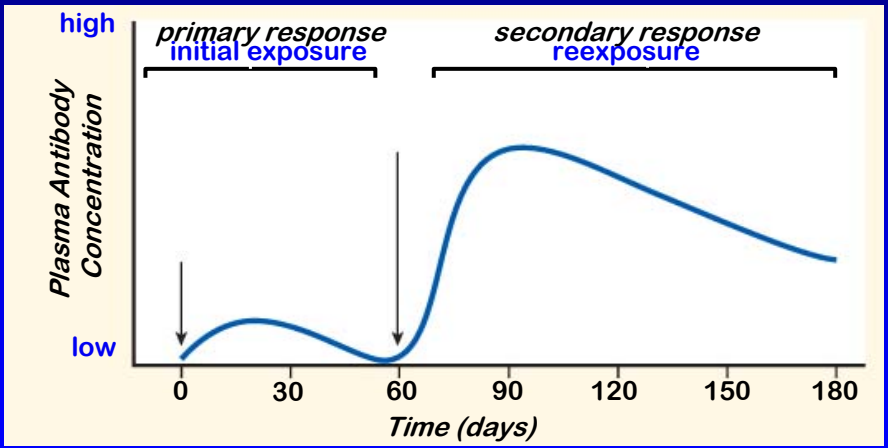


Immunobiology . Janeway, Charles A.; Travers, Paul; Walport, Mark; Shlomchik, Mark New York and London: Garland Science; c2001 . http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=imm&part


Overview of Lymphocyte
Activation, Proliferation, Differentiation
antigen stimulation
resting naïve
T cell & B cell
proliferation
differentiation
memory
T cell & B cell
(circulating)
effector
T cell & B cell
CD4+
T helper cell
(Th cell)
CD8+
cytotoxic T cell
(CTL)
plasma B cell
antibody-producing


immature
mDC
mDC maturation
CTL
Th cell
Afferent
lymphatics
Efferent
lymphatics
Lymph Node
naïve Th cell
mature
mDC
effector CTL
memory CTL
T cell differentiation
Antibody production
plasma cell
B cell differentiation
B cell
effector Th cell
memory Th cell
Viral Antigen Presentation
DC: dendritic cell Th cell: T helper cell CTL: cytotoxic T cell (lymphocyte)

Adaptive Immunity has Memory



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Positive Outcomes of an Immune Response

Protection from Infectious Disease (Positive Outcome)

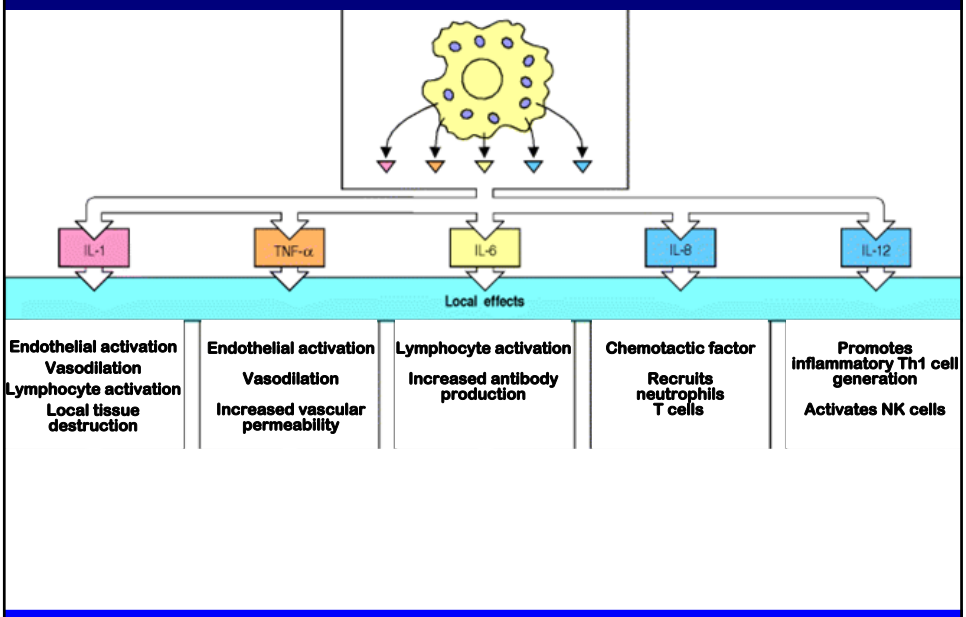
- natural immunity protects from reinfection
- vaccination protects from primary infection

Negative Outcomes of an Immune Response

Shock and Tissue Damage Negative Outcomes

- acute effects due to a "cytokine storm" / "cytokine surge"
  (endotoxic shock, SARS, Hanta, Dengue)
- chronic effects of cell mediated granuloma formation
  (Schistosomiasis)
- autoimmunity
  (Multiple Sclerosis, Systemic Lupus Erythematosus)

The Cytokine Storm Endotoxic Shock



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Chronic Schistosomiasis

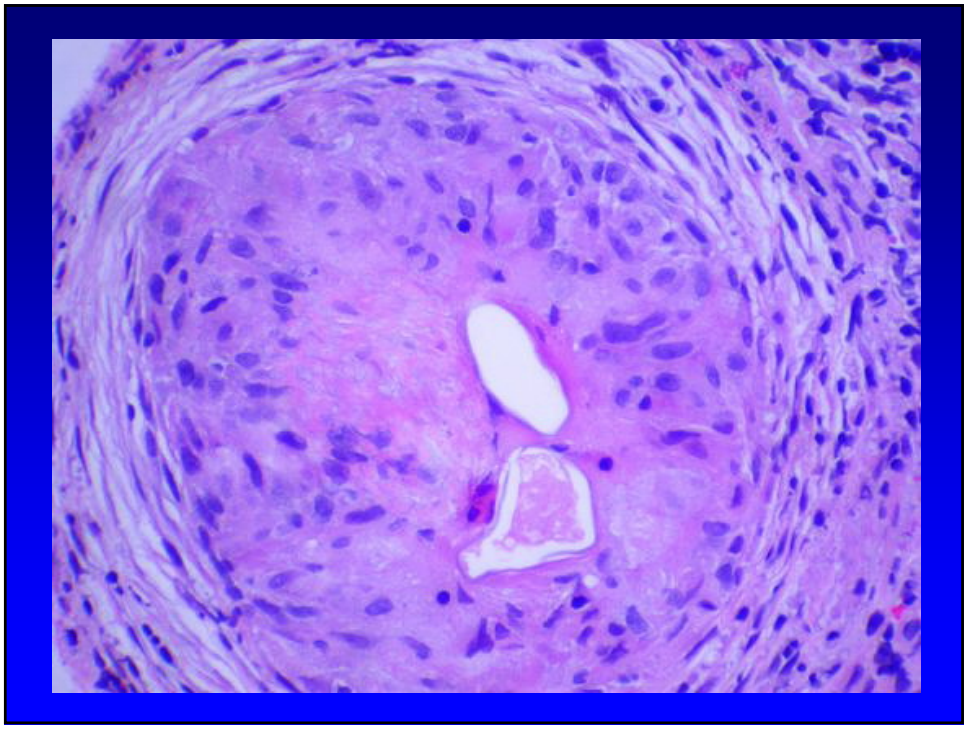
continuing infection causing
granulomatous reactions to schistosoma eggs
and
fibrosis in the affected organs

Egg → **Granuloma formation** → **Fibrosis** → **Disease**

Host Response

Collagen Deposition
Tissue Scarring

Circulatory Obstruction
Organ malfunction



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As early as 1989, Charles Janeway theorized that the innate immune system used specialized receptors to recognize infecting pathogens.

Approaching the Asymptote? Evolution and Revolution in Immunology
C.A. Janeway, Jr.

Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role of the Toll gene product.

Anderson KV, Jürgens G, Nüsslein-Volhard C.
Cell. 1985 Oct;42(3):779-89.

Toll Mutation Severely Reduces Survival of Adult Flies after Fungal Infection

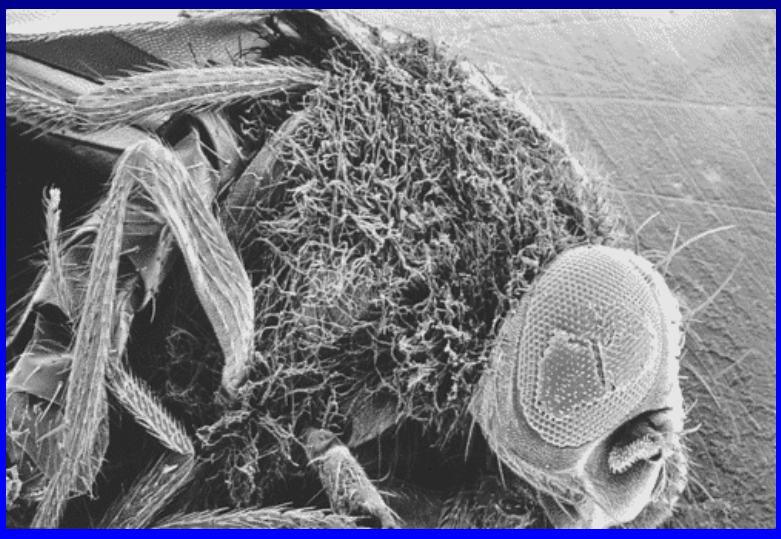
Cell, Vol. 86, 973–983, September 20, 1996, Copyright ©1996 by Cell Press

The Dorsoventral Regulatory Gene Cassette *spätzle/Toll/cactus* Controls the Potent Antifungal Response in Drosophila Adults

Table 1. Survival of Dorsoventral Mutant Adults to Bacterial and Fungal Infections

Genotype Tested	Fungal Infection	Bacterial Infection
Or^6	89 (4.2;9)	95 (5.3; 14)
dl^1/dl^1	94 (4.3; 5)	92 (3.0; 6)
$pll^{9/6}/pll^5$	4 (7.4; 5)	87 (8.5; 8)
tub^{ts}/tub^5	3 (5.3; 6)	71 (27; 4)
Tl^{ts}/Tl^{1-RXA}	8 (10.8; 8)	93 (6.6; 9)
spz^{rm7}/spz^{rm7}	3 (5.6; 7)	84 (11; 9)
ea^1/ea^8	98 (8.8; 5)	87 (5.7; 8)
imd/imd	93 (5.6; 5)	8 (7.4; 13)
imd/imd; Tl^{ts}/Tl^{1-RXA}	1 (2.3; 5)	3 (4.4; 6)

Germinating fungal hyphe on a drosophila deficient for a *Toll* receptor gene



Lemaitre, B. *Cell.* 86 (6) 973-983

Toll Receptors

- best-defined pattern recognition receptors of innate immune system
  (others include Nod-like receptors [NLRs] and RIG-like receptors [RLRs])
- *Toll receptor* stimulation triggers production of anti-fungal peptides in response to fungal infections
- different Toll family members are involved in activating an anti-bacterial and anti-viral responses

Toll-like Receptors

TLRs

Mammalian homologues of
drosophila Toll receptors

Toll-like Receptors (TLRs)

bacterial lipopolysaccharide, LPS

- **cell-wall component of gram-negative bacteria**
- **can induce a dramatic systemic reaction known as *endotoxic shock***

Mutant Mice with TLR4 Gene Mutation

- ***unresponsive to bacterial lipopolysaccharide, LPS***
  cell-wall component of gram-negative bacteria
- ***protected from endotoxic shock***

Defective LPS Signaling in C3H/HeJ and C57BL/10ScCr Mice: Mutations in *Tlr4* Gene

Poltorak et.al. Science. 1998 Dec 11;282(5396):2085-8.

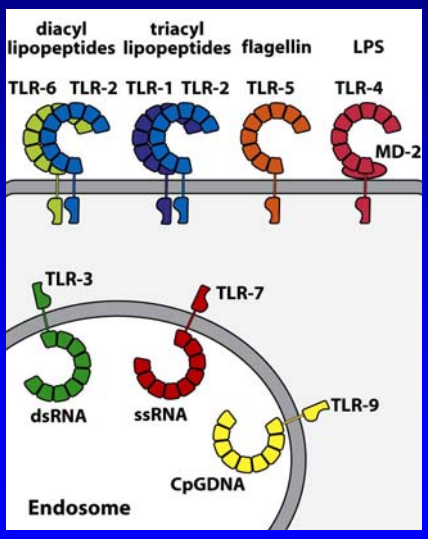
Human Toll-like Receptors

TLR	Exogenous Ligand; Pathogen
TLR1	tri-acetylated lipopeptides, porins; Gram positive and negative bacteria
TLR2	lipopeptides, peptidoglycans, glycolipids, polysaccharides; virus, Gram positive bacteria, yeast
TLR3	double-stranded RNA (dsRNA); viruses
TLR4	LPS (lipid A); Gram- negative bacteria
TLR5	flagellin; bacteria
TLR6	di-acetylated lipopeptides; Gram positive bacteria
TLR7	single-stranded RNA (ssRNA); viruses
TLR8	single-stranded RNA (ssRNA); viruses
TLR9	unmethylated CpG DNA; bacteria, viruses
TLR10	?

Toll-like Receptors TLRs

plasma membrane
TLRs 1, 2, 4, 5, 6, 10

endosome membrane
TLRs 3, 7, 8, 9



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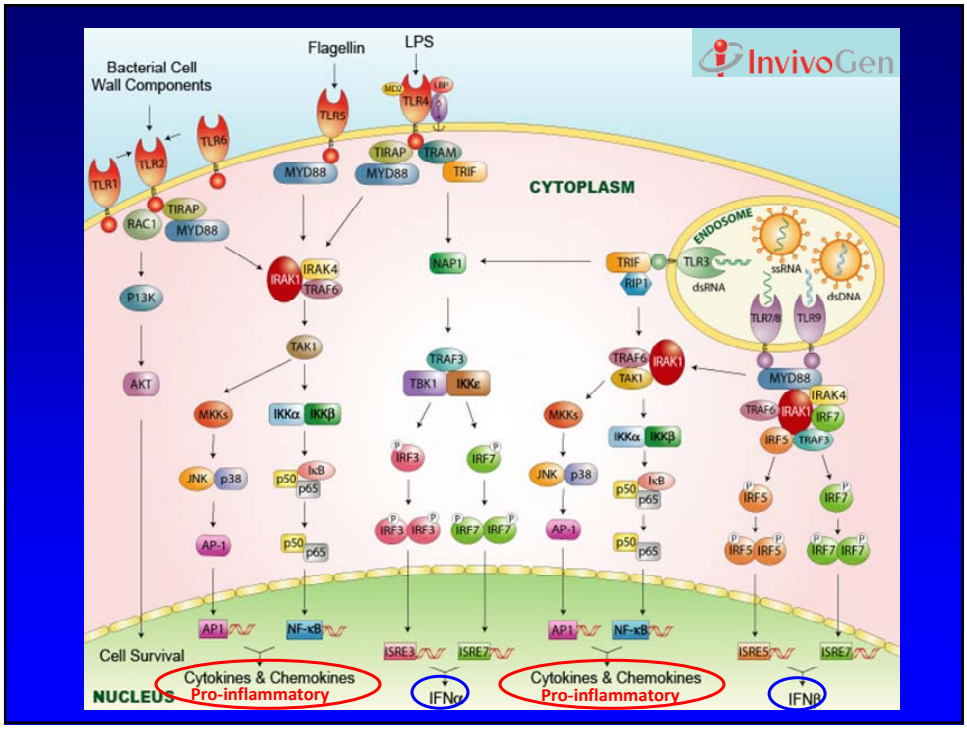
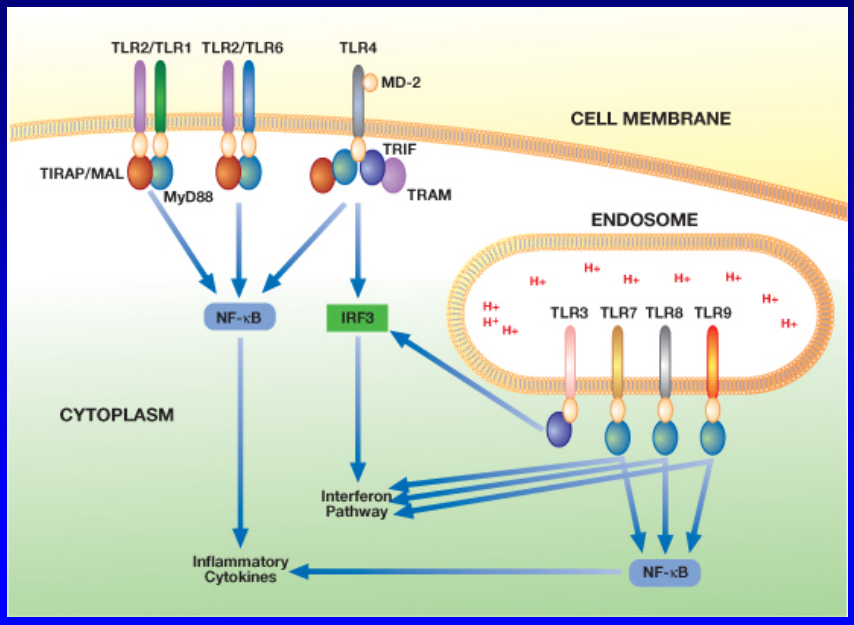
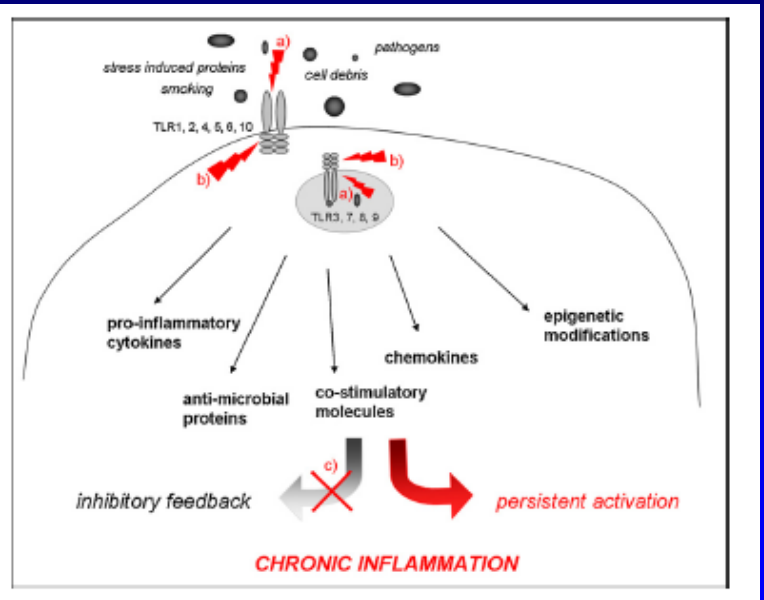




J Virol. 2004 August; 78(15): 7867–7873.

Human Toll-like Receptors

TLR	Endogenous Ligand
TLR1	
TLR2	Hsp60; Hsp70; Gp96; HMGB1
TLR3	double-stranded RNA (dsRNA)
TLR4	Hsp60; Hsp70; Gp96; HMGB1; Fibrinogen, Surfactant protein A, Fibronectin extra domain A, Heparansulfate, defensin 2
TLR5	
TLR6	
TLR7	single-stranded RNA (ssRNA)
TLR8	single-stranded RNA (ssRNA)
TLR9	DNA, DNA-containing immuncomplexes
TLR10	



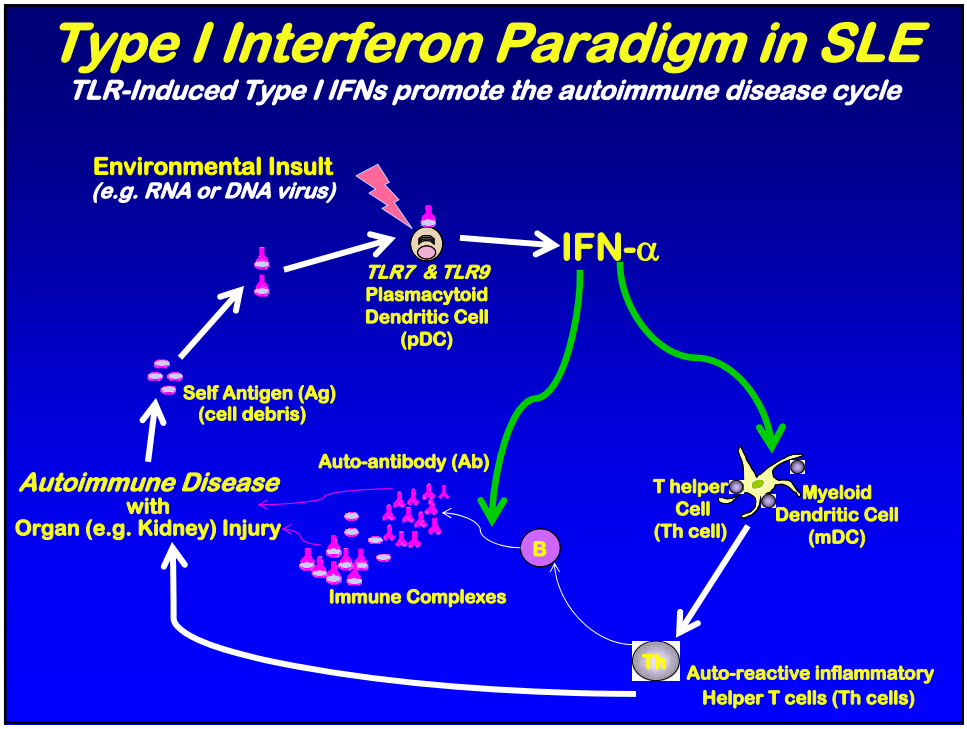
C. Ospelt, S. Gay / The International Journal of Biochemistry & Cell Biology 42 (2010) 495–505

Systemic Lupus Erythematosus (SLE, Lupus)

- progressively debilitating, systemic autoimmune disease
- affects >5 million people worldwide
- disproportionately affects women of childbearing age
- affected males often experience severe disease

Both B cells and T cells Mediate Tissue Damaging Inflammation in SLE

- auto-antibody (Ab) production by B cells & immune complex deposition result in tissue inflammation and destruction
- auto-reactive T cells also cause inflammatory tissue damage
- kidney damage (glomerulonephritis) leads to kidney (renal) failure



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Sites of Immune Privilege

- Eye
- Testis
- ~~CNS~~ X

Microglia

- resident innate immune cells of the CNS
- myeloid derived immune sentinels
- express variable levels of TLR2, TLR3, and TLR4

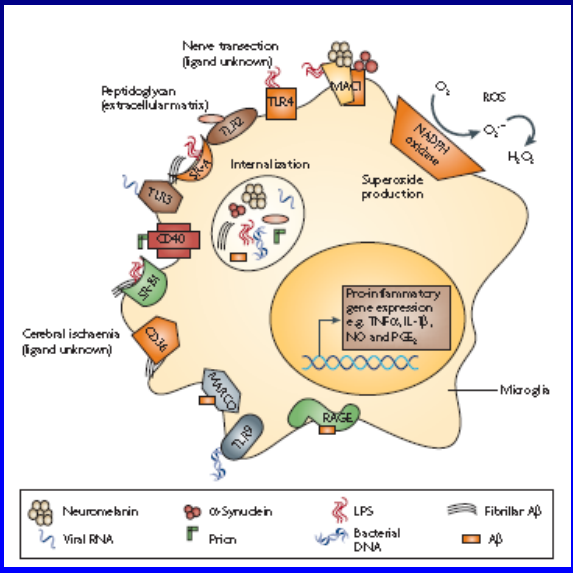
Microglia

- recognize both pathogen and host-derived ligands in the CNS

TLR-induced activation of microglia
- positive outcomes

CNS homeostasis and immunity

Microglial PRRs Recognize Neurotoxic & Pro-inflammatory Ligands



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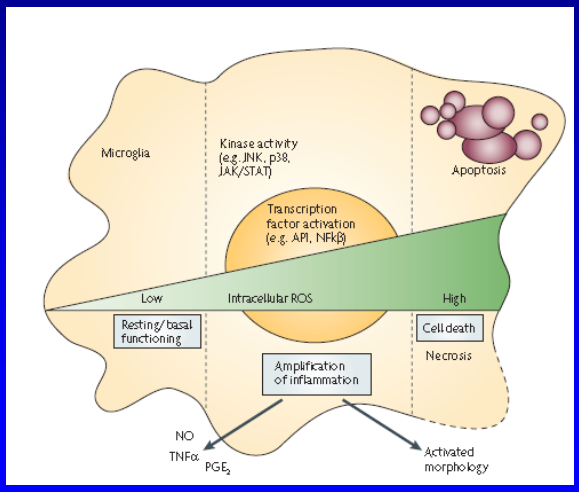
Microglial Activation Results in Generation of Reactive Oxygen Species (ROS)



NATURE REVIEWS | NEUROSCIENCE VOLUME 8 | JANUARY 2007

Microglia

- recognize both pathogen and host-derived ligands in the CNS

TLR-induced activation of microglia
- positive outcomes
CNS homeostasis and immunity

- negative outcomes:
neurotoxicity contributing to various CNS diseases
(chronic demylelinating diseases)