

Fundamentals of Host Defense and Innate Immunity

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Introduction

- Host defense (maintenance of identity) is a common feature of all metazoan animals; thus the need for an evolutionarily conserved system of humoral and cellular elements dedicated to this goal
- Immune system of vertebrates is highly complex, but lower animals exhibit remarkable abilities nonetheless
- Innate (or “natural”) immunity has historically been seen as inferior to adaptive – this is patently incorrect as new data are clearly demonstrating
- Innate immunity is not a discrete entity, but rather part of a larger continuum – a collection of integrated systems

First-line host defense

- Physical and chemical defenses
 - Skin (barrier function)
 - Respiratory system - coughing, sneezing, elevation in body temperature (also adjunct to mucosal immunity)
 - Digestive tract - pH; microorganisms in the intestines
- Inflammatory response
- Cellular defenses
 - Not strictly antigen-specific; relies on markers of pathogenicity (e.g., TLR) and tissue damage
 - Memory response is not invoked
 - Cellular elements include dendritic cells, granulocytes, macrophages, and NK cells

The inflammatory response

- Immediate danger signal following breach of the physical barrier
 - Interaction of pathogens with plasma molecules
 - Induction of tissue damage and release of mediators
 - DNA damage appears to be an important trigger
- Potentially dangerous to the host due to the vigor and magnitude of the immediate response (tight control crucial)
- Immediate responses include:
 - Vasodilation
 - Vascular permeability
 - Neutrophil recruitment and activation
 - Fever

Rubor et turgor, cum calor et dolor
(Redness and swelling with heat and pain)

Soluble mediators of inflammation

- Plasma proteases
 - Complement
 - 30 serum proteins acting in a sequential manner
 - Function to modify membranes of infectious agents (proteases) and promote the inflammatory response
 - Two pathways – one for innate and one for adaptive immunity
 - Kinens
 - Clotting and fibrinolytic proteins
- Lipid mediators
 - Prostaglandins
 - Leukotrienes
 - Platelet-activating factor
- Peptides and amines
 - Histamine and serotonin
 - Neuropeptides
 - Nitric oxide

Soluble mediators of inflammation

- Acute-phase reactants synthesized by hepatocytes: serum amyloid A, serum amyloid P, C-reactive protein, fibrinogen
 - Involved in opsonization
 - Bind bacteria to facilitate complement binding, resulting in uptake by phagocytic cells
- Proinflammatory cytokines
 - Too many to list!
 - A major initiator of both innate immunity and transition to adaptive immunity
 - This is arguably the key immunoregulatory subsystem

Recognition by innate immune response

- Recognition occurs via two mechanisms:
 - Microbial non-self
 - Missing self
- Microbial non-self depends on the presence of molecular structures that are unique to microorganisms (pathogen-associated molecular patterns, PAMPs)
- PAMPs are recognized by pattern recognition receptors (PRRs)
- Missing self depends on recognition of molecular structures that are expressed only on healthy cells of the host (lost on damage or infection)
- Recognition of missing self is important in NK cell function

Pattern recognition receptors – three basic types

1. PRRs that signal the presence of infection
 - Associated with cell surface or intracellularly
 - Engagement leads to activation of proinflammatory mediators including antimicrobial peptides and cytokines
2. Phagocytic PRRs
 - Engagement leads to phagocytosis by macrophages, dendritic cells and others
3. Secreted PRRs
 - Activate complement
 - Opsonization
 - Act as accessory molecules for other PRRs

The Toll-Like Receptor (TLR) system

- Example of PRR signaling the presence of an infection
- Plasma membrane-associated proteins with extracellular domains
- Highly conserved evolutionarily
 - Originally discovered in fruit flies
 - Appear to have both structural and defense roles
- Signaling tends to induce a TH1-type immune response

Triggers for TLRs

TLR	Natural Ligand
TLR1 (with TLR2)	Bacterial triacyl lipopeptides; parasite proteins
TLR2 (with TLR6)	Bacterial diacyl lipopeptides; lipoteichoic acid; zymosan
TLR3	DS viral RNA
TLR4	Gram-negative endotoxin
TLR5	Flagellin
TLR7	SS viral RNA
TLR8 (inactive in mice)	Same as TLR7
TLR9	CpG
TLR10 (present in mice, inactive)	Unknown
TLR11 (mice; form in human is truncated and assumed inactive)	Profilin (from <i>T. gondii</i>)
TLR12/TLR13 (mice, not human)	Unknown

Therapeutic manipulation of TLRs

- Nonspecific induction of innate immunity, particularly mucosal
 - May hold great promise in biodefense and protection against rapidly evolving infectious “events”
- Vaccine adjuvants

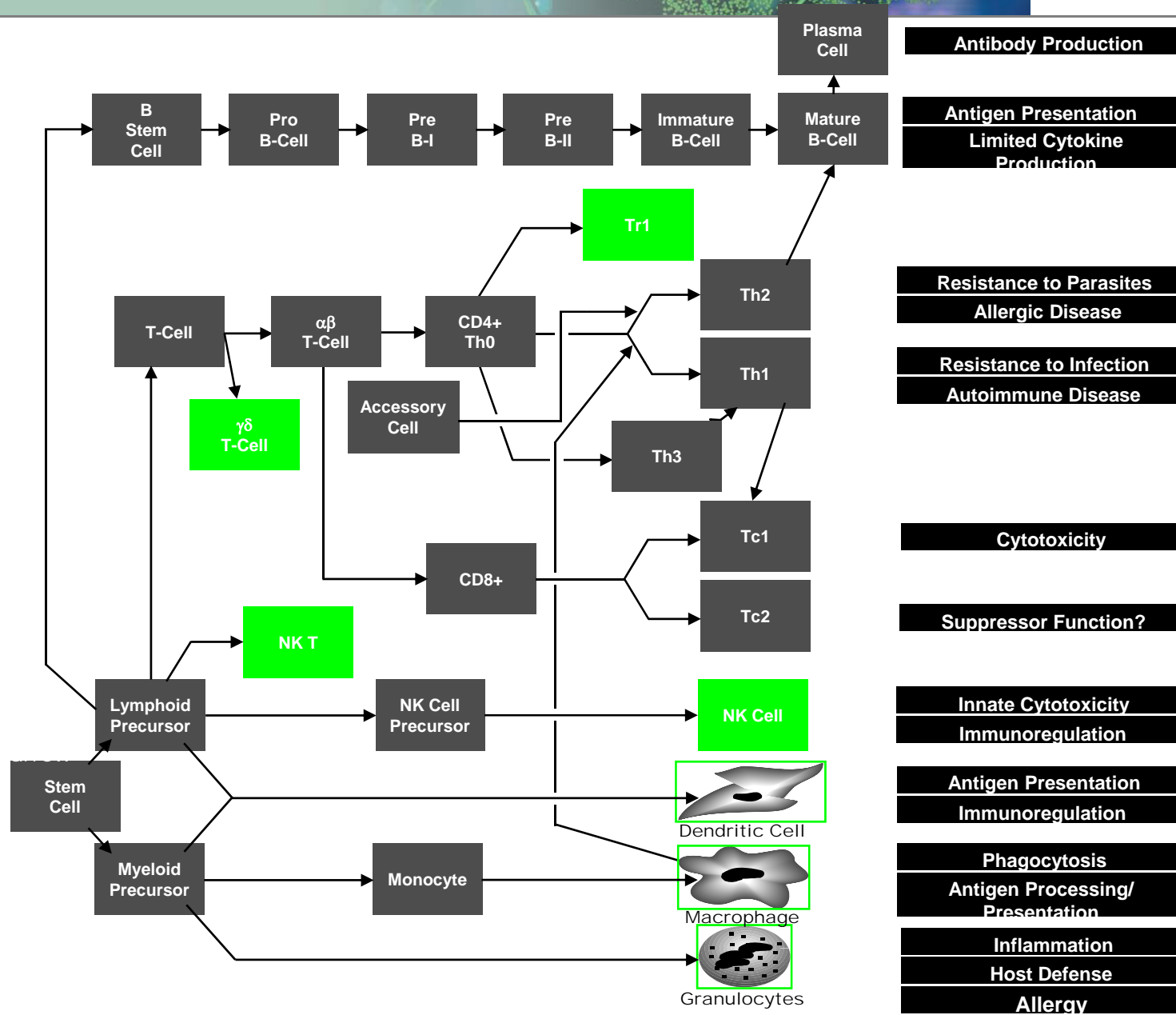
Adjuvants that act via TLRs

TRL ENGAGED	LIGANDS	ADJUVANTS
TLR1/TLR2	Triacyl lipopeptides	Pam3Cys
TLR2/TLR6	Diacyl lipopeptides	MALP-2; Pam2Cys
TLR2	Peptidoglycan	Neisserial porins; BCG; CFA
TLR3	Double-stranded RNA	Poly I:C
TLR4	LPS	MPL A; BCG; CFA; LPS analogs
TLR5	Flagellin	Flagellin
TLR7/8	Single-stranded RNA	Imiquimod; Resiquimod (synthetic TLR agonists)
TLR9	Bacterial/viral DNA	CpG ODN

Modified from Duin et al., 2005

What goes up...

- TRL modulation may be targeted toward immunosuppression, as well as immunostimulation
 - Allergy: binding of TLR9 agonist to an allergen can result in the destruction of CD4 T-cells with allergen specificity (Dynavax)
 - Autoimmunity: inactivation of TLR5 modulates lupus
 - Sepsis: Eritoran (Eisai) binds to TLR4 without activating it
- TLRs appear to have a role in plaque formation, so their modulation may have applicability to treating cardiac disease



The macrophage and its kin

- Primary cell of the innate immune system
- Monocytes circulate about one day then terminally differentiate into macrophages
- Play important roles in both innate and acquired immunity
- Activated by cytokines to be more effective killers
- Produce immunoregulatory cytokines
- Numerous non-immune housekeeping functions
- Macrophages - terminally differentiated monocytes
 - Kupffer cells - liver
 - Alveolar Macrophages – lung
 - Splenic Macrophages
 - Microglia - CNS

The granulocytes

- Primary defense against infectious agents
 - Defect of these cells (e.g., Chronic Granulomatous Disease) is associated with increased rate of infection with bacteria and fungi
- Pass through cell membrane of blood vessels
- Phagocytic activity enhanced by complement and antibodies on the surface of targets
- Involved in induction of the inflammatory response
- Multiple cell types
 - Neutrophils
 - Basophils
 - Eosinophils

Neutrophils

- Short-lived (about 24-48 hours)
- Live in circulation and are recruited to sites of infection (not tissue resident)
- Phagocytic and microbicidal
- Capable of both extracellular and intracellular killing
 - Granules released into extracellular environment
 - Reactive proteins released into the phagolysosome

Basophils

- Found primarily in connective and mucosal tissue
- Phagocytic
- Secrete inflammatory mediators important in leukocyte (neutrophil) recruitment
- Produce cytokines, mostly Type II cytokines

Eosinophils

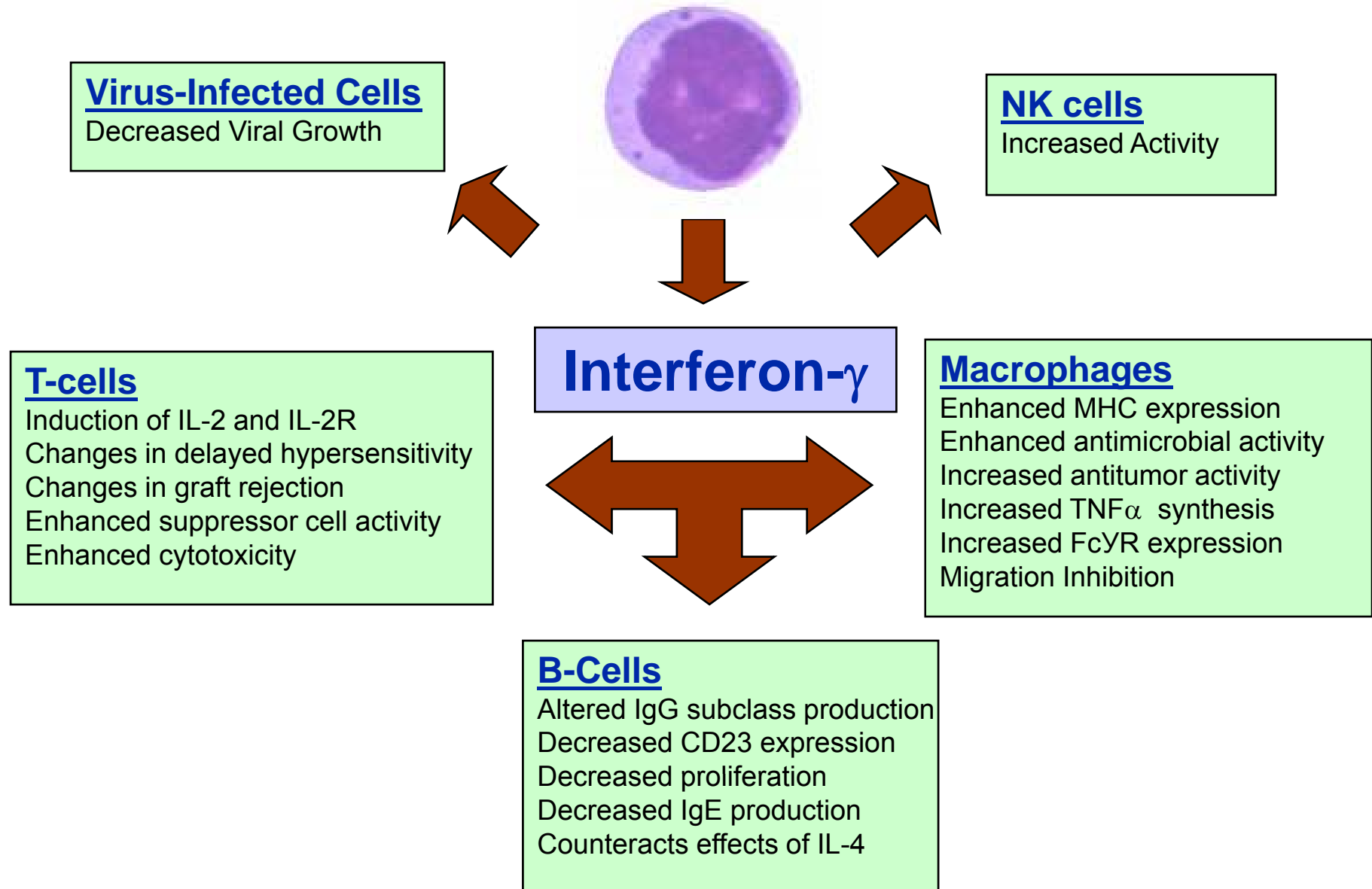
- Occur primarily in respiratory, intestinal and genitourinary tracts
- Highly granular; granules contain cationic effector proteins
- Produce cytokines and lipid mediators
- Poorly phagocytic; release granular contents into the extracellular space
- Appear to have evolved as an antiparasitic defense mechanism

Dendritic cells

- Found primarily in peripheral tissue
- Best known for their role in the induction of adaptive immunity, but are capable of direct antimicrobial activity as well

The natural killer (NK) cell

- Granule-containing, non-T/non-B lymphocytes
- Surface receptors recognize:
 - Tumor cells
 - Virally infected cells
 - Monocytes infected with bacteria
 - Fc portion of IgG on an Ab-coated target cell
- NK cell undergoes cytoplasmic reorientation
 - Cytolytic granules localize near the target cell
 - Induce apoptosis of the target cell
- Appears to be a key cytokine-producing immunoregulatory cell, particularly IFN- γ



NKT cells

- T-lymphocytes that express NK cell-related receptors
- Found in very small numbers (0.2% of total T-cell repertoire)
- Secrete large amounts of IFN- γ and IL-4
- Appear to have significant immunoregulatory function
- May not be directly activated by pathogens, but is activated by IL-12 secreted by dendritic cells following PAMP/PRR interaction; thus serve as a crucial link between innate and adaptive immunity



Biochemical effectors of innate immunity

- Lysozyme
- Chitinase
- Phospholipase A2
- Bactericidal permeability-increasing protein (BPI)
- Cathelicidins
- Serprocedins
- Lactoferrin and calprotectin
- **Defensins**



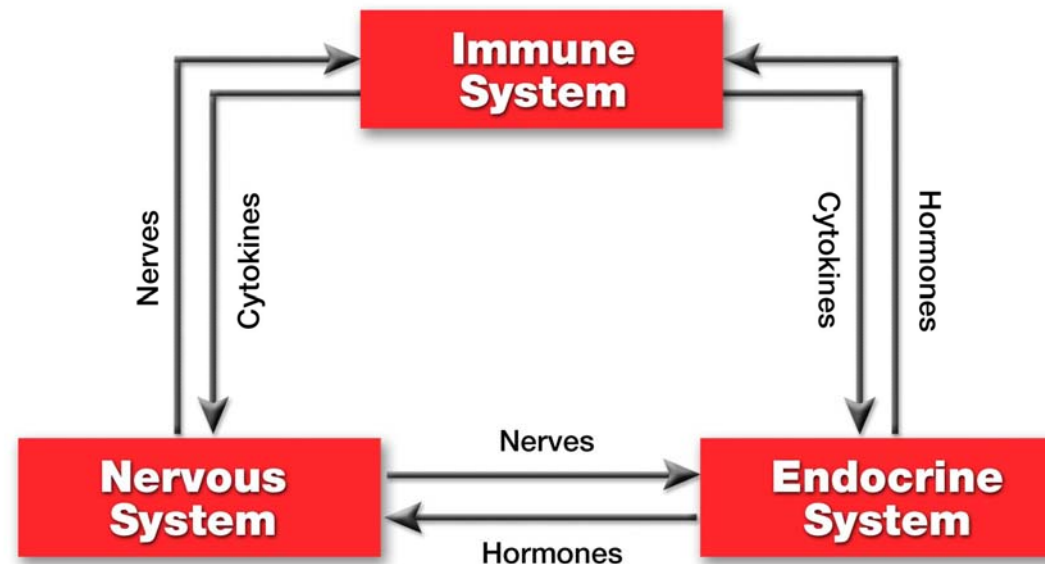
Good for frogs, good for us

The interferons

- Type I
 - IFN- α , IFN- β , IFN- ω and IFN- τ
 - Production triggered by engagement of certain PRRs
 - Strong antiviral activity via induction of Mx-A, Mx-B and GBP proteins
 - Modest immunoregulatory activity
- Type II
 - IFN- γ
 - Primarily immunoregulatory cytokine involved in adaptive immunity and serving as a principal bridge between innate and adaptive
 - Distinct from Type I in multiple attributes
- Type III
 - IFN- λ 1 (IL-29), IFN- λ 2 (IL-28A) and IFN- λ 3 (IL-28B)
 - Recently described, acceptance not yet universal

Regulation of the early innate immune response

- Innate immunity (as well as adaptive immunity) is controlled by the nervous and endocrine systems in a dynamic feedback loop mechanism
- Major system is the hypothalamic-pituitary-gonadal (HPA) axis
- Early manifestations of inflammation result from neural triggers
- HPA interactions are key in development and maintenance of inflammation



Assessing innate immune function

- Historically, innate immune function has received far less attention as a target for toxic insult than adaptive immune function
 - Innate immunity was perceived as “primitive” and thus less important in long-lasting and specific host defense
- Recent work has clearly demonstrated that innate immunity is a key regulator not only of initial host protection, but the development (and perhaps maintenance) of specific immunity
- Innate immune function defects (primarily NK cell function) correlate with immune deficits, so we know it’s “important”

Assessing innate immune function

- Increasing understanding of the complexity of this “subsystem” presents enormous challenges and opportunities for understanding both deliberate and unintentional immunomodulation
- Interpretation of results perhaps more difficult than we first imagined:
 - Decreased/altered function generally assumed to be deleterious only until the adaptive response took over, however...
 - Current recognition of the key regulatory function of the innate response **may represent an unexpected diversity of targets**
 - What happens when innate immune function is increased?
 - Inflammation is a dangerous tool
 - Global dysregulation of the immune continuum?

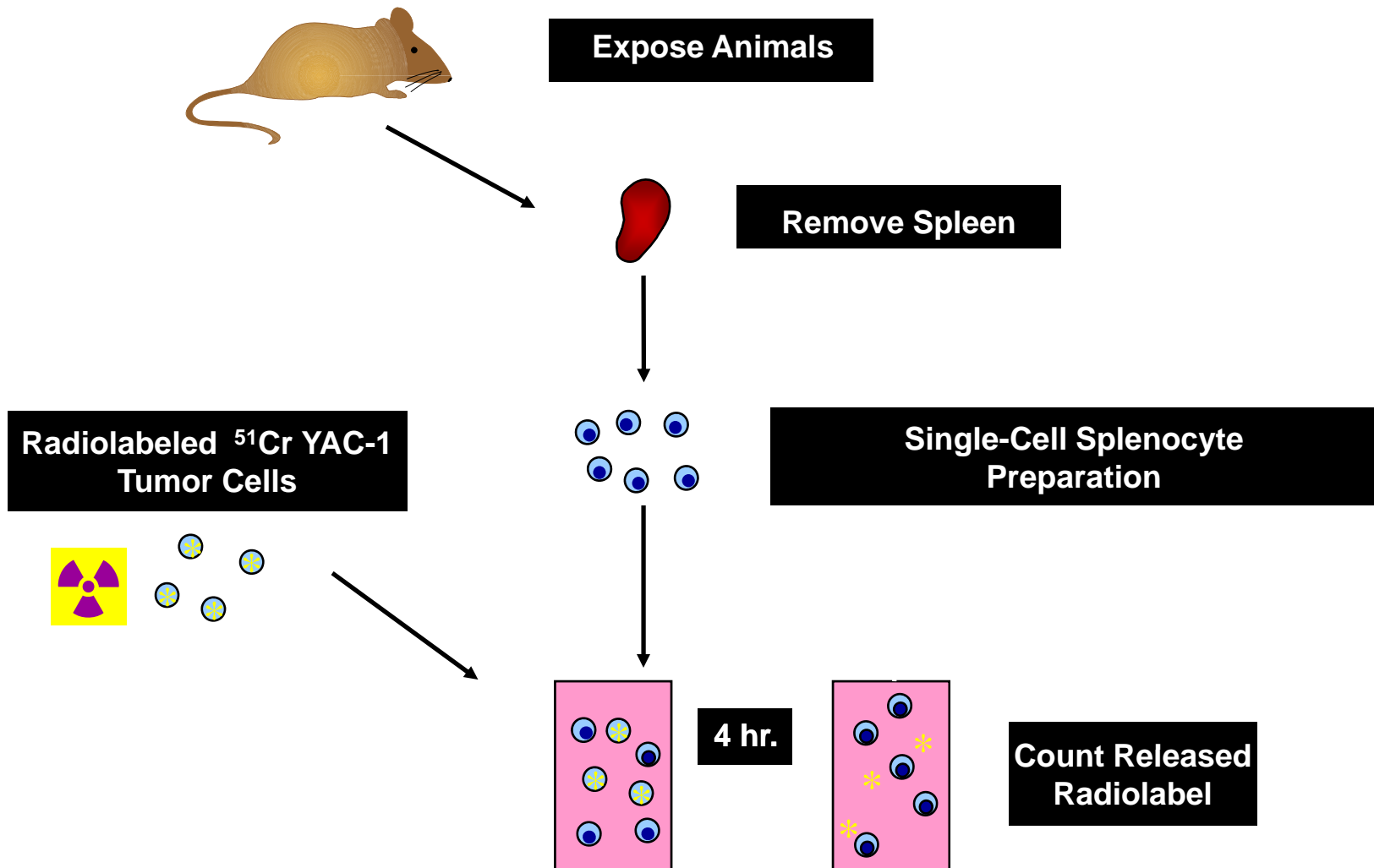
Assessing innate immune function

- Functional (cellular) assays are available to measure:
 - NK cell function
 - Macrophage activity
 - Granulocyte activity (very seldom used...damage or dysregulation probably indicative of a more significant toxicity)
- Next frontier in immunotoxicology may be understanding the key role of TLRs and how their regulation/dysregulation affects the entire immune response
 - Negative regulation of this subsystem increasingly understood to result in conditions such as inappropriate inflammatory response and other immune-mediated diseases
 - Future therapeutic modulation of the immune response via this subsystem will present significant challenges for safety assessment
 - Adequate assays not currently in place

Assessing NK cell function

- Traditional model has been the radiolabel release (*in vitro*) assay
 - Based on short-term destruction of tumor target cells (“missing self”) by homogeneous leukocyte preparation containing NK cells
 - Advantages:
 - Relatively simple to perform for appropriately equipped laboratories
 - Quick read-out
 - Disadvantages:
 - Requires the use of radioisotopes
 - Labor- and material-intensive
- Other models are increasingly being used, but are generally variants on this theme using alternative reporter systems (enzymatic, flow cytometric, etc.)

In vitro assessment of NK cell function



Macrophage function assays

- Not routinely used as part of immunotoxicology screening (Tier I-type) assessments, and rarely used even in mechanistic (Tier II-type)
 - Very labor-intensive
 - Requires finesse
 - Useful mainly for detailed studies on agents suspected of acting on these cells
- Representative assays
 - Phagocytosis
 - Microbial/tumor destruction
 - Respiratory burst
 - Cytokine production

Acquired (Adaptive) immunity

- Specificity - specific immune response to infectious agent
- Memory – protection from future infection
 - Specific memory cells remain in circulation
 - Antigen sequestration – chronic stimulation
- Humoral immunity - host response to soluble antigens
- Cell mediated immunity (CMI): host response to intracellular antigens
- Elements of the front-line defense (primarily macrophages and dendritic cells) and innate immunity (NK cells) continue to operate; there is not a clear line of demarcation separating innate from adaptive

Comparison of innate and adaptive immunity

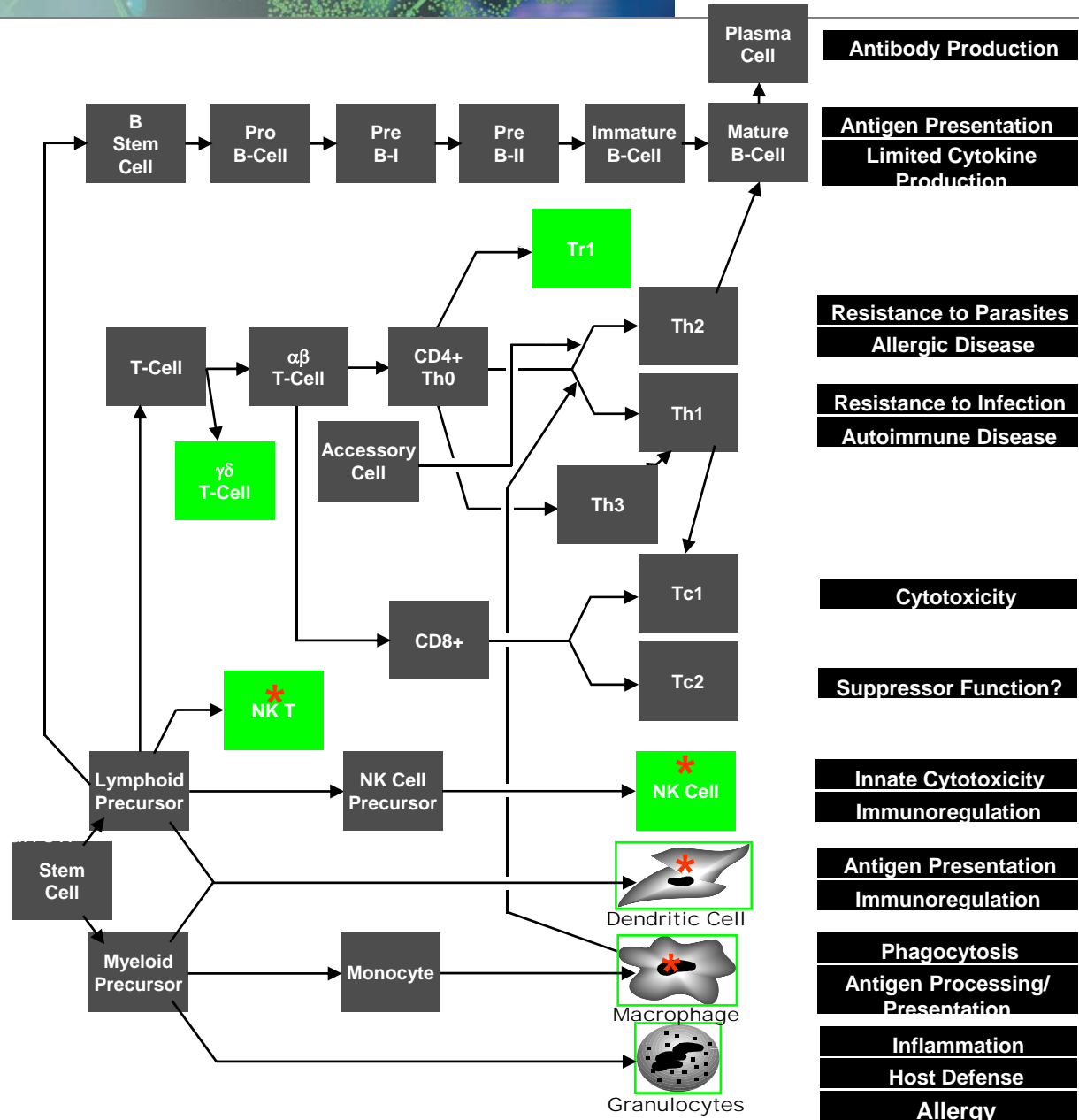
INNATE	ADAPTIVE
Targets are recognized by receptors encoded by the germline	Targets are recognized by receptors generated by genetic rearrangement
Receptors have relatively broad specificity (PAMPs)	Epitope recognition is highly specific
PAMPs are generally polysaccharides and polynucleotides; limited specificity between pathogens	Epitopes are generally highly specific polypeptides
Receptors are PAMPs	Receptors are T-cell receptor and B- cell receptor/Ig
Immediate response	Delayed response due to need for clonal expansion
No memory	Long memory
Metazoans	Vertebrates

Transition of innate to adaptive immunity

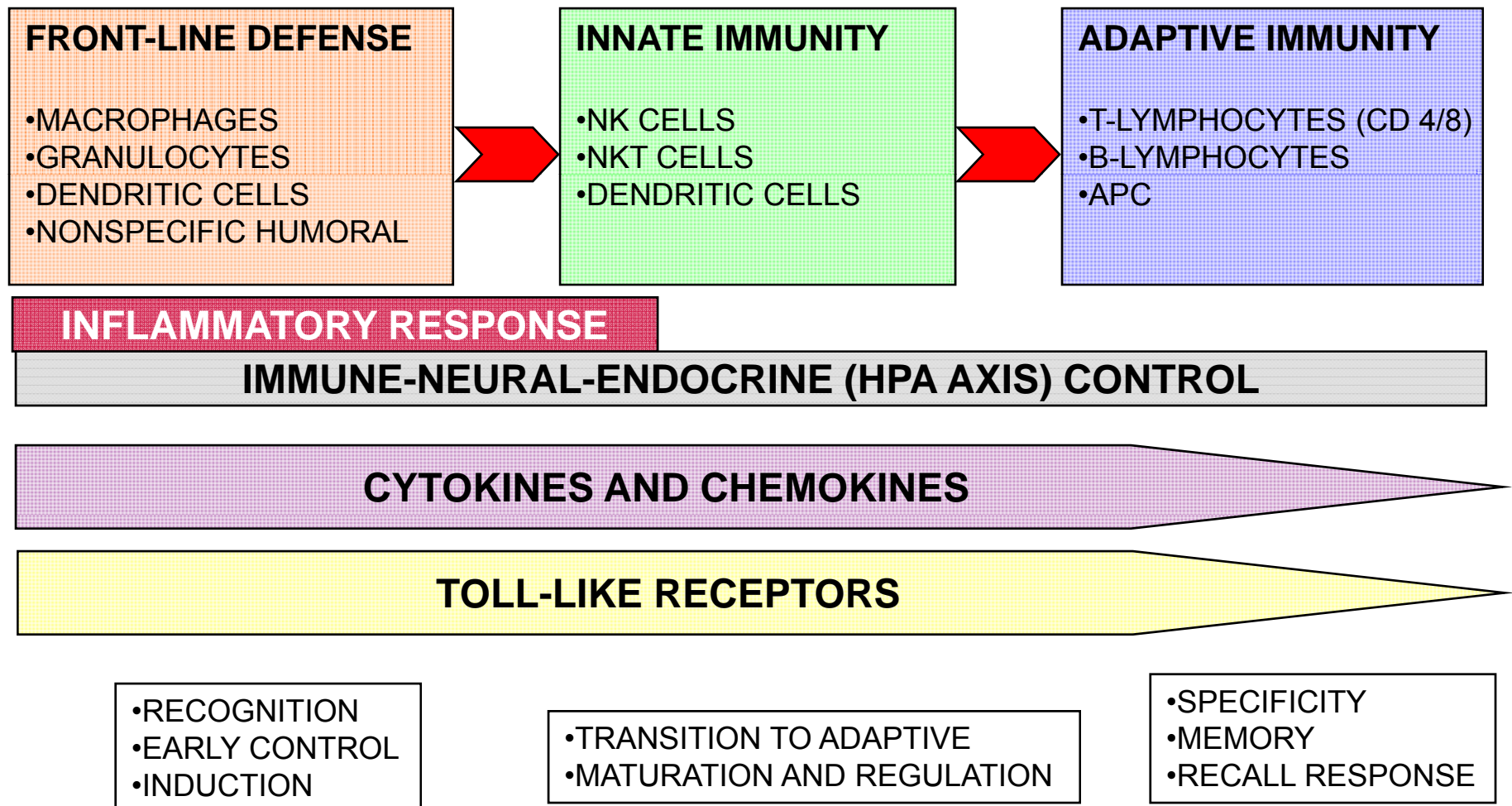
- Recognition of PAMPs induces inflammation through macrophages and endothelial cells
- Recognition of PAMPs leads to dendritic cell maturation and increase in expression of MHC II and accessory molecules
- Induction of innate immune response triggers release of cytokines that control subsequent immune responses, particularly IL-12
- Cytokines induced in the early stages of innate immunity operate at many subsequent stages of this transition process

Far from being a “primitive” mechanism, innate immunity is the key bridge between front-line host defense and the adaptive immune response

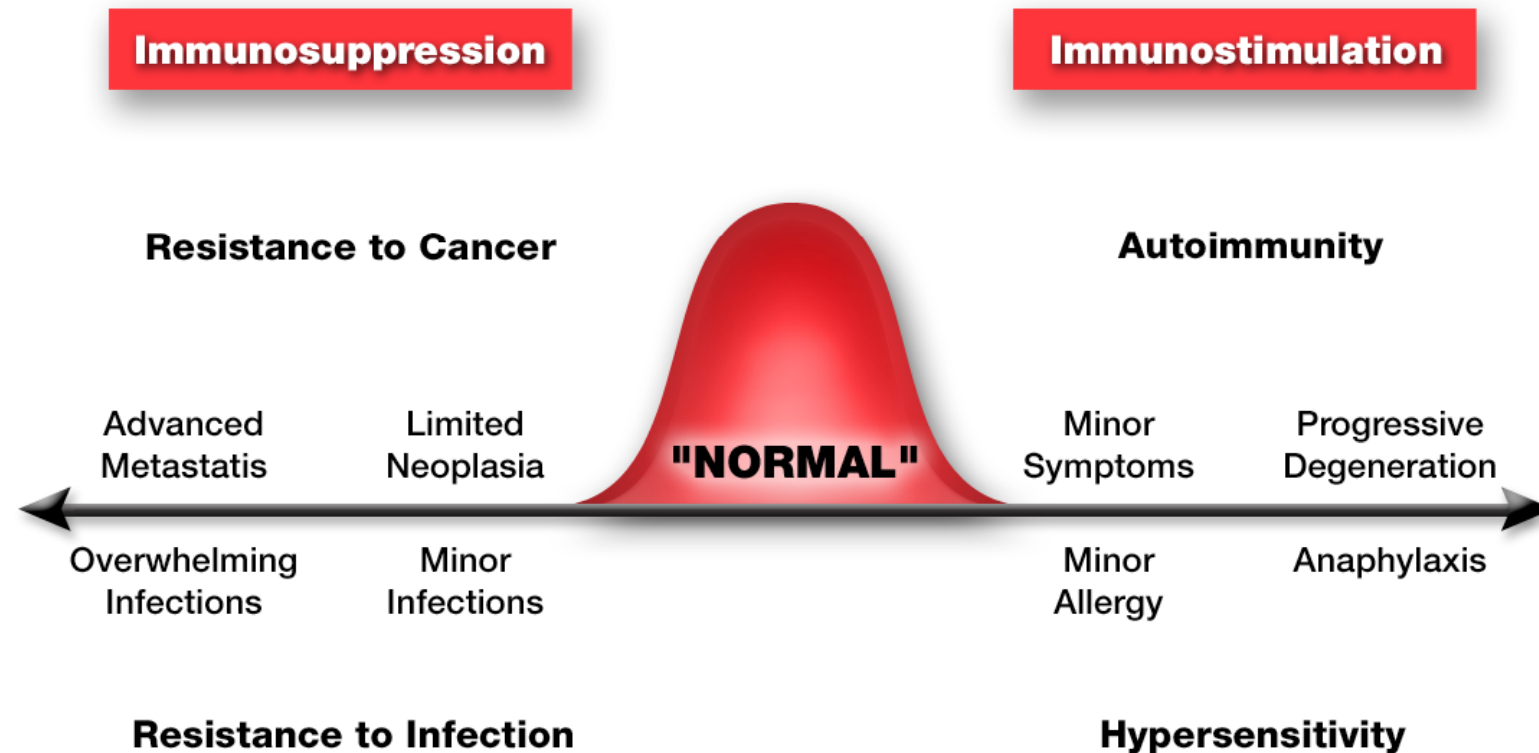
Damage to any component of the innate immune apparatus can have potentially significant ramifications due to the pivotal role this system has in regulation of the overall immune response.



Continuum of host defense

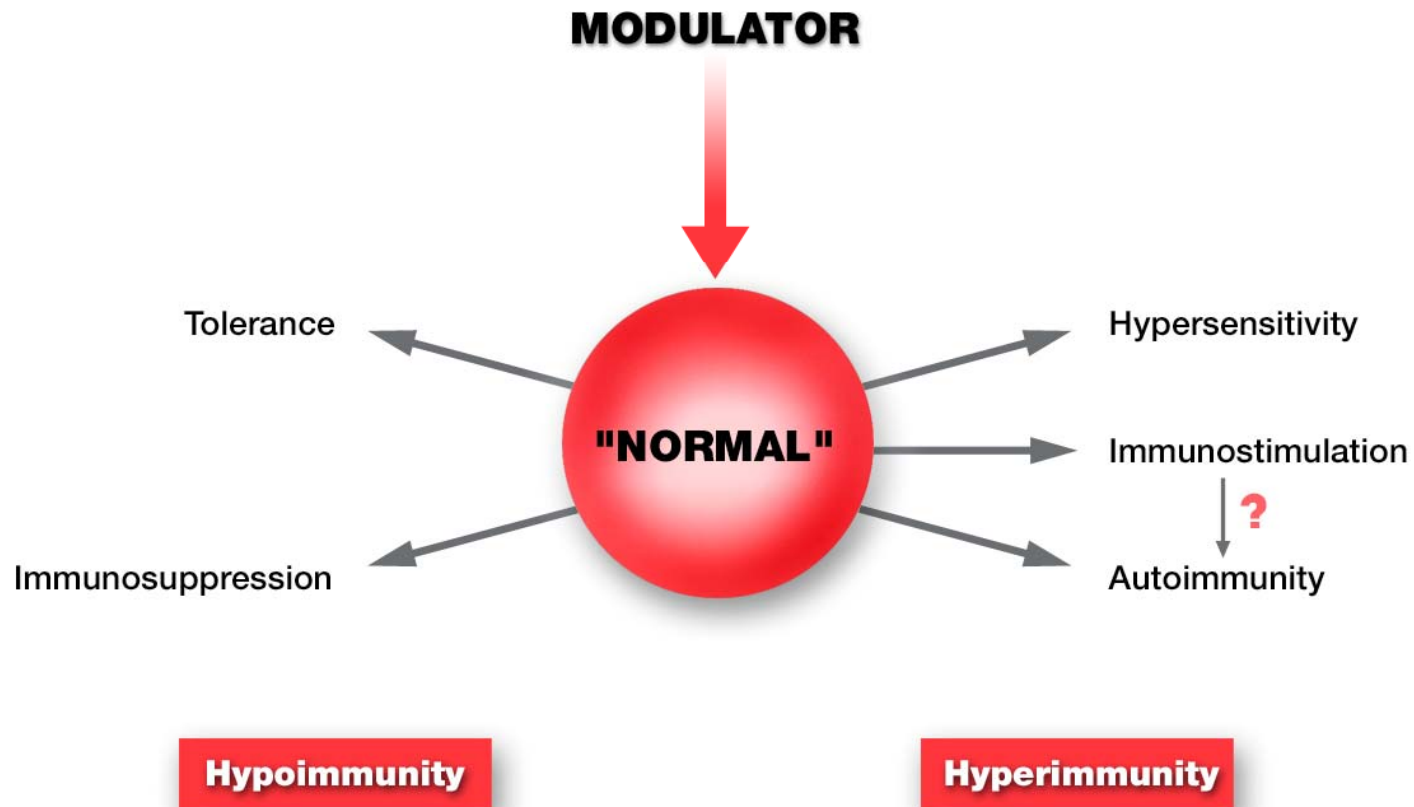


Standard model of immunomodulation



Standard model makes it difficult to identify detailed targets

Alternative model of immunomodulation



Revised model makes target identification easier