

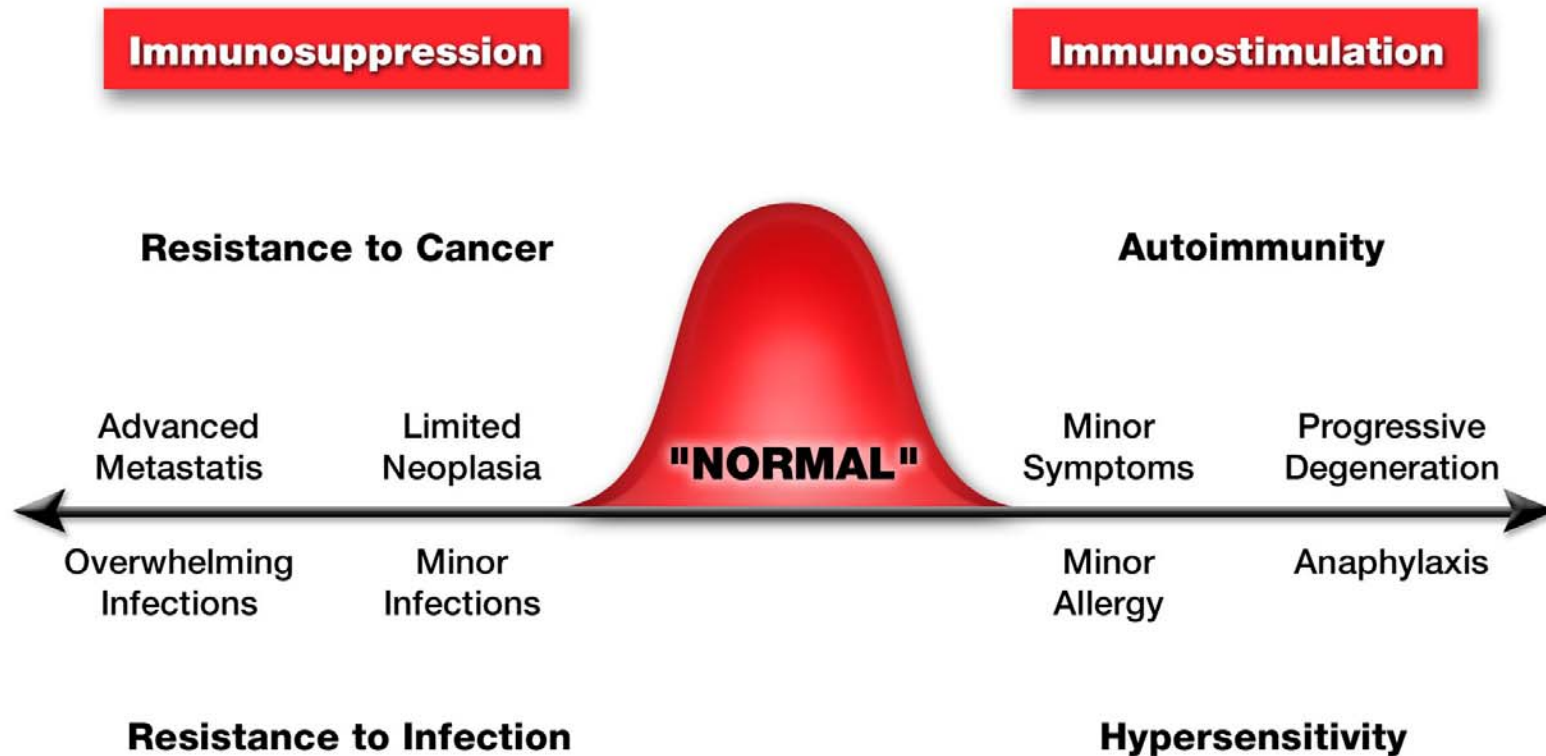
# Inadvertent immunostimulation: how it happens, what it means

**Robert V. House**

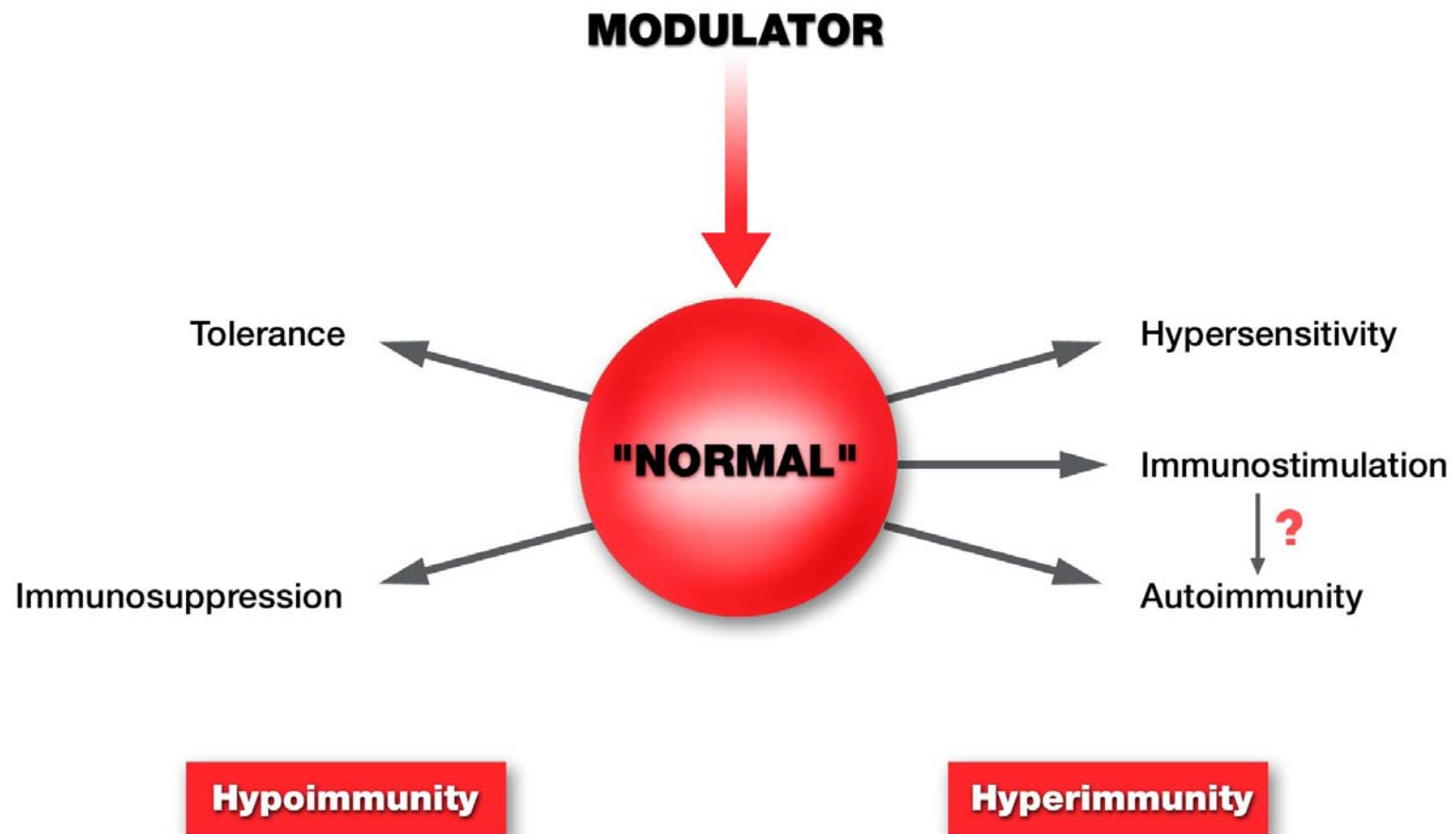
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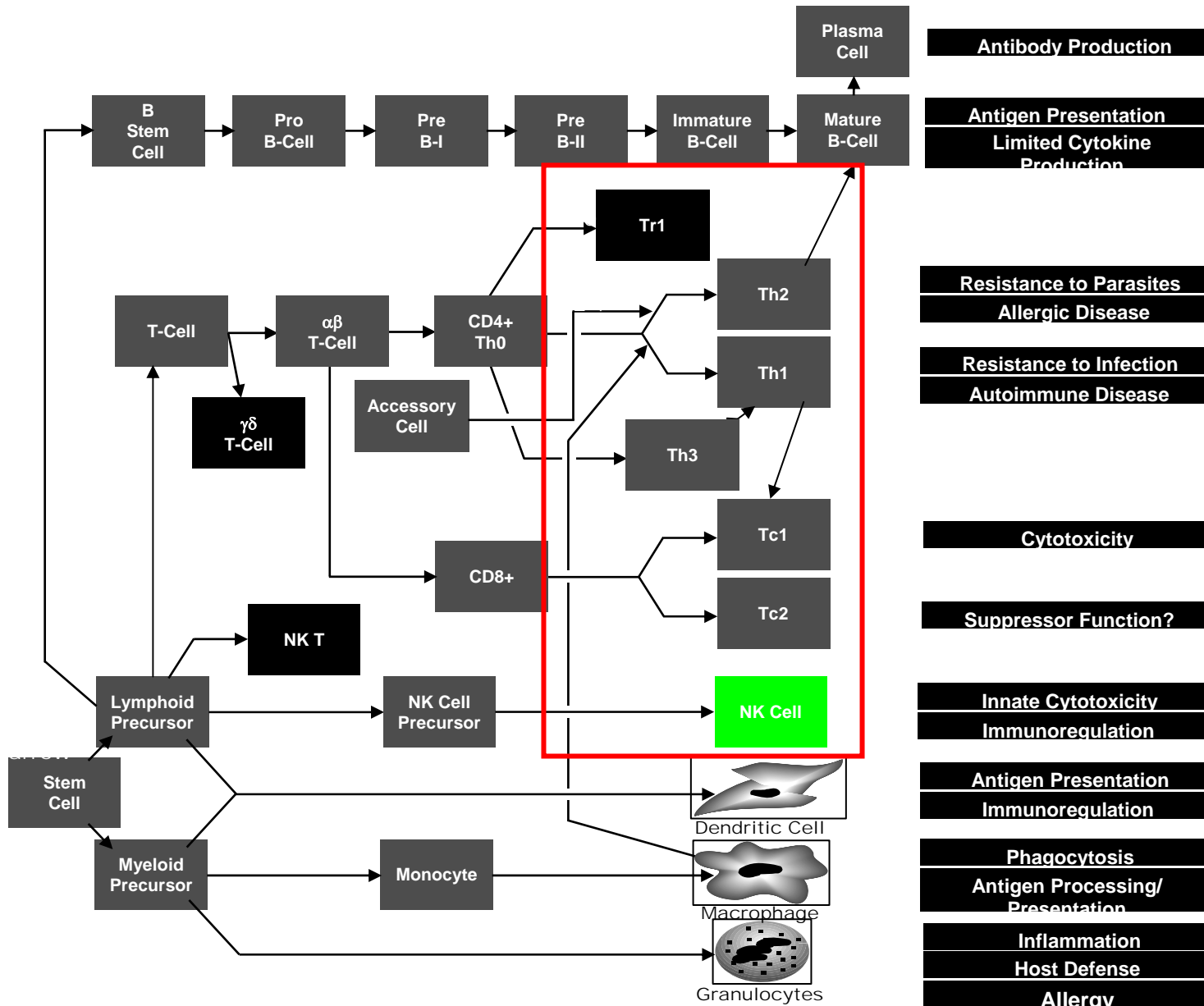


## Standard view of immunomodulation



## Alternative model of immunomodulation





## **Mechanisms for stimulating the immune system**

- Increased volume of effectors (primary mechanism for exogenous cytokines and growth factors)
- Stimulation or modification of intrinsic function
- Interruption of regulatory/suppressor circuits

## Targeting the immune system

- Biopharmaceuticals
  - Exogenous cytokines
  - Co-stimulatory molecules
  - TLR agonists
- Small molecule drugs
- Vaccination (including adjuvants)
- “Other”
  - Supplements
  - Exercise
  - Biofeedback

## Immunostimulant examples

Macrophage activators	BCG; Picibanil ( <i>S. pyogenes</i> ); Krestin; Lentinan; Biostim; muramyl dipeptide; trehalose dimycolate
Thymic peptides	Thymostimulin; T-activin; Thym-Uvocal
T-cell stimulants	Bestatin; Isoprinosine; Levamisole; Thymopentin; methylinosine monophosphate
Cytokine growth factors	Sargramostin (GM-CSF); Oprelvekin (IL-11); Alferon (IFN); Aldesleukin (IL-2); Filgrastim (G-CSF)
“Other”	Ampligen (synthetic DS RNA); CpG (TLR agonist); Multikine (cytokine cocktail)

## Clinical adverse events following cytokine treatment

<b>Agent</b>	<b>Effects</b>
Interferon-alpha	Flu-like syndrome; autoimmune disorders; immediate hypersensitivity; immune-related reactions (dermal, neurological)
Interferon-beta	Autoimmune disorders (limited)
Interferon-gamma	Flu-like syndrome
Interleukin-2	Autoimmune-like reactions; vascular leak syndrome; hypersensitivity; infectious complications
Colony-stimulating factors	Exacerbation of autoimmune diseases; complications associated with hematopoietic activation; immediate hypersensitivity

## A lesson from Nature

- Various microbes produce superantigens as a defense mechanism
- Bind to MHC molecules and then to TCR
- Mitogenically stimulate T-cells (up to approximately 20% of the total repertoire). The resulting nonspecific response preoccupies the adaptive immune response
- Cytokine production (mainly Th1) is induced, resulting in a cytokine storm and Toxic Shock Syndrome
- Exposure to superantigens can also result in increased IFN- $\alpha$  production, possibly leading to autoimmunity

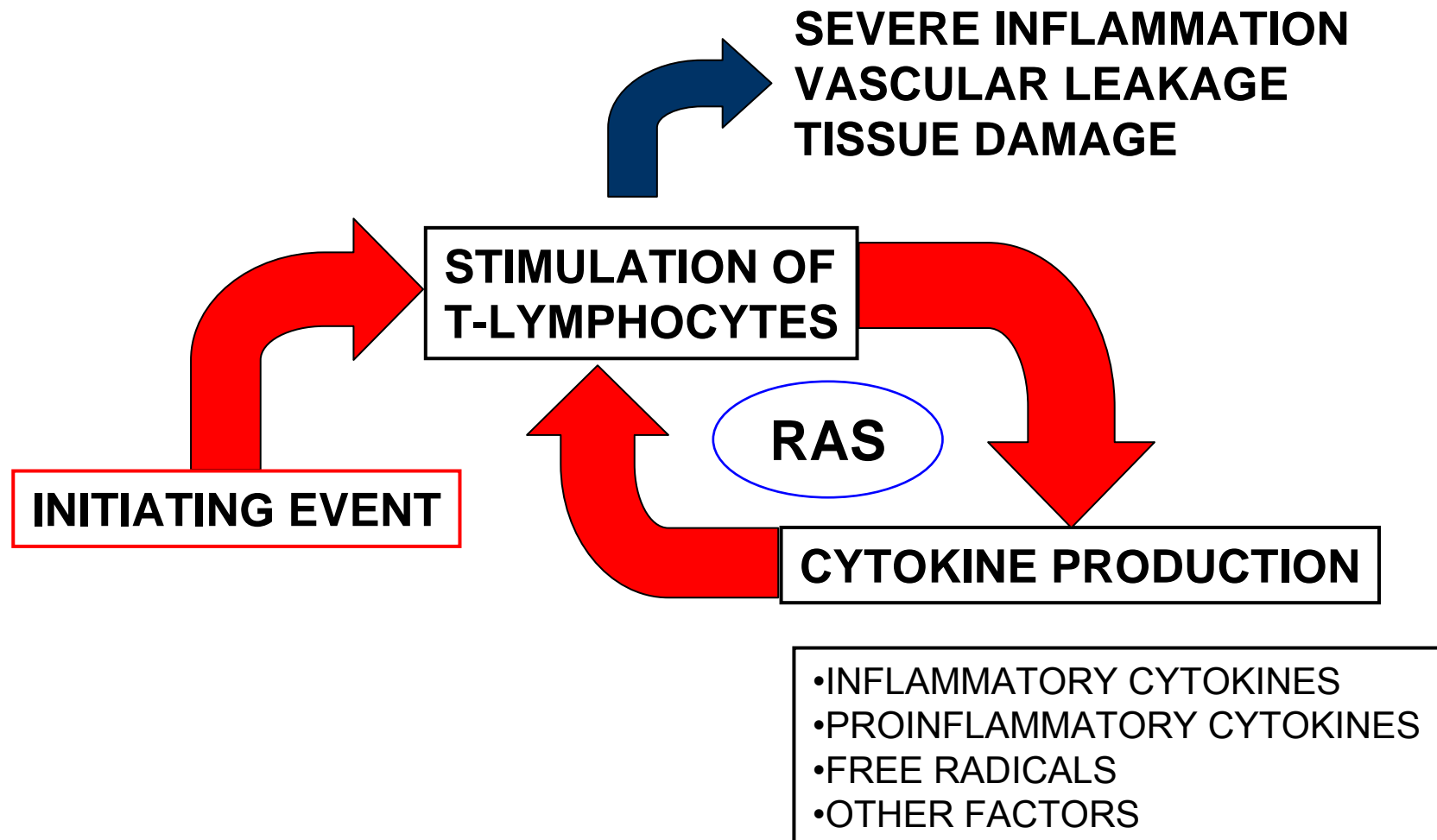
**The point?**

**Stimulation of the immune response can have significant adverse consequences.**



**SEB superantigen**

## The cytokine storm (hypercytokinemia)



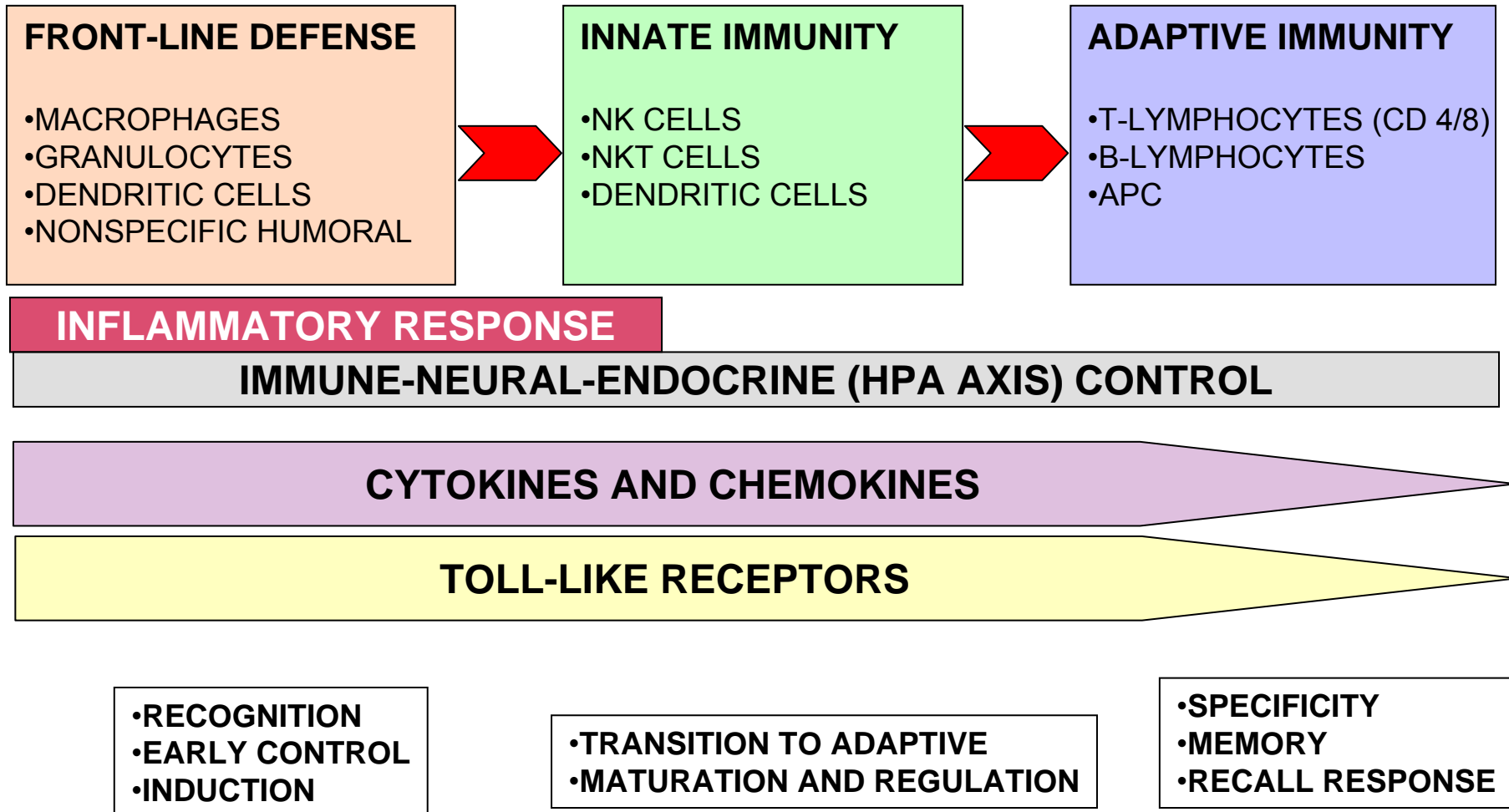
## Innate immunity: key to regulation/dysregulation?

- 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”

## Understanding the role of 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?

# Continuum of host defense



## 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

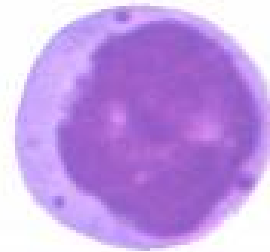
## Adjuvants that act via TLRs

<b>TRL ENGAGED</b>	<b>LIGANDS</b>	<b>ADJUVANTS</b>
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

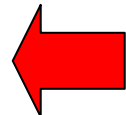
Modified from Duin et al., 2005

## Target of opportunity: the natural killer (NK) cell

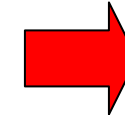
- Granule-containing, non-T/non-B lymphocytes
- Surface receptors recognize:
  - Tumor cells
  - Virally infected cells
  - Monocytes infected with bacteria
  - Fc portion of IgG on an Ab-coated target cell
- NK cell undergoes cytoplasmic reorientation
  - Cytolytic granules localize near the target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



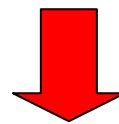
**Virus-Infected Cells**  
•Decreased Viral Growth



**Interferon- $\gamma$**



**NK cells**  
•Increased Activity



**T-cells**

- Induction of IL-2 and IL-2R
- Changes in delayed hypersensitivity
  - Changes in graft rejection
- Enhanced suppressor cell activity
  - Enhanced cytotoxicity

**Macrophages**

- Enhanced MHC expression
- Enhanced antimicrobial activity
- Increased antitumor activity
- Increased TNF $\alpha$  synthesis
- Increased FcYR expression
- Migration Inhibition

**B-Cells**

- Altered IgG subclass production
- Decreased CD23 expression
  - Decreased proliferation
  - Decreased IgE production
- Counteracts effects of IL-4

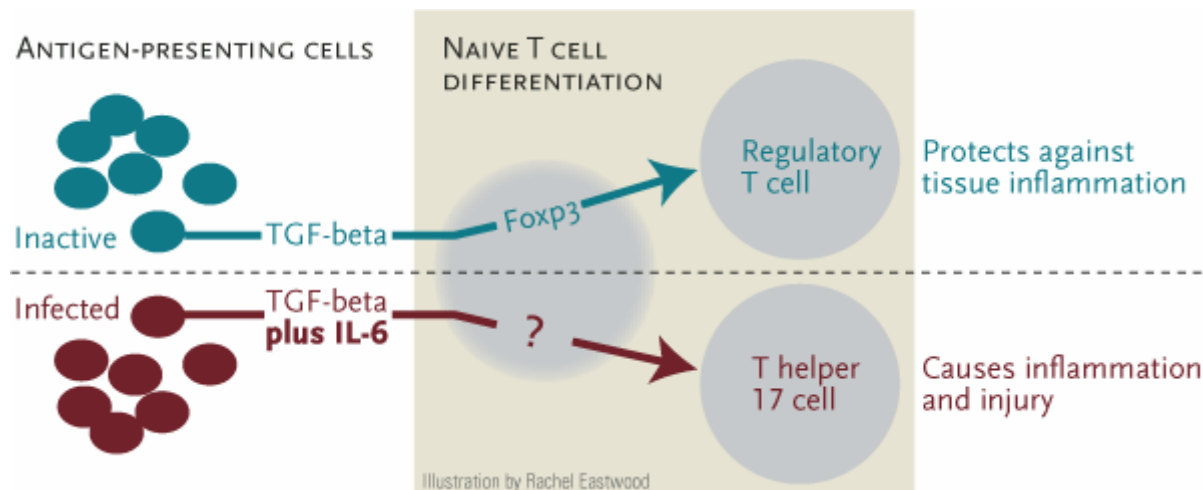
**The NK cell represents only one potential target with multiple downstream targets**

## Target of opportunity: the T-regulatory (Treg) cell

- CD4+CD25+ T-lymphocytes; coexpress FoxP3
- Not induced to proliferate following activation
- Act to suppress immune reactivity, particularly autoimmune reactions, in other CD4+ cells (mechanism unknown)
- Importance is confirmed by the IPEX syndrome (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome) linked to the dysfunction of FoxP3
  - Results in overwhelming autoimmunity and often death in the first year of life
- Little direct evidence from the literature that this has been studied in any detail

## Target of opportunity: the Th17 cell

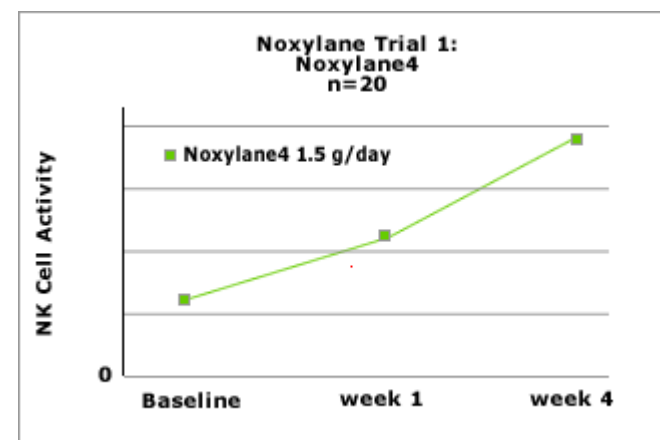
- CD4+ effector cell; secretes IL-17 and IL-6
- Currently thought to be a key regulator in autoimmunity
  - “Natural” role may be clearance of certain pathogens
- This concept replaces previous understanding that IFN- $\gamma$  produced by Th1 cells was the primary mechanism in autoimmune pathology
- Can induction/stimulation of Th17 by drugs lead to autoimmunity?



## “Do-it-yourself” immune stimulation

- A wide range of dietary supplements are targeted toward increasing immune function
- Some of these (particularly Asian herbals) have been tested in reputable scientific formats
- **How are the data to be interpreted?**
  - A “classic” example of the difficulties in immunotoxicology
  - In vitro results of isolated changes have not been adequately correlated to actual clinical benefits

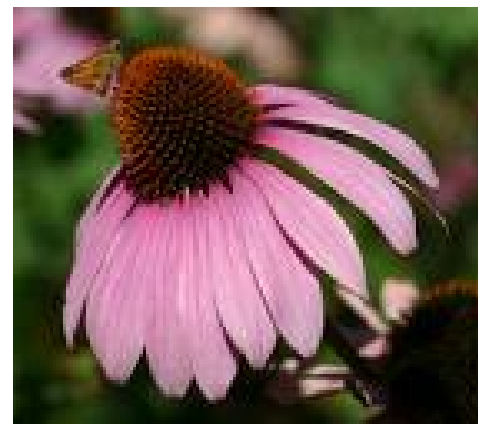
Clinically Shown  
To Increase  
NK Cell  
Activity



## “Do-it-yourself” immune stimulation: questions

- Is “more” necessarily “better”? Is more, in fact, less?
- What is the interaction of these immune boosters, so-called?
- If individuals are really boosted, what happens when allopathic immune stimulation is employed? What about immune suppression?
- What, if any, are the long-term effects of tweaking the immune system with extracts and tinctures?
- Why do it?

**Certain nutritional and physical exercise regimens have been shown to boost the immune system; this may be safer in the long run (“dangerous exercise” notwithstanding.)**



## Regulatory Guidance

- ICH S8 Immunotoxicity Studies for Human Pharmaceuticals
  - *...enhancing the immune response can stimulate the expansion of autoreactive immune cells and lead to autoimmune diseases.*
  - *The term immunotoxicity in this guideline will primarily refer to immunosuppression, i.e. a state of increased susceptibility to infections or the development of tumors.*
  - Opinion: Insufficient guidance for unintentional immunostimulation
- ICH S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
  - Better description than ICH S8
  - Probably more relevant than for pharmaceuticals
  - Section of immunotoxicology is vague (by design?)

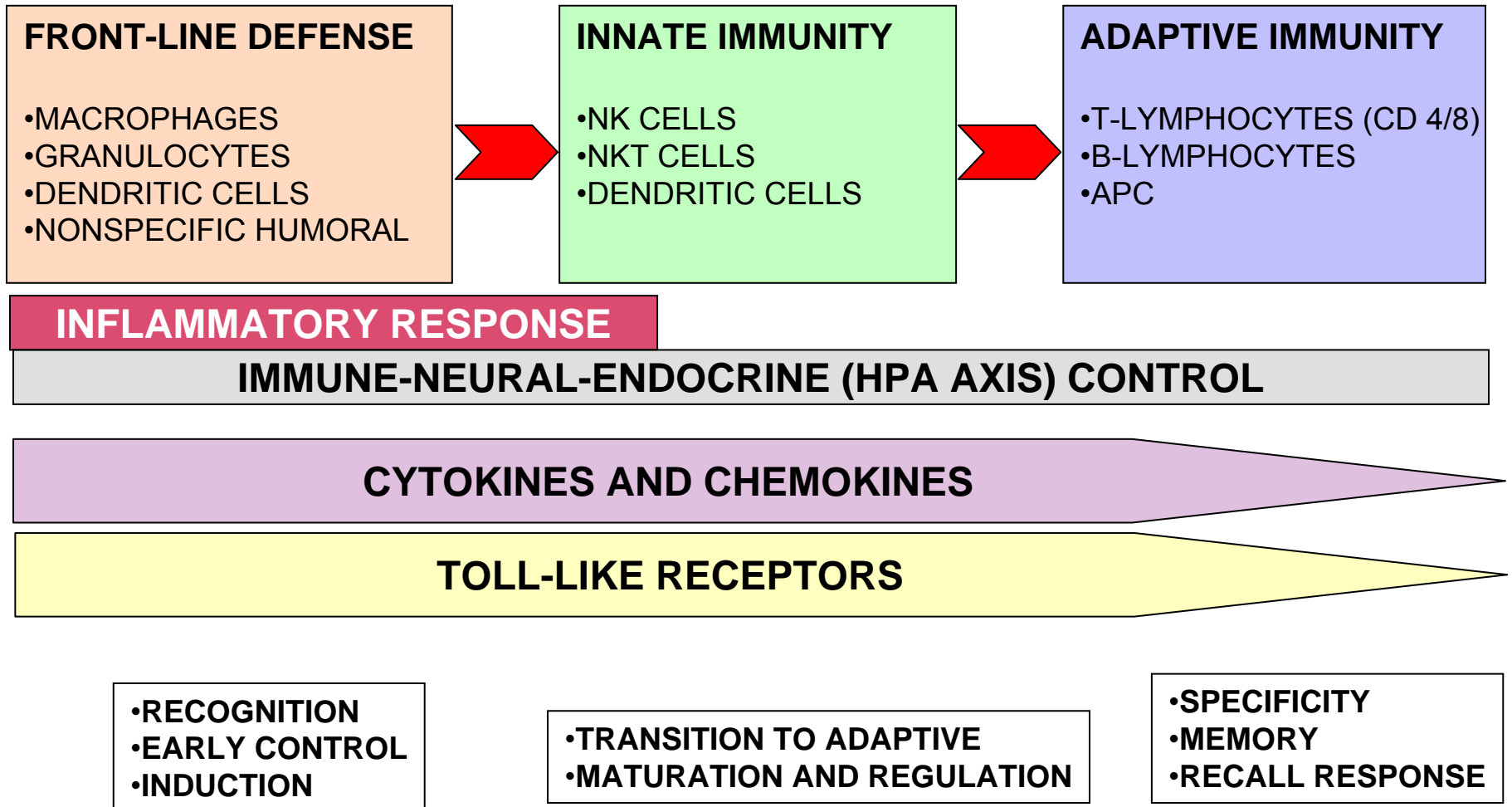
**Determining toxicity of immunostimulation is case-by-case.**

## How can we properly assess immunostimulation?

- Like immunosuppression, statistical significance tells only part of the story (maybe a very small part).
- Given the atypical nature of reactions observed to date, it is unlikely that standardized functional tests will ever be practical.
- Preclinical assessment must take into account species differences to a degree not often encountered in assessment of immunosuppression.
- Lack of data correlating changes with known pathology makes prediction of adverse reactions difficult.

**Determining toxicity of immunostimulation is case-by-case.**

# A plethora of targets for immunotoxicity



## Summary: Basic comparisons

<b>Immunosuppression</b>	<b>Immunostimulation</b>
Usually detrimental to host	Consequences unknown
Results from a variety of insults (drugs, environmental, physical and biological stressors)	Little explored outside of agents intended to cause this effect
Clearly established consequences	Consequences practically unknown
Many in vitro and in vivo assays available to measure changes	Unknown whether the same assays can be used, or new systems required
Loss of some components may be compensated for by other mechanisms; not all-or-nothing	Unknown how stimulation of one component affects other subsystems