

**Lecture 1.** Defining Innate Immunity  
Recognition and effector mechanisms (I)

Key concepts

- Hard wired specificity
- Multiple molecular mechanisms
- Tightly integrated with adaptive immune responses

Endotoxin and its receptors

- 1890 Richard Pfeiffer describes a heat stable intrinsic poison derived from Gram negative bacteria which is called endotoxin
- Endotoxin is identified as lipopolysaccharide, a component of the bacterial cell wall
- Administration of small amounts leads to fever, shock and death ...
- 1960s ...except in C3H/HeJ mice which can be filled up with endotoxin and still remain happy. They are, however, very susceptible to bacterial infection.
- 1997 Medzhitov and Janeway identify TLR4 as mediating an activating immune signal
- 1998 Bruce Beutler uses a positional cloning strategy to identify the LPS receptor as TLR4

<http://esi-topics.com/tlr/interviews/BruceBeutler.html>

Endotoxin (lipid A) activation of the inflammatory response provides a simple paradigm of innate immunity. But the reality is more complex.

Defining Innate Immunity is not straight forward.

We might define it in evolutionary context; ancient mechanisms of immunity found in organisms from which we diverged before bony fish.

We might define in a negative way; those parts of the immune response that are not adaptive.

We might define it by classifying specific cells and effector mechanisms that are part of the innate immune response.

Each of these approaches has problems.

The innate immune system continues to evolve in parallel with the adaptive immune system.

The adaptive and the innate immune responses are an integrated whole; many innate responses potentiate adaptive responses

New cell types/subtypes/cytokines are discovered on a regular basis, so this is an intrinsically less complete approach.

How do others define the innate immune response?

*Janeway, Travers, Walport and Shlomchik*

Immunobiology 5<sup>th</sup> Edition

Defense mechanisms that do not require a prolonged period of induction because they do not rely on the clonal expansion of antigen-specific lymphocytes are the mechanisms of innate immunity

Innate immunity depends upon germline-encoded receptors to recognize features that are common to many pathogens

Infectious disease occurs when a microorganism succeeds in evading or overwhelming innate host defenses to establish a local site of infection and replication that allows its further transmission.

**Summary 1:** The Innate Immune system is an integrated part of the immune response characterised by mechanisms and molecules that respond rapidly to infection and adapt on an evolutionary timescale, rather than over a single lifespan.

### **What's the trigger?**

The problem the successful immune system solves is, what do you recognise?

foreign patterns

danger

missing self

Foreign Patterns      Original idea attributed to Charlie Janeway.  
1989 Cold Spring Harb. Symp. Quant. Biol.  
54:1-13

“The immune system has evolved specifically to recognize and respond to infectious microorganisms, and that this involves recognition not only of specific antigenic determinants, but

also of certain characteristics or patterns common on infectious agents but absent from the host”

At a first impression, you might think this is restricted to molecular patterns that are just there, at the surface of the bug, but actually some of these molecular signatures can undergo quite sophisticated processing before presentation, eg association with specific binding protein such as LPS binding protein.

PAMPs: Pathogen associated molecular patterns

MAMPs: Microbe associated molecular patterns

Danger: Matzinger Ann. Rev. Immunol. 12:991-1045 1994:  
Cells die in different ways, some ways are safer (apoptosis), some ways imply trauma (in the widest sense) (necrosis). Polly Matzinger proposed that the immune system should be more interested in the latter than the former, but how? One proposal was distinguishing apoptotic death from necrotic death, but the data is controversial. Results both ways.

Shi and Rock

Adding cells and cell fragments enhanced immune responses (adjuvanticity). In Nature 2003 425:516 they identified crystal of uric acid as an endogenous adjuvant released by stressed cells. What it is doing is activating APCs i.e. enhancing adaptive responses.

Confounding problem is presence of LPS. Other candidates include fragments of extracellular matrix, heat shock proteins.

DAMPs (Danger associated molecular patterns)

May use common PRR (pattern recognition receptors)

e.g. TLR-2 (Immunity 27:321 – 333 2007)

## **Extracellular Pattern Recognition**

TLR-4

In combination with LPS binding protein, CD14 and MD2, recognise the Lipid A (endotoxin) component of LPS (lipopolysaccharide; from gram negative bacteria). This leads to activation of NF- $\kappa$ B transcription factor which activates the pro-inflammatory response inducing IL-6, IL-1, TNF $\alpha$

etc. In humans, TLR4 polymorphisms have been associated with susceptibility to a number of diseases (septic shock, premature birth, ulcerative colitis, atherogenesis). The TLR family is extremely important in sensing infection and different members are found in different locations both extracellularly and intracellularly.  
( J Allergy Clin Immunol 2006;117:979-87.)

Mannan-binding lectin (MBL): Binds mannose on pathogen surface and triggers complement cascade. People deficient in mannan-binding lectin experience a substantial increase in infections during early childhood, indicating the importance of the MB-lectin pathway for host defense. The age window of susceptibility to infections associated with mannan-binding lectin deficiency illustrates the particular importance of innate host defense mechanisms in childhood, before the child's adaptive immune responses are fully matured and after maternal antibodies transferred across the placenta and in colostrum have been lost.

Dectin

Brown, Gordon D.

Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nature Reviews. Immunology.* 6(1):33-43, 2006.

“Dectin-1 is a natural killer (NK)-cell-receptor-like C-type lectin that is thought to be involved in innate immune responses to fungal pathogens. This transmembrane signalling receptor mediates various cellular functions, from fungal binding, uptake and killing, to inducing the production of cytokines and chemokines. These activities could influence the resultant immune response and can, in certain circumstances, lead to autoimmunity and disease.”

Invariant TCRs:

Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature.* 434:525-9, 2005.

“CD1d-restricted natural killer T (NKT) cells are innate-like lymphocytes that express a conserved T-cell receptor and contribute to host defence against various microbial pathogens. However, their target lipid antigens have remained elusive. Here we report evidence for microbial, antigen-

specific activation of NKT cells against Gram-negative, lipopolysaccharide (LPS)-negative alpha-Proteobacteria such as Ehrlichia muris and Sphingomonas capsulata.”

### Intracellular Pattern Recognition

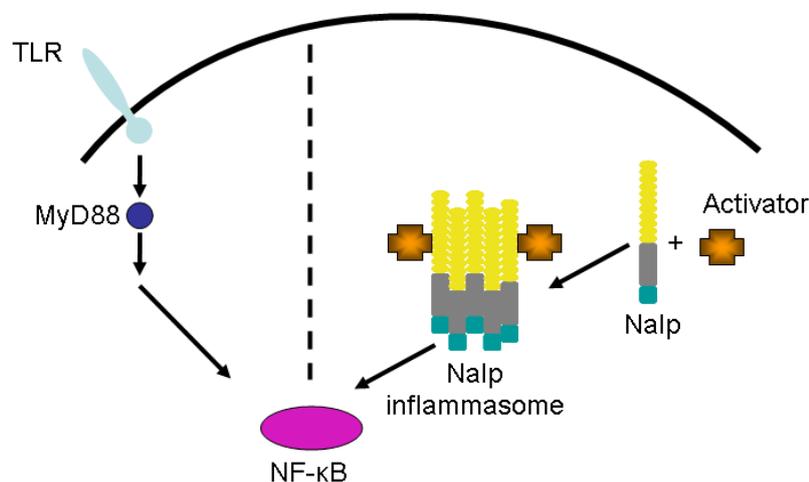
Toll-like receptor 9 (TLR9)  
Intracellular sensing of unmethylated CpGs in monocytes, dendritic cells and lymphocytes

NOD2 (CARD15)  
An intracellular protein that senses muramyl dipeptide, a component of Gram positive and negative bacteria and activates NF- $\kappa$ B. Mutations of NOD2 have been associated with an increased risk of developing Crohn’s disease and Blau’s syndrome, both chronic granulomatous conditions. These mutations appear to reduce the sensing ability of this protein.

Nalp1 (NACHT, leucine rich repeat and pyrin domain containing 1)  
Intracellular sensing of anthrax lethal toxin (LT); caspase-1 activation of IL-1 $\beta$  etc.

RIG-1  
Intracellular sensing of RNA viruses e.g. Hepatitis C. Induces type I interferons.  
Immunity 27: 549-559 (2007)

## Extracellular and intracellular pattern sensing



Extracellular sensing

Intracellular sensing

## Missing self

- Viruses target MHC I molecules
  - Adenovirus
  - Herpes viruses
- Tumours downregulate MHC I molecules
  - Failure of immunosurveillance
- NK cells
- Large lymphocytes that kill tumours (1973)
- NK cells in mice (1977)
- Require constitutive inhibition (1992)
- Mouse and humans use different families of inhibitory molecules
- NK cells
- Inhibited from killing by the presence of MHC molecules interacting with KIRs
- When pathogens or transformation interfere with the expression of MHC, NK cells kill the deficient cell
- They also release cytokines which stimulate other parts of the immune response

Annual Review of Immunology. 2005 23:225-274

## Summary 2

Innate immune activation uses a number of different strategies to trigger responses to pathogens: Foreign pattern recognition; Recognition of danger; Absent self. These processes are mediated by a number of different specific receptor ligand interactions (some known and some as yet unknown) and commonly lead to the expression of a large number of proteins induced by the NF- $\kappa$ B transcription factor family.

The end result is an **adjuvant effect**

It takes more than **just antigen specificity** to get an immune response going.

Adjuvants lower the threshold for productive immune activation

The effector mechanisms of the innate immune response are essentially the mechanisms of acute inflammation. A cell autonomous sensing system can trigger systemic activation. This triggering can arise from a very wide ranges of causes e.g. cardiopulmonary bypass.

The cells that are primarily responsible for this are macrophages and neutrophils.

Macrophage products of acute activation (Nat 420:885-891 2002)

Group	Examples
Cytokines	IL-1, IL-6, IL-12, IL-15, IL-18, TNF $\alpha$ , MIF, IL-10
Chemokines	IL-8, MIP-1a, MIP-1b, MCP-1, MCP-3
Lipid mediators	PAF, eicosanoids (prostaglandins, leukotrienes, thromboxane, etc), tissue factor
Oxygen radicals	Superoxide and hydroxyl radical, nitric oxide
Killer cell products	Perforin, caspase activators, FasL.

In extremis, these mediators can be fatal. The immune system contains within it the seeds of its own destruction. But usually, they are contained and regulated by countervailing forces. (IL-10, TGF $\beta$ , TNF-binding protein, IL-1Ra, glucocorticoids etc)

What do defects in innate immunity tell us?

Deficiency	Infection
Complement C3 (direct or indirect)	Pyogenic bacteria. H. influenzae and S. pneumoniae
Complement C5-C9	Neisseria species
Complement mannose binding lectin	Pyogenic bacteria in childhood
NK cells	Herpes viral infections
IRAK-4 (Toll and IL-1 signalling)	Pyogenic bacteria

That although these are defects in a general system of protection, they lead to specific patterns of disease, emphasising the co-evolution of these responses.

### Summary 3

Innate immune responses entrain powerful inflammatory processes with the potential to prevent the spread of infection. But the same signals also integrate adaptive immune responses into the ongoing process by activating professional antigen presenting cells (dendritic cells and macrophages), thus providing an ongoing adjuvant effect. Deficiencies in different parts of the innate immune response reveal that the detectable effects relate to specific micro-organisms, emphasising the co-evolution that occurs between infection and immunity, and the

specificity of the innate immune response. Therefore the innate immune system is adaptable, albeit over evolutionary time scales, not individual lifetimes. Mutations may focus on ligand binding sites, for example in the human TLR-4 receptor.

### ***Additional notes on Innate immune system deficiencies***

#### *Complement*

N Engl J Med, Vol. 344, No. 14· 2001

#### Pyogenic Infections

Increased susceptibility to pyogenic bacteria such as *Haemophilus influenzae* and *Streptococcus pneumoniae* occurs in patients with defects of antibody production, complement proteins of the classical pathway, or phagocyte function. Study of these patients shows that the normal pathway of defense against pyogenic bacteria is opsonization with antibody, followed by the activation of complement, phagocytosis, and intracellular killing. The most important complement opsonins in the defense against bacterial infection are C3b and iC3b, the covalently-bound cleavage fragments of C3

Deficiencies in complement components C5–C9 have been associated with susceptibility only to *Neisseria* species

The importance of factor I can be seen in people with genetic factor I deficiency. Because of uncontrolled complement activation, complement proteins rapidly become depleted and such people suffer repeated bacterial infections, especially with ubiquitous pyogenic bacteria.

#### *NK Cells*

NEJM 320:1731 1989

NK cells critical in viral infections especially herpes viruses.

#### *IRAK-4 deficiency*

SCIENCE 299:2076 2003

Members of the Toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) superfamily share an intracytoplasmic Toll–IL-1 receptor (TIR) domain, which mediates recruitment of the interleukin-1 receptor–associated kinase (IRAK) complex via TIR-containing adapter molecules. We describe three unrelated children with inherited IRAK-4 deficiency. Their blood and fibroblast cells did not activate nuclear factor κB and mitogen-activated protein kinase (MAPK) and failed to induce downstream cytokines in response to any of the known ligands

of TIR-bearing receptors. The otherwise healthy children developed infections caused by pyogenic bacteria. These findings suggest that, in humans, the TIR-IRAK signalling pathway is crucial for protective immunity against specific bacteria but is redundant against most other microorganisms.

**Further reading**

Rock, K. L., Hearn, A., Chen, C. J., and Shi, Y., Natural endogenous adjuvants. Springer Semin. Immunopathol. 26 (2005) 231-246. A good discussion of danger and pattern recognition