分子医学
(Molecular Medicine)

炎症、感染与相关疾病

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Inflammation

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process.

Four Major Symptoms of Inflammation:

1. Redness
2. Pain
3. Heat
4. Swelling

May also observe:
5. Loss of function
Functions of Inflammation

1. Destroy and remove pathogens (as well as damaged self tissues and cells).

2. If destruction is not possible, to limit effects by confining the pathogen and its products.

3. Repair and replace tissue damaged by pathogen and its products.
Inflammatory trigger

Physiological purpose
- Host defence against infection
- Tissue-repair response

Pathological consequences
- Autoimmunity, inflammatory tissue damage and sepsis
- Fibrosis, metaplasia and/or tumour growth

Inflammation

Tissue stress and malfunction
- Adaption to stress, and restoration of a homeostatic state
- Shift in homeostatic set points, development of diseases of homeostasis and/or autoinflammatory diseases
Stages of Inflammation

1. **Vasodilation**: Increase in diameter of blood vessels. Triggered by chemicals released by damaged cells: histamine, kinins, prostaglandins, and leukotrienes (组胺，激肽，前列腺素，白三烯).

2. **Phagocyte Migration and Margination**: Margination is the process in which phagocytes stick to lining of blood vessels. Diapedesis (Emigration): Phagocytes squeeze between endothelial cells of blood vessels and enter surrounding tissue. Phagocytes are attracted to site of infection through chemotaxis. Phagocytes destroy microbes, as well as dead and damaged host cells.

3. **Tissue Repair**: Dead and damaged cells are replaced.
Steps of the Inflammatory Response

The inflammatory response is a body's second line of defense against invasion by pathogens. Why is it important that clotting factors from the circulatory system have access to the injured area?

1. Damaged tissues release histamines, increasing blood flow to the area.

2. Histamines cause capillaries to leak, releasing phagocytes and clotting factors into the wound.

3. Phagocytes engulf bacteria, dead cells, and cellular debris.

4. Platelets move out of the capillary to seal the wounded area.
Definition of Infectious Diseases

Also known as **communicable diseases**, or **transmissible diseases** comprise **clinically evident illness** resulting from the infection, presence and growth of **pathogenic biological agents** in an individual host organism.
• Immunity is the body's solution to protect itself from Infectious Diseases

• **Commensal flora (共生菌群):** staph, strep, pneumonia (葡萄球菌，链球菌，肺炎)

• **Opportunistic pathogens (条件致病菌):** candida, Clostridium difficile (念珠菌，艰难梭状芽胞杆菌)

• **True pathogens (致病菌):** Influenza

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Figure 1.3 The Immune System, 3ed. (© Garland Science 2009)
Contents

1. Brief introduction of Infectious Diseases

2. Bacteria infection and Innate Immunity against bacteria infection

3. Viral infection and Innate Immunity against viral infection
**Brief introduction of Infectious Diseases**

**THROUGHOUT HISTORY, INFECTIOUS DISEASE WAS THE PRIMARY AGENT OF POPULATION CONTROL.**

1951年中国城市居民前10位死因:

肺炎;中枢神经系之血管病变;胃炎,十二指肠炎,肠炎及大肠炎;心脏疾病;恶性肿瘤;周产期之死因;结核病;意外灾祸;自杀及自伤;肾炎及肾水肿
Infectious diseases is still a major primary causes of death worldwide

Many Cancers are also related to viral infection

- Hepatitis B and/or Hepatitis C: 81% of Liver Cancers
- HIV/HHV-8
- Helminths
- EBV
- HTLV-1
- *Helicobacter pylori*: 56% of Stomach Cancers
- Human Papilloma Virus: 89% of Cervical Cancer

15.6% of All Cancers
Emerging and re-emerging infectious diseases

Viral Evolution and Pathogen-Host relationship:
Evolutionary pressure on immunity

Many viruses, in particular RNA viruses, have short generation times and relatively high mutation rates. This elevated mutation rate allows viruses to quickly adapt to changes in their host environment.

Viral evolution causes problems in the development of successful vaccines and antiviral drugs, as resistant mutations often appear within weeks or months after the beginning of the treatment.
Innate immunity against Pathogenic Microbes

- Bacteria:
- Virus
- Fungi
Bacteria Infection

Bacteria FACTS:

1. Large numbers of single-celled prokaryote microorganisms.

2. Typically a few micrometers in length;

3. Everywhere in earth: water, soil, as well as in live bodies of plants and animals.

4. 10 million in a gram of soil; a million in 1 L water, $5 \times 10^{30}$ on earth; 10 times cell numbers in human flora (菌群).
Bacteria FACTS:

5. The vast majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, and a few are beneficial.

6. A few species of bacteria are pathogenic and cause infectious diseases, including cholera, syphilis, anthrax, leprosy and bubonic plague (霍乱, 梅毒, 炭疽, 麻风病和鼠疫). The most common fatal bacterial diseases are respiratory infections, with tuberculosis alone killing about 2 million people a year.

7. Antibiotics are used to treat bacterial infections and in agriculture, so antibiotic resistance is becoming common.

8. In industry, bacteria are important in sewage treatment, the production of cheese and yogurt through fermentation, as well as in biotechnology, and the manufacture of antibiotics and other chemicals.
Pathogenesis of Bacteria Infection

**Endotoxin:** Endotoxins are toxins associated with certain Gram-negative bacteria which are structural molecules of the bacteria that is recognized by the immune system. The prototypical examples of endotoxin are lipopolysaccharide (LPS).

**Exotoxin:** Toxins excreted by a microorganism, including bacteria, fungi, algae and protozoa. An exotoxin can cause damage to the host by destroying cells or disrupting normal cellular metabolism. They are highly potent and can cause major damage to the host.
### Exotoxins of Bacteria Infection

#### 表36-3 常见细菌外毒素的作用方式和致病机制

<table>
<thead>
<tr>
<th>作用方式</th>
<th>毒素名称</th>
<th>毒素来源</th>
<th>作用部位</th>
<th>致病机制</th>
</tr>
</thead>
<tbody>
<tr>
<td>改变宿主细胞通透</td>
<td>大肠杆菌溶血素A</td>
<td>E. coli hemolysin A</td>
<td>多种 G+菌</td>
<td>肠道黏膜</td>
</tr>
<tr>
<td>干扰信号转导</td>
<td>肉毒毒素</td>
<td>botulinus toxin, BT</td>
<td>神经组织</td>
<td>其中的水肿因子是腺苷酸环化酶，可增加胞内cAMP浓度，促进IL-6基因的表达。致死因子具有蛋白水解酶活性，可水解MAPKK或MEK的N-端使之灭活。此外，LT可诱导巨噬细胞大量表达IL-1和TNF-α。</td>
</tr>
<tr>
<td></td>
<td>梅毒杆菌</td>
<td>梅毒杆菌</td>
<td>肠道黏膜</td>
<td>可作用于G蛋白的αS亚基，使之发生ADP-核糖化修饰，抑制其自身GTPase活性，从而持续激活Gsα，最终造成Cl-通道开放。细胞在释放Cl-的同时伴随大量的失水。</td>
</tr>
<tr>
<td></td>
<td>炭疽毒素CT</td>
<td>炭疽杆菌</td>
<td>吞噬细胞</td>
<td>其中热稳定性毒素α的裂解产物可激活内原性鸟苷酸环化酶，促进cGMP的生成，最终引起Cl-的释放。</td>
</tr>
<tr>
<td></td>
<td>霍乱毒素CT</td>
<td>霍乱弧菌</td>
<td>肠道黏膜</td>
<td>PT作用与霍乱毒素相似，对G蛋白进行ADP-核糖化修饰，但其作用靶点是Gi，因而最终引起细胞内cAMP水平下降。</td>
</tr>
<tr>
<td></td>
<td>百日咳毒素</td>
<td>百日咳鲍特菌</td>
<td>呼吸道</td>
<td>A亚基为RNA糖苷酶，可以使核糖体28S rRNA失活，从而抑制蛋白质合成。B亚基可以特异地对肽链延长因子2（EF-2）进行ADP-核糖化修饰，从而抑制宿主细胞的蛋白质合成。</td>
</tr>
<tr>
<td></td>
<td>肠毒素</td>
<td>大肠杆菌</td>
<td>肠道黏膜</td>
<td>影响基因表达</td>
</tr>
<tr>
<td></td>
<td>志贺毒素ST</td>
<td>志贺菌</td>
<td>小肠黏膜</td>
<td>白喉毒素DT</td>
</tr>
</tbody>
</table>
Diseases from Bacteria Infection

Overview of Bacterial infections

**Bacterial meningitis**
- Streptococcus pneumoniae
- Neisseria meningitidis
- Haemophilus influenzae
- Streptococcus agalactiae
- Listeria monocytogenes

**Otitis media**
- Streptococcus pneumoniae

**Pneumonia**
Community-acquired:
- Streptococcus pneumoniae
- Haemophilus influenzae
- Staphylococcus aureus
Atypical:
- Mycoplasma pneumoniae
- Chlamydia pneumoniae
- Legionella pneumophila
- Tuberculosis
- Mycobacterium tuberculosis

**Eye infections**
- Staphylococcus aureus
- Neisseria gonorrhoeae
- Chlamydia trachomatis

**Sinusitis**
- Streptococcus pneumoniae
- Haemophilus influenzae

**Upper respiratory tract infection**
- Streptococcus pyogenes
- Haemophilus influenzae

**Gastritis**
- Helicobacter pylori

**Food poisoning**
- Campylobacter jejuni
- Salmonella
- Shigella
- Clostridium
- Staphylococcus aureus
- Escherichia coli

**Skin infections**
- Staphylococcus aureus
- Streptococcus pyogenes
- Pseudomonas aeruginosa

**Sexually transmitted diseases**
- Chlamydia trachomatis
- Neisseria gonorrhoeae
- Treponema pallidum
- Ureaplasma urealyticum
- Haemophilus ducreyi

**Urinary tract infections**
- Escherichia coli
- Other Enterobacteriaceae
- Staphylococcus saprophyticus
- Pseudomonas aeruginosa
Bacteria Infection

**Antibiotics:** Antibiotics is a compound or substance that kills or slows down the growth of bacteria.

**Resistance**
The emergence of resistance of bacteria to antibacterial drugs is a common phenomenon.
Emergence of resistance often reflects evolutionary processes that take place during antibacterial drug therapy. The antibacterial treatment may select for bacterial strains with physiologically or genetically enhanced capacity to survive high doses of anti-bacterials. Under certain conditions, it may result in preferential growth of resistant bacteria while growth of susceptible bacteria is inhibited by the drug.

**Misuses:** The first rule of antibiotics is try not to use them, and the second rule is try not to use too many of them.

—Paul L. Marino, *The ICU Book*
**Microbial recognition in innate immune receptors**

**Innate immunity**

- Phagocytes
  - Macrophages
  - Dendritic cells
  - Neutrophils
- Receptors
  - Toll-like receptors
  - C-type lectin receptors
- Cytosolic
  - NOD proteins
  - RNA helicases
  - DNA recognition
- Mediators

**Recognition**

- Opsonization, phagocytosis
- Destruction

**Adaptive immunity**

- Humoral
  - Antibodies
- Cellular
  - T cells

**Invertebrates/Vertebrates**

**Memory**
Innate immunity is critical to adaptive immune response.

**Innate Immunity**
- Pathogen
- Toll-like receptors recognize PAMPs

**Adaptive Immunity**
- Antigen-presenting cell (macrophage or dendritic cell)
- Cytokines (IL-12, IL-6, TNFα)
- Costimulatory signals
- Naïve T cell
- Effector T cell

固有免疫

适应性免疫
Recognition of Microbe products by PAMPS

Pathogen-associated molecular patterns, or PAMPs, are molecules associated with groups of pathogens recognized by cells of the innate immune system.

These molecules can be referred to as small molecular motifs conserved within a class of microbes.

They are recognized by Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) in both plants and animals.
PAMPs from bacteria

Bacterial Lipopolysaccharide (LPS) on bacterial cell membrane (Gram−)

Bacterial flagelin

Peptidoglycan and lipoteichoic acid (磷脂壁酸) from Gram positive bacteria

Unmethylated CpG DNA
**Pattern Recognition Receptors (PRRs)**

- Toll-like receptors (TLRs; transmembrane receptors)
- RIG-I-like receptors (RLRs; cytoplasmic RNA helicases)
- NOD-like receptors (NLRs; cytoplasmic sensors)
- C-type lectin receptors (CLRs; transmembrane receptors)
Recognition of pathogen-associated molecular patterns by PRRs
Innate Immunity to extracellular Bacteria

**TLRs: Toll-like Receptors**

- **diacyl-triacyl-lipopeptide**: 酰基脂肽
- **flagellin**: 鞭毛蛋白
- **LPS**: 脂多糖 (Lipopolysaccharides)
Drosophila Toll

- Identified a protein called “Toll” meaning “weird”

- Helps the Drosophila embryo to differentiate its top from its bottom
  (Neural tube development)

http://www.nature.com/genomics/papers/drosophila.html
Toll and Inner Part of Human IL-1R is Similar

• Searching for proteins similar to Toll
• Shows cytoplasmic domain of Toll related to that of hIL-1R
• Identity extends for 135 aa
• Didn’t make sense

Why does a protein involved in human inflammation look like one involved in fly neural tube development?
Flies use Toll to Defend from Fungi

- Infected Tl-deficient adult flies with *Aspergillus fumigatus*
- All flies died after 2-3 days
- Flies use Toll to defend from fungi
- Thus, in Drosophila, Toll seems to be involved in embryonic development and adult immunity
Survival rate of adult *Drosophila* infected with *Aspergillus fumigatus* in *Toll*^-^
Flies use Toll to Defend from Fungi

- Drosophila has no adaptive immune system
- Therefore needs a rapid antimicrobial peptide response
- Two distinct pathways to activate antimicrobial peptide genes in adults
- Mutations in Toll pathway reduce survival after fungal infection
Human Toll Discovery

- In insect, IL-1 receptor and the Toll protein are only similar in the segments within the cell.

- Human proteins that totally resemble to Toll were later discovered.
Human Toll Discovery

- Alignment of the sequences of human and Drosophila Toll proteins
- Homology over the entire length of the protein chains
- hToll gene most strongly expressed in Spleen and PBL (peripheral blood leukocytes)
## TLR Recognition of Microbial Components

### Bacteria

<table>
<thead>
<tr>
<th>Microbial Components</th>
<th>Species</th>
<th>TLR usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS</td>
<td>Gram-negative bacteria</td>
<td>TLR4</td>
</tr>
<tr>
<td>Diacyl lipopeptides</td>
<td><em>Mycoplasma</em></td>
<td>TLR6/TLR2</td>
</tr>
<tr>
<td>Triacyl lipopeptides</td>
<td>Bacteria and mycobacteria</td>
<td>TLR1/TLR2</td>
</tr>
<tr>
<td>LTA (脂磷壁酸)</td>
<td>Group B <em>Streptococcus</em></td>
<td>TLR6/TLR2</td>
</tr>
<tr>
<td>PG (肽聚糖)</td>
<td>Gram-positive bacteria</td>
<td>TLR2</td>
</tr>
<tr>
<td>Porins</td>
<td><em>Neisseria</em></td>
<td>TLR2</td>
</tr>
<tr>
<td>Lipoarabinomannan</td>
<td>Mycobacteria</td>
<td>TLR2</td>
</tr>
<tr>
<td>Flagellin</td>
<td>Flagellated bacteria</td>
<td>TLR5</td>
</tr>
<tr>
<td>CpG-DNA</td>
<td>Bacteria and mycobacteria</td>
<td>TLR9</td>
</tr>
<tr>
<td>ND</td>
<td>Uropathogenic bacteria</td>
<td>TLR11</td>
</tr>
</tbody>
</table>
The Nobel Prize in Physiology or Medicine 2011 was divided, one half jointly to Bruce A. Beutler and Jules A. Hoffmann "for their discoveries concerning the activation of innate immunity" and the other half to Ralph M. Steinman "for his discovery of the dendritic cell and its role in adaptive immunity".

LPS is the ligand for TLR4

Large molecules found in outer membrane of Gram-negative bacteria

Comprised of a lipid and saccharide component
Highly immunogenic

Recognized by TLR4

Can cause septic shock and lead to death

Often referred to as Endotoxin since it is not secreted but is a byproduct of bacterial lysis
TLR4 Activated by LPS

- Normal mice die of sepsis after being injected with LPS
- C3H/HeJ mice have defective response to LPS and survive
- Missense mutation affecting the cytoplasmic domain of TLR4
- Major breakthrough in the field of sepsis - molecular mechanism that underlies inflammation revealed
Figure 3-9
Kuby IMMUNOLOGY, Sixth Edition
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LPS

Bacterial Cell (E. coli)

Cell Wall Organisation

Lipopolysaccharide Architecture

Lipid A Structure
Cellular location of TLRs

TLR4  
TLR5  
TLR1  
TLR2  
TLR6  
TLR10

Gm- Bacteria  
LPS  
Flagellin  
Gm+ Bacteria  
Mycobacteria  
Yeast  
Various Membrane/Wall Components

ENDOSOME  
dsRNA  
Viral and Bacterial Nucleic Acids

INNATE IMMUNE RESPONSE  
ssRNA  
ssRNA  
dsDNA
Ligand Recognition

TLR3 - dsRNA
Ligand Recognition

TLR4/MD2 - LPS

A

B

a

b
MyD88-dependent and independent pathway

TLR4

MyD88

p50 p65
Early-phase NF-κB

Inflammatory cytokines
MyD88-dependent response

Late-phase NF-κB

IFN-β, IFN-inducible gene products
MyD88-independent response

IRF3

Nature Reviews | Immunology
TLRs and TIR Domain

- Cytoplasmic tails of TLRs show similarities to IL-1 receptor (TIR)
- Common adaptor to TLRs is MyD88
- Crucial proline residue in all TLR TIR domains, except TLR3
- If mutated or deleted, no signaling occurs
- All TLRs likely have a MyD88 pathway (TLR3 is an exception)
- TLR4 has a MyD88 independent pathway as well

<table>
<thead>
<tr>
<th>Protein</th>
<th>Start</th>
<th>End</th>
<th>Accession Number</th>
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<tbody>
<tr>
<td>MyD88</td>
<td>190</td>
<td>209</td>
<td>NP_002459</td>
</tr>
<tr>
<td>IL18R</td>
<td>411</td>
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<td>IL-18RacP</td>
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<td>422</td>
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<td>IL-1RacP</td>
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<td>455</td>
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<td>671</td>
<td>690</td>
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</tr>
<tr>
<td>TLR4</td>
<td>704</td>
<td>723</td>
<td>000206</td>
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</table>
MyD88 Adaptor

- MyD88 knockout mice have no response to LPS
- MyD88 is essential to all inflammatory signaling pathways
- MyD88s, a splice variant of MyD88 down regulates the inflammatory response
- MyD88 interacts with TIR domain of TLR and recruits IRAK-4, IRAK-1 and TRAF-6

IRAK and TRAF6

- 4 IRAKs known, IRAK1, IRAK-2, IRAK-M and IRAK-4
- IRAK are serine/threonine kinases
- IRAK-4 phosphorylates IRAK-1
- IRAK-M plays an inhibitory role in TLR signaling

- TRAF6 is a member of the TNF receptor associated factor (TRAF) family
- TRAF6 interacts with IRAK-1 and gets activated
- Release of TRAF6/IRAK-1 ensues subsequent signaling

IL-1RI-associated protein kinases (IRAKs)
tumor necrosis factor receptor-associated factor 6 (TRAF6)
**IRAK and TRAF6 Release**

- TRAF6/IRAK-1 complex associates with 3 proteins
  - TAK1 (TGF-B activated kinase)
  - TAB1 (TAK1 binding proteins)
  - TAB2 (TAK1 binding proteins)

- Large complex associates with membrane
- Eventually IRAK-1 stays in membrane while TRAF6/TAK1/TAB1/TAB2 move to cytosol
- E2 Ligases such as Ubc13 and Uev1A join further enlarging complex
TAK-1 Activation

- The enlarged complex that includes
  - TRAF6, TAK1, TAB1, TAB2, Ubc13, Uev1A activate TAK1
- Activated TAK1 phosphorylates IKK complex
- Activated TAK1 can also phosphorylate MAP Kinases
- IKK complex consists of IKK\(\alpha, \beta\) and \(\gamma/NEMO\)
- I\(\kappa\)B phosphorylation results in NF-\(\kappa\)B translocation to nucleus
Overview of MyD88-dependent pathway
Step by Step view of MyD88-mediated signaling

1. Ligand binding to TLR triggers association of MyD88 with TIR domain and assembly of IRAK1/IRAK4 complex

2. IRAK4 phosphorylates IRAK1, creating a binding site for TRAF6

3. The IRAK1-TRAF6 complex dissociates and activates the protein kinase TAK1 complex

4. The active TAK1 activates two distinct signal transduction pathways

5a. TAK1 phosphorylates IKK to activate the NFκB pathway; IKK then phosphorylates IκB, causing it to release NFκB

5b. TAK1 also phosphorylates and activates a component of the MAP kinase (MAPK) pathway

6a. The freed NFκB translocates from the cytoplasm into the nucleus, where it serves as a transcriptional activator for NFκB-dependent genes

6b. The MAPK cascade results in translocation of a transcriptional activator from the cytoplasm into the nucleus, where it activates transcription of MAPK-dependent genes

Figure 3-14
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MyD88 Independent Pathway

- MyD88 Knock out mice do not produce inflammatory cytokines such as TNF-α

- However with TLR4 stimulation NF-κB and JNK delayed activity occurs

- This strongly suggests the existence of 2 pathways in TLR signaling
  - a MyD88 dependent pathway
  - a MyD88 independent pathway

- TLR3 stimulation also exhibits a MyD88 independent pathway
Overview of TLR signaling

The diagram illustrates the signaling pathways of TLRs (Toll-like receptors) and their downstream effects. The TLRs, such as TLR7, TLR9, TLR1, TLR2, TLR4, and TLR3, are activated by their respective ligands and trigger a cascade of events involving adaptor proteins like MyD88 and TRAF6. This leads to the activation of IKKα and IKKβ, which in turn phosphorylate and activate NF-κB, leading to the expression of inflammatory cytokines and IFN-β. The pathway also involves TBK1 and IRF-3, which play roles in the activation of NF-κB in the late phase of signaling.
LPS

TLR4

Inflammatory cytokines
Chemotactic factors and receptors
Coagulation factors
Antimicrobial effector functions
Pathogen recognition and phagocytosis
Antigen processing and presentation
Tissue repair factors
Metabolic regulators
Inflammation

Bacteria trigger macrophages to release cytokines and chemokines

Inflammatory cells migrate into tissue, releasing inflammatory mediators that cause pain

Cytokines

Chemokines

Bacteria trigger macrophages to release cytokines and chemokines

Vasodilation and increased vascular permeability cause redness, heat, and swelling

Cytokines

Chemokines

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Inflammatory cells migrate into tissue, releasing inflammatory mediators that cause pain

Bacteria trigger macrophages to release cytokines and chemokines

Vasodilation and increased vascular permeability cause redness, heat, and swelling

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Infl
Inflammation mediated by cytokines

– Low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune system

– Innate immune system
  • Macrophages and Dendritic cells
    – Tumor necrosis factor-alpha (TNF-α)
    – Interleukin-1 (IL-1)
    – Interleukin-12 (IL-12)

– Adaptive immune system
  • .................
Inflammation

- Pro-inflammatory cytokines (TNF, IL-1) signal to endothelial cells to make them:
  - Leaky to fluid (influx of plasma; containing antibodies, complement components, etc.)
  - Sticky for leukocytes, leading to influx of neutrophils first, then monocytes, lymphocytes
  - Systemic effects: fever, acute phase response
Sepsis (Septic shock)

- Bacterial septicemia leads to activation of TLRs on monocytes in the blood
- Systemic release of TNF and IL-1 leads to “inflammation” all over the body
- Shock from loss of blood pressure (vasodilation and leakage of fluid into tissues)
- The combination of effects can lead to multi-organ failure and death

- Bacteria Infection after injury — Sepsis in Emergency Room
- Neonatal Sepsis
Neonatal Sepsis

Neonatal sepsis is defined as a clinical syndrome of bacteremia (presence of bacteria in the blood) with systemic signs and symptoms of infection in the first 4 weeks of life. When pathogenic bacteria gain access into the blood stream, they may cause overwhelming infection without much localization (septicemia) or may get predominantly localized to the lung (pneumonia) or the meninges (meningitis).

Neonatal sepsis is the single most important cause of neonatal deaths in the community, accounting for over half of them. If diagnosed early and treated aggressively with antibiotics and good supportive care, it is possible to save most cases of neonatal sepsis.
# Negative Regulation of Toll-like receptor signaling

<table>
<thead>
<tr>
<th>Regulator</th>
<th>Expression and induction</th>
<th>Affected TLR</th>
<th>Possible mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTLR2</td>
<td>Constitutively expressed in breast milk and plasma</td>
<td>TLR2</td>
<td>TLR2 antagonist</td>
<td>52</td>
</tr>
<tr>
<td>sTLR4</td>
<td>ND</td>
<td>TLR4</td>
<td>Blocks interaction of TLR4 and MD2</td>
<td>51</td>
</tr>
<tr>
<td>MyD88s</td>
<td>LPS-induced expression, mainly in spleen</td>
<td>TLR4</td>
<td>MyD88 antagonist</td>
<td>55,56</td>
</tr>
<tr>
<td>IRAKM</td>
<td>LPS-induced expression by monocytes</td>
<td>TLR4,9</td>
<td>Inhibits phosphorylation IRAK1</td>
<td>60</td>
</tr>
<tr>
<td>SOCS1</td>
<td>LPS- and CpG-induced expression by macrophages</td>
<td>TLR4,9</td>
<td>Suppresses IRAK</td>
<td>65,66</td>
</tr>
<tr>
<td>NOD2</td>
<td>ND</td>
<td>TLR2</td>
<td>Suppresses NF-κB</td>
<td>73</td>
</tr>
<tr>
<td>PI3K</td>
<td>Constitutively expressed by most cells</td>
<td>TLR2,4,9</td>
<td>Inhibits p38, JNK and NF-κB function</td>
<td>80</td>
</tr>
<tr>
<td>TOLLIP</td>
<td>Constitutively expressed in most tissues</td>
<td>TLR2,4</td>
<td>Autophosphorylates IRAK1</td>
<td>81–83</td>
</tr>
<tr>
<td>A20</td>
<td>LPS-induced expression by macrophages</td>
<td>TLR2,3,4,9</td>
<td>De-ubiquitylates TRAF6</td>
<td>89</td>
</tr>
<tr>
<td>ST2L</td>
<td>LPS-induced expression by macrophages</td>
<td>TLR2,4,9</td>
<td>Sequesters MyD88 and MAL</td>
<td>100</td>
</tr>
<tr>
<td>SIGIRR</td>
<td>Mainly expressed by epithelial cells and immature dendritic cells but downregulated by activation</td>
<td>TLR4,9</td>
<td>Interacts with TRAF6 and IRAK</td>
<td>110,111</td>
</tr>
<tr>
<td>TRAILR</td>
<td>Constitutively expressed by most cells</td>
<td>TLR2,3,4</td>
<td>Stabilizes IkBα</td>
<td>114</td>
</tr>
<tr>
<td>TRIAD3A</td>
<td>Constitutively expressed by most cells and tissues</td>
<td>TLR4,9</td>
<td>Ubiquitylates TLRs</td>
<td>116</td>
</tr>
</tbody>
</table>

IRAK, interleukin-1 receptor-associated kinase; LPS, lipopolysaccharide; ND, not determined; NF-κB, nuclear factor-κB; NOD2, nucleotide-binding oligomerization domain protein 2; PI3K, phosphatidylinositol 3-kinase; SIGIRR, single immunoglobulin interleukin-1-related receptor; SOCS1, suppressor of cytokine signaling 1; sTLR, soluble decay TLR; TOLLIP, Toll-interacting protein; TRAF, tumour-necrosis factor receptor-associated factor; TRAILR, tumour-necrosis factor-related apoptosis-inducing ligand receptor.
## Soluble TLRs

<table>
<thead>
<tr>
<th>Regulator</th>
<th>Expression and induction</th>
<th>Affected TLR</th>
<th>Possible mechanism</th>
</tr>
</thead>
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<td>ND</td>
<td>TLR4</td>
<td>Blocks interaction of TLR4 and MD2</td>
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</tbody>
</table>

**Diagram:**

- TLR2 ligand
- Soluble TLR2
- TLR2
- IRAKs
- TRAF6
- NF-κB
- Inflammatory gene expression
- sTLR4
- Soluble TLR4
- LPS
- CD14
- MD2
- TRAM
- IRAKs
- TRAF6
- NF-κB
- Inflammatory gene expression
Membrane-associated Regulators

**Diagram:**
- **TRAILR**
- **SIGIRR**
- **TLR2, TLR4, or TLR9**
- **ST2L**

**Table:**
<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST2L</td>
<td>LPS-induced expression by macrophages</td>
</tr>
<tr>
<td>SIGIRR</td>
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<td>TLR2,4,9</td>
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<td>Interacts with TRAF6 and IRAK</td>
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### Feedback Regulation of Intracellular Signaling

**Diagram:**

- **TLR**
- **MyD88s**
  - LPS-induced expression, mainly in spleen
  - TLR4
  - MyD88 antagonist
- **IRAKM**
  - LPS-induced expression by monocytes
  - TLR4,9
  - Inhibits phosphorylation IRAK1
- **SOCS1**
  - LPS- and CpG-induced expression by macrophages
  - TLR4,9
  - Suppresses IRAK
- **A20**
  - LPS-induced expression by macrophages
  - TLR2,3,4,9
  - De-ubiquitylates TRAF6

**Inflammatory gene expression**
TLRs can also signaling to apoptosis
NLR

The **NOD-like receptors** (NLRs) are cytoplasmic proteins that have a variety of functions in regulation of **inflammatory** and **apoptotic** responses;

The official definition of NLR is currently "**Nucleotide-binding domain, Leucine-Rich repeat containing**" protein since these proteins are composed of conserved "modules" including a central **nucleotide-binding oligomerization domain** and a series of tandem **leucine-rich repeats**.

NLRs are encoded by a large **gene families** in many different animal species; there are more than **20 NLR genes in humans**. Many are thought to serve as **pattern recognition receptors** (PRRs) which sense microbial products in the cytoplasm of cells, although some members have different functions.
NOD1 & NOD2 recognize peptidoglycan substructures and promote innate immune responses.

NOD1 and NOD2 are intracellular molecules and resemble some plant disease resistance proteins; best understood of the "NOD-like receptors" or NLRs.
NLR signaling

CARD Domains: Caspase recruitment domains
Processing of IL-1 and related cytokines by inflammasome: an important regulatory step

• Some “NLRs” assemble to form the “inflammasome” which proteolytically processes IL-1 and related cytokines to their active, secreted forms.

• Inflammasome is activated by cellular stress or recognition of microbial components in the cytoplasm.

• Genetic periodic fever syndromes are due to activating mutations in inflammasome.
NOD-like receptor (NLR) family members such as NALPs, NAIP, and IPAF. The LRR of NALP3 or IPAF sense the activating signals leading to the oligomerization of the NACHT region. The exposed PYD and CARD domain can recruit adaptor protein ASC and CARD-containing caspases to form a donut shape complex. The IL-1β-processing caspase activity most likely face the inside of the donut (lower panel).
Two signals are essential for IL-1 secretion
Innate Immunity against Viral infection
Viral Infection

Viral infection: A virus is a small infectious agent that can replicate only inside the living cells of organisms.

Entry mediated by Cell surface Receptors:

<table>
<thead>
<tr>
<th>Virus</th>
<th>receptor</th>
<th>cell type</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>CD4</td>
<td>Th cells</td>
</tr>
<tr>
<td>EBV</td>
<td>CR2</td>
<td>B cells</td>
</tr>
<tr>
<td>Influenza sialic</td>
<td>CR2, ICAM-1</td>
<td>many cell types</td>
</tr>
<tr>
<td>acid</td>
<td></td>
<td>neurons</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>ICAM-1, poliovirus receptor</td>
<td>many cell types</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>CD46</td>
<td>neurons</td>
</tr>
<tr>
<td>Measles</td>
<td>CD46</td>
<td>many cell types</td>
</tr>
<tr>
<td>HHV6</td>
<td>CD46</td>
<td>many cell types</td>
</tr>
</tbody>
</table>
Viral Infection

General Process of Viral Infection:

Attachment
Penetration
Uncoating
Replication
self-assembly
Release
Viral Infection

Cell damages mediated by viral infection—Effects on the host cell

Most virus infections eventually result in the death of the host cell. The causes of death include cell lysis and apoptosis. Often cell death is caused by cessation of its normal activities because of suppression by virus-specific proteins.

Some viruses cause no apparent changes to the infected cell (latent infection). This causes persistent infections and the virus is often dormant for many months or years. This is often the case with herpes viruses.

Some viruses, such as Epstein-Barr virus, can cause cells to proliferate without causing malignancy, while others, such as papilloma viruses (HPV 乳头状瘤病毒) are established causes of cancer.
Viral Infection

Overview of Viral Infections

- Encephalitis/meningitis
  - JC virus
  - Measles
  - LCM virus
  - Arbovirus
  - Rabies

- Pharyngitis
  - Adenovirus
  - Epstein-Barr virus
  - Cytomegalovirus

- Cardiovascular
  - Coxsackie B virus

- Gingivostomatitis
  - Herpes simplex type 1

- Parotitis
  - Mumps virus

- Eye infections
  - Herpes simplex virus
  - Adenovirus
  - Cytomegalovirus

- Pneumonia
  - Influenza virus, Types A and B
  - Parainfluenza virus
  - Respiratory syncytial virus
  - Adenovirus
  - SARS coronavirus

- Hepatitis
  - Hepatitis virus types A, B, C, D, E

- Skin infections
  - Varicella zoster virus
  - Human herpesvirus 6
  - Smallpox
  - Molluscum contagiosum
  - Human papillomavirus
  - Parvovirus B19
  - Rubella
  - Measles
  - Coxsackie A virus

- Sexually transmitted diseases
  - Herpes simplex type 2
  - Human papillomavirus
  - HIV

- Myelitis
  - Poliovirus
  - HTLV-I

- Gastroenteritis
  - Adenovirus
  - Rotavirus
  - Norovirus
  - Astrovirus
  - Coronavirus

- Pancreatitis
  - Coxsackie B virus
Viral Nucleic Acids recognized by TLRs

• TLR3, TLR7 and TLR8 detect viral nucleic acids

• Found in intracellular membranes since viral nucleic acids are endogenously generated
**Viral Nucleic Acids recognized by TLRs**

<table>
<thead>
<tr>
<th>Virus genome</th>
<th>TLR2</th>
<th>TLR6</th>
<th>TLR4</th>
<th>TLR7/8</th>
<th>TLR9</th>
<th>TLR3</th>
</tr>
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<tbody>
<tr>
<td>DNA</td>
<td>HSV</td>
<td>VZV</td>
<td>MMTV</td>
<td>HSV</td>
<td>CMV</td>
<td>MCMV</td>
</tr>
<tr>
<td></td>
<td>VZV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>HCMV</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Double stranded RNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reo</td>
</tr>
<tr>
<td>Positive stranded RNA</td>
<td>Measles</td>
<td></td>
<td></td>
<td>Coxsackie B</td>
<td></td>
<td>West Nile</td>
</tr>
<tr>
<td>Negative stranded RNA</td>
<td>VSV</td>
<td></td>
<td>RSV</td>
<td>Sendai VSV influenza</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambi sense RNA</td>
<td>LCMV</td>
<td>LCMV</td>
<td></td>
<td>LCMV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Viral Nucleic Acids recognized by TLRs
Recognition of cytosolic viral RNAs by RLRs

**RIG-I-like receptors** (RLRs), also known as RIG-I-like helicases (RLHs) constitute a family of cytoplasmic RNA helicases that are critical for host antiviral responses.

**RIG-I** (retinoic-acid-inducible protein 1, also known as Ddx58) and **MDA-5** (melanoma-differentiation-associated gene 5, also known as Ifih1 or Helicard) sense double-stranded RNA (dsRNA), a replication intermediate for RNA viruses, leading to production of type I interferons (IFNs) in infected cells.
RIG-I and MDA-5 contain a DExD/H box RNA helicase and two caspase recruiting domain (CARD)-like domains. The helicase domain interacts with dsRNA, whereas the CARD domains are required to relay the signal.

RIG-I participates in the recognition of Paramyxoviruses (Newcastle disease virus (NDV), Sendai virus (SeV)), Rhabdoviruses (vesicular stomatitis virus (VSV)), Flaviviruses (hepatitis C (HCV)) and Orthomyxoviruses (Influenza). MDA-5 is essential for the recognition of Picornaviruses (encephalomyocarditis virus (EMCV)) and poly(I:C), a synthetic analog of viral dsRNA.

Notably, RIG-I binds specifically to single stranded RNA containing 5'-triphosphate such as viral RNA and in vitro-transcribed long dsRNA [4]. Mammalian RNA is either capped or contains base modifications suggesting that RIG-I is able to discriminate between self and non-self RNA.

RIG-I binds preferentially to short dsRNA while MDA-5 recognizes preferentially long dsRNA.
Viral dsRNA is also recognized by **Toll-Like receptor 3 (TLR3)** which is expressed on the cell surface membrane or endosomes.

Recognition of dsRNA by RIG-I/MDA-5 or TLR3 is cell-type dependent. Studies of RIG-I- and MDA-5-deficient mice have revealed that conventional dendritic cells (DCs), macrophages and fibroblasts isolated from these mice have impaired IFN induction after RNA virus infection, while production of IFN is still observed in plasmacytoid DCs (pDCs).

Thus in cDCs, macrophages and fibroblasts, RLRs are the **major sensors for viral infection**, while in pDCs, TLRs play a more important role.
**Interferons**

**Table 14.5** The interferons: antiviral cytokines

<table>
<thead>
<tr>
<th>Interferon&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Producer cells</th>
<th>Inducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ifn-α</td>
<td>Leukocytes</td>
<td>Virus infection, dsRNA</td>
</tr>
<tr>
<td>Ifn-β</td>
<td>Fibroblasts, epithelial cells</td>
<td>Virus infection, dsRNA</td>
</tr>
<tr>
<td>Ifn-γ</td>
<td>T cells, NK cells</td>
<td>Antigens, mitogens, Il-2, Il-12</td>
</tr>
</tbody>
</table>

Ifn-α and Ifn-β are induced by viral infection of any cell type.

Ifn-γ is induced only when certain lymphocytes are stimulated to replicate and divide after binding a foreign antigen.
Interferons

1. IFN is induced by viral infection.

2. IFN binds to specific cell surface receptors and activate intracellular signaling pathways.

3. IFN signal induces gene expression at the transcriptional level.

4. A cell that is bound to interferon undergo an antiviral state.

5. IFN induces expression of more than 100 genes, products of many of these genes possess broad spectrum antiviral activity.

6. They also lead to cell death by apoptosis or programmed cells death, limiting cell to cell spread of virus.

7. Production of large amounts if IFN causes common symptoms such as fever, chills, nausea, etc.
Amplification of interferon response
IFN-stimulated genes
Interferon induced antiviral responses

• Both viral and cellular protein synthesis stops in IFN treated cells.

• This is due to two cellular proteins, ds-RNA activated protein kinase (PKR) and ribonuclease L (RNase L).

• PKR is a serine/threonine kinase that has antiviral properties, as well as antiproliferative and antitumor functions.

• Activated PKR phosphorylates the alpha subunit of the translation initiation factor eIF2, inhibiting translation.
Interferon induced antiviral responses:

- RNase L is a nuclease that can degrade cellular and viral RNA; its concentration increases after Ifn treatment.

- RNase L concentration increases 10-1,000 fold after Ifn treatment, but is inactive unless 2′-5′-oligo(A) synthetase is produced.

- 2′-5′-oligo(A) synthetase produces 2′, 5′ oligomers of adenylic acid, only when activated by dsRNA.

- These poly(A) oligomers then activate RNase L, which degrades all host and viral mRNA in the cell.

- RNase L participates not only in IFN-mediated antiviral defense, but also in apoptosis.
Anti-viral effects of interferon α/β

- IFN-α/β binds to IFNAR1 and IFNAR2.
- TYK2 and JAK1 are activated.
- STAT1 and STAT2 are phosphorylated and translocated to the nucleus.
- STAT1 and STAT2 form dimers and activate transcription factors.
- dsRNA induces PKR activation.
- eIF2α phosphorylation inhibits translation.
- dsRNA activates 2',5'-oligo A synthetase.
- 2',5'-oligo A activates RNase L.
- mRNA degradation is induced.
- 2',5'-oligo A activates Mx proteins.
- Mx proteins inhibit transcriptional inhibition, inhibition of virus assembly.
<table>
<thead>
<tr>
<th>Type of modulation</th>
<th>Representative virus</th>
<th>Viral protein</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of IFN synthesis</td>
<td>Epstein-Barr virus</td>
<td>BCRF-1</td>
<td>IL-10 homolog, inhibits production of IFN-γ</td>
</tr>
<tr>
<td></td>
<td>Vaccinia virus</td>
<td>A18R</td>
<td>Regulates dsRNA production</td>
</tr>
<tr>
<td></td>
<td>Human papillomavirus type 16</td>
<td>E6</td>
<td>Binds Ifr3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E7</td>
<td>Binds Ifr1</td>
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<tr>
<td></td>
<td>Influenza A virus</td>
<td>NS1</td>
<td>Mechanism under study</td>
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<td>Hepatitis B virus</td>
<td>OrfC and terminal protein</td>
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<td>Measles virus</td>
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<tr>
<td></td>
<td>Foot-and-mouth disease virus</td>
<td>L</td>
<td>Host protein synthesis block</td>
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<td>IFN receptor decoys</td>
<td>Myxoma virus</td>
<td>M-T7</td>
<td>Soluble IFN-γ decoy receptor</td>
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<td></td>
<td>Vaccinia virus</td>
<td>B18R</td>
<td>Soluble IFN-α/β decoy receptor</td>
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<tr>
<td>Inhibition of IFN signaling</td>
<td>Adenovirus</td>
<td>E1A</td>
<td>Decreases quantity of Stat1 and p48; blocks Isgf3</td>
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<td></td>
<td></td>
<td></td>
<td>formation; interferes with Stat1 and Chp/P300 interactions</td>
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<td></td>
<td>Epstein-Barr virus</td>
<td>EBNA-1, EBNA-2</td>
<td>?</td>
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<td></td>
<td>Herpes simplex virus type 1</td>
<td>?</td>
<td>Inhibits phosphorylation of Stats</td>
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<td></td>
<td>Vaccinia virus</td>
<td>VH1</td>
<td>Viral phosphatase reverses Stat1 activation</td>
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<td></td>
<td>Human papillomavirus type 16</td>
<td>E7</td>
<td>Binds p48</td>
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<td></td>
<td>Polyomavirus</td>
<td>Large T antigen</td>
<td>Binds and inactivates Jak1</td>
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<td></td>
<td>Human cytomegalovirus</td>
<td>?</td>
<td>Reduces levels of Jak1 and p48</td>
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<td>Hepatitis B virus</td>
<td>Terminal protein</td>
<td>?</td>
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<td></td>
<td>Hepatitis C virus</td>
<td>NS5a</td>
<td>Blocks formation of Isgf3 and Stat dimers</td>
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<td>Sendai virus</td>
<td>C proteins</td>
<td>Block Stat phosphorylation</td>
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<td></td>
<td>Simian virus (Virus)</td>
<td>rprotein V</td>
<td>Degrades Stat1</td>
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<tr>
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<td>Bovine respiratory syncytial virus</td>
<td>NS1, NS2</td>
<td>Block IFN induction</td>
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<tr>
<td></td>
<td>Parainfluenza virus type 2</td>
<td>?</td>
<td>Degrades Stat2</td>
</tr>
<tr>
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<td>Parainfluenza virus type 3</td>
<td>?</td>
<td>Blocks Stat1 phosphorylation</td>
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<td>Nipah virus</td>
<td>V protein</td>
<td>Prevents Stat1 and Stat2 activation and nuclear</td>
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<td>accumulation</td>
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<tr>
<td>Block function of IFN-induced proteins</td>
<td>Adenovirus</td>
<td>VA-RNA 1</td>
<td>Binds dsRNA, blocks Pkr</td>
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<td>Simian virus 40</td>
<td>?</td>
<td>Blocks RNase L</td>
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<td>Baculovirus</td>
<td>PK2</td>
<td>Blocks eIF2α kinases and Pkr</td>
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<td>Epstein-Barr virus</td>
<td>EBER RNA</td>
<td>Binds dsRNA, blocks Pkr</td>
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<td>Herpes simplex virus type 1</td>
<td>US11</td>
<td>Blocks Pkr activation</td>
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<td>ICP34.5</td>
<td>Redirects protein phosphatase 1α to dephosphorylate</td>
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<td>eIF2α; reverses Pkr action</td>
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<td>Produces 2'-5' derivatives that antagonize 2'-5'</td>
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<td>oligo(A) synthetase</td>
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</table>
Viral evasion of interferon: PKR

Viral protein binds-blocks dimerization (HCV)

inactive PKR

Viral dsRNA binding proteins (vaccinia, influenza)

Viral decoy RNA antagonists (adenoviruses, EBV, HIV)

Viral protein promotes degradation (polio)

Viral pseudosubstrates (HCV, RV, HIV, vaccinia)

Vaccinia: 豆苗病毒
Fungal infections recognized by pattern recognition receptors (PRRs)

O-linked mannann RNA DNA

N-linked mannann β-glucan α-glucan Chitin

β-mannosides C3b- or C3d-coated β-glucan BAD1 β-glucan

TLR2 TLR3 TLR6 TLR9

MYD88 TRIF

ERK p38

Canonical or non-canonical NF-κB

IRF3

Defensins, phagocytosis, chemokines, cytokines and IDO

Nucleus

Cytoplasm

Defensins, phagocytosis, chemokines, cytokines

ROS

NLRP3 ASC

Pro-IL-1β Pro-IL-18 IL-1β IL-18

Fungal killing and cytokines

Phagocytosis and cytokines

Phagocytosis, chemokines and cytokines

Galectin 3

CR3

CD36
The key point of today’s talk

The molecular Mechanism used by the innate immune system to recognize and defend against infectious microbes.

Thank You!