Immunology

Innate Immunity — Pattern Recognition Receptors (PRRs), Inflammasome, Complement System

See online here

Various infectious agents are constantly attacking and posing a threat to human health. Under normal conditions, our body manages to remain healthy. Our innate immune system provides the first line of protection against such pathogens and harmful agents. Read on to find out the different components of the innate immune system and how they interact with each other to form the body’s primary line of defence.

The Immune Response

The innate immune system

Immune responses are classified into innate and adaptive immunity. The innate immune system is non-specific to infectious agents and consists of mechanisms and
molecules which have a rapid response to infection. The innate immune system is an evolutionarily older defense strategy, and is the dominant immune system found in plants, fungi, insects, and primitive multicellular organisms.

Whenever there is potential microbial or viral infection, innate immunity acts as the first line of defense. This defence mechanisms include physical barriers such as skin, chemicals in the blood, and the immune system cells also attack foreign cells in the body. Innate immunity is inherent to an individual and does not have any memory to respond to the infection. Earlier exposure to any particular antigen does not alter the response. Since it is not stimulated by any specific antigens, innate immunity is generally nonspecific and in contrast to acquired immunity.

Innate immunity examples:

- Cough reflex
- Enzymes in tears and skin oils
- Mucus, which traps bacteria and small particles
- Skin
- Stomach acid

Innate immunity is made up of anatomical barriers, such as physical, chemical and biological barriers in the form of specialized cells and soluble molecules. The epithelial surfaces form a physical barrier that is impermeable to most infectious agents, acting as the first line of defense against invading organisms. Desquamation (shedding) of skin epithelium also helps remove bacteria and other infectious agents that have adhered to the epithelial surfaces. Lack of blood vessels and inability of the epidermis to retain moisture, presence of sebaceous glands in the dermis provides an environment unsuitable for the survival of microbes.

In the gastrointestinal and respiratory tract, movement due to peristalsis or cilia, respectively, helps remove infectious agents. Also, mucus traps infectious agents. The gut flora can prevent the colonization of pathogenic bacteria by secreting toxic substances or by competing with pathogenic bacteria for nutrients or attachment to cell surfaces. The flushing action of tears and saliva helps prevent infection of the eyes and mouth.

These barriers collectively eliminate or identify the potential threat i.e. the microbial or viral infection and consequently, the innate immune system cells and complement system are activated for effective elimination of invading pathogen and its products. Invasion caused activation of the innate immune system and the result is infection.

Inflammation is one of the first responses of the immune system to infection or irritation.

Inflammation is stimulated by chemical factors released by injured cells and serves to establish a physical barrier against the spread of infection, and to promote healing of any damaged tissue following the clearance of pathogens.

The process of acute inflammation is initiated by cells already present in all tissues. The main effector cells, which include neutrophils, macrophages, dendritic cells, histiocytes, Kupffer cells and natural killer (NK) cells, rapidly move to the site of infection or tissue damage and cause resolution of infection and tissue repair. However, the innate immune system does not confer long-lasting protection against repeated invasions by the same agent.
Events occurring during the innate response

- Recognition of a threat — infection, toxins, tissue damage
- Activation of innate immune cells and the complement system
- Production of cytokines, chemokines, acute phase proteins and defenses
- Upregulation of cell adhesion molecules
- Recruitment of cells to the site of infection or tissue damage
- Elimination of the stimulus
- Resolution of the response
- Tissue repair

Adaptive immune system

The adaptive immune system, also known as the acquired/specific immune system, as the words dictate, and unlike the innate immune system, it is a specialized system made up of cells and their secretions that are made to kill/ inactivate pathogens. Adaptive immunity is broadly classified as:

1. Antibody mediated immunity that largely comprises of B cells that once presented with an antigen/ activated by t regulatory cells then, they mount an immune response by release of antibodies that bind to the antigen to destroy them via the antigen-antibody reaction pathway.

2. Cell mediated immunity (CMI) which entails: a. Cytotoxic CD8+ T cells are capable of engulfing and killing the antigens; b. Th Type 1 cells that cannot destroy antigens but activate other cells of the immune system to do so; c. Th Type 2 cells that form part of the memory cells.

3. The adaptive immune system is thus capable of keeping memory of the inciting event and thus can mount an enhance response when challenged with a similar antigen in the future a feature known as immunity. Immunity can be broadly classified into:
   - Active immunity where the body makes antibodies to a specific antigen. It can either be natural such as immunity developed after acquiring disease say measles or acquired such as vaccination where administration of inactivated/dead antigens triggers antibody release for future response without necessarily causing disease status.
   - Passive immunity where antibodies are transferred to the body without a trigger antigen. Antibodies may be acquired naturally such as in mother to child transfer of antibodies or artificially administered to prevent disease once antigens are within the body such as in anti-venom antibodies administration during treatment of poisonous bites.

PRR: Pattern Recognition Receptors

PRRs are receptors of the innate immune system that are on a lookout for pathogenic infections and are specialized in the recognition of pathogens such as bacteria and viruses. PRRs are protein molecules encoded in the human genome that cannot be changed throughout the lifespan of any individual and are mainly present on important immune cells like macrophages, monocytes, neutrophils, epithelial cells and dendritic cells.
**PAMPs** are pathogen-associated molecular patterns, such as lipopolysaccharide, bacterial or viral DNA or RNA, mannose, bacterial peptides, peptidoglycans and lipoteichoic acids and many others. PAMPs are involved in the damage of antigens from microbial disease processes.

**DAMP** stands for damage-associated molecular patterns. Examples are uric acid and extracellular ATP, among many other compounds. They are involved in the damage of toxic products of the body's functional processes. Both PAMPs and DAMPs are highly conserved motifs.

PAMPs lead to **cytokine and chemokine production** in response to a pathogenic infection. Through an assortment of proteins and by recognition of PAMPs, PRRs can activate **inflammation**, **clotting** and **complement pathways**, **opsonisation** and **apoptosis**.

There are many receptor families (PAMPs), and diverse receptors in every family strengthen the innate immune response and decrease chances of pathogen evasion. These receptors are capable of bringing about a synchronized reaction to pathogenic infections due to extensive communication among different signaling pathways.

**Note:** Recognition is structurally-specific but **what is recognized is common to whole groups of organisms or host cells**.

On the other hand, in case of certain **autoimmune diseases**, **trauma**, **acute myocardial infarction**, **atherosclerosis** and **cancer**, sterile inflammation is induced by the release of DAMPs. These are endogenous molecules which are released following tissue injury or tissue stress.

PRR are divided into 3 categories: **cellular** (e.g. CD14, MARCO), **intracellular** (e.g. NODs, PKR) and **soluble forms** (e.g. C-reactive protein, mannan-binding lectin). Cellular receptors are able to respond to all bacteria having common components in their cell walls but are not protein-specific to any micro-organism.
Trans-membrane **Toll-like receptors** (TLRs) and **C-type lectin receptors** (CLRs) reside on the cell surface, while **NOD-like receptors** (NLRs) and **RIG-I-like receptors** (RNA-LRs) are cytoplasmic proteins that regulate inflammatory and apoptotic responses. **AIM2-like receptors** (ALRs) detect foreign DNA.

**Pro-inflammatory cytokines, chemokines, and antiviral molecules** are expressed as a result of triggering of the intracellular signal transduction cascades due to the recognition of ligands by PRRs.

**A rigid regulation of PRR signaling is necessary** for elimination of pathogens and simultaneous prevention of excessive PRR activation as it can cause **autoimmune and inflammatory disorders** to develop.

**PRRs which recognize PAMPs**

<table>
<thead>
<tr>
<th>PRR</th>
<th>PAMP</th>
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<tbody>
<tr>
<td><strong>Endosomal</strong></td>
<td></td>
</tr>
<tr>
<td>TLR3 (toll-like receptor 3)</td>
<td>Viral double-stranded RNA</td>
</tr>
<tr>
<td>TLR7 and TLR8</td>
<td>Viral single-stranded RNA</td>
</tr>
<tr>
<td>TLR9</td>
<td>Bacterial unmethylated CpG DNA</td>
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<tr>
<td><strong>Cytosolic</strong></td>
<td></td>
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<tr>
<td>NOD-1 (nucleotide-binding oligomerization domain-containing protein-1) and NOD-2</td>
<td>Bacterial peptidoglycan</td>
</tr>
<tr>
<td>RIG-1 (retinoic acid-inducible gene 1)</td>
<td>Viral double-stranded RNA</td>
</tr>
<tr>
<td>NLRP3 (NOD-like receptor family, pyrin domain containing 3) (inflammasome)</td>
<td>Bacterial lipopolysaccharide (LPS)</td>
</tr>
<tr>
<td><strong>Cell surface</strong></td>
<td></td>
</tr>
<tr>
<td>TLR2</td>
<td>Bacterial lipopeptides and lipoproteins</td>
</tr>
<tr>
<td>TLR4</td>
<td>Bacterial lipopolysaccharide (LPS)</td>
</tr>
<tr>
<td>TLR5</td>
<td>Bacterial flagellin</td>
</tr>
<tr>
<td><strong>Soluble</strong></td>
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<tr>
<td>Mannose-binding lectin</td>
<td>Mannose</td>
</tr>
<tr>
<td>Ficolin</td>
<td>N-acetylglucosamine</td>
</tr>
<tr>
<td><strong>Cell surface</strong></td>
<td></td>
</tr>
<tr>
<td>RAGE (receptor for advanced glycation endproducts)</td>
<td>Advanced glycation endproducts</td>
</tr>
<tr>
<td>RAGE, TLR2, TLR4</td>
<td>HMGB1 (high mobility group box 1)</td>
</tr>
<tr>
<td><strong>Cytosolic inflammasome</strong></td>
<td></td>
</tr>
<tr>
<td>NLRP3</td>
<td>Uric acid</td>
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Innate Immune Signaling Overview

The activation and signaling PRR system is a multifactorial, intricate process resulting in comparable outcomes. Due to activation of PRR, proteins undergo conformational changes and intracellular signaling pathways are activated leading to amplification of signals and initiation of innate immune response. Signaling complex assembly is dependent on the specific **adapter proteins** which further engage various signaling components. Activation of PAMPs by PRRs takes place.

**Endogenous ligand molecules** like ATP and **heat shock proteins** are known as damage-associated molecular patterns (DAMPs). PRRs use of specific adaptors and different adaptor proteins results in the activation of different signaling pathways. e.g: **family transcription factors NFκB** (Nuclear Factor kappa B) and **IRF** (interferon regulatory factor) upregulation; **mitogen-activated protein kinases (MAPK)** i.e. **stress kinase pathways stimulation**; and **caspase-1 activation**.

Eventually, the pro-inflammatory cytokines, chemokines, and anti-viral proteins are upregulated.

**Inflammasome**

The **inflammasome** is basically a multiprotein, high molecular weight complex which leads to activation of inflammatory caspases and cytokines of the interleukin-1 family. Inflammasomes act as immune guardians of the **cytosol**, which in turn act as a watch guard by identification of intracellular pathogens with the help of PAMPs. **Multiprotein inflammasome complexes** are formed due to activation of some ALRs and NLRs which provide a platform for cleavage and activation of **caspase-1**.

The actions of inflammasomes have been implicated in an immediate inflammatory response, both physiological and pathological. The cells of the innate immune system secrete mature forms of **caspase-1** and **interleukin-1β (IL-1β)** which is characteristic of the activation of the inflammasome.

Sensor proteins containing various inflammasomes have been described, those being
NLRP1 (NALP1), NLRP3 (NALP3), IPAF (NLRC4), NLRP6 (NALP6), NLRP12 (NALP12), RIG-I and AIM-2 (absent in melanoma 2). Asc (apoptosis-associated speck-like protein), an adapter protein, is necessary for most of the inflammasomes for the purpose of recruitment of caspase-1 to inflammasome complex.

There is cleavage and activation of caspase-1 when there is binding to the inflammasome. Thus, its different targets are cleaved which lead to maturation and secretion of pro-inflammatory IL-1β.

Activation of inflammasomes can take place due to several different signals like bacteria and microbial toxins, xeno-compounds, cytoplasmic pathogen-associated molecular patterns (PAMPs) and/or endogenous danger signals (DAMPs). Beneficial or harmful inflammatory responses are associated with the inflammasome activity. Maintenance of homeostatic tissue function gives rise to beneficial responses while sterile inflammation due to host-derived particles like monosodium urate (MSU) crystals (involved in the pathogenesis of gout) and environmental particles like asbestos, silica as well as metallic nanoparticles (involved in lung inflammation) lead to harmful inflammatory responses.

The activity of inflammasomes is also concerned with several diseases like cancer and metabolic diseases (such as type 2 diabetes, atherosclerosis), certain neurodegenerative diseases (such as Parkinson, Alzheimer, Prion), autoimmune diseases (like multiple sclerosis) along with inflammatory bowel diseases.

### Acute Phase Inflammatory Response

*Inflammare* in Latin means to set on fire and it is usually self-limiting and controlled. Acute phase inflammation is an immediate, short duration inflammatory response of a tissue to injury that involves host cells, blood vessels as well as proteins.

Acute inflammation can be considered as the first line of defense against injury. Changes in the microcirculation, which include fluid exudation and leukocyte migration from the blood vessels to the area of injury, are the hallmark of acute inflammation. Acute inflammation takes place prior to establishment of the immune response and it is mainly intended for removal of the injurious agent.

Acute inflammation leads to an elimination of early causes of cell injury and...
removes **necrotic cells** as well as **necrotic tissues**. It also causes the **initiation of repair**, which may be potentially harmful. **Inflammatory components** that cause the destruction of pathogenic microorganisms can also destroy the normal bystander tissue. **White blood cells**, as well as plasma proteins, are the components of the inflammatory process. These are carried to the site of infection or tissue damage. Production of **chemical mediators** like **cytokines** leads to induction of inflammation.

Various stimuli of acute inflammation are:

1. **Bacterial, viral, fungal, parasitic infections**
2. Microbial toxins
3. Tissue necrosis due to ischemia, trauma, physical or chemical injury like thermal injury, irradiation or certain environmental chemicals
4. Foreign bodies like splinters, dirt, sutures
5. Immune reactions or **hypersensitivity reactions**

**Cardinal signs of acute inflammation**

Clinically, there are five cardinal signs that are the hallmark of acute inflammation:

- Rubor (redness)
- Calor (increased heat)
- Tumor (swelling)
- Dolor (pain)
- Functio laesa (loss of function).

The first four signs were described by **Celsius** while the fifth one was added later by **Virchow**.

### Cells vs. Molecules

<table>
<thead>
<tr>
<th>Cells</th>
<th>Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>Proinflammatory cytokines</td>
</tr>
<tr>
<td>Endothelium</td>
<td>Chemokines</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Acute phase proteins</td>
</tr>
<tr>
<td>Basophils</td>
<td>Adhesion molecules</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Complement</td>
</tr>
<tr>
<td>Platelets</td>
<td>Histamine</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Prostaglandins</td>
</tr>
<tr>
<td></td>
<td>Leukotrienes</td>
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**Consequences of acute inflammation**

In case of an acute inflammatory response, various natural anti-inflammatory factors are released. These include **cytokines**, **protease inhibitors**, **antioxidant enzymes**, **chemokines**, **prostaglandins**, and **leukotrienes**.
lipoxins, glucocorticoids, kinases, phosphatases and transcriptional factors.

By and large, the cytokines are pro- or anti-inflammatory factors. The equilibrium between these factors decides the result of an inflammatory response. Pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8, and IFN-γ are implicated in early inflammatory response as well as amplification of inflammatory reactions whereas IL-4, IL-10 and IL-13 are anti-inflammatory cytokines that lead to limitation of inflammatory responses.

Chronic Inflammation

Chronic inflammation, as the name suggests, is of a longer duration. It occurs when the preliminary acute inflammatory response cannot eliminate the inflammatory agent. It is necessary to measure the effects of different cytokines in order to understand the communication amongst the inflammatory cells.

This inflammatory process involves lymphocytes, plasma cells and macrophage infiltration. These inflammatory cells lead to tissue destruction. Resolution of the inflammation takes place by angiogenesis and fibrosis.

Chronic inflammation, if unresolved, is a central component of various chronic conditions such as autoimmune diseases as well as neurodegenerative diseases. Examples include tuberculosis, silicosis, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, Alzheimer's disease and cancer.

Contrasting acute and chronic inflammation

<table>
<thead>
<tr>
<th></th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predominant cell type</td>
<td>Neutrophil</td>
<td>Macrophage and T-lymphocyte</td>
</tr>
<tr>
<td>Time course</td>
<td>Rapid onset, short lived</td>
<td>Slow onset, long lived</td>
</tr>
<tr>
<td>Nature of the response</td>
<td>“Physiological”</td>
<td>“Pathological”</td>
</tr>
<tr>
<td>Tissue damaging</td>
<td>Usually mild and resolves quickly</td>
<td>Often severe and progressive</td>
</tr>
</tbody>
</table>
The complement system is a key element of the innate immune system that enhances the ability of antibodies and confers protection particularly against the invading infectious agents like bacteria, viruses, and protozoa.

Using different complement pathways, it produces biologically active products. These result in pro-inflammatory mediators which bring in the PMNs and lead to the production of opsonins and lytic factors for bacteria as well as nucleated cells. Opsonins promote phagocytosis.

The three pathways of complement activation are the classical pathway, alternative pathway, and lectin pathway. The complement system is made up of a complex of different proteins. Nine different proteins have been recognized, namely C1 to C9. 

C1 is a heat-labile component that forms the major bulk of the complement system and consists of 3 sub-proteins, C1q, C1r, C1s which are bound together by calcium.

C2 is also a heat-labile component whereas C3 is a heat stable component that has an ability to combine with yeast cell wall and leads to its inactivation.

C4 is a heat stable component that is inactivated by ammonia and hydrazine. C5 to C9 are the components that cause cell lysis by punching holes in the cell membrane thereby leading to impairment of function. C5 to C9 are the terminal components of the classical as well as the alternate pathway.

The Classical pathway (See Complement System and Chronic Inflammation) is dependent on all 9 proteins acting in a sequence. It is activated when IgM or IgG combine with the cell membrane in the presence of complement. The Alternate pathway bypasses the activation of C1, C4, and C2 and directly activates C3.

The binding of C3b to antigen-antibody complexes, antibody sensitized cells and viruses make them adhere to cells that possess immune adherence receptors i.e. PML, macrophages, some B-lymphocytes, primate RBCs etc. C1-C5 also enhance the susceptibility to phagocytosis. Complement is also responsible for the death of tumor cells.
Type I interferon response

Interferons are named so because they ‘interfere’ with viral replication. They were the first cytokines to be discovered.

The type I IFN family is made up of many IFN members, single IFN, and subtypes that are present in swine and ovine species. Type I interferons induce antiviral responses by binding to a common receptor, the type I IFN/receptor (IFNAR) that is expressed on an extensive array of cell types. These are induced quickly and perform a critical role in defense against viral infection. At every stage in the life cycle of the virus, they have a defensive role.

Type I IFNs not only induce proteins with their direct anti-viral effects but also regulate various aspects of the innate as well as adaptive immunity. Type I IFN promotes the survival and proliferation of NK cell by induction of production of IL-15. They upregulate the MHC surface expression and also that of CD80, CD86, and CD40.

Type I IFN activate naïve CD8+ T cells, and play a role in the survival of activated CD4+ as well as CD8+ cells along with the development and proliferation of B cells. In most cases, there is recognition of the viral infections by host PRRs that leads to type I IFN production.

When the viruses are recognized, various Toll-Like Receptors such as the TLR3, TLR4, TLR7, TLR8, and TLR9 as well as some intracellular Pattern Recognition Receptors (PRRs) like RIG-I and PKR induce the production of type I IFN. There is an induction of plentiful amounts of proteins on activation of PRRs that leads to triggering of complex signaling pathways.

Along with viruses, type I IFN production is also significant during bacterial infections and in the treatment of several malignant disorders such as myeloma, renal cell carcinoma, and melanoma, where IFN is used as chemotherapeutic agent in deep melanomas.

NK cells activating and inhibitory response

Natural killer cells are large, non-phagocytic, granular lymphocytes that constitute 5-10% of total lymphocytes. They are not formed in response to Ag but are naturally present. Their action is nonspecific and they do not require Ab. They can kill a multitude of transformed and virally infected cells.

Their activity is increased by INF and interleukin-2. They cause cell lysis by the release of cytolytic factors such as perforins, lymphotoxin, TNF, and NKCF. NK cells
are involved in an early response to infection by certain viruses and bacteria. They are essential for immune surveillance and natural defense against virus-infected cells and malignant mutant cells.

References

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Robins, Textbook of pathology

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