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Host-Microbiota Interface on Innate Mucosal Immune Responses

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ABSTRACT

The diverse microbial population interacts with their host and together maintains immune homeostasis whereas infection with pathogens generates inflammation and tissue damage. For example, 4-6 million people die of enteric infections each year. The human has a vast amount of gut microbiota for which they have tolerance whereas they get enteric diseases following infection with Gram-negative pathogenic bacteria, such as enteropathogenic *Escherichia coli, Salmonella enterica,* and *Shigella flexneri*. The situation of infection is critical because of increasing antimicrobial resistance. In response to these infections, phagocytes are recruited into the mucosa and become activated leading to innate responses that transit to adaptive immunity. The innate immune response is crucial in the maintenance of immunological homeostasis. However, nothing is known about how innate signals in phagocytes react to pathogenic versus nonpathogenic microbes to generate differential inflammatory host responses.

Microbial products are recognized by multiple receptors known as Pattern Recognition Receptors (PRRs). The well-known examples are Toll-like receptors (TLR), C-type lectin receptors (CLRs) and cytosolic sensors such as nucleotide-binding oligomerization domain-containing protein 1 and 2 (NOD1 and NOD2) that recognizes pathogen-associated molecular patterns (PAMPs) or host-derived damage-associated molecular patterns (DAMPs) and activate inflammatory signals. PRRs can bind microbial ligands from common flora and pathogens. For example, Brain Angiogenesis Inhibitor 1 (BAI1) is a new pattern recognition receptor that binds

lipopolysaccharide (LPS) of pathogenic and non-pathogenic Gram-negative bacteria. Therefore, the host immune system needs to discriminate between beneficial and pathogenic bacteria to maintain immune homeostasis. BAI1 interacts with the cytosolic effector, ELMO1 (engulfment and cell motility 1) in phagocytes. ELMO1 interacts specifically with pathogenic effectors to generate inflammatory responses after encountering commensals as well as pathogens. Several phagocytic PRRs also mediate significant crosstalk with other immunoregulatory signaling components that regulate the inflammatory milieu. This chapter will discuss cutting-edge knowledge about the phagocytic receptors utilized by microbes, their signaling responses and the onset of inflammatory diseases. A more comprehensive understanding of the molecular mechanisms governing microbial recognition and innate responses can lead to new therapies that limit antimicrobial resistant and immune-mediated tissue damage.

Keywords: Microbiota; Mucosal immune responses; Intrecellular receptors; Macrophages; Dendritic cells; neutrophils.

INTRODUCTION

The mucosal epithelium in the gastrointestinal tract or the respiratory tract is the first line of defense against attack from pathogens. After crossing the epithelia, the microbes gain access to the underlying tissues where phagocytes such as macrophages, dendritic cells and neutrophils reside. However, our understanding about how phagocytes interact, clear, and generate inflammation uniquely from the pathogenic microbes yet can remain unresponsive to the vast complex populations of common flora, is incomplete.

Bacterial recognition by host cells is fundamental for the initiation of mucosal immune responses during the infection process. Macrophages express several receptors including Toll like receptor, C type lectin receptor, Nod like receptor, RIG like receptors. These receptors are known as Pattern Recognition Receptors or PRRs. The PRRs bind pathogen associated molecular patterns (PAMPs), Microbial associated molecular patterns (MAMPs), or Danger associated molecular patterns (DAMPs) (Figure 1). PRRs also bind infected and dead cells and toxic components. As a consequence of this interaction, signaling cascades are activated in host cells that lead to inflammatory responses and/or phagocytic clearance of attached microbes. PRRs are present in non-immune cells and immune cells such as macrophages, dendritic cells and neutrophils. After phagocytosis, PRRs process the antigen and present it on MHC class I or class II molecules that link innate to adaptive immunity. The ligand binding capacity and the function is discussed in the Table 2. The importance of PRRs was recently recognized in 2011 when the Nobel Prize in Physiology and Medicine was awarded to Bruce Beutler, Jules Hoffmann and Ralph Steinman who worked extensively on characterizing PRR function in immunity.

Pattern Recognition Receptors can be found on the cell surface to enable the cell to respond to surrounding PAMPs. They are also found within the endolysosomal membrane, where they contribute to sensing of PAMPs within the endosome throughout the process of pathogen degradation. Finally, some PRRs are found freely in the cytoplasm where they are important for sensing of PAMPs that enter the cell through direct invasion strategies or those that escape the endosomal compartments. In that sense, PRRs connect innate immune responses to adaptive immune responses. The immune cells had several PRRs and often had a cross talk to make the system efficient from microbial attack.

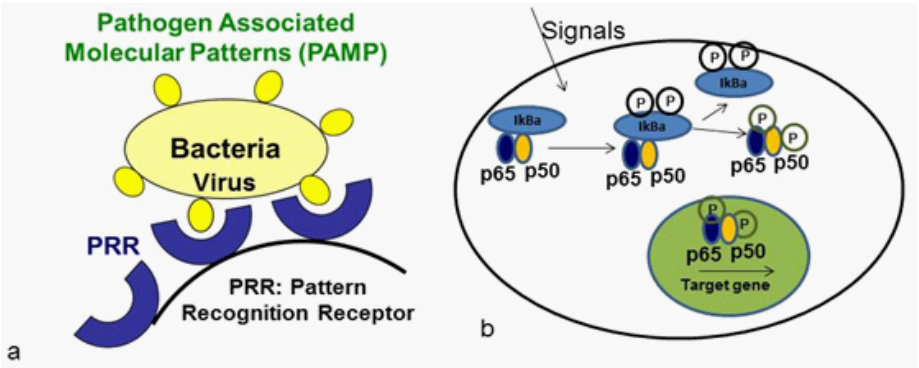

Figure 1: Binding and recognition of microbes with the Pattern Recognition Receptors and the associated signaling. (a) The microbial ligands [Pathogen Associated Molecular Pattern (PAMPs) or Microbial Associated Molecular Patterns (MAMPs) from bacteria or virus] bind the Pattern Recognition Receptors (PRRs). (b) The signals from the binding of PRRs with PAMPs activate the NFκB signaling pathway. In the schematic, the IκBα, p65 and p50 are present in the complex. After phosphorylation of IκBα, it will degrade and the phosphorylated p65 and p50 will go to nucleus from the cytosol, bind to the κB binding site of target gene and that in turn starts the transcription of the pro-inflammatory genes such as TNF-α.

Toll like Receptors

Jules Hoffman and his group [1] first discovered the Toll receptor in Drosophila melanogaster. They showed that antimicrobial peptides are generated after activation of signaling cascades by Toll. Mammals have 11 TLRs that bind unique microbial ligands as shown in Table 1. TLR4 is the first TLR that was discovered and binds Gram-negative LPS with the help of adapter protein MD-2 and CD14. The TLRs are type I transmembrane proteins. It has leucine-rich repeats in the N terminal and the cytoplasmic tail has homology with the interleukin-1 receptor family (IL-1R). TLR has adaptor molecules such as myeloid differentiation primary response gene 88 (MyD88), TIR-containing adaptor protein (TIRAP), TIR-containing adaptor-inducing IFN-γ (TRIF), TRIF-related adaptor molecule. The TLR mediated downstream signaling cascades activates signal transduction pathways resulting in activation of the transcription factor NF-κB, Jun N-terminal kinase, MAP kinases and interferon regulatory factors. TLRs are either on the cell surface or are

found in intracellular endosomes. After the synthesis from the endoplasmic reticulum, the TLR traffics through the golgi and is recruited to the membrane or the endosomes. TLR3, TLR7/TLR8 and TLR9 are localized in the endosomal membrane and are specialized for the recognition of viral double-stranded RNA, single-stranded RNA, and nonmethylated DNA, respectively.

All of the TLR receptors are G protein-coupled receptors (GPCRs) which couple to adaptor molecules within the cytoplasm to activate signaling, the best characterized of these is myeloid differentiation factor 88 (MyD88). This adaptor protein is linked with both cell surface and endosomal TLRs and signals via secondary molecules, ultimately triggering transcription of pro-inflammatory genes via transcription factors, nuclear factor kappa B (NF-κB) and activation protein 1 (AP-1).

MyD88 dependent pathway: After binding the ligand, TLR adapter MyD88 forms a complex with IRAK kinase family members known as Myddosome. The activation steps involve several phosphorylation steps that leads to the activation of the IKK complex-NFκB, IKKα, IKKβ and NEMO are present in the IKK complex. TAK1 binds to the IKK complex and phosphorylates IKKβ. The IKK complex phosphorylates, the NFκB inhibitory protein IκBα, which is then targeted for proteasomal degradation, allowing the p65 to translocate to nucleus and induce pro-inflammatory cytokine gene expression. TAK1 activation also induces MAP kinase family members such as $ERK_{1/2}$, p38 and JNK.

TRIF Dependent Pathway

TRIF interacts with TRAF6 and TRAF3 and recruits the kinase RIP1. The activation of TAK1 complex activates NFκB and MAPKs. TLR4 can activate MyD88 dependent and TRIF-dependent pathways depending on the pattern of the ligands.

Scavenger Receptors

The Scavenger Receptors (SRs) are diverse class of transmembrane cell surface glycoproteins present on macrophages and dendritic cells. The SRs were identified functionally as they bind and endocytose low density lipoproteins (LDL; oxidized or acetylated but not the native LDL) [2]. SRs are important in atherosclerosis for foam cell formation, in Alzheimer's disease and in adhesion and tissue maintenance [3]. SRs play a major role as phagocytic receptors and for the uptake of non-opsonized microbes. Some SRs act in concert with other PRRs such as TLRs to generate pro-inflammatory responses. SRs are divided in several classes according to their structural domains.

Class A SR

SRA

SRA is a trimeric type II transmembrane glycoprotein. SRA binds purified Lipid A of LPS of Gram negative bacteria and LTA of Gram positive bacteria. SRA KO mouse models have highlighted the importance of these receptors in host defense against bacterial infection.

MARCO

MARCO is structurally similar to SRA. They are present only in some populations of macrophages and the expression is upregulated after the interaction of microbial ligand in a TLR dependent manner.

Class B SR

CD36

CD36 is a type III transmembrane receptor present predominantly on macrophages, platelets and some endothelial and epithelial cells. It has two transmembrane domains, an extracellular loop with multiple glycosylation sites and two short intracellular tails. CD36 binds LTA and diacylated lipopeptide. It is a phagocytic receptor of *S. aureus* and cooperates with TLRs. The TLR2/6 response to whole bacteria depends on CD36 mediated internalization of *S. aureus* into the phagosome. The role of CD36 in Gram-negative infection is not clear and it is not a major phagocytic receptor for *E. coli* [4].

SRB1

SR-BI structure is similar to that of CD36. SR-BI is primarily expressed on macrophages and dendritic cells.

Several pathogens utilize their virulence factors to avoid recognition and phagocytosis by scavenger receptors. Therefore, studies investigating the interactions between SR and pathogenic microbes are important to understand microbial pathogenesis in these cases.

C type Lectin Receptor

C type lectin receptors (CLRs) are either transmembrane or soluble receptors that bind the carbohydrate moiety of a glycan structure [5]. Other than pathogen recognition, CLRs helps in cellular communication, antigen processing and presentation to T cells. Three well known CLRs are the Mannose receptor (MR), dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin (DC-SIGN), and Dectin-1. Most of these PRRs couple to intracellular immunoreceptor tyrosine-based activation like motif (ITAM) or ITAM-containing adaptor proteins, which then recruits and activates spleen tyrosine kinase (SYK) and activates pro-inflammatory responses.

Mannose Receptors

The MR is unique with multiple C type Lectin domains (CTLD) in the N terminal, a transmembrane region and a short tail. The CTLD is involved in pathogen recognition.

DC-SIGN

This receptor is expressed mainly on DCs and partly on macrophages. DC-SIGN interacts with ICAM-3 (intracellular adhesion molecule 3) and helps the interaction of DCs with T cells.

DC-SIGN binds several pathogenic microorganisms, including *M. Tuberculosis, S. Pneumoniae, H. pylori and C. Albicans*.

Dectin 1: These are important innate immune receptors involved in defense against fungi. It binds β glucan, a major component of fungal cell walls. It can cooperate with TLR2 to generate pro-inflammatory responses [6]. It suggests several PRRs act synergistically for host defense against microbial infection.

INTRACELLULAR PATTERN RECOGNITION RECEPTORS

Nod like receptors: Nod like receptors or NLRs are intracellular receptors. NLRs are conserved from sea urchin to humans as well as in plants to protect from pathogens. There are more than 20 genes identified in NLR families, some of them are associated with inflammatory diseases. NLRs consist of a variable N-terminal domain, a central nucleotide-binding domain (NBD) and a variable C-terminal leucine-rich repeats (LRRs). NLRs are cytosolic and considered to be the checkpoint of inflammation. NLRs are subdivided in four subfamilies: NLRA, NLRB, NLRP and NLRC; depending on their evolution and domain structures. NLRP and NLRC subfamilies have amino terminal CARD domains that recruit caspase-1 for downstream signaling.

The canonical Nod like receptor Nod1 and Nod2, recognizes bacterial peptidoglycan motifs (di-amino-pimelic acid and muramyl dipeptide respectively) from Gram-negative and Gram-positive bacteria. These receptors transmit the signal through the common adapter Rip2, a serine-threonine kinase and activate host inflammatory signaling by NFκB. These proteins are expressed strategically to the intestine to maintain immune homeostasis. Mutations and polymorphisms in the Nod1 and Nod2 genes are associated with development of Crohn's disease and inflammatory bowel diseases. After the delivery of bacterial ligands to cytosol, it binds the Nod receptors and activates immune responses. Nods also act in concert with other receptors such as TLRs to provide responses to complex stimuli. For example, the pro-inflammatory cytokine IL-23 in dendritic cells is poorly induced by TLR2 or Nod2 ligands alone but increases significantly when these receptors are activated in synergy [7]. Though most of the work of Nod1 and Nod2 has focused on recognition of bacterial ligands, these receptors are also known to have a major role in viral infection. Nod2 deficient mice have increased viral burden and are more susceptible to infection with respiratory syncytial virus (RSV) [8].

Inflammasome

NLR gene family members assemble macromolecular protein complexes, called inflammasomes that activates the inflammatory cysteine protease, caspase-1 (interleukin-1 converting enzyme or ICE). The NLRs NLRP3, NLRP1 and NLRC4 form inflammasome complexes which result in the caspase 1 dependent proteolytic production of the inflammatory cytokine, IL-1β. The inflammasome complex consists of a PRR connected to procaspase 1 usually via the adaptor protein apoptosis associated speck-like protein containing a CARD (ASC).

When activated pro-caspase 1 is processed to caspase 1 which then subsequently activates the production of IL-1β from pro-IL1β and IL-18 from pro-IL-18 and secretes the mature cytokines [9]. IL-1β induces leukocyte migration to the site of infection, and activates adaptive immunity. IL-18 triggers T helper 1 (Th1) polarization and Th2 and Th17 cell responses. In the presence of infection, NLRs sense the pathogen by their C terminal LRRs and the Nod domains unfold and exhibit oligomerization. In turn, it recruits the adapter (such as ASCs) and initiates cell signaling. ASCs recruit the inflammasome-forming proteins such as NLRP3 and NLRP1 that leads to pro-caspase-1 autocleavage and formation of the active caspase-1 [10]. Several bacterial toxins and viruses can activate the NLRP3 inflammasome.

Retinoid Acid Inducible Gene-I (RIG)-like Receptors (RLRs)

RLRs (RIG-I, MDA5, and LGP2) are cytosolic proteins that sense various viral RNA species and generate antiviral cytokines such as Type1 interferon (IFN). RIG-I and MDA5 contain two N terminal caspase recruitment domains (CARDs) and an RNA helicase domain in the C terminal. After ligand binding it recruits IκB kinases (IKKs) and activates NF-κB and IRF transcription factors that generate anti-viral responses. RIG-I and MDA5 have different ligand binding specificity but induce similar pro-inflammatory cytokines and type I IFN. STING and TRIM are two cofactors that contribute an important role in signaling.

Other PRRs

BAI1

BAI1 is a recently identified PRR that recognizes Gram-negative bacteria by interacting with lipopolysaccharide (LPS). Unlike TLR4 (Toll like receptor 4), which binds the Lipid A region of LPS, BAI1 recognition of LPS is via the core oligosaccharide [11]. Previously BAI1 was identified as a phagocytic receptor involved in apoptotic cell recognition through its thrombospondin repeats (TSRs) that triggers engulfment through the ELMO1 (Engulfment and cell motility protein 1) /Dock/Rac1 signaling molecule. Similar to apoptotic cells, enteric bacteria bind the TSR region of BAI1 for attachment and subsequent internalization into macrophages and possibly other phagocytes. ELMO1 is a cytosolic protein that controls the induction of inflammation following enteric infection by activating NF-κB and MAP Kinase signaling and it discriminates between pathogenic and non-pathogenic microbes.

Other than binding the microbial products, PRRs also bind infected or damaged host cell products known as Danger Associated Molecular Patterns (DAMPs). These interactions regulate cell death, differentiation and secretion of inflammatory or anti-inflammatory mediators. Examples of DAMPs are High-mobility group protein B1 (HMGB1) and Adenosine triphosphate (ATP).

High-mobility group protein B1 (HMGB1): HMGB1 is a nuclear protein that binds to nucleosomes and helps in the binding of DNA. HMGB1 is released by necrotic dying cells. HMGB1 is involved in endothelial cell activation, stem cell migration and recruitment and activation of innate immune effectors through TLR2 and 4.

Adenosine Triphosphate (ATP)

ATP belongs to the purine family and is primarily involved in energy transport and cell metabolism. ATP is also a pro-inflammatory immune mediator released from dying cells, which can stimulate monocyte cell migration.

LOCALIZATION AND FUNCTION OF MACROPHAGES

Macrophages and dendritic cells are the two populations present on the mononuclear phagocytes and share several receptors with distinct functions [12]. Macrophages are different from DCs in their expression of markers such as F4/80, CD11b and Fc receptors.

Macrophages are divided in two subtypes; M1 macrophages or classically activated macrophages, induced by Toll-like receptors signaling and Interferon-γ pro-inflammatory cytokines such as IL-1, IL-6, IL-12, IL-23 and TNF-α. Sometimes macrophages are unique in their location and maintain diverse function as mentioned in table 1.

Alveolar macrophages are unique as they present in between the airway epithelium and the blood vessels. These specialized macrophages encounter a different level of oxygen pressure, numerous microbes and environmental pollutants from inhaled air.

Table 1: Classification of macrophages according to locations.

Macrophages	Location	Function
Osteoclast	Bone	Multinucleated giant cells, of myeloid origin, that are responsible for bone resorption
Microglia	Brain	
Alveolar macrophages	Lung (between the airway epithelium and the blood vessels)	Important for taking environmental challenges due to their strategic location in lung, encounter a different level of oxygen pressure, infection and pollutants.
Histiocytes	Interstitial connective tissue	
Kupffer cells	Liver	A specialized macrophage that lines the sinusoidal vessels of the liver. These cells regulate local immune responses, and remove microbial particles, endotoxin and other noxious substances that penetrate the portal venous system.
Intestinal macrophages	Intestine	
Splenic macrophage	Marginal zone metallophilic macrophage	A type of macrophage that surrounds the splenic white pulp, adjacent to the marginal sinus, and is involved intrapping particulate antigens. Generally CD11b expression is low.

It is important to understand phagocyte diversity and the differences in their responses to develop phagocyte targeted therapeutic strategies.

DENDRITIC CELLS (DCS)

DCs are present throughout the body and represent around 2% of white blood cells. These cells can induce self-tolerance and trigger immunological responses. DCs are the most efficient antigen

presenting cells (APCs) that orchestrate T cells differentiation and expansion and therefore is an important link connecting innate immunity to adaptive immunity. DCs engulf extracellular particles, microbes, activate signaling and expression of cytokines, chemokines and continuously sample surrounding antigens for presentation to T cells.

DCs are generated from bone marrow-derived CD34+ hematopoietic stem cells (HSC), which maintains the function and subsets of DCs. Other subsets of DCs are CD141+ blood DCs that cross present viral antigens; the monocyte derived CX3CR1+ non-migrating DCs associated with the gut epithelium and CD103+ DC present in the gut lamina propria and lymph nodes [13].

NEUTROPHILS DURING INFECTION

Neutrophils are a class of granulocyte leukocyte cells that are found abundantly in the circulation. They have a characteristic multi-lobed nucleus and a cytoplasm full of multiple, distinct granules (azurophilic, specific, gelatinase and secretory). These granules are important for most neutrophils functions, which are primarily to aid in defense against pathogens, particularly bacteria but also some fungi and viruses. In fact, the neutrophil response is considered to be the first line of defense against invading pathogens and this is due to their rapid recruitment to sites of infections where the proteolytic and histotoxic granule cargo are released to kill surrounding pathogens. The first role of the neutrophil is migration to affected tissues. They do this in response to chemotactic gradients which initiate changes in the neutrophil which allows for their adhesion and transmigration through the endothelium to the underlying tissue. The neutrophil membrane quickly increases expression of important adhesion receptors, chemokine receptors and receptors which recognize formyl peptides. These proteins are contained within the granules and are rapidly transported to the neutrophil membrane allowing the neutrophil to bypass the need for lengthy protein transcription pathways. Once at the site of the infection, neutrophils encounter pathogens resulting in rapid degranulation, which releases an array of anti-microbial peptides, proteolytic molecules and inflammatory mediators. Neutrophils are also phagocytic, having the ability to recognize and internalize pathogens that are transported into intracellular compartments for lysosomal degradation. The non-discriminatory and non-specific actions of release of ROS and potent granule contents mean that neutrophil responses can often come with collateral damage to surrounding tissues. This is particularly deleterious in conditions of chronic inflammation such as arthritis and inflammatory bowel disease where tissue damage contributes to worsening disease outcomes. The neutrophil, although thought to be transcriptionally silent, especially when compared to macrophages, actually release small amounts of important chemokines and cytokines. When considering the sheer abundance of neutrophils at sites of infection, one can see how these small signals could be amplified, and thus have important functions in the orchestrating the next phase of the inflammatory response. The recruitment of macrophages to the site of infection is one such function that is pivotal to the successful resolution of the neutrophil response. In normal circumstances, when inflammation resolves and infections

are cleared, the neutrophil undergoes controlled cell death or apoptosis. This process is a tightly regulated event, which normally occurs soon after neutrophils have released their anti-microbial arsenal and are ready to be decommissioned. This process is an extremely important part of the neutrophil life cycle as the cell undergoes changes that allows for its recognition and removal by other professional phagocytes, the macrophage. The removal occurs in a non-phlogistic fashion, meaning that neutrophil membrane integrity is maintained throughout the process, containing the further release of damaging granule contents. The removal of neutrophils by macrophages also confers further anti-inflammatory effects through the skewing of the macrophage phenotype to favor resolution through reducing macrophage production of pro-inflammatory cytokines and stimulating release of factors associated with dampening the immune response and promoting tissue repair.

Due to the short life cycle of neutrophils and the apparent non-specific nature of microbial attack, these cells have not elicited much attention in the host/pathogen interaction arena. However, it has been shown that these cells express a diverse array of pathogen recognition receptors (PRRs), which stimulate an equally diverse array of neutrophil signaling pathways. The ability of neutrophils to engage a wide range of receptors and signaling molecules means that these cells are equipped with a dynamic response repertoire that reflects the pathogenicity of the encountered microbe. This is an important feature especially when considering the damaging consequences to the host from inappropriate or prolonged exposure to neutrophil products. A more recent neutrophil phenomenon has been described adding another caveat to this cells function. This process is a specialized form of cell death occurring in response to acute neutrophil activation. This death results in the eruption of a mesh of DNA and histones coated with anti-microbial peptides, which have been shown to capture microbes. These structures have been called Neutrophil Extracellular Traps or NETs and the importance of these events in the pathology of diseases such as Cystic Fibrosis is starting to be realized.

Although the neutrophil is central to the innate immune response to microorganisms, some microorganisms are able to evade this response by impacting neutrophil functions. Most commonly, neutrophil apoptotic responses can be delayed or enhanced to create an environment more suitable for pathogen survival. For example, the bacterium *Leishmania*, not only delays apoptosis of the neutrophil but it is able to survive in intracellular compartments until the neutrophil is taken up by macrophages where it replicates and disseminates- a Trojan horse type infection. Pathogens can alter neutrophil signaling through a diverse array of PRRs and release of virulence factors that engage signaling events that are critical in the determining the ultimate fate of the pathogen as well as the ensuing inflammatory response. Below is a summary of the most widely known PRRs expressed by neutrophils.

Neutrophil Pathogen Recognition Receptors

Neutrophils are endowed with many pathogen recognition receptors, allowing for the recognition of a multitude of pathogen associated molecular patterns (PAMPs). The three main classes of PRR are the Toll like receptors (TLRs), the C-type lectin receptors (CLRs) and the nucleotide oligomerization domain (NOD)-like receptors (NLRs). Other receptors involved in pathogen sensing in the neutrophils are the retinoic acid-inducible gene (RIG)-like helicases and the formyl peptide receptors (FPRs).

Toll like receptors

The Toll like receptors are the most widely researched class of PRRs in neutrophils. Among the multiple distinct subtypes (named TLR1 through TLR11, summarized in the table), the only subtype not found in neutrophils is TLR3. Although TLR7 is found in murine neutrophils, it is absent in human neutrophils. The major pro-inflammatory factors released from neutrophils following TLR activation are interleukin 8 (IL-8) and tumor necrosis factor alpha (TNF-α), these cytokines act in both a paracrine and autocrine manner to influence immune cell functions. The engagement of TLR receptors is responsible for other functions including degranulation, migration, NET formation, respiratory burst, phagocytosis and apoptosis (Table 2). The adaptor TIR-domain containing adaptor protein inducing interferon β (TRIF), which is normally downstream of TLR4 activation is absent in neutrophils echoing the lack of IFN type responses produced by these cells. The dynamic nature of neutrophil responses is further demonstrated by the ability of receptors from different classes to synergize to produce an enhanced response. For example, TLR4 activation by Lipopolysaccharide (LPS) in concert with formyl peptide receptor activation enhances neutrophil ROS production.

C-type lectin receptors

The CLRs are a broad class of receptors found at the neutrophil surface, which recognize carbohydrate moieties in a calcium dependent manner. The best characterized CLRs in neutrophils are Dectin-1, Dectin-2 and Mincle. The major signaling pathways in CLRs converge on NF-κB to increase production of pro-inflammatory cytokines. Activation of CLRs also stimulates ROS generation, phagocytosis activation and degranulation responses.

NOD-like receptor

The NLRs are intracellular receptors found in the cytoplasm. Activation of these receptors results in stimulation of various neutrophil effector functions (ROS production, phagocytosis, degranulation, migration, CD62L shedding) and activation of NF-κB and MAPK pathways to increase pro-inflammatory cytokine production. The release of ROS from neutrophils is also linked with NLRP3 inflammasome activation, linking the activation of other PRR receptor classes to NLR activation.

THE COORDINATED FUNCTION OF NEUTROPHILS, MACROPHAGES AND DENDRITIC CELLS

The ability of a pathogen to infect the host is a balance between the immune response to the infection and the microbial virulence factors that alter immune cell functions. Inhibiting early neutrophil functions such as migration and ROS production is one tactic employed by pathogens such as uropathogenic *Escherichia coli* (UPEC), which is important in understanding how these pathogens can subvert the immune response to promote survival. For example, UPEC overcome the defense strategies of the host, which includes urine flow, exfoliation of urothelial cells, antimicrobial factors and incoming neutrophils. UPEC uses fimbriae and Type 1 pilus for adherence and invasion, flagella and toxin for dissemination and an iron acquisition system to survive in the low iron environment of the urinary tract. UPEC also modify the pro-inflammatory responses generated after the immune recognition of bacterial ligands by Toll like receptors.

Many more examples of pathogen immune evasion exist in the other phagocytic cells of the immune system, the macrophage and dendritic cells. These cells have a more direct role in stimulating the adaptive immune response, which results in specific and efficient pathogen clearance. Furthermore, these cells are longer lived and often migrate to sites distant from the site of infection, thus offering more opportunity for microbial replication and dissemination.

The Discrimination of Pathogenic and Non Pathogenic Microbes: Intestinal Pathogen vs Commensal or Common Flora

The human intestine is associated with trillions of commensal flora that maintain immune homeostasis while the interaction of host cells with enteric pathogens generates inflammation. Globally, 4-6 million people die of enteric infections each year. Even within the United States, *Salmonella* and other gastroenteric infections are responsible for millions of illnesses, thousands of deaths, billion-dollar expenditures from food recall, and hospital-associated expenses. Therefore, it is necessary to know the pathogenesis associated with enteric bacteria and how the immune system discriminates between pathogenic micobes and non-pathogenic commensal flora.

So far, our understanding about disease is about the interaction of pathogen with hosts. Following recognition of microbial ligands the host generates pro-inflammatory responses and tissue damage. However, the millions of microbiota are present in the oral and intestinal cavity, the upper respiratory tract, the vagina, and the skin and show tolerance towards the immune cells. The important question is how does the host immune cell discriminate between pathogenic and non-pathogenic microbes?

It is possible by the following ways:

- The non-pathogenic microbe generate signals or anti-inflammatory cytokines that make them tolerogenic. For example, the avirulent strains or the commensals dampen the pro-inflammatory signals by inhibiting IκBα degradation, followed by down regulation of NFκB activation.

- Microbial ligands from commensals undergo modifications that protect them from the binding to PRRs and associated immune responses. For example, the Lipid A of symbiotic microorganism is tetra or penta acetylated which is not sensed by host TLR4.

- Pathogens have effectors or pathogenic factors that generate inflammatory responses that provide danger signals and alert the host to the microbial attack.

A large part of the microbiota resides strategically in the mucus layer where the vast complex of microbes protect the epithelium and microbiota derived products can cross talk with the epithelium.

Self vs Non-Self

The discrimination of self versus non-self is important. For example, host DNA methylation protects the host DNA from cleavage by restriction enzymes but destroys the foreign DNA. In mammalian immunity, restriction and inhibition of self recognition is crucial. Using this strategy NK cells detect infected and stressed cells, utilizing major histocompatibility complex class I molecules on target cells to avoid killing healthy uninfected cells.

The interesting point in immunology is the discrimination of self vs pathogen by immune receptors. The PRRs such as TLR4 binds LPS present in non-pathogenic commensals as well as on pathogens. Most of the PRRs are present in the membrane and some of them in the endosomes. For example, TLR3,7,9 binds nucleic acids (NA), phospholipids and fatty acids. Interestingly the endolysosomal TLRs can interact with host or commensal derived metabolites to produce type 1 IFNs. In this way, metabolites from food products can cause non-infectious inflammatory disease and their level is crucial for homeostasis [14].

Patterns of Pathogenesis

In addition to sensing microbial ligands, PRRs responds to signals so pathogens can live, invade, replicate and spread inside the infection; also known as Patterns of pathogenesis. Cytosolic pattern recognition is important to discriminate between pathogen from non-pathogen. The examples of "pattern of pathogenesis" are: attachment of pathogens to host cells, generation of extracellular enzymes (e.g., proteases, phospholipases), important in sensing of certain microbes.

Importance in Health

PRR-mediated microbial sensing in the innate immunity directs the adaptive immune responses. PRRs determine antigen presentation by the antigen receptors expressed on immune

cells depending on the infection encountered and help lymphocytes to generate the appropriate immune response.

Intestinal macrophages are the body's largest population of phagocytes present in the sub epithelial lamina propria in close proximity to luminal bacteria and antigenic stimuli. Human gut macrophages are relatively nonresponsive although they utilize several phagocytic receptors that confer avid scavenger capacity and host defense function. There is much yet to be learned about how intestinal macrophages use their cell surface receptors to limit microbial attack and regulate intestinal inflammation.

The macrophages clear apoptotic cells and cellular debris and contribute to the host immune responses. Generally, the clearance of apoptotic cells is anti-inflammatory but the response is pro-inflammatory following a microbial encounter [15]. A more complete knowledge of these aspects of microbial interactions with the innate immune system will lead to targeted therapies for antimicrobial resistant infections and broadly, to limit inflammation-linked diseases.

Generally, the viruses and intracellular bacteria provide Th1 responses and extracellular microbe such as protozoa at mucosal surfaces provide Th2 responses and are involved in allergic reaction.

The recognition of microbes by pattern recognition receptors and associated inflammatory signaling in the infection and immunity field is vital to understand the pathogenesis of infections and inflammatory diseases and in the development of new therapeutic strategies.

Table 2: Pattern recognition receptor families in phagocytes.

Receptors	Microbial Ligands/locations	Function	References
TLR Family:		In general the TLR family members recruits adapters (such as MyD88), activate NF-κB and MAP kinase signaling and pro-inflammatory gene expression	Toll-Like Receptors (TLRs) and Their Ligands [16]
TLR1	Works in conjunction with TLR2		
TLR2	Lipoproteins, Peptidoglycans, Lipoteichoic acids		
TLR3	Viral double stranded RNA		
TLR4	LPS		
TLR5	Flagellin		
TLR6	Works in conjunction with TLR2		
TLR7	Single stranded RNA, present in endosomes	endosomal	
TLR8	Viral bacterial RNA		
TLR9	Single stranded RNA, present in endosomes	endosomal	
TLR10			
TLR11	Flagellin		
CLR family			
MGL	N-acetylgalactosamine (GalNAc) residues		
Mannose receptor	Mannose, Fucose, N-acetyl glucosamine or glucose	MR binds Mycobacterium using the terminal mannose residues that blocks phagolysosomal fusion.	[17]
Dectin 1	Binds beta glucan of fungal cell wall	Phagocytose live yeast and zymosan from fungi	[18]
Scavenger Receptor			
SRA	Lipid A of LPS; soluble LTA from Streptococcus pyogenes	Phagocytic receptor helps in the non-opsonic uptake of bacteria	
MARCO	Soluble LPS and LTA, CpG DNA, intact Gram-positive and -negative bacteria	Host defense against S pneumonae infection in mouse model	[19]
CD36			
SRB1			
Nod like receptor (NLR)			
Nod1	Di-amino-pimelic Acid	Cytosolic sensor, activates host signaling by Rip2	
Nod2	Muramyl dipeptide		
Naip	Flagellin		
NLRC3	Unknown		
NLRC4	Flagellin		
NLRP1	Muramyl dipeptide		

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