UNDERSTANDING OF COMPLEX SIGNALING PATHWAYS OF IMMUNE SYSTEM: A REVIEW

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ABSTRACT

Cell surface receptors receive the initial signals that activate immune responses. Immune response is very complex process which involves a number of cell types that function as initiators, regulators, and effectors. These cells interact with and cross-regulate each other, and the target cells respond using signal transduction pathways to mediate gene expression. Innate immune responses to pathogens such as bacteria and viruses are triggered by recognition of specific structures of invading pathogens called pathogen-associated molecular patterns (PAMPs) by cellular pattern recognition receptors (PRRs) that are located at plasma membrane or inside cells. Stimulation of different PAMPs activates Toll-like receptor (TLR)-dependent and -independent signaling pathways that lead to activation of transcription factors nuclear factor-κB (NF-κB), interferon regulatory factor 3/7 (IRF3/7) and/or activator protein-1 (AP-1), which collaborate to induce transcription of a large number of downstream genes. In case of Adaptive immune response the signal will be processed MHC antigenic peptide complexes on APCs. This event catalyzes a series of intracellular events resulting in the transcription of genes that drive the differentiation of the T cell. The innate and the adaptive immunity signaling pathways are different, but they usually work together to eliminate pathogen. In this review, we discuss our current understanding of complex signaling pathways of immune system.

KEYWORDS: Toll like receptors; dendritic cells; NFκB; CD markers; T cell.
1. INTRODUCTION

The immune system is a complex network, including lymphoid, reticular, dendrite and epithelial cells, interacting by cell to cell contacts and communicating through cytokines. There are two systems of immunity - innate immunity and adaptive immunity, which collaborate to protect the body \(^{[1,2,3]}\). Before an infection, innate immunity has been developed to prevent or eliminate the pathogens within hours. Thus, innate immune system acts as a first line of defense against pathogens. Adaptive immunity which is second form of immunity develops in response to infection and adapts to recognize, eliminate, and then remember the invading pathogen \(^{[1,4,5]}\).

Innate immune cells recognize pathogens by evolutionarily conserved receptor system of pattern recognition. Such pattern-recognition receptors (PRRs), which include Toll-like receptors (TLRs), nucleotide-binding oligomerization domain receptors (NOD-like receptors NLRs) and RIG-I like receptors (RLRs), are germline encoded and do not undergo gene rearrangement \(^{[3,6]}\). So, they are not specialized to distinguish small differences in foreign molecules. In adaptive immunity, T and B lymphocytes recognize non-self through antigen-specific receptors, such as T cell receptors (TCRs) and immunoglobulins. These receptors are generated by gene rearrangement, which allows the recognition of a vast number of different antigens.

The innate responses uses phagocytic cells (neutrophils, monocytes and macrophages), cells that release inflammatory mediators (basophils, mast cells and eosinophils) and natural killer cells. The molecular components of innate responses include complement, acute phase protein and cytokines such as interferon. Adaptive responses are generated in the lymph nodes, spleen and mucosa-associated lymphoid tissue. It involves the proliferation of antigen-specific B and T cells. Specialized cells, called antigen-presenting cells, display the antigen to lymphocytes and collaborate with them in response to antigen. B cells secrete immunoglobulin. T cells help B cells to make antibody. The adaptive immunity is a pathogen-specific host defence based on clonally expanded B and T lymphocytes generated first through random somatic recombination of immunoglobulin genes and then through positive and negative selections. These pathogen-specific B and T lymphocytes not only serve as effectors cells for pathogen eradication, but they also function as memory cells for rapid expansion upon re-encountering these pathogens. The adaptive immunity is highly specific and effective against evolving pathogens and takes days or weeks to develop \(^{[1]}\).
Cell surface receptors receive the initial signals that activate complex immune responses. In case of innate immunity, the signal will be a microbial product, the receptor will be PRR on a leukocyte and the signal will be transduced by the interaction of specific intracellular molecules results in the clearance of the invading organism. In case of adaptive immunity, the signal will be processed MHC antigenic peptide complexes on APCs \[^{[4,7]}\]. This event catalyzes a series of intracellular events resulting in the transcription of genes that drive the differentiation of the T cell. Innate and adaptive responses usually work together to eliminate pathogen. In this review, we discuss the role of complex signalling pathways of immune system which protect the body against infection. In addition, we discuss the recognition systems of innate and adaptive immune system.

2. Innate immune System Signalling pathways

Innate immune responses to pathogens such as bacteria and viruses are triggered by recognition of specific structures of invading pathogens called pathogen-associated molecular patterns (PAMPs) by cellular pattern recognition receptors (PRRs) that are located at plasma membrane or inside cells.

2.1 Soluble and membrane-bound mediators signaling pathways

Detection of pathogen-associated molecular patterns by soluble and membrane-bound mediators of innate immunity results in activation of multiple components of immune system. The soluble mediators include initiators of the complement system, such as mannose-binding lectin (MBL) and C-reactive protein (CRP). Mannose-binding lectin (MBL), C-reactive protein (CRP), and serum amyloid protein (SAP) are secreted pattern recognition molecules produced by the liver during the acute phase response at the early stages of infection \[^{[8,9,10,11,12]}\]. If pathogen bears PAMPs recognised by MBL and CRP, the complement system will be activated. The complement system form membrane attack complex that puch holes in the cell membranes of targeted microbes, killing the cells by lysis as shown in figure 1. The complement system also include serum glycoproteins that, when activated, promote uptake of microorganisms by phagocytes (opsonisation).

2.2 Neutrophil or Macrophages signalling pathways

After adherence of the microbe to the surface of the neutrophil or macrophage through recognition of a PAMP, the resulting signal initiates the ingestion phase by activating an actin-myosin contractile system which extends pseudopods around the particle. The cytoplasmic granules fuse with the phagosome and discharge their contents around the
imprisoned microorganism \(^{10,13}\). Destruction of pathogen takes place by oxygen dependent as well as oxygen independent pathways. Oxygen dependent pathways use superoxide anion, hydrogen peroxide, singlet oxygen and hydroxyl radicals to kill the pathogens. Oxygen independent pathways utilize lysozyme, lactoferrins and cytokines as a microbicidal agents. Dendritic cells utilize phagocytosis to direct antigens to both MHC 1 and MHC 2. Thus, phagocytosis serves a dual role: as an innate immune effector as well as a bridge between the innate and adaptive immune responses \(^{11,17}\).

Phagocytes also remove the body’s own dead or dying cells. Dying cells in necrotic tissue release substances that trigger an inflammatory response, whereas cells that are dying as a result of apoptosis (programmed cell death resulting in the digestion of DNA by endonucleases) express molecules on their cellsurface, such as phosphatidylserine, that identify them as candidates for phagocytosis \(^{12,18}\).

2.3. TLR Signaling pathways

Toll-like receptors (TLRs) are important sensors of foreign microbial components as well as products of damaged or inflamed self tissues. Toll-like receptors (TLRs) are transmembrane proteins that detect invading pathogens by binding conserved, microbially derived molecules and that induce signalling cascades for proinflammatory gene expression. TLRs are widely expressed in many cell types such as macrophages, neutrophils and dendritic cells. Thus far, 13 mammalian TLRs, 10 in humans and 13 in mice, have been identified \(^{13,19}\). TLRs 1–9 are conserved among humans and mice, yet TLR10 is present only in humans and TLR11 is functional only in mice. The biological roles of TLRs 10, 12, and 13 remain unclear, as their expression patterns, ligands, and modes of signalling have yet to be defined \(^{17}\). Among the characterized TLRs, TLR1, 2, 4, 5 and 6 are represented on the cell surface and seem to specifically recognize bacterial and fungal products that are not made by the host, whereas TLR3, 7, 8 and 9 reside in intracellular endosomes and specialize in the detection of nucleic acids of pathogens \(^{2}\). For example, lipopolysaccharide (LPS), a common structure of the cell wall of Gram-negative bacteria, is recognized by TLR 4 \(^{18}\); double stranded RNA (dsRNA), which has long been considered a viral PAMP, triggers TLR3 signalling \(^{19}\).

Toll-like receptor (TLR) activate MyD88-dependent and independent signaling pathways that lead to activation of transcription factors nuclear factor-κB (NF-κB), interferon regulatory factor 3/7 (IRF3/7) and/or activator protein-1 (AP-1), which collaborate to induce transcription of a large number of downstream genes \(^{20}\).
2.3.1 MyD88-Dependent Signaling
Microbial products bind the extracellular portion of the TLR as shown in figure 2. On the cytoplasmic side, adaptor protein (MyD88) interact with the TIR domain of TLRs. Adaptor protein promotes the association of two protein kinases, IRAK1 and IRAK4 [21, 22, 23, 24]. The protein kinase IRAK4, of the IRAK1:IRAK4 complex, phosphorylates its partner, IRAK1 and provide docking site for TRAF6, which binds and form an complex resulting in the activation of the TAK1 kinase activity [28]. TAK1 activates mitogen-activated protein kinase (MAP kinase) pathway and NFκβ pathway [25, 26, 27].

2.3.1.1 MAP kinase pathway
MAP kinases are a group of highly conserved serine/threonine protein kinases in eukaryotes [28]. They play pivotal roles in a variety of cellular processes including proliferation, differentiation, stress response, apoptosis, and host immune defense. In innate immune cells, MAP kinases are critical for the synthesis of numerous cytokines, chemokines, and other inflammatory mediators that mobilize the immune system to combat pathogenic infections [29]. The activated MAP kinases can translocate to the nucleus and phosphorylate proteins that control chromatin structure as well as numerous transcription factors such as AP-1, thereby influencing the transcription of MAP kinase-regulated genes [30].

2.3.1.2 NFκβ pathway
The nuclear factor-kB (NF-kB) family of transcription factors is comprised of a collection of structurally related proteins that modulate multiple physiological processes, ranging from immune responses to cell death and survival [31]. The eukaryotic NF-κB transcription factor family regulates the expression of a large variety of genes that are involved in a number of processes like inflammatory and immune responses of the cell, cell growth, and development. NF-κB transcription factors are activated as a response to a variety of signals, including cytokines, pathogens, injuries, and other stressful conditions. Activation of NF-κB proteins is tightly regulated, and inappropriate activation of the NF-κB signaling pathways has been linked to autoimmunity, chronic inflammation, and various cancers [32, 33]. In unstimulated cells, NF-κB is bound to an inhibitory protein, IκB. Binding to IκB masks the nuclear localization signal (NLS) of NF-κB, sequesters the NF-κB-IκB complex in the cytoplasm, and prevents NF-κB from binding to DNA.

Activation of NF-κB signaling is initiated by extracellular stimuli. These stimuli are recognized by receptors and transmitted into the cell, where adaptor signaling proteins initiate
a signaling cascade. These signaling cascades culminate in the activation of IkB kinase (IKK). IKK phosphorylates the inhibitory IkB subunit of the NF-κB·IkB complex in the cytoplasm. This phosphorylation marks IkB for degradation by the proteasome and releases NF-κB from the inhibitory complex \[^{[34,35,36,37,38]}\]. The freed NF-κB proteins are then transported into the nucleus where they bind to their target sequences and activate gene transcription. NF-κB is itself a critical transcriptional activator of cytokines involved in the innate immune response, including TNFα and IL-1. In addition to its prominent role in innate immunity, NF-κB exerts important functions in the adaptive immune system \[^{[39,40]}\].

2.3.2 MyD88-Independent/TRIF Dependent Signaling

TLR4 and TLR3 stimulation results in the MyD88-independent activation of IRF3, a key transcription factor necessary for IFNβ production and the delayed-phase NFκB activation via TLR4 \[^{[41,42]}\]. Although it has been studied extensively, the mechanism by which TRIF activates NFκB and IRF3 is not completely understood. The N terminus of TRIF is believed to form a complex with TBK-1, IRF3, and possibly IKK for the specific phosphorylation and activation of IRF3 \[^{[43]}\]. Type I IFNs (IFN-a/β) are integral components of innate antiviral responses, and their expression is governed by IRF transcription factors. Two members of this family, IRF3 and IRF7, are absolutely required for transcription of IFN-a/β genes \[^{[44,45]}\]. Upon viral infection, latent IRF3 is phosphorylated at C-terminal serine residues, which leads to its dimerization and subsequent translocation into the nucleus. Upon entering the nucleus, IRF3 synergizes with coactivator molecules and binds DNA elements at the IFN-β promoter to induce gene transcription. In contrast, IRF7 is basally present only in plasmacytoid dendritic cells, which are specifically adapted to detect viruses and synthesize IFNα upon infection, but is strongly induced in many cells after viral infection and subsequent type I IFN autocrine/paracrine signaling \[^{[45,46]}\].

3. TLR signalling and adaptive immunity

Upon stimulation, innate immune cells, such as DCs and macrophages, phagocytose pathogens and present the pathogen derived antigens to naive T cells. TLR signaling functions in the processes of phagocytosis of microbial pathogens and phagosome maturation and TLR-mediated expression of genes such as costimulatory molecules facilitates to mount adaptive immune responses \[^{[10,47,48]}\].
4. Connection between innate and adaptive immune system

Among the cells that bear innate immune or germline-encoded recognition receptors are macrophages, dendritic cells (DCs), mast cells, neutrophils, eosinophils, and the so-called NK cells. These cells can become activated during an inflammatory response, which is virtually always a sign of infection with a pathogenic microbe. Such cells rapidly differentiate into short-lived effector cells whose main role is to get rid of the infection; in this they mainly succeed without recourse to adaptive immunity. However, in certain cases, the innate immune system is unable to deal with the infection, and so activation of an adaptive immune response becomes necessary. In these cases, the innate immune system can instruct the adaptive immune system about the nature of the pathogenic challenge [49, 50, and 51].

Dendritic cells act as a bridge between the innate and adaptive immune system. [52, 53]. Endogenous danger signals, such as the release of interferon-α from virally infected cells or an increase in heat shock proteins as a result of necrotic cell death, also activate dendritic cells. Molecules that act as pattern-recognition receptors on dendritic cells include the lipopolysaccharide receptor, the mannose receptor, and members of a family of molecules called toll. Pathogen-associated molecular patterns include yeast-cell-wall mannans, lipopolysaccharides on the surface of gram-negative bacteria, and teichoic acids, which are present on gram-positive bacteria [54, 55].

Naïve T-cell activation requires at least two signals: one signal triggered by MHC–peptide complex, and another from co-stimulatory molecules. Although there are many families of costimulatory molecules, the B7 family members are the first to be identified as playing a crucial role in T-cell activation. The most studied B7 family members are CD80 and CD86; their interaction with CD28 and CTLA-4 (cytotoxic T-lymphocyte-associated antigen-4) on T cells is also well studied. CD28 is expressed constitutively on human and murine T cells; and upon ligation with CD80 or CD86, CD28 delivers a positive co-stimulatory signal [56, 57, 58]. Activation causes dendritic cells to up-regulate the expression of B7 costimulatory molecules (also known as CD80 and CD86) on their surface. Costimulatory molecules are molecules that provide the signals necessary for lymphocyte activation in addition to those provided through the antigen receptor. These activated dendritic cells migrate to the local draining lymph node, where they present antigen to T cells. The antigen is processed intracellularly into short peptides by means of proteolytic cleavage before it is presented by major histocompatibility complex (MHC) molecules on the surface of dendritic cells [59].
When activated, naïve CD4+ T cells can acquire effector functions by differentiating into T-helper (Th) subsets. These subsets are distinguished as Th1 and Th2 T cells, and they are characterized by their varying ability to produce cytokines and to express surface receptors \[^{[60]}\]. It can take several rounds of activation for T cells to differentiate terminally to either Th1 or Th2 \[^{[61]}\], which suggests that T cells can be activated and expanded in a non-polarized manner.

5. Adaptive immune System Signaling pathways

Adaptive immune system signalling pathways are divided into two parts:

1. T cell signalling pathways
2. B cell signalling pathways.

5.1. T cell signalling pathways

T cell signalling pathways is initiated by interaction of TCR-CD3 complex with a processed antigenic peptide bound to either a class 1 (CD8+ cells) or class 2 (CD4+cells). In a resting T cell, p56 lck, a protein tyrosine kinase essential for the initiation of TCR signalling, is sequestered from the TCR complex. On activation this complex phosphorylates the immune receptor tyrosine based activation motifs (ITAMs) of the CD3 component polypeptides \[^{[62]}\]. Phosphorylated tyrosines in the ITAMs of the zeta chain provide docking sites to which another protein tyrosine kinase called ZAP-70 attaches and become active. This event catalyzes a series of intracellular events beginning at the inner surface of the plasma membrane and culminating in the nucleus, resulting in the transcription of genes that drive cell cycle and differentiation of the T cell.

5.2. B cell signalling pathways

B cell signalling pathway is activated by the T\(_H\) cells and BCR. After a T\(_H\) cell recognizes and interacts with an antigen-MHC class 2 molecule complexes, the cell is activated. It undergoes a metabolic transformation and begins to secrete various cytokines. The secreted cytokines play an important role in activating B cells, T\(_C\) cells, macrophages and various other cells that participate in the immune response. B cell interacts with antigen and then differentiates into antibody-secreting plasma cells. The secreted antibody binds to the antigen and facilitates its clearance from the body.
5.2.1. Signal transduction pathways activated by the BCR

The antigen-mediated cross-linking of BCRs initiates signal transduction processes that result in B-cell activation. Binding of antigen leads to phosphorylation and activation of various molecules result in activation of various small G protein pathways (Rho, Rac and Ras) as well as calcium dependent phospholipase pathway and protein dependent kinase pathways. All of these pathways are also engaged during T-cell activation.

Fig 1. Innate immune response signalling pathways.
Extracellular

Microbial product

TLR3, 4.

Cytoplasm

MyD88 dependent pathways

MyD88 independent pathways

Fig 2. TLRs Signaling Pathways.
Fig 3. Connection between innate and adaptive immunity.

6. CONCLUSION
The immune system is a complex system responsible for protection against pathogenic agents. The more we learn about the immune system, the more amazed we are by its complexity. Pathogens interact with immune cells and initiate signalling pathways. Both innate and adaptive immune signalling pathways are very complex and different from each other but finally they work together for elimination of pathogen.

7. ACKNOWLEDGEMENT
Author gratefully acknowledges the DST, New Delhi, India for providing financial assistance in the form of a DST-INSPIRE fellowship.

8. Declaration of Interest statement
There is no conflict of intersect.

9. REFERENCES


