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Review

Toll-like receptors in immunity and inflammatory diseases: Past, present, and future

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ABSTRACT

The immune system is a very diverse system of the host that evolved during evolution to cope with various pathogens present in the vicinity of environmental surroundings inhabited by multicellular organisms ranging from achordates to chordates (including humans). For example, cells of immune system express various pattern recognition receptors (PRRs) that detect danger via recognizing specific pathogen-associated molecular patterns (PAMPs) and mount a specific immune response. Toll-like receptors (TLRs) are one of these PRRs expressed by various immune cells. However, they were first discovered in the *Drosophila melanogaster* (common fruit fly) as genes/proteins important in embryonic development and dorso-ventral body patterning/polarity. Till date, 13 different types of TLRs (TLR1-TLR13) have been discovered and described in mammals since the first discovery of TLR4 in humans in late 1997. This discovery of TLR4 in humans revolutionized the field of innate immunity and thus the immunology and host-pathogen interaction. Since then TLRs are found to be expressed on various immune cells and have been targeted for therapeutic drug development for various infectious and inflammatory diseases including cancer. Even, Single nucleotide polymorphisms (SNPs) among various TLR genes have been identified among the different human population and their association with susceptibility/resistance to certain infections and other inflammatory diseases. Thus, in the present review the current and future importance of TLRs in immunity, their pattern of expression among various immune cells along with TLR based therapeutic approach is reviewed.

1. Introduction

The innate immune system is the primary defense entity of the host to protect against invading pathogens and thought to be evolutionarily conserved and phylogenetically ancient arm of the immune system [1,2]. In humans innate immune system mainly comprises of innate immune cells (i.e. monocytes/macrophages, neutrophils, dendritic cells (DCs), natural killer (NK) cells, mast cells (MCs), eosinophils, basophils along with newly identified innate lymphoid cells (ILCs) and mucosal associated invariant T (MAIT) cells, γδT cells, NKT cells etc.) [3–9] and its humoral components that is circulating complement system proteins/components, cytokines and chemokines secreted by innate immune cells along with various antimicrobial peptides (AMPs) (i.e. LL37, Bactericidal/permeability increasing protein (BPI) etc. [10–16]. Innate immune cells express various pattern recognition receptors (PRRs) including Toll-like receptors (TLRs,) responsible for the recognition of pathogen-associated molecular patterns (PAMPs) and induction of inflammatory immune response [17–21]. Thus this recognition of pathogens by PRRs plays a very important role in the generation of an effective innate immune response. TLRs are one of highly conserved PRRs and have been identified in animals as low as nematodes that is *Caenorhabditis elegans* (*C. elegans*) and in ascidian called *Ciona intestinalis* (*C. intestinalis*) [22–25]. The first identification of TLRs in 1988 in *Drosophila melanogaster or D. melanogaster* [26] and then subsequent recognition of its one homolog called TLR4 in humans in 1997 [27] revolutionized the field of innate immunity. This novel discovery of TLR4 in humans filled the great gap stayed long in the field of immunology that is how pathogens and microbes are recognized by host immune system. However, a variation in TLR4 expression and function in different animal species is also observed [28]. In addition to the variation in expression of TLR4 among different animals, a great variation in expression of the number of TLRs in the animal kingdom is observed [29]. For example, Purple sea urchin or *Strogylocentrotus purpuratus* expresses most that is 222 TLRs, Amphioxus or

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Branchiostoma floridae expresses 42 TLRs, Xenopus or *Xenopus tropicalis* expresses 19 TLRs, while Zebra fish or *Danio rerio* expresses 17 TLRs [30,31]. This can be explained on the basis of the evolutionary primitiveness of the animal. This is because TLRs are involved in the recognition of almost every pathogen including bacteria, viruses, fungi, and parasites in animals as soon as they come in contact with the host via any route of pathogen exposure [32–34]. Thus, TLRs are very important PRRs of immune system required to initiate an effective innate immune response at an early stage of infection [35,36]. While at later stages these TLRs regulate the generation of adaptive immune response [37–39]. Thus, TLRs are still sitting over the top of the immune system pyramid since their first discovery in *D. melanogaster* in 1988 and will they be remain sitting at this position in the ever-changing and evolving field of innate immunity and immunology. This review is designed to highlight the past, present, and future of TLRs in immunity in terms of their pattern of expression in various immune cells, recognition of various TLR SNPs in humans making them resistant/susceptible to various infections and inflammatory disease and development of various TLR agonists and antagonist as pharmacological therapeutics and/or vaccine adjuvants.

2. Recognition of pathogens by TLRs and generation of inflammatory immune response

2.1. Discovery of TLRs and their recognition as PRRs

The Toll protein was first identified in *D. melanogaster* or common fruit fly as an integral membrane protein with a cytoplasmic domain and a large extra cytoplasmic domain with a role in dorso-ventral body patterning during embryonic development as a maternal effect gene [26]. The further study established that maternal expression of *toll* genes plays an important role in the correct spatial organization of lateral and ventral structures of Drosophila embryo [40]. While expression of *toll* gene in the embryo is an essential factor for the survival of embryo and this zygotic Toll protein exhibits similar biochemical activity as shown by maternal Toll protein [40]. Thus, Toll proteins were first identified as very important proteins responsible for the viability of the insect embryo and their development along with patterning.

In 1991, Gay and Keith showed that cytoplasmic domain of Toll protein of *Drosophila* was related to interleukin-1receptor (IL-1R) of humans [41]. These Toll proteins were further shown to exert antifungal action in *D. melanogaster* via regulating the gene responsible for synthesis of an antifungal peptide called drosomycin [42]. Thus, an era of recognition of TLRs as PRRs was about to begin as later in 1997 human homolog of Toll protein was identified by the group led by a prominent immunologist Charles A Janeway Junior, which is now known as toll-like receptor 4 (TLR-4) [27]. Thus, similar to *Drosophila* Toll, human Toll is also a type I transmembrane protein having an extracellular domain comprising of a leucine-rich repeat (LRR) domain, and a cytoplasmic domain homologous to the cytoplasmic domain of the human IL-1R [27]. Both *Drosophila* Toll and the IL-1 receptor (IL-1R) signal through NF-κB pathway [27,41].

This identification of human Toll protein revolutionized the field of immunology and led to the development of the concept of PRRs and innate immunity [43]. This is because after their first identification and characterization in humans that is human TLR4, different *Drosophila* homologs of Toll have been identified, causing an activation of NF-κB upon stimulation. This was further strengthened by the view of Gay and Keith (1991) that TLRs and IL-1Rs are related to each other and trigger similar signaling responsible for the inflammatory pathway [27,41,44]. By 1998, five human TLRs (i.e.TLR1, 2, 3, 4 and 5) had been identified as direct homologs of *Drosophila* Toll protein along with their genetic location on human chromosomes [44]. For example, *tlr* 1, 2, and 3 genes for humans are located on chromosome 4, *tlr4* gene is located on chromosome 9 and *tlr5* gene sits on the chromosome 1 [44]. Subsequently in 1998 the receptor for identification of lipopolysaccharide (LPS) in mouse was identified by a group of researchers, called TLR4 belonging to IL-1R family [45] as a mutation in this gene was making certain mice strains (i.e. C3H/HeJ and C57BL/10ScCr mice) more resistant to LPS but still they were highly prone to get gram-negative bacterial infections [46,47]. Thus, identification of TLRs and the TLR4 as a potential and major receptor for bacterial LPS in mice and humans revolutionized the biology of the mammalian immune system [43,48]. This discovery proved to be a milestone in the evolution of the innate immune system as an important component of the immune system responsible for recognizing potential pathogens, inflammatory immune response and regulating the adaptive immune response. This is because, before the identification of PRRs and concepts regarding innate immunity given by late CA Janeway Junior [49,50], immunologists were mainly focussed on adaptive immunity that is T cell and B cell-based immune response. Thus, the discovery of TLRs as PRRs played an important role in the establishment of the innate immune system as a separate and important branch of immunology. Furthermore, both the arms of immune system regulate each other depending on the cause, duration, and intensity of the associated immune response and its outcome.

3. Current scenario in TLR biology, regulation of innate immune response by TLRs during infection and generation of pro-inflammatory immune response

TLRs have been considered evolutionarily conserved proteins and the oldest TLR has been identified in nematodes (i.e. *Caenorhabditis elegans* or *C. elegans*) [51,52]. These are essentially characterized by an extracellular leucine-rich repeat (LRRs) domain, which mediates recognition of PAMPs, a transmembrane domain along with its cytosolic or intracellular Toll/IL-1R-like (TIR) domains required for downstream signaling pathways [27,51–53]. Thus, Toll signaling is present from primitive life that is nematode to the most advanced form of life that is modern human and plays a very important role in the development and immune response [52]. Till date, 10 functional TLRs (i.e. TLR1-TLR10) in humans and 13 active TLRs in laboratory mice have been identified [54]. Whereas, *D. melanogaster,* has 9 different Toll proteins (i.e. Toll, 18 Wheeler (18W) or Toll-2, Toll-3-Toll-9) [55]. These Toll receptors have two or more characteristic cysteine-rich motifs flanking LRRs. However, TLR-9 of humans and toll-9 of *D. melanogaster* have only single cysteine-rich motif between the transmembrane domain and LRRs [55]. Even toll-9 from *D. melanogaster* has a great homology with mammalian TLR1, 2, 4 and 6 [56].

All TLRs expressed by host cells are synthesized in the endoplasmic reticulum (ER) and are transported to Golgi complex and from there these TLRs are transported to either cell membrane or intracellular compartments (i.e. endosomes) [57]. The trafficking of intracellular TLRs (i.e. TLR3, TLR7, TLR8, and TLR9) to endosomes is controlled and regulated by a multi-pass transmembrane protein called UNC93B1 (Unc-93 homolog B1) [57]. The excessive activation of TLR7 is also controlled by UNC93B1 by employing TLR9 to counteract the exaggerated activation of TLR7 [57,58]. Protein associated with TLR4 (PRAT4A) is another ER resident protein molecule controlling TLR trafficking of TLR1, TLR2, TLR4, TLR7 and TLR9 from ER to their site of location that is plasma membrane and endosomes [59]. gp96 (a member of Hsp90 family) in ER acts as a general chaperone for most of TLRs including TLR1, TLR2, TLR4, TLR5, TLR7 and TLR9 [60]. Proteolytic cleavage of nucleic acid-sensing TLRs by Cathepsin B, S, L, H, and K and asparaginyl endopeptidase is required for the functional maturation of TLRs to recognize their competent ligands and mount an effective innate immune response [61–63].

TLRs are either expressed extracellularly on the cell surface (For example, TLR1, TLR2, TLR4, TLR5, TLR6, and probably TLR11 and TLR12 of mice and TLR10 of humans are expressed largely on the cell surface of innate immune cells) or intracellularly in the cytosolic