Roles of Toll-Like Receptors in Natural Interferon-Producing Cells as Sensors in Immune Surveillance

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ABSTRACT: Natural IFN- α/β producing cells (IPCs) play a central role in innate immunity against microbial infections. In primary immune responses, toll-like receptors (TLRs), as major pattern-recognition receptors, are essential for IPCs as well as other antigen presenting cell (APC) subsets to recognize microbes. IPCs unequivocally express TLR7 and TLR9, and can respond to the respective ligand to produce IFN- α/β and to rapidly differentiate into dendritic cells (DCs). Thereby, IPCs can not only activate innate immune system but also provoke T cell responses. Thus, IPCs link innate and adaptive immunity through TLR system. In addition, recent work has revealed the regulatory system of DC subsets in response

to microbial invasion. In this context, by the different but complementary expression profile of TLRs, IPCs together with myeloid APC subsets constitute a rational system of immune surveillance that can cover a wide variety of pathogens and enlarge immune adjuvant effects. *Human Immunology* 63, 1120–1125 (2002). © American Society for Histocompatibility and Immunogenetics, 2002. Published by Elsevier Science Inc.

KEYWORDS: IFN- α/β producing cells; dendritic cells; Toll-like receptors (TLRs); TLR7; proinflammatory cytokines

INTRODUCTION

Natural IFN- α/β producing cells (IPCs), so-called plasmacytoid cells, are a unique hematopoietic cell type that differentiate into mature dendritic cells (DCs) [1,2]. IPCs are suggested to be of lymphoid origin [3, 4] and, accordingly, IPC-derived DCs are different from myeloid-lineage DCs such as monocyte-derived DCs or blood CD11c⁺ myeloid DCs (mDCs) in terms of their phenotypical and functional properties [5–7]. Upon viral infection, IPCs are activated and produce not only proinflammatory cytokines but also large amounts of IFN- α/β , thereby activating innate immunity and provoking adaptive immunity [8]. Therefore, IPCs can link innate and adaptive immunity during the process of their differentiation.

Expression of TLRs in IPCs

Interferon producing cell activation is triggered by pathogen recognition. How do IPCs recognize microbial

pathogens? Recent research on Toll-like receptors (TLRs) will provide the answer. TLRs, which are phylogenetically well conserved, are type I transmembrane proteins that are expressed on antigen presenting cells (APCs) including macrophages and DCs, and are major pattern-recognition receptors for various pathogen-associated molecular patterns (PAMPs) in the innate immune system [9–13]. TLR signaling leads to activation of NF- κ B through the adaptor protein MyD88-dependent pathway and induces a battery of immune adjuvant effects. The expression of TLR family members (TLR1–10) in human immune cells including IPCs and other DC subsets has been described by several groups (Table 1) [14–18]. IPCs unequivocally express both TLR7 and TLR9, and low TLR1, TLR6, and TLR10. On the other hand, blood immature CD11c⁺ mDCs and monocytes are shown to have a similar TLR expression profile. Although the expression level is variable, both blood mDCs and monocytes express TLR1, TLR2, TLR4, TLR5, TLR6, and TLR8. Notably, blood mDCs selectively express TLR3. There is controversy surrounding in TLR7 expression in these blood myeloid APCs; the expression is undetectable or at low level. Thus, IPCs and myeloid APCs express a different TLR repertoire.

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TABLE 1 Functional TLR repertoire in human DC subsets

	IPCs (plasmacytoid DC precursors)	Blood CD11c +DCs (immature myeloid DCs)	Monocytes (myeloid DC precursors)
Expression of TLRs			
TLR1	low	+	+
TLR2	—	+	+
TLR3	—	+	—
TLR4	—	low	+
TLR5	—	low	+
TLR6	low	low	low
TLR7	+	low*	low*
TLR8	—	low	+
TLR9	+	—	—
TLR10	low	low	—
Cytokine production in response to ligand for TLRs			
PGN (ligand for TLR2)	—	IL-12/IL-6/TNF- α	IL-6/TNF- α
Poly I:C (ligand for TLR3)	—	IL-12/IFN- α	—
LPS (ligand for TLR4)	—	IL-12/IL-6	IL-6/TNF- α
Flagellin (ligand for TLR5)	?	?	?
Imidazoquinoline compounds (ligands for TLR7)	IFN- α	IL-12	?
CpG-ODNs (ligands for TLR9)	IFN- α	—	—

* Expression level in the subsets is in an argument among the report (Ref. 14–18); undetectable or in low level.

Abbreviations: DC = dendritic cells; IPC = interferon producing cells; LPS = lipopolysaccharide; PGN = peptidoglycan; TLR = Toll-like receptor.

Differential Responsiveness Through TLR Signaling in IPCs and Myeloid DC Subsets

TLR2 and TLR4 are essential for the recognition of peptidoglycan (PGN) from gram-positive bacteria and lipopolysaccharide (LPS) from gram-negative bacteria, respectively [19, 20]. Together with TLR2, TLR1 is associated with the recognition of bacterial or spirochetal lipoproteins [21, 22] and TLR6 co-recognizes mycoplasma lipopeptide [12]. TLR5 and TLR9 recognize bacterial flagellin and bacterial DNA containing unmethylated CpG motifs, respectively [23–25]. TLR3 is involved in recognition and signal transduction induced by double-stranded (ds) RNA synthesized from viruses [26]. In accordance with the TLR expression, IPCs recognize CpG-oligonucleotides (ODNs), but not PGN or LPS, and thereby rapidly produce IFN- α/β and develop into mature DCs [14, 27–29]. By contrast, blood CD11c⁺ mDCs and monocytes respond to PGN or LPS, but not CpG-ODNs, to produce different sets of proinflammatory cytokines (i.e. TNF- α , IL-6, or IL-12) [14, 16]. In addition, polyinosinic-polycytidylic acid (poly I:C), a mimic of viral RNA, selectively stimulates blood mDCs to produce both IFN- α/β and IL-12 and to undergo their maturation [27]. Thus, depending on the differential expression of cognate TLRs, IPCs, blood CD11c⁺ mDCs, or monocytes evoke distinct responsiveness in the production of different sets of proinflammatory cytokines in response to the respective TLR ligands from invading pathogens. Therefore, they may induce appropriate in-

nate immune responses and also dictate the type of adaptive immune responses (Figure 1).

TLR7, a New Member of TLRs, Involvement in IPC Functions

Among the ten members of TLRs, the natural ligands for TLR7, TLR8, and TLR10 are unknown at present. However, a search for ligands of TLRs using gene-deficient mice has recently shown that synthetic compounds with antiviral activity are the ligands for TLR7 [30]. These are imidazoquinoline compounds, imiquimod and its derivative, resiquimod (R-848), and have potent antiviral and antitumor properties in animals [31–34]. In fact, imiquimod has been clinically approved for the treatment of genital warts caused by human papillomavirus [35]. It has been demonstrated in experiments using gene-deficient mice that these antiviral compounds activate immune cells, including macrophages, bone marrow-derived DCs, and splenocytes, via the TLR7-MyD88-dependent signaling pathway. Human TLR7 can confer R-848 responsiveness in experiments using transfected human embryonic kidney (HEK) 293 cells [30].

Several studies using a quantitative PCR method have shown that TLR7 is expressed in IPCs substantially, but in blood mDCs or in monocytes only at low levels [15–18]. However, not only IPCs but also blood mDCs can respond to imidazoquinoline compounds with enhanced survival and upregulation of costimulatory molecules such as CD40, CD80, and CD86 [18]. These

FIGURE 1 Functions of two blood dendritic cell (DC) subsets, interferon producing cells (IPCs) and blood CD11c⁺ mDCs, by sensing microbial components through toll-like receptors (TLRs). IPCs and blood mDCs can respond to a wide variety of pathogens through different, but complementary, sets of TLRs in immune surveillance. These subsets produce the different sets of proinflammatory cytokines to the respective TLR ligands from invading pathogens, and may cooperate in immune responses against specific microbial pathogens. Thereby, they may induce appropriate innate immune responses and also dictate the type of adaptive immune responses.

effects of TLR7 ligands on IPCs are equivalent to those of CpG-ODNs through TLR9. Accordingly, TLR7 expression on IPCs and on blood mDCs might be enough to transmit the biological effects. Interestingly, TLR7 ligands exhibit differential cytokine inducing effects in different DC subsets: IFN- α in IPCs versus IL-12 in mDCs [18]. The TLR7 ligands, when injected into mice, can also elevate serum levels of IFN- α and IL-12 [30]. It is of note that TLR7 is phylogenetically closely related to TLR9. In addition to TLR9, TLR7 is a possible new member of the TLR family which has the potential to evoke IFN- α production from IPCs. Viral infection enhances TLR7 mRNA expression in human macrophages, but the presence of neutralizing anti-IFN- α/β Abs blocks this effect, while exogenous IFN- α directly up-regulates TLR7 mRNA in macrophages [36]. Also in IPCs, TLR7 mRNA is upregulated in response to IFN-inducing CpG-ODNs, but not to IL-3 [17]. These findings suggest that TLR7 expression on IPCs is positively modulated by IFN- α/β , and that a positive feedback mechanism may control IFN- α production through

TLR7 signaling. Thus, the TLR7 system in IPCs may efficiently function in the event of microbial invasion.

Though there is debate regarding the ability of IPCs to produce IL-12 in response to some stimuli, it is known TLR7 is unable to induce a significant amount of IL-12 [18]. TLR7 ligands have also no capacity to induce IFN- α production from blood mDCs [18]. Thus, signaling through the same receptor results in the induction of subset-specific cytokine production. So far there has been a question whether cytokine production from a given DC subset is determined by cell lineage or by pathogenic stimuli that DCs receive. TLR9 and TLR2/TLR4 are expressed in either IPCs or myeloid APC subsets, and each subset differentially responds to the respective TLR ligands, as mentioned above. These findings favor the idea that cytokine production is determined by TLR expression on distinct DC subsets. However, the differential cytokine production from TLR7-stimulated IPCs and blood mDCs may indicate that DC lineage as well as TLR expression are decisive factors affecting DC response.

It remains unclear why TLR7 exerts differential outcomes in IPCs and blood mDCs. In response to LPS, DC-derived cytokine induction depends on MyD88, whereas DC maturation can proceed without MyD88, in the gene-deficient mice model [37]. These results indicate that MyD88 is a bifurcation point in TLR4 signaling. However, the effects of TLR7 signaling completely depend on MyD88 [30]. Therefore, it is likely that there is another bifurcation point leading to the synthesis of IFN- α or IL-12. Furthermore, it has been recently reported that the activation of NF- κ B is observed in

HEK293 cells transiently transfected with human TLR8 in response to imidazoquinoline R-848 [38], as in the case of HEK293 cells transfected with human TLR7. Blood mDCs are shown to express not only TLR7 but also TLR8 at low levels, although the expression of TLR8 is not detected in IPCs at all. It remains unknown how TLR8 signaling is involved in blood mDC response to the imidazoquinoline compounds. Considering that TLR7 and TLR8 show high homology in their molecular structures [39], it is possible that TLR7 or TLR8 just work redundantly in blood mDCs. Identification of TLR7- or TLR8-specific ligands could provide clearer answers.

In B cells, TLR7 is also detected [17]. B cells show *in vitro* blast formation in response to TLR7 ligands [40], but *in vivo* roles of TLR7 signaling in B cells are so far unknown. Future identification of natural ligands for TLR7 will be important to clarify the pathophysiologic roles of this receptor in immunity. Until then, however, the imidazoquinoline compounds are useful tools to study the biological functions as well as the signaling pathway via TLR7.

Rational Repertoire of TLRs in DC System in Innate Immunity

Because IFN- α/β is essential in antiviral and antibacterial innate immunity [41], IPCs may play a central role in the immune responses against infections by sensing microbial invasion. The limited TLR expression on IPCs suggests that IPCs are incapable of responding to all microbial components. However, the different, but complementary, expression profiles of TLRs among IPCs and other myeloid APC subsets may cover a wide variety of pathogens. It is very likely that a given pathogen can activate not only IPCs but also myeloid APC subsets simultaneously through multiple TLR pathways (Figure 1). For example, upon invasion of Gram-negative bacteria, the bacteria-derived LPS and CpG DNA can simultaneously activate myeloid APCs and IPCs, respectively, and may contribute to the establishment of antibacterial immune responses. Similarly, in antiviral immunity, intracellular viral infection directly stimulates IPCs and virus-synthesized dsRNA also can activate immature mDCs via TLR3. Thus, the TLR network between IPCs and other myeloid APCs constitutes a kind of rational fail-safe system and thereby, these subsets may cooperate to enlarge innate immune responses against diverse microbial pathogens.

IPC-Derived DCs in Adaptive Immunity, Possible Involvement in TLRs

It is unclear how TLR-mediated microbial recognition by DC results in antigen processing and presentation. However, the downregulation of TLR expression during

DC differentiation [16] implicates a functional shift; DC precursors act as particular cytokine producers in innate immunity through microbial recognition, and mature DCs act as APCs in priming T cells in adaptive immunity. Furthermore, after pathogen recognition through TLRs, either IPCs or blood mDCs differentiate into mature DCs that can prime naive T cells [27]. Recent progress has clarified a functional plasticity of DCs in adaptive immunity [5–7, 42]. In this context, DCs mainly regulate the quality of Th cell responses by DC-derived cytokines, in particular IL-12 or IFN- α/β . Most myeloid DCs have some capacity to produce IL-12 and, thereby, to induce Th1 development. However, this capacity varies with the conditions of their maturational stage or stimulation delivered to DCs [43–48]. A similar functional plasticity is observed in IPC-derived DCs [8, 49–51]. When IPCs sense viruses, they differentiate into mature DCs that can direct naive Th cells toward Th1-like (IFN- γ and IL-10 producing) cells through the IPC-derived IFN- α/β . When IPCs develop into DCs upon exposure to IL-3, which is mainly produced under allergic conditions, these DCs induce Th2 cells and have no capacity to produce IFN- α/β and/or IL-12. In response to pathogenic stimuli through TLRs, myeloid DC subsets can skew naive Th cells into Th1 cells. In fact, every susceptible TLR ligand can induce IL-12 production from immature mDCs and monocytes [16]. Also, when IPCs respond to ligands for TLR7 [18] or TLR9 [29], IPC-derived DCs can induce IFN- γ producing Th cells through IFN- α/β suggesting that IPCs acquire Th1 (or Th1-like) inducing activity upon microbial invasion. In addition, the production of IL-12 by myeloid DCs triggered by some microbial stimuli [52, 53] and the production of IFN- α by IPCs stimulated by CpG-ODN [15] is amplified by CD40 ligation. These findings suggest that their Th1-inducing activities through the TLR system can be well performed in the T-cell priming process. Taken together, to eliminate invading pathogens, both types of DCs are programmed to make different innate responses in terms of cytokine production and can eventually support anti-microbial Th1 responses in a subset-specific cytokine dependent manner (Figure 1).

In this article, we have focused on the role of TLRs in the functions of the DC system, including IPCs. However other cell surface receptors of DC such as mannose receptors, CD1 family, or DC-SIGN may be important for pathogen recognition. The interesting points are how signalings from TLRs and those from the phagocytic receptors are connected at the molecular level, and how these signals contribute to the functions of DCs as APCs. These points should be clarified, to provide a better understanding of DC-mediated immunity.

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