



TLRs, NLRs and RLRs: Innate sensors and their impact on allergic diseases – A current view

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ABSTRACT

Charles Janeway first wrote 1989 about how important recognition of “certain characteristics or patterns common on infectious agents but absent from the host” would be for our immune response [1]. Surprisingly, it almost took 10 years before his ideas would lead to the revolutionary findings that fundamentally changed the view of the innate immune system over the past decade. Recognition of invading microorganisms belongs to the primary tasks of the innate immune system and is achieved through different families of innate immune sensors. Among these, Toll-like receptors (TLRs), nucleotide-binding domain and leucine-rich repeat containing receptors (NLRs) and Rig-I-like receptors (RLRs) have drawn major interests over the last decade. These receptor families are targeted by overlapping classes of pathogens and share functional domains and signal transduction pathways (see Fig. 1 and Table 1 for an overview of their structural organization, ligands, adaptors and activated pathways).

This current view describes our present knowledge about these three main innate immune receptor families and their importance for adaptive immune responses such as asthma and allergy.

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1. Introduction

Charles Janeway first wrote 1989 about how important recognition of “certain characteristics or patterns common on infectious agents but absent from the host” would be for our immune response [1]. Surprisingly, it almost took 10 years before his ideas would lead to the revolutionary findings that fundamentally changed the view of the innate immune system over the past decade. Recognition of invading microorganisms belongs to the primary tasks of the innate immune system and is achieved through different families of innate immune sensors. Among these, Toll-like receptors (TLRs), nucleotide-binding domain and leucine-rich repeat containing receptors (NLRs) and Rig-I-like receptors (RLRs) have drawn major interests over the last decade. These receptor families are targeted by overlapping classes of pathogens and share functional domains and signal transduction pathways (see Fig. 1 and Table 1 for an overview of their structural organization, ligands, adaptors and activated pathways).

This current view describes our present knowledge about these three main innate immune receptor families and their importance for adaptive immune responses such as asthma and allergy.

2. Innate immune sensors

2.1. Toll-like receptors (TLRs)

The name-giving receptor of this family, the Toll protein, has been first identified in *Drosophila* as an essential receptor during embryogenesis, controlling the dorsoventral polarization during the development of the fly larvae [2]. However, it took more than 10 years before its second essential role, the central receptor in the antifungal immune response, was identified [3]. Today, 13 mammalian TLRs are known and usually divided into cell-surface and intracellular TLRs.

2.2. Cell-surface TLRs

2.2.1. TLR2, TLR1, TLR6, and TLR10

The main ligands for the heterodimeric complexes of TLR2/TLR1 or TLR2/TLR6 are tri- and diacylated lipopeptides, respectively [4–9], although this restriction may not be true for all lipopeptides [10,11]. Interestingly, despite the different heterodimer formations of TLR2, the induced signaling through TLR2 appears to be identical [12]. In addition, a vast number of other ligands, such as peptidoglycan, LTA [13], or heat shock proteins, have been described as ligands for TLR2 [14]. However, in particular for TLR2- and TLR4-dependent signaling, minute contaminations with lipopeptides or lipopolysaccharide, respectively, may account for many of the described ligands of these TLRs [15].

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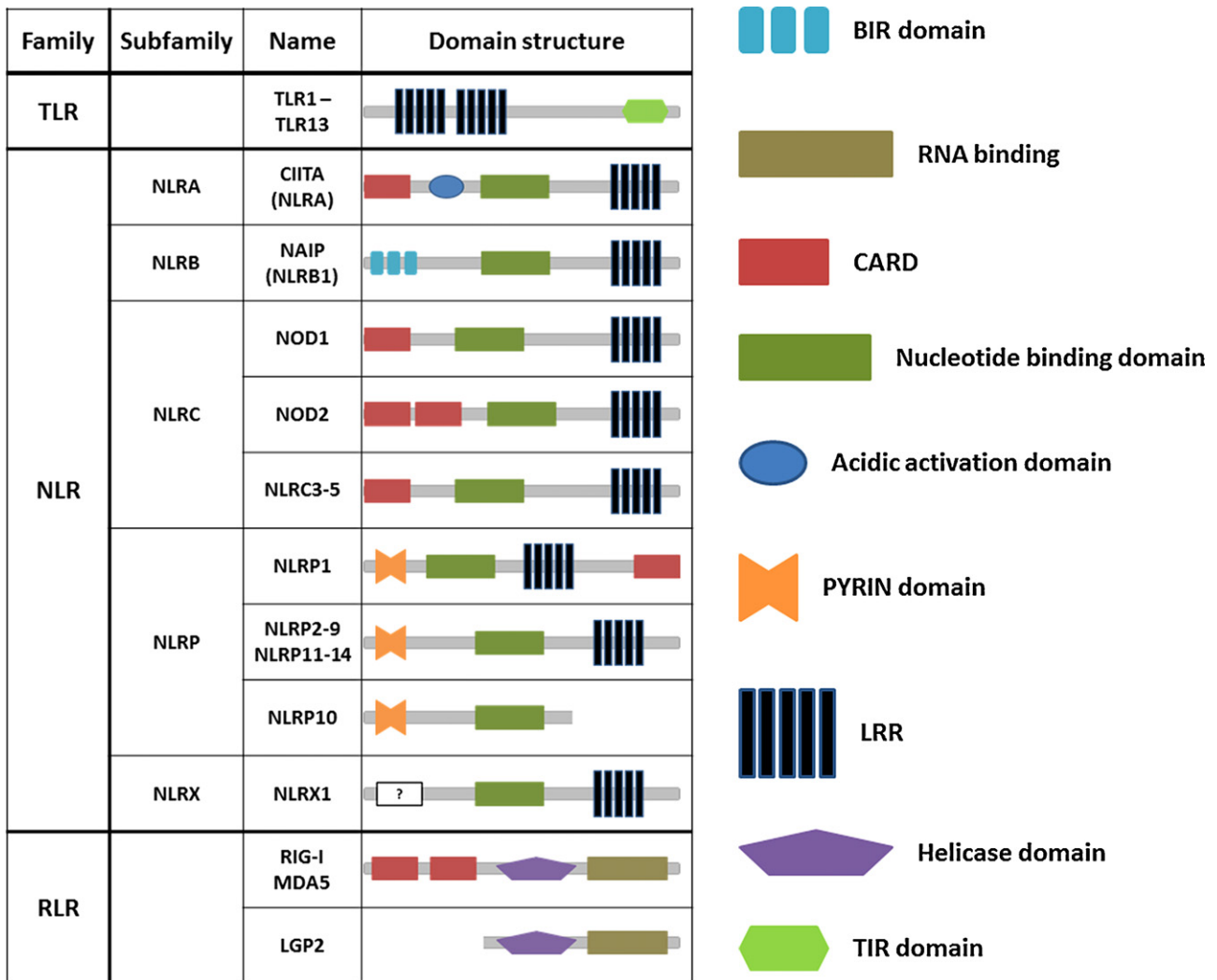


Fig. 1. Nomenclature and domain structure of the TLR, NLR, and RLR innate immune receptor families.

Binding of lipopeptides to TLR2 heterodimers has been shown for the synthetic triacylated lipopeptide Pam₃CysSK₄. Unexpectedly, this binding to the TLR2/TLR1 heterodimer occurs in a lipid-binding pocket at the convex face of the heterodimer [16].

TLR10 belongs into the same family and is most closely related to TLR1 and TLR6 [17], and was the only remaining human orphan TLR until Guan et al. could show in 2010 that TLR10 apparently shares ligand specificity with TLR1, but, for reasons not understood so far, is not capable to induce the same signaling [18].

2.2.2. TLR4

The first human Toll receptor (later renamed TLR4) was identified in 1997 by Medzhitov et al. [19], without knowing its ligand. However, the results already show the capability to activate adaptive immune responses. Two years later, the first microbial TLR ligand was identified, the Gram-negative main cell wall component lipopolysaccharide (LPS) [20]. Ironically, it was the wrong TLR (TLR2) that was thought to be required for LPS-induced signaling. Later, it became clear that TLR2 was dispensable for LPS responses [21] and that it was not the LPS itself, which induced TLR2-signaling, but a contamination within the often used commercial LPS preparations, namely bacterial lipoprotein [22,23].

During the same time Bruce Beutlers long-lasting approach to identify the LPS receptor by positional cloning came to end [24] and TLR4 was identified as the LPS receptor [25]. However, LPS

recognition and signaling has an additional twist: a small secreted endogenous protein, MD2, is absolutely required for LPS-induced signaling [26] and is actually the true LPS binding partner [27,28]. In contrast to TLR2, where the actual binding side is located “on top” of the TLR2 heterodimer, LPS-MD2 binding to TLR4 takes place at the concave face [27].

Similar to TLR2, a number of additional microbial and endogenous compounds have been found to signal through TLR4 [29]. As an example, heat shock proteins (hsp) 60 and 70 were thought to serve as endogenous danger signals and have been shown to induce cytokines in a TLR2- and TLR4-dependent manner [30,31]. Although in most cases the possible contamination with LPS was carefully considered, recent publications cast doubts about the normally used procedures to rule out LPS-contamination as responsible for the detected TLR4 activity. With respect to hsps, this activity has been later traced to residual LPS [32–34].

2.2.3. TLR5

TLRs are not only capable of recognizing lipid structures but also proteins. The first protein that has been identified as TLR ligand is flagellin, an essential protein in motile bacteria [35]. The amino acids that are required for TLR5 activation are not easily accessible, since they are hidden in the tertiary structure of the flagellin protein [36]. Thus, it is believed that monomeric flagellin is the activator, although the specific mode of action, i.e., how monomeric

Table 1

Main ligands (or inducers), adaptors and activated signaling pathways of mammalian innate immune sensors.

Family	Subfamily	Member	Main ligands (or Inducers)	Adaptors	Signaling pathway
TLR	TLR1	TLR1	Lipopeptides	MyD88, Mal	NF- κ B, MAPK
		TLR2			
		TLR6			
		TLR10			
	TLR3	TLR3	dsRNA	TRIF	NF- κ B, IRF3/7, MAPK
		TLR4	Lipopolysaccharide	MyD88, Mal; TRIF, TRAM	NF- κ B, IRF3/7, MAPK
		TLR5	Flagellin	MyD88	NF- κ B, MAPK
		TLR7	ssRNA	MyD88	NF- κ B, IRF7, MAPK
		TLR8	ssRNA	MyD88	
		TLR9	CpG-DNA	MyD88	
	TLR11	TLR11	<i>Uropathogenic bacteria</i> , profilin	MyD88	
		TLR12		MyD88	
		TLR13		MyD88	
NLR	NLRA	CIITA (NLRA)	<i>Legionella pneumophila</i>	ASC	MHCII expression Inflammasome formation
		NAIP (NLRA)			
	NLRB		<i>Pseudomonas aeruginosa</i>		
			ie-DAP	RICK/Rip2	NF- κ B, MAPK
			MDP		
	NLRC	NOD1 (NLRC1)	Flagellin, T3SS	ASC (?), NAIP	Inflammasome formation
		NOD2 (NLRC2)			
		NLRC3			
		NLRC4			
		NLRC5			
	NLRP	NLRP1	<i>Anthrax lethal toxin</i> , MDP	ASC	Inflammasome formation
		NLRP2	<i>Bacterial DNA and RNA</i> , extracellular ATP, silica, alum, amyloid- β , gout-associated crystals, monosodium urate crystals	ASC	Inflammasome formation
		NLRP3			
RLR	NLRX	NLRP4-14			
		NLRX1			
		RIG-I	ss/dsRNA	MAVS, STING	NF- κ B, IRF3/7
		MDA5	ss/dsRNA	MAVS	
		LPA2	ss/dsRNA	?	

flagellin is made available to the cell, is not yet clear. Interestingly, a second, intracellular receptor for flagellin exists (NLRC4, s. below) which allows the innate immune system to distinguish between flagellated bacteria in general and those, which are capable to reach the cytosol of cells by means of various secretion systems [37].

3. Intracellular TLRs

3.1. TLR3

As seen above, the cell-surface TLRs recognize mainly structural bacterial molecules, such as lipopeptides, LPS or flagellin. The intracellular TLRs are expressed on endosomal membranes and mostly recognize nucleic acids, originating from viruses and bacteria. TLR3 is a receptor for double-stranded RNA molecules [38] and therefore recognizes a number of different viruses [39–41].

3.2. TLR7 and TLR8

The first ligands that were described for these TLRs were synthetic imidazoquinoline compounds used for antiviral therapy as well as guanosine nucleotide analogues [42–44]. Later, it was shown that single-stranded (ss)RNA is the natural ligand for TLR7 and TLR8 [45,46]. Since ssRNA *per se* is not specifically a microbial structure, compartmentalization of this specific immune response to the endosomal/phagolysosomal compartment is required in order to avoid autoimmune reactions. Since TLR8 knockout mice responded normally to ssRNA and mouse TLR8 could not confer responsiveness to R-848 [43,45] it was believed that mouse TLR8 is non-functional. However, a recent report shows TLR-8 dependent activation of plasmacytoid dendritic cells by vaccinia virus, a find-

ing which is highly disputed [47]. In addition, mouse TLR8 might also have a function in the regulation of TLR7 expression [48].

3.3. TLR9

TLR9 is the only DNA-recognizing member of the TLR family [49] (for other DNA sensors see below). To “avoid” unwanted stimulation by host DNA, TLR9 recognizes unmethylated ssDNA-containing CpG motifs often found in bacteria and viruses [50]. Mammalian DNA is highly methylated and thus only a very weak, if any, stimulator of TLR9. Interestingly, it depends on the type of DNA (e.g., A-type or B-type) if more type I interferon or inflammatory cytokines are produced [51].

3.4. TLR11, TLR12, and TLR13

According to Roach et al. [17] vertebrate TLRs are evolutionarily divided into 6 major families: TLR1 (containing TLR1, TLR2, TLR6 and TLR10), TLR3, TLR4, TLR5, TLR7 (containing TLR7, TLR8 and TLR9), and finally TLR11 (containing TLR11, TLR12, and TLR13). The latter ones are expressed in the mouse, but in humans only represented by a pseudogene. The first report on ligand-specificity of TLR11 came in 2004, when Zhang et al. could show that TLR11 is required for combatting infections with uropathogenic bacteria [52]. In addition, TLR11 was the first TLR to be involved the detection of parasites by detecting profilin from *Taxoplasma gondii* [53]. Recently, it was also shown that this detection essentially depends on UNC93B [54], an ER protein which was until then only known to be required for the expression of the endosomal TLRs 3, 7, 8, and 9 [55]. Therefore, it appears very likely that not only the latter TLRs are intracellular expressed, but TLR11–TLR13 as well. Whereas for TLR12 a ligand still has to be found, TLR13 has now been linked to the recognition of vesicular stomatitis virus [56].

3.5. TLR signaling

The major signaling pathways emanating from activation of TLRs are the NF- κ B pathway (induction of inflammatory cytokines), activation of interferon-response factors (induction of Type I interferon response) and the activation of MAP kinases [57].

Upon binding of their respective ligands, conformational changes in the TLR homo- and heterodimers lead to the recruitment of certain intracellular adapter molecules [58,59] to their TIR domain [60]. The most important one, and used by all TLRs but TLR3, is MyD88 [61], a molecule that has already been known from studies on IL-1/IL-18 receptor signaling [62–64]. For TLR5, TLR7, TLR8, TLR9, TLR11 and TLR13, MyD88 appears to be the sole adapter protein recruited to the TIR domain. Instead of MyD88, TLR3 uses TRIF [65,66]. TLR2 and TLR4 are using an additional adaptor named MAL/TIRAP to recruit MyD88 [67–69]. Finally, with the help of yet another adapter molecule called TRAM [70,71] TLR4 (as the only TLR) has the ability to signal through TRIF as well, giving TLR4 therefore the unique competence to activate MyD88-dependent as well as MyD88-independent signaling pathways.

After the binding and activation of MyD88, the next step involves the recruitment of the IRAK family members IRAK-1, -2, -4, [63,72] and formation of a large signaling complex [73]. Since IRAK-4 is the first kinase of this family recruited to the TIR domain, it has an essential role in IL-1/IL-18 as well as TLR signaling [74]. Subsequently, IRAK1 becomes phosphorylated, which increases its own kinase activity and leads to autophosphorylation of its N-terminus [75]. Hyperphosphorylated IRAK-1 then associates with TRAF6 [76]. Due to sequence homology TRAF6 was originally identified as a member of the TNF-receptor associated factor (TRAF) family, but instead of TNF responded to IL-1 with NF- κ B activation [77]. Activated TRAF6 is an ubiquitin protein ligase (E3) and catalyzes the additions of an K63-linked ubiquitin chain on itself and on NEMO/IKK γ . Polyubiquitination of proteins and thus targeting them for proteosomal degradation was long thought to be the most important role of ubiquitin. However, it has become increasingly clear over the last few years that depending on the residue that is polyubiquitinated (i.e., K48 vs. K63) ubiquitination is a major reversible signaling mechanism [78]. Once TRAF6 is polyubiquitinated, it activates an additional complex consisting of transforming growth factor- β (TGF- β)-activated kinase (TAK1), TAK1 binding protein 1 (TAB1), and TAB2/TAB3. IRAK-1 also becomes K63-polyubiquitinated and binds to NEMO/IKK γ [79]. Finally, the TRAF6/IRAK-1/TAK1/TAB2/NEMO complex leads to phosphorylation of IKK α / β as well as the activation of mitogen activated protein kinases (MAPK) [57]. After activation of IKK α / β , I κ B is phosphorylated, K48-linked ubiquitinated and degraded by the ubiquitin-dependent proteasome. After proteolysis of the I κ B proteins, NF- κ B is liberated and able to translocate into the nucleus, where it is involved in the transcription of inflammatory cytokines and other immune genes [80,81].

The MyD88-independent signaling of TLR3 and TLR4 activates both IRF3 [82] and NF- κ B. For the induction of NF- κ B translocation, TRAF6 is recruited to TRIF and the TAK1 activation is facilitated in a similar manner as has been shown for the MyD88-dependent pathway [83]. The other proteins involved are Receptor-interacting protein 1 (RIP1), TRADD and Pellino-1 [84,85]. Again, a large protein complex consisting of TRIF/TRAF6/RIP1/TRADD/Pellino-1 facilitates full NF- κ B and MAP kinase activation by TAK1. For the activation of Type I interferon through IRF3, TRIF recruits the non-canonical IKKs TBK1 and IKK ϵ to its N-terminal domain [86]. This recruitment depends on TRAF3, which is also required for other nucleic-acid sensors. Subsequently, IRF3 is phosphorylated and translocates as dimer into the nucleus where it facilitates interferon- β transcription [87].

Since the other nucleic-acid sensing TLRs (TLR7–9, TLR13) use only MyD88 as primary signaling adaptor, one has to ask how the activation of the antiviral gene program and induction of Type 1 interferons is achieved. IRF7 is constitutively expressed in plasmacytoid DCs (pDCs), the cell type particularly involved in the rapid interferon response to foreign nucleic acids [88]. Due to the IRF7 expression, MyD88 can form a complex consisting of MyD88/IRAK4/IRAK1/TRAF6/TRAF3/IKK α and IRF7, leading to the IRF7 phosphorylation by IRAK1, dissociation from the complex and translocation into the nucleus [89–92].

4. Nucleotide-binding domain and leucine-rich repeat containing family (NLRs)

NLRs comprise a family of cytosolic receptors (22 in humans, <33 in mouse), consisting of three functional domains: C-terminal leucine-rich repeats (LRR), a central NACHT nucleotide-binding (NB) domain and a N-terminal effector domain (e.g., CARD, PYRIN, and BIR domain). Intriguingly, the NB-LRR formation resembles that of plant disease resistant genes, the so-called R-proteins [93], and thus NLRs represent a truly ancient defense mechanism. Members of this family have been studied and characterized for a long time and not only the different genes/proteins but also the family and subfamilies have been given many different names (e.g., NOD-like receptors, CATERPILLAR; NLRP3 is also known as NALP3, cryopyrin, PYPAF1, CLR1.1 and CIAS1). Therefore, a new and unifying nomenclature was required and has been recently agreed upon [94]. Depending on the effector domain, the subfamilies are named NLRA (NLR with acidic domain, 1 member), NLRB (NLR with BIR domain, 1 member), NLRC (NLR with CARD domain, 5 members), NLRP (NLR with PYRIN domain, 14 members) and NLRX (NLR with no strong homology to any other N-terminal domain, 1 member). Due to the enormous amount of published literature, four members (CIITA, NAIP, NOD1 and NOD2) were not renamed.

4.1. NOD1 and NOD2

The first NLRs involved in innate immune sensing were NOD1 and NOD2 (similar to TLRs, the first reports regarding the actual ligands were inaccurate: recognition of LPS through NODs has now been traced to contaminations of LPS preparations with peptidoglycan). NOD1 recognizes small fragments of peptidoglycan from Gram-negative bacteria containing meso-diaminopimelic-acid (ie-DAP) [95,96], whereas NOD2 recognizes the muramyl-dipeptide (MDP) motif of peptidoglycan [97,98]. Recently, cytoplasmic flagellin and a part of the type III secretion system have been described as ligands for NLRC4 (IPAF) [99–101]. However, as with many other activators and thus potential ligands for other NLRs (s. below), direct interaction has yet to be shown.

The earliest signaling pathways characterized upon NOD1 and NOD2 triggering were the NF- κ B pathway and activation of MAP kinases [102]. The first and essential step of the activation process is the recruitment of RICK/RIP2 [103,104]. Eventually, as already been seen with MyD88-dependent and -independent signaling, TAK1 and NEMO are activated through phosphorylation and K63-linked polyubiquitination mechanisms [105,106], thus enabling the final steps of NF- κ B translocation. However, in contrast to TLR signaling, NOD-dependent MAPK activation requires the adaptor protein CARD9 that can directly associate with NOD2 via its CARD domain [107].

4.2. Inflammasome formation by NLRs

Over the last few years, one additional feature of NLR activation has gained tremendous interest: the regulation of IL-1 β and IL-18 release through the inflammasome complex, a name that has been

coined by Tschoopp and colleagues in their seminal paper of 2002 [108]. The inflammasome is a multi-protein complex assembled to cleave and thereby activate the proinflammatory caspase-1. To date, at least four different inflammasome complexes are known, three of which involving the NLRs NLRP1, NLRP3, and NLRC4. NLRP1 was the first inflammasome activator, but, due to the lack of a CARD domain, unable to bind directly to Caspase-1. The adaptor protein apoptosis-associated speck-like protein ASC consists of a CARD and a PYRIN domain [109], thus capable of bridging NLRP1 and Caspase-1. In addition, Caspase-5 is also involved in the NLRP1 inflammasome.

The best-studied inflammasome however, is the NLRP3-containing complex, not only because so many different activators seem to exist but also because genetic variations in NLRP3 are particularly involved in a number of human diseases [110]. The NLRP3 inflammasome consists out of NLRP3, ASC and caspase-1. Various microbes and microbial ligands such as *Shigella flexneri*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Candida albicans*, Sendai and Influenza virus, bacterial DNA, bacterial and viral RNA and others are reported to trigger the NLRP3 inflammasome [111–118]. Even more astounding, a number of very different compounds, either endogenous or foreign origin, are activators as well: extracellular ATP, monosodium urate crystals, gout-associated uric acid crystals, asbestos, silica, alum or amyloid- β [119–125]. It is not yet clear, how NLRP3 can be activated by such different molecules. However, since direct binding has not been detected for any of the above mentioned substances, an indirect activation is much more likely. One model that has been put forward is the so-called “frustrated” phagocytosis which also involves reactive oxygen species production [124], the other model favors lysosomal damage after normal phagocytosis [126,127].

The third NLR inflammasome requires NLRC4 (IPAF), which can directly bind pro-caspase-1 through CARD–CARD interactions [128]. As already mentioned, just like TLR5, NLRC4 is activated by flagellin, however, the inflammasome activation of NLRC4 by flagellin is independent of TLR5 [99]. Despite the direct interaction of NLRC4 and pro-caspase-1, the adaptor molecule ASC still seems to be required for caspase-1 activation through NLRC4 [129–131], although the exact molecular mechanisms and composition of the inflammasome are not yet clear. Furthermore, in some cases, e.g., infection with *Legionella pneumophila*, another NLR termed NAIP5 (a mouse homolog of NAIP, which is the only human member of the NLRB subfamily) is crucially involved [132–134].

5. RIG-I-like receptors (RLRs)

The detection of viral RNA/DNA in dendritic cells is mainly achieved via TLR7, TLR8 and TLR9. Most other cell types, however, use different receptors for this detection, the so-called RLRs. The RLR family is named after the retinoic acid-inducible gene I (RIG-I), which was first shown to be involved in the innate immune response against double-stranded RNA by Yoneyama et al. [135,136] and comprises RIG-I, melanoma differentiation-associated gene 5, and laboratory of genetics and physiology (LPG) 2 [137]. Whereas the DExD/H helicase domain is shared by all three members, only RIG-I and MDA5 contain two CARD-like domains through which further signal transduction can occur. Generation of RLR knockout mice revealed that RIG-I and MDA5 are not redundant receptors: whereas RIG-I detects viruses such as hepatitis C virus, Sendai virus, rabies virus or Newcastle disease virus, MDA5 recognizes a number of picornaviruses and the synthetic ligand poly I:C [138,139]. How do RLRs distinguish between host and foreign RNA? Viral RNA usually contains a modified 5' triphosphate end, which is not present in capped or processed host RNA [140–142]. Interestingly, the absence of this

modification could enable viruses to escape immune detection by RLRs [143].

Because of the CARD-deficiency, LGP2 was believed and experimentally shown to act as a negative regulator of RIG-I and MDA5 [137,144–147]. However, independent generation of LGP2 knockout mice in two laboratories showed actually a more positive role in antiviral signaling [148,149], which could be explained by a mechanism where LGP2 works upstream of RIG-I and MDA5 [150].

RLR-induced signaling leads to NF- κ B, MAP kinase and IRF activation, which together are required for a robust type I interferon response. The molecule that bridges the recognition by RLRs with these signaling events was independently discovered by multiple groups and is known as mitochondrial antiviral signaling (MAVS), CARD adaptor inducing interferon (Cardif), interferon beta promoter stimulator-1 (IPS-1), or virus-induced signaling adaptor (VISA) [151–154]. MAVS does not only contain an N-terminal RIG-I-like CARD domain required for association with either RIG-I or MDA5 but also a C-terminal transmembrane domain required for mitochondrial targeting, implying critical involvement of mitochondria in this signaling pathway [152]. The generation of MAVS knockout mice resulted in a lack of viral-induced NF- κ B and IRF3 activation in numerous cell types except for pDCs, which use TLR7/TLR8 and thus were not affected [155,156]. The activation of MAVS through RIG-I and MDA5 causes its dimerization, initiating a platform for the recruitment of numerous additional adaptor and signaling proteins [157]. One arm include the activation of IRF3 and IRF7 and involves TRAF3, TANK, NEMO, NAP-1, IKK ϵ and TBK1 [86,158–162]. The other arm includes a complex of RIP-1/TRAFF6/TRAFF2 and the death domain adaptors TRADD and FADD, that eventually activates caspases-8/-10 and leads to activation of NF- κ B [151,163–165].

Recently, a new molecule involved in the activation of the Type-I interferon pathway has been described and named stimulator of interferon genes (STING) [166]. As so often, this gene has also been independently discovered by other groups and is therefore known as MITA, MPYS or ERIS as well [167–169]. Since STING-deficient mice were particularly sensitive to some viruses such as VSV or sendai virus, and MDA5 could not be co-immunoprecipitated with STING [166], it appears that STING is specifically required for RIG-I-mediated (and not MDA5 or TLR-mediated) interferon production. In addition, STING has also been shown to be required for intracellular DNA-mediated type I interferon production, however it does not contain a known DNA binding site and thus presumably works downstream of yet to be defined further cytosolic DNA sensors [170].

Finally, the NLR protein NLRX1 has also been shown to be involved in the regulation of RIG-I-mediated antiviral response [171]. NLRX1 is unique among the NLRs in that it does not contain an N-terminal effector domain such as CARD, PYRIN or BIR, but instead a mitochondrial-targeting sequence [172]. Since overexpression of NLRX1 results in decreased NF- κ B and type-I interferon production [171], NLRX1 was believed to act as a negative regulator. However, other reports find NLRX1 involved in positive regulation of reactive oxygen species generation, NF- κ B and JNK signaling [173,174].

6. Influence of innate sensors on allergy and asthma

The prevalence of allergies and asthma substantially increased in the last decades of the 20th century, in particular in developed countries [175,176]. Concomitantly, westernized lifestyle led to an increased urbanization, higher hygiene standards and diminished rural living conditions. One explanation for that increase in allergic diseases was the so-called “Hygiene hypothesis” [177], which was based on the idea that a lesser degree of infections during childhood increases allergic diseases in later life. In the

beginning of this century, a vast number of studies showed that growing up on farms significantly reduces the risk of developing allergic diseases such as asthma, hay fever, or atopic sensitization [178–184]. Strikingly, exposure to cell-wall components of Gram-negative bacteria (LPS) [185,186] or Gram-positive bacteria (MDP) [187] has been shown to be inversely correlated with allergic sensitization or asthma and wheeze, respectively. These data clearly demonstrate that activation of the innate immune response in early life (or during pregnancy, for that matter [188]), has a tremendous impact on adaptive immunity later on. Conceptionally, two different models of how to explain the hygiene hypothesis in immunological terms are discussed: “missing immune deviation” (i.e., no shift from Th2 to Th1) or “reduced immune suppression” (no activation of regulatory T cells) [189]. Investigations on specific bacterial strains isolated from cow sheds that took part in the above mentioned farmer studies in rural parts of Austria, Bavaria and Suisse clearly favored the “missing deviation” model [190,191].

Asthma and allergy are multifactorial and complex diseases, with a strong genetic and environmental background. To identify genes that are crucially involved in asthma and allergy, different genetic approaches have been used over the last decade: positional cloning based on family studies and linkage analysis, candidate gene approach based on testing of genetic variants, and, due to the rapid progress of high-throughput genotypic technologies, genome-wide association studies [192]. The first “innate immune” gene that was associated with modified risk of allergy was CD14, a GPI-linked molecule on monocytes/macrophages and also as soluble form in serum [193,194]. CD14 is not only is particularly involved in LPS/TLR4 signaling [195], it also binds other microbial molecules and enhances their signaling [196,197]. A polymorphism identified in the CD14 gene (rs2569190) was in the promoter region and modified the expression level of the soluble form of CD14. The relevance of this polymorphism for allergic diseases, however, was controversial and depended highly on the specific setting of the studies [198–204]. Apparently, the different outcomes were caused by different levels of endotoxin (LPS) exposure: high endotoxin plus polymorphism led to less allergy [205,206], whereas low endotoxin plus polymorphism led to an opposite outcome, which exemplifies the remarkable importance and complexity of gene-environment interactions [207–209].

6.1. TLRs and asthma/allergy

Not surprisingly, the first two TLRs investigated for their impact in asthma and allergy were TLR4 and TLR2. One of the TLR4 SNPs, rs4986790, leads to a decreased responsiveness towards LPS [210,211], but with respect to the impact on asthma and allergy, as has already been seen with CD14, the results were conflicting and ranging from association with higher prevalence of asthma over no effects to a reduced prevalence of hay fever and atopy [212–219]. The relevance of TLR2 gene polymorphisms were first seen in farmer's children, where a SNP in the TLR2 promoter was significantly associated with a lower risk of asthma, atopy and hay fever [220], but in addition, a number of other associations have been found [221,222]. As TLR1 or TLR6 are essential for TLR2-mediated signaling, it does not surprise that in these two genes polymorphisms were found to be involved in asthma as well [223], however, an association to allergy or atopic diseases has not been found [224]. Interestingly, also polymorphisms in the TLR10 gene have been identified and linked to asthma [223,225]. As already mentioned (s. above), TLR10 appears unable to induce signal transduction upon ligand binding [18], thus, as of today, the functional relevance of engaging TLR10 (that is, the TLR2/TLR10 heterodimer) and the reason for its impact on asthma is unclear. Whereas there

are currently no data on associations with TLR5, TLR7 and TLR8 have been recently identified as novel risk genes not only for asthma, but also for atopic disorders such as rhinitis or atopic dermatitis [226]. The SNPs showing the most consistent associations were presumably of functional relevance, affecting either the processing of the protein (TLR7) or the splicing of the mRNA (TLR8). In a small study, a promoter polymorphism in TLR9 has been found to be associated with asthma [227], and similar findings were found in another study as well [228]. However, larger studies revealed that this polymorphism has apparently no role in asthma susceptibility [229–233].

It might be tempting to speculate that polymorphisms in genes coding for adaptor proteins in TLR signaling pathways should be even more likely associated with asthma and allergy. Strikingly, no associations have been reported for MyD88, TRIF, or NF- κ B yet. Further downstream pathway molecules have also been investigated and some associations have been identified: polymorphisms in IRAK-M, a regulatory protein in the TLR pathway, are associated (either as protective or predisposing SNP) with early-onset persistent asthma [234]. Another IRAK molecule, IRAK-4, was linked to serum IgE levels, one of the hallmarks of asthma and other allergic diseases [235]. Interestingly, in the same study there was no association of SNPs in TLRs 1, 2, 3, 4, 6, 9, or 10, CD14, or MD2 with respect to serum IgE. An additional regulatory TLR-pathway genes that was implicated in asthma or allergy is IL1RL1 (also known as ST2). IL1RL1 is an inhibitor of IL-1R and TLR4 receptor signaling [236], involved in inducing Th2-associated cytokines [237], and SNPs have been found associated with atopic dermatitis [238] and asthma [239–241]. The relevance of the IL1RL1–IL18R1 gene locus on chromosome 2 has recently been confirmed in a “large-scale, consortium-based genome-wide association study of asthma”, genotyping 10,365 asthmatics and 16,100 unaffected controls [242].

6.2. NLRs and asthma/allergy

The involvement of NLRs in inflammatory disorders and diseases is well established [243,244]. With respect to asthma and allergy, relevant SNPs have only been identified in NOD1, but also in NOD2 and NLRC4. Polymorphisms in NOD1 are associated with increased risk of developing asthma, atopic dermatitis and total IgE levels [241,245–247]. As it has already been seen with CD14-SNPs and different levels of LPS exposure (s. above), also SNPs in NOD1 contribute significantly to the protective effect of farming environment [248]. NOD2 polymorphisms are involved total IgE levels [241] and in atopic disorders, but not in asthma [249]. Besides NOD1 and NOD2, only few NLR gene polymorphisms have been investigated with respect to asthma and allergy. However, given the increasing importance of this family, this likely will change in the future. Recently, an association with susceptibility to food-induced allergy and aspirin-induced asthma has been found for functional SNPs in NLRP3 [250].

6.3. RLR and asthma/allergy

Viruses and virus infections are undoubtedly involved in asthma. They contribute a great deal to asthma in infants and children, and up to 85% of acute asthma exacerbations are caused by viruses. This being said, one would assume that polymorphisms in the receptors that are responsible for the detection such as the RLRs are likely to be associated with asthma or allergy. However, as of today, no SNPs have been found in RLRs that are linked to allergic diseases. On the other side, this family of receptors is relatively young and, without question, the number of investigations of this connection will greatly increase in the future.

7. Conclusion

Innate immune receptors such as the TLR, NLR and RLR families are primordial innate immune receptors. Not only do they recognize essential microbial structures, they also are able to detect intrinsic danger molecules. In particular when it comes to allergic diseases, the close connection and interrelation of innate and adaptive immune reactions is now clearly appreciated. In order to design therapeutical approaches that are able to trigger the innate immune system in early-childhood in an “allergy-protective” way, a better knowledge of the molecular mechanisms of how innate immunity shapes the adaptive response is still needed.

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