

Editorial

Toll-like receptors: sensors of the innate immune system

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The major role of the innate immune system is to react rapidly to infectious agents with the initiation of an inflammatory response, and to shape subsequent adaptive immune responses. Activation of the innate immune system is mediated by pattern recognition receptors (PRRs) on dendritic cells (DCs), macrophages and polymorphonuclear granulocytes that recognize pathogen-associated molecular patterns (PAMPs). The best characterized signalling PRRs so far are the toll-like receptors (TLRs) that are present in plants, invertebrates and vertebrates and represent a primitive host defence mechanism against bacteria, fungi and viruses (1–3).

At least 10 mammalian TLRs have been described, all transmembrane proteins possessing repeat leucine-rich motifs in their extracellular domains. The cytoplasmic domain of the TLR family shares extensive homologies with the interleukin (IL)-1 receptor superfamily and is called the toll-interleukin receptor (TIR) domain (4, 5). Ligand binding to TLRs leads to a common signal transduction pathway involving TIR which couples to adaptor molecules including MyD88 (6), which binds to TLR2, TLR4 and TLR9 (7), and MAL/TIRAP, an MyD88 homologue that binds to TLR4 (8, 9). Subsequently, a signalling complex is formed including the IL-1R-associated kinases (IRAKs), Tollip and tumour necrosis factor (TNF) receptor-associated factor 6 (TRAF-6), and from this signalling complex, at least three additional kinases are activated, ultimately resulting in the phosphorylation of inhibitor of nuclear factor κ B (NF- κ B) (I κ B) and activation of the NF- κ B pathway. This is of great significance with regard to human disease because the NF- κ B pathway is involved in the regulation

of about 40% of the human genes (10) and is particularly involved in the production of inflammatory cytokines including IL-1 and TNF- α . The NF- κ B pathway is also important in cell stimulation by these cytokines, resulting in production of tissue-degrading enzymes and co-stimulatory molecules necessary for induction of B- and T-cell responses (11, 12). It is interesting, however, that activation of different TLRs can result in different reactions within the cell. For example, TLR2 signalling not only activates proinflammatory gene transcription, but can also activate proapoptotic pathways resulting in inflammatory cell death (13, 14). The ways in which different TLRs couple to cause various cellular signalling outputs remain to be fully explained, but are the major subject of current research.

To better characterize the aetiology of innate immune system activation it is of particular interest to know the ligands of distinct TLRs. TLR2 being expressed on myeloid blood dendritic cells and macrophages appears to be the primary regulator of responses to Gram-positive organisms such as peptidoglycans (PGs) from *Staphylococcus aureus*, and lipoproteins from *Borrelia burgdorferi*, *Mycobacteria*, *Listeria* and *Mycoplasma*, and may also react with endogenous heat-shock protein and necrotic cells. TLR4 is the receptor for lipopolysaccharide (LPS) as part of a complex with LPS-binding protein from serum, soluble or membrane-bound CD14, and another membrane protein called MD2 (15). CD14 may also play an important role in the innate immune system as a receptor on macrophages for apoptotic cells (16). Apoptotic cells are thought to express a series of specific membrane structures called apoptosis-cell-associated

molecular patterns (ACAMPs) that bind to PRRs on phagocytic cells. Whereas LPS stimulation of macrophages is proinflammatory, resulting in the production of IL-1, TNF- α , and other cytokines, ingestion of apoptotic cells is anti-inflammatory (17). Furthermore, plasmacytoid DCs present TLR9 which binds oligodeoxynucleotides with unmethylated cytosine-guanosine-containing motifs (CpG DNA) and immature DCs are presumed to bind viral double-stranded RNA like poly:IC by TLR3. The function of other mammalian TLRs, whether different PAMPs bind only to specific TLRs, and what signal transduction pathways are involved are as yet less well characterized.

There is growing evidence that endogenous molecules can also activate innate immune cells through TLR4 binding. These are heparan sulphate, hyaluronan, heat-shock proteins, fibrinogen, fibronectin and defensins (18, 19). The latter are small antimicrobial peptides of the innate immune system produced in response to microbial infection of mucosal tissue and skin. At least in mice the β -defensin 2 has been shown to act directly on immature dendritic cells as an endogenous ligand for TLR4 inducing upregulation of co-stimulatory molecules and dendritic cell maturation. These events, in turn, trigger robust, type 1 polarized adaptive immune responses *in vivo* (19).

In the August 2003 issue of *Allergy*, Claeys et al. (20) presented the results of a detailed study measuring the expression of human β -defensins (HBD) 2 and 3 and of TLR2 and 4 in various upper airway tissues and in different disease states. They found a strong HBD-2 expression only in tonsillar tissues in contrast to adenoid or nasal mucosa in accordance with Weise et al. (21) and they observed no significant difference in HBD-2 mRNA

expression between patient groups with either idiopathic hypertrophic tonsils or recurrent tonsillitis. The absence of HBD-2 and HBD-3 mRNA in adenoids and paranasal mucosa underlines that normal bacterial triggering in these tissues is insufficient to stimulate defensin expression and production possibly due to the relatively short contact time with environmental microorganisms because of the intensive mechanical clearance in the nose and nasopharynx. In contrast to HBD-2 and HBD-3 expression the authors found no significant difference of expression of TLR2 and TLR4 between the different patient groups with tonsillar and sinonasal disease. That may reflect the fact that microbial or inflammatory triggering in these upper airways conditions provokes the same TLR2 or TLR4 expression pattern and that only invasive pathogens might induce a strong innate immune response by influencing the TLR expression. Finally, TLR expression as found in this study illustrates a possibly important immunological sentinel function of upper airway mucosa.

The ability to mount a prominent inflammatory response to exogenous microbial pathogens or to endogenous stimuli may confer at the same time an advantage in innate immune defence and may signal an increased risk of inflammatory damage to affected tissues. Functional polymorphisms in human TLR4 have been described and appear to influence airway reactivity (22) and atherogenesis (23). Therefore, it is a very important current research issue to see if such polymorphisms in human TLRs correlate with risks of various chronic inflammatory diseases. This is particularly relevant because once allergic, nonallergic or even autoimmune disease are established, infectious triggers may worsen inflammation by TLR signalling.

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