TLRs and chronic inflammation

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Abstract:

After the discovery of Toll-like receptors (TLRs), innate immune mechanisms came back in the focus of scientific research. With more and more mechanisms of TLR biology known, it has become clear that these and also other innate immune receptors are not only of crucial importance in the immune response to invading pathogens, but also play a role in the homeostasis of commensal flora and in the response to stress and danger signals. In this respect, increasing evidence is found that inappropriate quantity or quality of TLR ligands or aberrant response to TLR activation plays a role in a variety of chronic inflammatory diseases. In this review, an overview of the currently known TLRs and their signaling pathways is given and reports about their expression and activation in chronic inflammatory diseases are recapitulated.

INTRODUCTION

The innate immune system responds to invading pathogens by the activation of a pro-inflammatory cascade aiming at eradication of the infectious agents. Pattern recognition receptors (PRR) are crucial parts of this innate immune reaction. A variety of intracellular and extracellular PRRs are known up to date. Among others the growing family includes Toll-like receptors (TLR), Nod-like receptors (NLR), and RIG-like receptors (RLR). Out of this intricate system of innate immune receptors, TLRs are the best characterized. Each PRR specifically recognizes conserved fragments of pathogens, so-called pathogen associated molecular patterns (PAMPs), which can be found on Gram-positive and -negative bacteria, DNA and RNA viruses, fungi and protozoa. Thereby a signaling cascade is started that leads to the production of pro-inflammatory cytokines, type-1 interferons and chemokines, and promotes direct killing of the pathogen, activates phagocytosis and influences adaptive immune responses.

In recent years it has become clear that activation of TLRs is not restricted to the initiation of innate and adaptive immune reactions. In the gastrointestinal tract for instance, TLRs and their interaction with commensal microflora seem to be required for the maintenance of normal homeostasis (1). Aberrant activation of TLR pathways on the other hand, has been implicated in various chronic and autoimmune diseases affecting the gastrointestinal tract, the central nervous system, kidneys, skin, lungs and joints. Thereby, not only exogenous but rather endogenous ligands have been suggested to act as TLR activators. Evidence that intracellular proteins or products of protein cleavage can act as endogenous ligands for TLRs supported the hypothesis that TLRs are not only of importance in mediating a response to infections but to stress, damage and death of cells in general (2-4). For instance, expression of IL-8 by macrophages in response to cigarette smoke extract was found to be mediated by TLR4 signaling (5).

In this review, the biology of TLRs and their most important signaling pathways are summarized. Furthermore, we discuss what is known and what is hypothesized about the role that TLRs might have in the development and maintenance of chronic inflammatory and autoimmune diseases.

TOLL-LIKE RECEPTORS AT A GLANCE

The theoretical concept that the innate immune system uses specialized receptors to recognize invading pathogens was already developed before TLRs were found to be connected to innate immune mechanisms (6). The first descriptions of the Toll protein in *Drosophila* related its function to the establishment of dorsoventral polarity in the developing *Drosophila* embryo (7, 8). Due to structural and functional similarities between the IL-1/NF-κB inflammatory response pathway of mammals and dorsoventral patterning pathways in *Drosophila* embryos, mutations affecting dorsoventral patterning were analyzed in regard to the expression of antifungal and antibacterial peptides. It was found that the Toll ligand Spz (Spätzle) controlled the expression of antifungal genes in adult flies and that mutation of Toll drastically reduced survival of these insects after fungal infection (9). Thereby for the first time the Toll proteins were connected with immune responses. In search for human homologues of Toll, at the same time two independent groups identified human TLRs and could show that they play a role in the activation of innate as well as adaptive immune response in mammals (10, 11). To date, ten different TLRs have been discovered in humans and for most of them also their specific ligands are known. They all have the same basic structure of a type 1 transmembrane glycoprotein receptor. However, whereas some TLRs are localized at the cell membrane (TLRs 1, 2, 4, 5, 6, 10), others are anchored in the endosome (TLRs 3, 7, 8, 9) and therefore lie intracellularly. The N-terminal end of all TLRs contains a leucine-rich repeat (LRR) motif and mediates ligand binding. The cytoplasmic domain is highly conserved and termed Toll-IL-1R (TIR) domain according to the strong similarity

between the *Drosophila* Toll and the mammalian IL-1R1 protein (12). The TIR domain functions as binding site for downstream adaptor molecules. It is understood that after binding of a ligand, TLRs homodimerize or heterodimerize and then the signal is mediated via adaptors to downstream signaling proteins. A variety of these adaptors and signaling molecules has already been discovered (for extensive review see (13)). Differential usage and combinations of these downstream molecules allow the adjustment of the response according to a specific PAMP. However, the mechanisms that provide diversification of TLR effector functions are not known in detail. Roughly, two main adaptor pathways can be distinguished (Figure 1). In one pathway, the main adaptor protein is MyD88, which binds directly to the cytosolic domain of TLR5, 7, 8, and 9. To TLR2 and TLR4 MyD88 is recruited to the TLR-TIR domain via Mal (MyD88 adaptor-like) also called TIRAP (TIR-domain-containing adaptor protein) (14). Depending on the downstream signaling molecules, activation of the MyD88 pathway can lead to induction of gene expression via the transcription factors NF-κB, AP(activating protein)-1 or IRF(interferon-response factor) 1, 5 and 7 (15-17). In the other pathway, recruitment of TRIF (Toll-receptor-associated activator of interferon) transmits the signal downstream, which mainly leads to activation of IRF3, but can also induce activation of NF-κB (18, 19). Whereas TLR3 is the only TLR that exclusively uses the TRIF-pathway, TLR1, 2, 5, 6, 7, 8, 9 and 10 are restricted to the MyD88 pathway. Exceptionally, TLR4 can signal via both pathways. Recent data propose that TLR4-ligand-binding first leads to activation of the MyD88 pathway at the plasma membrane. Subsequently, TLR4 gets internalized and in a second step activates the TRIF pathway via TRAM (TRIF-related adaptor molecule) at the endosomes (20). These findings nicely fit the hypothesis that endosomal TLRs like TLR3, 7, 8, 9 and probably TLR4 rather induce a type-1 interferon response by activation of the IRF transcription factors, whereas TLRs located at the plasma membrane like TLR1, 2, 6, 5, 10 and 4 mainly lead to activation of the NF-κB pathway (21, 22).

The exact mechanism by which TLRs differentiate between the diverse PAMPs and also by which one TLR can sense more than one PAMP is not yet fully understood. It is assumed that specific insertions in LRR of each TLR provide specific ligand binding sites (23). Due to technical hindrances, crystallographic pictures elucidating TLR/PAMP binding sites are hardly available. Even though the molecular structure of the TLR3 ligand-binding site has been solved, the results allow different conclusions about its ligand binding. Whereas one group suggests the convex, outside face of the horseshoe-shaped receptor as potential binding site, another group favors the inner, concave surface as site of ligand interaction (24, 25). A study showing the structure of mouse TLR3 binding double stranded RNA actually suggests that both groups are right and that there is one binding site on the convex and one on the concave face of the molecule (26).

Various pathogens have developed mechanisms by which they prevent the activation of the TLR cascade after recognition of their PAMPs. Viruses and bacteria have been found to produce proteins with strong similarity to the TIR domain of TLRs. By their binding of MyD88 instead of TIR, they block further signaling, prevent activation of the innate immune system and increase virulence. Examples for such virulence factors are A46R produced by vaccinia virus, TIR-like protein A from *Salmonella enteritica* and TcpC from *Escherichia coli* CFT373 (27-29).

But not only pathogens try to repress TLR activation, also a variety of endogenous mechanisms exist to contain the pro-inflammatory TLR cascade. A soluble form of TLR2 was found to be expressed in humans and soluble TLR4 was measured in mice (30, 31). These soluble forms might function as decoy receptors, blocking TLR responses by binding to co-receptors or ligands. In the past years a variety of intracellular inhibitors of TLR pathways were described, which attenuate TLR responses in a negative feedback loop. For instance, the dual specificity phosphatase MKP-1 (MAPK phosphatase) was shown to be acetylated after stimulation of TLR4. Acetylation of MKP-1 blocks the MAPK pathway and inhibits innate

immune signaling (32, 33). Other negative regulators of TLR signaling are SOCS1, TOLLIP (Toll-interacting protein) or IRAKM.

TLRs are expressed in a wide variety of immune as well as on non-immune cells (table 1). Whereas dendritic cells (DCs), neutrophils or macrophages express an almost complete panel of the different TLRs, other cells only express a restricted repertoire. Naturally, expression of TLRs is of particular importance in cells that lie at sites of high host-pathogen interactions such as intestinal or airway epithelial cells or keratinocytes. However, in line with the above mentioned hypothesis, that TLRs are activated in response to danger signals in general, functional TLR expression has also been found in cell types which do not fulfill such an obvious barrier function. Thus, functionally active TLRs were found to be expressed in different cells of the central-nervous system (CNS) and kidney, in cardiomyocytes and synovial fibroblasts and interestingly also seem to be involved in non-infectious pathologies such as ischemic or traumatic injury or autoimmunity (34-38). Also, a relation between expression of TLRs and vasculopathies has been suggested based on the fact that different arterial segments such as aorta, subclavian or temporal arteries express a distinct pattern of TLRs (39).

TOLL-LIKE RECEPTORS IN DETAIL

TLR1: TLR1 is anchored in the plasma membrane and mainly seems to signal as heterodimer together with TLR2 (40, 41). In this complex TLR1/2 recognize tri-acetylated lipopeptides, which are coupled to the peptidoglycan layer of bacteria such as *M. tuberculosis* or *B. burgdorferi* (40, 42). It was also found that porins of outer membranes of Neisseria need the TLR1/2 complex to be recognized (43). Interestingly, also TLR10 has found to heterodimerize with TLR1 (44). The ligand of this complex is however not yet known. TLR1 is ubiquitously expressed in leukocytes but is also found in non-immune cells such as astrocytes, fibroblasts, keratinocytes (45), endothelial (46) and epithelial cells (47).

TLR2: The expression pattern of TLR2 resembles the one of TLR1 but with a remarkably high expression in monocytes and low expression in lymphocytes (47). Interestingly regulation of TLR2 expression was shown to differ between various cell types according to their specific function. During wound healing, expression of TLR2, but not TLR1, TLR4 or TLR6 was found to be up regulated in keratinocytes by a 1,25-dihydroxy-vitamin D_3 dependent mechanism, probably to prevent wound infection (48). In monocytes on the other hand, 1,25-dihydroxy-vitamin D_3 down regulates TLR2, containing the activated immune cascade (49).

TLR2 signals as a heterodimer with TLR1, TLR6 or TLR10 (44). The ligand for TLR2/TLR10 is currently not known. As mentioned above TLR1/2 recognizes tri-acetylated lipopeptides. In contrast TLR6/2 recognizes di-acetylated lipopeptides (50). Additionally numerous further ligands for TLR2 have been found, making TLR2 to the one TLR with the broadest spectrum of ligands. For instance, bacterial cell wall constituents such as lipoteichoic acid or peptidoglycans, mycobacterial cell wall components like lipomannans and the yeast cell wall component zymosan were reported to induce TLR2 activation (50-52). Also proteins from parasites such as *Trypanosoma cruzi* or *Schistosoma mansoni* are recognized by TLR2 (53-55), and it was suggested that TLR2 might play a role in the response to infection with viruses from the herpes family (56-58). In addition a variety of endogenous ligands have been proposed such as eosinophil derived neurotoxin (59), HMGB1 (high mobility group box) (60), and different heat shock proteins (61, 62). The versatility of TLR2 in binding such variable ligands has been explained by its ability to build heterodimers with other TLRs and to use co-receptors for the recognition of certain molecules. In this respect, CD14 was shown to enhance the response to TLR2 ligands (63, 64), and also CD36 was found to influence TLR2 dependent response to di-acetylated lipopeptides and lipoteichoic acid (65). Furthermore, the beta-glucan receptor dectin-1 is required for the recognition of zymosan by TLR2 (66). Some concern however was raised whether such “promiscuity” of a PRR does not

contradict the need for specificity, and it was postulated that lipopeptides are actually the only activating ligands for TLR2 and that most of the tested ligands contained contaminations of lipopeptides (67). After all, also the TLR4 ligand lipopolysaccharide (LPS) was first wrongly identified as TLR2 ligand due to contaminations of lipoprotein in the preparations (68, 69). Whether TLR2 is really capable of binding such a variety of ligands or not, future studies hopefully will tell.

TLR3: TLR3 differs from all the other TLRs since, as mentioned above it is the only TLR not to signal via MyD88 but exclusively via TRIF, mainly leading to the production of type I interferon regulated genes. In leukocytes, TLR3 is expressed by DCs, macrophages, mast cells and natural killer (NK) cells. But it was also found to be strongly expressed in non-immune cells like fibroblasts, keratinocytes, astrocytes, oligodendrocytes, epithelial and endothelial cells (45, 70, 71). Even though in most of these cells TLR3 is located in the endosomes, in some cells e.g. fibroblasts TLR3 was also described to be expressed on the cell surface (72). TLR3 binds double stranded (ds) RNA from viral sources but also as endogenous ligand from necrotic cells (70, 73). Similar to TLR2 and TLR4, TLR3 response seems to be enhanced by the co-receptor CD14 (74). There is uncertainty as to which role TLR3 plays in response to viral infections in particular in the light of the newly discovered, cytoplasmic PRRs RIG-I (retinoic acid-inducible gene) and MDA5 (melanoma-differentiation-associated gene), which also sense viral RNA (75, 76). Also, in some viral infections TLR3 signaling seems to have detrimental rather than protective effects. TLR3 deficient mice, for instance have more chance to survive an infection with Influenza A virus and Punta Toro virus than wild type mice (77, 78). And West Nile virus use the TLR3-induced inflammatory response to enter in the host cells (79). As discussed in more detail below, TLR3 signaling has been associated to the development of various autoimmune diseases like autoimmune liver and kidney diseases, diabetes or rheumatoid arthritis (70, 80-82). Together with its opposing roles in different viral infections these findings suggest that

TLR3 might play an important role in the delicate balance between tolerance and immune response.

TLR4: TLR4 was the first of the human TLRs to be identified. It responds to LPS from Gram-negative bacteria. The actual part of the LPS molecule that is necessary to be recognized by TLR4 is the lipid A component. Recognition of lipid A by TLR4 leads to production of a wide range of immunostimulatory cytokines and chemokines mediated by MAPK (mitogen-activated protein kinases), AP-1, NF-κB and IRF5 pathways. For signaling, TLR4 is dependent on the presence of CD14 and MD-2 with which it forms a complex (83). Similar to TLR1, 2 and 3, TLR4 was found to be expressed by monocytes, but also by polymorphonuclear (PMN) cells and DCs, at low levels in B cells, on fibroblasts, astrocytes, keratinocytes, myocytes, endothelial cells and epithelial cells (47, 84-86). A number of endogenous ligands for TLR4 have been described. However, since a lot of these experiments were conducted with recombinant proteins from *E.coli,* in hindsight it is probable that some of the results stem from the usage of preparations contaminated with endotoxin (LPS). Heat shock protein Hsp70 for instance has been described as endogenous TLR4 ligand by different research groups (61, 87, 88), but later studies could not confirm these results and showed that tested, LPS-free Hsp70 had no TLR4 dependent effects (89-92). Further endogenous ligands have since then been described for TLR4. However, not in all cases it was tested whether their activating properties might be due to LPS contaminants. Most interestingly various extra-cellular matrix proteins like fibrinogen, fibronectin, heparan sulfate, and hyaluronan were also shown to activate TLR4 as endogenous ligands (93-96).

TLR5: The only known ligand for TLR5 is flagellin, the major protein of the flagella of Gram-negative bacteria (97). TLR5 is widely expressed in different cell populations. It can signal as a homodimer, but it can also build a heterodimer with TLR4, which results in the activation of an alternative downstream signaling pathway. Whereas binding of flagellin to

TLR5 homodimers stimulates activation of NF-κB, its binding to heterodimers leads to the production of type-1 interferons and nitric oxide (98).

TLR6: As described above TLR6 heterodimers with TLR2 and binds specifically to di-acetylated lipopeptides. Expression of TLR6 is found in similar cell populations as TLR1, or TLR2. Interestingly both TLR6 and TLR1 are highly expressed in B-cells, whereas TLR2 is only little expressed (47).

TLR7: The expression of TLR7 is much more limited than the expression of the previously described TLRs and seems to be restricted to DCs and B cells. Remarkably, TLR7 shares its ligand with TLR8; both receptors recognize single stranded (ss) RNA from viruses or from endogenous sources (99). Even though the exact activating RNA sequences remain elusive, it is known that sequences rich in uracil (U) and guanosin/uracil (GU) are stimulators of TLR7 and TLR8 signaling (99). In addition, sequence specific recognition of small interfering RNA occurs independently of the content of uracil or guanosin (100). Despite the close relation of TLR7 and TLR8, studies with specific TLR7 and TLR8 agonists revealed differences in their signaling pathways and expression profiles. Whereas TLR7 agonists lead to the activation of interferon pathways in plasmacytoid DCs, activation of TLR8 lead to the production of pro-inflammatory cytokines in myeloid dendritic cells, monocytes, and monocyte-derived dendritic cells (101).

TLR8: Even though, both TLR7 and TLR8 recognize ssRNA, recent findings indicate that each of these receptors responds to specific RNA sequences (102). Using the distinct expression and cytokine pattern of TLR7 and TLR8, it was found that synthetic single-stranded oligoribonucleotides containing GU-rich sequences lead to an interferon and TNF response on all tested cells, reflecting activation of TLR7 and TLR8. In contrast, oligoribonucleotides containing adenosine(A)/U rich regions provoked production of TNF but not interferon in monocytes and myeloid DCs, but not in plasmacytoid DCs, hinting to a

selective activation of TLR8. This sequence specificity of TLR7 and TLR8 might have been evolved to modulate the innate immune response according to the invading virus.

TLR9: Together with TLR3, 7 and 8, TLR9 belongs to the family of nucleic acid recognizing TLRs located in the endosome. The ligand of TLR9 is DNA containing unmethylated CpG motifs (103). This type of DNA is quite common in bacteria and viruses; in contrast in mammalians CpG rich sequences are rare and mostly methylated. Nevertheless, it is described that DNA-containing immuncomplexes activate TLR9, which could be an important component in the pathogenesis of autoimmune diseases (104, 105). The expression profile of TLR9 in immune cells resembles the one of TLR7, being expressed in B cells and plasmacytoid DCs, but can additionally be found in intestinal epithelial cells and keratinocytes. Activation of these cells via TLR9 leads to their maturation and induces the expression of co-stimulatory molecules and interferon-inducible cytokines and chemokines (106, 107).

TLR10: The last discovered human TLR is TLR10. It has no rodent homologue and until now it is the only orphan receptor among TLRs. Its structure is highly related to TLR1 and 6 and consequently it signals in homodimers and heterodimers with TLR1 and TLR2. As for TLR7 and TLR9, its expression is only described in B cells and plasmacytoid DCs (44).

TOLL-LIKE RECEPTORS IN CHRONIC INFLAMMATION

Given the crucial role TLRs but also other innate immune receptors play in the initiation of innate as well as adaptive immune responses, their involvement in the pathogenesis of chronic inflammatory or autoimmune diseases is not surprising. Thereby, pathological reactions might arise from inappropriate response of the receptor due to a particular genetic background or from inappropriate quantity or quality of ligands (Figure 2). Evidence for both pathogenetic mechanisms has been found and will be discussed in the following for a selected set of diseases. Due to the variety of animal models used in each discussed disease, comparisons

and conclusions are often hard to draw. Therefore, we mainly concentrated on data obtained from patients. The mentioned animal models are summarized in table 2.

TLRs in inflammatory bowel disease (IBD): IBD, with its two main representatives, Crohn's disease and ulcerative colitis are chronic inflammatory diseases of the gastrointestinal tract which also include extra-gastrointestinal symptoms particularly in joints and skin. Taking the close contact the gastrointestinal tract constantly has to a variety of bacteria, the maintenance of tolerance against commensal flora but at the same time recognition of pathogens is of utmost importance. As mentioned above, it could be shown that intestinal TLRs, such as TLR2, and TLR4 contribute to intestinal homeostasis (108, 109). Their reaction to commensal bacterial products results in the production of protective factors such as TGFβ, defensins, keratinocyte-growth factors and cyclooxygenase-2, and thereby allows constant proliferation and differentiation of intestinal epithelial cells (1, 110). MyD88 -/- mice for instance have been found to produce very low levels of IL-6, TNF and KC-1, factors that are believed to support repair mechanisms after intestinal injury. These factors were also not inducible by injury in these mice. Furthermore, protection from experimental colitis after TLR9 and TLR3 stimulation has been described (111, 112). Both stimulations could protect IL-10 knock-out mice from the development of spontaneous colitis, indicating an IL-10 independent manner of this effect. Instead, the beneficial effect of TLR3 and TLR9 activation appears to be mediated by increased production of type I interferons (113). In accordance to these animal data, a small therapeutic trial with interferon β showed promising results in patients with ulcerative colitis (114). In the healthy intestine, epithelial cells mainly expresse TLR3 and 5. Interestingly, one study showed that in Crohn's disease but not in ulcerative colitis expression of TLR3 is downregulated as compared to healthy intestinal epithelial cells, whereas expression of TLR2 remains low in both diseases (115); however these data could not be confirmed by another study where levels of TLR3 were unchanged and levels of TLR2 were induced in patients with inflammatory bowel disease (116). On the other hand, in both

studies, expression of TLR4 was induced in patients with Crohn's disease as well as in ulcerative colitis. These data clearly show that expression of TLRs is regulated in a disease specific manner in inflammatory bowel disease. The cause and consequences of this regulation however remains unclear. A role for TLRs in the development of inflammatory bowel disease is also suggested by genetic studies, showing a positive or negative association of various TLR polymorphisms with disease development (117-119).

TLRs in psoriasis: Similar to the gastro-intestinal tract, also the skin is confronted with a vast amount of different bacteria. Dermal mechanisms to prevent constant activation of the immune system by the microflora include low TLR expression levels and diminished responsiveness of the specialized dendritic cells of the skin, the Langerhans cells (120). Keratinocytes express all known human TLRs except TLR7 and 8 (45, 121). Studies on the location of TLR expression in the epidermis showed that in normal skin TLR2 is expressed in keratinocytes of the basal level, whereas in psoriatic lesions expression of TLR2 is additionally found in more outer levels of the epidermis (121). Whether this aberrant expression is connected to epidermal hyperproliferation and disturbance of keratinocyte-differentiation typical for psoriatic skin has however not yet been studied. Similar to Crohn's disease also in psoriasis, TLRs seem differentially regulated. Similar to TLR2, expression of TLR1 is upregulated in upper epidermal layers. In contrast, levels of TLR5 are lower in psoriatic than in nonlesional epidermis. Most interestingly, several cases are described in literature where topical applications of the TLR7 agonist imiquimod lead to aggravation of psoriatic plaques (122-125).

TLRs in inflammation of the CNS: TLRs were found to be expressed in various cells of the CNS. Microglia, as immune cells express mRNA for TLRs 1-9, but not TLR10 (126). The main TLRs expressed in astrocytes and oligodendrocytes are TLR2 and TLR3 (86). Interestingly, also neurons are able to express TLR3 and its expression is induced in different CNS pathologies (127, 128). In particular, enhanced expression of TLR2 and 3 was found in

brains of patients with multiple sclerosis (MS). MS is a chronic inflammatory disease of the CNS which leads to progressive demyelination and neuronal injury. The role of TLR3 in MS is controversial. On the one hand, stimulation of TLR3 (and TLR4) in cultured microglia and astrocytes lead to the production of the chemokine CXCL10, a major chemoattractant for Th1 cells (126, 129). Levels of CXCL10 are high in the cerebrospinal fluids of MS patients and this chemokine is regarded as one of the major attractants of lymphocytes to the brain in MS (130). On the other hand, in the mouse model of MS, experimental autoimmune encephalomyelitis (EAE), TLR3 activation had immunoregulatory effects and suppressed demyelination by upregulation of interferon β (131). This finding is in line with the successful use of interferon β in the treatment of MS and the strong induction of interferon pathways by TLR3 via TRIF. Since MyD88 deficient mice are resistant to the development of EAE, a key role of MyD88 signaling TLRs in the pathogenesis of this model is undoubted (132).

TLRs in diabetes mellitus (DM): Recently also the etiopathogenesis of type 1 DM has been connected to pathological innate immune reactions. Polymorphisms of the genes coding for TLR2 and TLR3 have been found to be associated with the development of DM (133, 134). However these studies were done in Korean and South African Zulu study populations and it is not clear in how far these associations are true for other populations. Nevertheless, monocytes of patients with type 1 DM have increased expression of TLR2 and TLR4. Furthermore, in vitro activation of TLR3 by dsRNA leads to apoptosis of pancreatic β cells, a hallmark of type 1 DM in vivo (135). This is of special interest since onset of type 1 DM has repeatedly been suggested to be a sequela of viral infections.

TLRs in systemic lupus erythematodes (SLE): In recent years intensive research has been done to elucidate the role of innate immune reactions in the development of SLE. SLE is characterized by chronic inflammation that can involve the skin, joints, kidneys, the nerval system, lungs and serosal membranes accompanied by the occurrence of a typical set of auto-antibodies. It is known that in patients with SLE increasing amounts of debris of apoptotic

cells accumulate. This debris contains nucleic acids at high concentrations, creating the previously mentioned situation of disease pathogenesis due to inappropriate quantity of ligands. Indeed increased levels of circulating DNA have been found in patients with SLE and DNA isolated from sera of SLE patients activated mononuclear cells (136). Moreover, auto-antibodies against DNA or chromatin, a common finding in SLE, bind DNA released from dying cells, forming complexes which are most effective in stimulating DCs via TLR9 (137). Also, an increased number of B cells, particularly memory B cells and plasma cells, express TLR9 in active disease and in vitro serum from patients with SLE upregulated expression of TLR9 on plasma cells (138). Similar to DNA and DNA containing immune complexes, RNA and RNA containing immune complexes have been implicated in the pathogenesis of SLE via activation of TLR7 signaling pathways (139). Increased levels of interferon α have long been known to occur in SLE and have been associated with disease activity. It is feasible to assume that increased production of interferon α in SLE is a reaction to constant activation of TLR pathways. In mouse models of SLE some data suggest a role of TLR3 in aggravation of lupus nephritis (81). However up to now this hypothesis on the role of TLR3 in nephritis was not tested in human SLE. A genetic study revealed that a polymorphism in the stop codon of TLR5 abrogating TLR5 signaling conferred increased risk of infection but protected against the development of SLE (140). Unfortunately, further data on the role of TLR5 in SLE is missing, and whereas expression of mRNA for TLR2, 7 and 9 were found to be elevated in peripheral blood mononuclear cells of patients with SLE, TLR3, 4, 5 and 8 were not (141).

TLRs in rheumatoid arthritis (RA): RA is a chronic inflammatory joint disease, where the activated and hyperplastic synovium invades and destroys cartilage and bone. Activated synovial cells, in particular synovial fibroblasts secrete pro-inflammatory and matrix-degrading effector molecules. The trigger of this aberrant activation is unknown. In animals, injection of TLR ligands such as peptidoglycan, CpG DNA or dsRNA alone leads to the development of arthritis (142-144). Since studies in search for bacterial or viral remnants in

human affected joints were unsuccessful, it is of special interest that many of the endogenous ligands described for TLRs can be found in joints of arthritis patients, including fibrinogen, hyaluronan, and HMGB1. Furthermore, the heat shock protein B8 was found to be a ligand for TLR4 and is elevated in RA joints (145). Finally, necrotic synovial fluid cells releasing dsRNA were shown to activate TLR3 on synovial fibroblasts (70). In the synovium of patients with RA, TLR2, 3, 4 and 7 were found to be upregulated and stimulation of synovial fibroblasts with TLR ligands leads to expression of pro-inflammatory cytokines and chemokines (38, 146-148). A surprising insight in possible pathogenic mechanisms of TLR activation in arthritis was gained by using a spontaneous autoimmune arthritis mouse model (IL-1 receptor antagonist knock-out). The fact that TLRs in general play role in the development of arthritis in this model was suggested by the observation that germ-free mice did not show any signs of disease. Surprisingly, deficiency of TLR2 leaded to more severe disease, whereas TLR4 knock-out was protective. Abolished expression of TLR9 had no effect on the course of the disease (149). These findings suggest that TLR2 might have a protective role in rheumatoid arthritis, whereas TLR4 signaling seems to be detrimental. Nevertheless, treatment of RA patients with chaperonin 10 lead to a marked improvement of RA symptoms in 65% of treated RA patients (15/23 patients) (150). Chaperonin 10, also called heat shock protein 10 inhibits both, TLR2 and TLR4 signaling by blocking their endogenous ligand heat-shock protein 60. Of note, the anti-malaria drug hydroxychloroquine is one of the oldest drugs used successfully in the treatment of RA. Its disease modifying properties might stem from its inhibition of acidification of endosomes and thereby blocking signaling of TLRs located at the endosome (TLR3, 7, 8, 9) (151).

FUTURE PROSPECTS

In the past couple of years a vast amount of new insights could be gained about the role of TLRs in health and disease. More information about exogenous and endogenous ligands of

TLRs has been collected and the field of TLR functions has been widened from innate immune reactions to sensing of danger and stress signals in general. Even though studies in human disease and animal models strongly suggest a pathogenic role of TLRs in a variety of chronic inflammatory and autoimmune diseases, direct connections and functional pathways are largely still in the dark. With more studies to come in future, the clinical relevance of altered activation of TLRs and their pathways will hopefully arise. Furthermore, therapeutic modifications of TLR pathways hold a big potential that has to be explored. Up to now, TLR agonists have been mainly pharmacologically used due to their immunostimulatory properties. Thus, the application of TLR agonists as vaccine adjuvants, and as adjunct cancer therapies has been tested (for review see (152). Also, TLR9 agonists together with allergens have been shown useful in the immunotherapy of allergic hypersensitivities. TLR antagonists on the other hand, are produced for the treatment of severe sepsis and are in the development process for therapy of autoimmune diseases. At least in one mouse model of SLE, a dual inhibitor of TLR7 and 9 ameliorated disease symptoms (153). Indirect modulators of TLR pathways like the above mentioned chaperonin 10 or chloroquine have already proven their good efficacy in the treatment of RA.

To find therapeutic possibilities to modulate TLR functions it is also essential to learn how TLR signaling is regulated and conducted in the cell. In this respect some interesting studies were published recently. A study by Asagiri et al for instance showed that cathepsin K, a cysteine protease is crucial for the production of interleukins after TLR9 activation and that inactivation of cathepsin K leads to disruption of all downstream signaling pathways of TLR9. Accordingly, adjuvant-induced arthritis in rats, a model which is dependent on TLR signaling pathways after recognition of PAMPs could be ameliorated by inhibition of cathepsin K. Rats treated with the cathepsin K inhibitor had markedly less paw swellings and bone erosions (154). The exact mechanism of this regulation of TLR9 pathways by cathepsin K is not determined yet. Other interesting studies could show that the induction of gene

transcription by TLR pathways is regulated by epigenetic modifications, for instance by histone deacetylases (HDACs) (155, 156). HDACs are enzymes that remove acetyl groups from histones. Thereby usually gene expression is shut down. In contrast, the production of the interleukin 12 subunit p40 in DCs after TLR stimulation was found to be positively regulated by HDACs, probably by facilitating recruitment of transcription factors (157). Another group found that LPS induced TLR4 signaling at an early time point leads to differential chromatin modifications of promoters of TLR4 responsive genes, thereby influencing their expression during persistent LPS stimulation. Whereas some genes such as pro-inflammatory cytokines, the constant expression of which might be detrimental, are silenced, the expression of other genes that are still needed for anti-microbial defense is facilitated and magnified (158). By this way, the innate immune system prevents excessive inflammation, but maintains defense against persistent pathogens. In future, further studies linking TLR signaling to epigenetic modifications will possibly bring some more insights in how TLR activation changes the pattern of gene expression of a cell.

It should be kept clearly in mind that TLRs are not the only PRRs known. Whereas TLRs are responsible to recognize extracellular ligands, there is a group of PRRs specialized in sensing intracellular ligands such as NLRs and RLRs. Even though less data exists on these receptors, evidence for their involvement in the development of chronic inflammatory diseases increases. For instance, the expression of RIG-I and MDA5 is elevated in psoriatic lesions (159). Furthermore, NALP3, a NLR that assembles with ASC and caspase-1 to form the NALP3 inflammasome was shown to be activated by urate crystals. After activation, the inflammasome processes pro-IL-1β to active IL-1β, which is then released from the cells and leads to the inflammatory process seen in gout (160). Finally, polymorphisms of NOD2, a NLR recognizing intracellular peptides derived from peptidoglycans (murayml dipeptide; MDP), are strongly correlated with high risk to develop Crohn's disease (161). Similar to the suggested role of TLRs in intestinal homeostasis, also NOD2 seems to be crucial to maintain

microbial balance in the gut. Evidence for this is given by the fact that the most common of the NOD2 mutations, leading to insensitivity of NOD2 to MDP, is associated with low expression of defensins. A consequence of this lack of microbial defense might be bacterial overgrowth and mucosal inflammation (162). As the TLR family also the NLR and RLR families comprise various members differing in their ligand specificity and signaling pathways. A challenge for future research will be to resolve the intricate interactions of these three PRR families and to analyze how they contribute to chronic inflammation.

Table 1:

TLR	**Expression**	**Exogenous ligands**	**Endogenous ligands**
TLR1	Monocytes, macrophages, B cells, T cells, DCs, PMN, NK cells, non-immune cells (fibroblasts, astrocytes, epithelial cells, keratinocytes)	Tri-acetylated lipopeptides, porins	
TLR2	Monocytes, macrophages, DCs, PMN, non-immune cells (fibroblasts, astrocytes, epithelial cells, keratinocytes)	Lipopeptides, peptidoglycans, glycolipids, polysaccharides, viruses, whole bacteria	Hsp60; Hsp70; Gp96; HMGB1
TLR3	DCs, macrophages, mast cells, NK cells, non-immune cells (fibroblasts, astrocytes, epithelial cells, keratinocytes)	dsRNA	dsRNA
TLR4	Monocytes, macrophages, DCs, PMN, non-immune cells (fibroblasts, astrocytes, epithelial cells, keratinocytes)	LPS (lipid A)	Hsp60; Hsp70; Gp96; HMGB1; Fibrinogen, Surfactant protein A, Fibronectin extra domain A, Heparansulfat, β -defensin 2
TLR5	Monocytes, macrophages, T cells, DCs, PMN, non-immune cells (fibroblasts, astrocytes, epithelial cells, keratinocytes)	flagellin	
TLR6	Monocytes, macrophages, B cells, T cells, DCs, PMN, NK cells, non-immune cells (fibroblasts, astrocytes, epithelial cells, keratinocytes)	di-acetylated lipopeptides	
TLR7	B cells, plasmacytoid DCs	ssRNA	ssRNA
TLR8	Monocytes, myeloid DCs	ssRNA	ssRNA
TLR9	B cells, plasmacytoid DCs, GI epithelial cells, keratinocytes	CpG DNA	DNA, DNA-containing immuncomplexes
TLR10	B cells, plasmacytoid DCs	?	

Table 2:

	Animal model	Protective	Aggravating	references
IBD	IL-10 -/-	TLR3, TLR9		(111, 112)
	DSS colitis	TLR3, TLR9, TLR2, TLR4		(1, 111, 112)
MS	EAE	TLR3, MyD88 -/-	TLR9-/-, TLR4-/-	(131)
SLE	MRL(lpr/lpr)		TLR3	(81)
RA	IL-1Ra-/-	TLR2	TLR4	(149)

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Figure legends:

Figure 1: TLR signaling pathways

Figure 2: Activation of extra-and intracellular TLRs by various PAMPs or DAMPs leads to initiation of a signaling cascade which physiologically ends in the resolution of the pathological state. Inappropriate quantity or quality of activating TLR ligands (a), aberrant TLR signaling (b) or disturbances in inhibitory feed-back mechanisms (c) might be responsible for the persistent activation leading to chronic inflammation.