FEBS Letters 585 (2011) 3660–3666

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Toll-like receptors in rheumatic diseases: Are we paying a high price for our defense against bugs?

K.C.M. Santegoets, L. van Bon, W.B. van den Berg, M.H. Wenink, T.R.D.J. Radstake *

Department of Rheumatology, Radboud University Nijmegen Medical Centre, The Netherlands

ARTICLE INFO

Article history:
Received 18 March 2011
Revised 11 April 2011
Accepted 12 April 2011
Available online 16 April 2011

Edited by Richard Williams, Alexander Flügel and Wilhelm Just

Keywords:
Toll-like receptor
Rheumatologic disease
Autoimmunity
Innate immunity

ABSTRACT

In the last decade Toll-like receptor (TLR) research has led to new insights in the pathogenesis of many rheumatic diseases. In autoimmune diseases like systemic lupus erythematosus, rheumatoid arthritis and systemic sclerosis TLR signaling is likely to be involved in tolerance breakthrough and chronic inflammation via combined Fc gamma receptors and TLR recognition of immune complexes. Furthermore, inflammatory diseases like psoriatic arthritis and gout also show more and more evidence for TLR involvement. In this review we will discuss the involvement of TLR signaling in several rheumatic diseases and stress their similarities and differences based on recent findings.

1. Introduction

In daily clinical practice a rheumatologist oversees a wide spectrum of diseases, being (auto) inflammatory like gout and psoriatic arthritis (PsA) or more autoimmune like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and systemic sclerosis (SSc). Although these diseases differ considerably in their clinical symptoms, overlapping features identified over the past few years suggest a shared background of inflammatory cascades. Notable examples are the involvement of the intracellular signaling molecule myeloid differentiation primary response protein 88 (MyD88), the activation of the type 1 interferon (IFN) pathway and the presence of (endogenous) Toll-like receptor (TLR) ligands. These aberrant immune pathways generate severe inflammation of joints, skin and visceral organs. This severe inflammation may be mainly autoimmune related like in RA, SLE and probably in SSc. An autoimmune disease develops as soon as tolerance for self is lost and the adaptive immune response is aberrantly directed to host tissue. In most diseases there is a strong association with autoantibodies, but up till now intensive research has failed to explain the direct implication of these autoantibodies in the development of autoimmune diseases such as RA and SSc. Next to autoimmune diseases, with their clear involvement of the adaptive immune system and presence of autoantibodies, another important group of diseases involving innate immunity are the auto-inflammatory diseases. These are characterized by the absence of a clear involvement of the adaptive immune system and paucity in autoantibodies [1]. Gout is a well-established auto-inflammatory disease induced by the presence of urate crystals activating the innate immune system. Other diseases like PsA and Crohn's disease are also characterized by a clear involvement of the innate immune system and a lack of autoantibodies. The adaptive immune system, however, does seem to play a role in these diseases placing them in between auto-immune and auto-inflammatory diseases. Because of the absence of autoantibodies and autoreactive T cells these diseases appear to be more auto-inflammatory in character. It seems to be the unique combination of a genetically susceptible host and specific environmental triggers which enable these immunopathological events to develop.

The immune system is equipped with multiple pattern recognition receptors that recognize a wide range of pathogens and endogenous ligands the human body can be confronted with. The main pattern recognition receptors are TLRs, C-type lectins,

Abbreviations: SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; SSc, systemic sclerosis; PsA, psoriatic arthritis; TLR, Toll-like receptor; FcγR, Fc gamma receptor; IFN, interferon; DC, dendritic cell; pDC, plasmacytoid dendritic cell; MSU, monosodium urate

* Corresponding author. Address: Department of Rheumatology, Nijmegen Centre for Molecular Life Sciences (NCMLS), Nijmegen Institute for Infection, Immunity and Inflammation, Radboud University Nijmegen Medical Centre, Geert Grooteplein 8, 6500 HB Nijmegen, The Netherlands.

E-mail address: TRadstake73@gmail.com (T.R.D.J. Radstake).

0014-5793
doi:10.1016/j.febslet.2011.04.028

NOD-like receptors (NLRs), RIG-1-like receptors (RLRs) and cytosolic DNA sensors. Most pathogens express several TLR ligands and numerous host-derived TLR ligands are generated in inflamed or degenerated tissue. The activation of multiple TLRs leads to the abundant release of pro-inflammatory cytokines creating a volatile situation. In genetically predisposed individuals this may eventually lead to a breach in tolerance, culminating in autoimmune disease [2]. In this context, the study of the control of TLR responses is of high relevance in diseases such as RA, SLE and SSc. However, in other rheumatic conditions that are not necessarily considered autoimmune such as PsA and gout, TLRs also play a more important role than previously thought.

2. Toll-like receptors

TLRs are pattern-recognition receptors capable of potently activating many different cells. TLRs are highly expressed on most immune cells, but also on other cells including fibroblasts, chondrocytes, keratinocytes and endothelial cells. TLRs recognize both endogenous molecules, released upon cell activation/damage (damage-associated molecular patterns; DAMPs), and a wide range of conserved constituents from pathogens (pathogen-associated molecular patterns; PAMPs). At present, 10 TLR subtypes have been identified in humans, each having its specific ligands, cellular localization and expression profiles. TLR2 (as heterodimer in combination with TLR1 or TLR6) and TLR4 are extracellular receptors that are designed to recognize lipid-based structures both from gram-positive and gram-negative bacteria including lipopeptides and lipopolysaccharides (LPS), respectively. For TLR5, also expressed extracellular, the identified ligand is flagellin, a component of flagellated bacteria. For TLR10, which is believed to originate from the TLR1/TLR6 precursor, no ligand has been described thus far. TLR3, TLR7, TLR8 and TLR9 are generally addressed as intracellular receptors located in endosomal compartments and are involved in the recognition of nucleic acids derived from viruses, bacteria and the host. TLR3 is activated by double stranded RNA (dsRNA) and TLR7 and TLR8 by single stranded (ssRNA). Double stranded RNA (dsRNA) activates TLR3 and single stranded RNA (ssRNA) activates TLR7 and TLR8. DNA and more specifically unmethylated CpG DNA is recognized by TLR9 (a more extended overview is presented in [3]). Downstream TLR signaling involves a family of five adaptor proteins known as MyD88, MAL/TIRAP, TRIF, TRAM and SARM. All TLRs except TLR3 signal via MyD88, either directly or via MAL/TIRAP in the case of TLR2 and 4. TLR3 signals via TRIF, which can also be used by TLR4 via binding of the TRAM adaptor protein to TRIF. The fifth member of the adaptor protein family is SARM, which is an inhibitor of TRIF-dependent transcription factor activation. Through these adaptor proteins protein kinases are activated, such as the mitogen activated protein kinases (p38, ERK1/2, JNK), ultimately leading to activation of transcription factors among which activator protein-1 (AP-1), nuclear factor κB (NF-κB) and members of the interferon regulatory factor (IRF) family are the most thoroughly investigated nowadays (reviewed in [4,5]). These transcription factors induce the expression of various inflammatory cytokines including TNFα, (pro)IL-1β and IL-6, type I IFNs, and chemokines. The TLR system is highly specific in that distinct cellular responses are observed depending on the activated TLR with important effects on ensuing inflammatory and adaptive immune responses. Much of this specificity is likely to result from the use of various co-molecules and down-stream adaptor pathways by the various TLRs. For example CD14 and MD-2 function as co-receptors for LPS, fine-tuning cell-type specific effects of TLR4 and influencing the threshold for TLR4 signaling. In the last few years it has also become clear that TLRs not only induce cell activation, but can also modulate inflammatory responses. While simultaneous stimulation of some TLRs results in synergistic induction of cytokine production, TLR2 has been demonstrated to inhibit cytokine production induced by TLR4 or TLR7/8 in dendritic cells (DC) with clear effects on T cell responses induced [6,7]. The regulatory role of TLR2 also extends to regulatory T cells, which proliferate upon contact with TLR2 (in mice). This results in a temporal reduction of suppressive function that recovers after removal of the TLR2 ligand [8]. Human regulatory T cells are also modulated by TLR2, although this has resulted in more suppressive regulatory T cells in the absence of proliferation [9].

3. Systemic lupus erythematosus

SLE is a prototypic systemic autoimmune disease in which virtually every organ of the human body can be involved, often including the skin, kidneys, joints and the central nervous system. Autoantibodies in SLE are generally directed against dsDNA or proteins bound to DNA or RNA, such as the Sm proteins (a protein complex that binds small nuclear RNAs). Levels of anti-dsDNA antibodies reflect disease activity in SLE, especially the lupus nephritis activity [10], suggesting an important role for these autoantibodies in the disease pathogenesis. Although the pathogenesis of SLE still remains unclear, the defective apoptotic clearance seen in many SLE patients could help us explain the excessive release of host DNA and RNA, which in combination with autoantibodies could lead to continuing immune cell activation via TLRs [11].

Normally, apoptotic cells are rapidly cleared by phagocytic cells and they have an immunosuppressive effect on macrophages/DCs, preventing autoimmunity. However, in approximately 40% of the SLE patients the clearance of apoptotic cells is impaired, resulting in the release of nucleosomes tightly bound to high-mobility group box 1 protein (HMGB1, a DNA binding protein). While nucleosomes normally have low immunogenicity, binding of HMGB1 during late apoptotic processes promotes immune cell activation. In non-autoimmune mice, HMGB1-containing nucleosomes from apoptotic cells have been shown to induce anti-dsDNA and anti-histone antibodies in a TLR2 dependent manner (reviewed in [11]). The release of HMGB1–nucleosome complexes from late apoptotic cells could therefore also play an important role in the induction of autoantibody production via TLRs in SLE patients. In addition, it was shown that monocytes cultured in the presence of SLE serum develop into functional DCs that efficiently present autoantigens from apoptotic cells to autologous T cells [12]. It appeared that the high level of IFNα present in SLE serum was responsible for this derailed response to apoptotic cells. Increased levels of IFNα have been correlated with disease severity and PBMCs from SLE patients clearly show an increased level of type I IFN regulated genes [13,14]. This IFN profile might be induced by the presence of DNA or RNA containing immune complexes. DNA/RNA released from apoptotic/necrotic cells is normally unable to trigger the nucleic acid recognizing TLRs (TLR3, 7, 8 and 9), because their expression is limited to the endosomal compartment. However, in the presence of autoantibodies against DNA or DNA/RNA binding proteins, DNA and RNA will become incorporated into immune complexes. These immune complexes can be recognized by Fc gamma receptors (FcγRs) present on APCs, inducing the uptake of the immune complexes and transport of RNA and DNA to the endosomal compartment, in turn triggering the intra-cellular TLR7, 8 or 9. This leads to the production of pro-inflammatory cytokines, such as type I IFNs, the upregulation of IFN regulated genes, the maturation of DCs and the presentation of self peptides to autoreactive T cells. Subsequently these autoreactive T cells can help B cells in the production of autoantibodies [15]. These immune complexes can also directly bind to B cells via the B cell

receptor and promote further autoantibody production [16]. It has been shown that immune complexes containing dsDNA or protein–RNA complexes are taken up by plasmacytoid dendritic cell (pDCs) via FcγRIIa, are translocated to the endosome and then stimulate TLR9 or TLR7, resulting in a massive release of IFNα [17,18]. Monocytes, myeloid DCs and macrophages also express high levels of FcγRs and respond well to TLR7/8 ligands. Upon stimulation with TLR7/8 ligands myeloid DCs secrete high levels of IL-12p70 and induce the proliferation of IFNγ producing Th1 cells, which can be even further enhanced by the presence of IFNα or IFNγ [19,20]. The combined stimulation of pDCs and mDCs by nucleic-acid containing immune complexes could induce a vicious circle, inducing Th1 cell differentiation and the release of IFNγ, which itself can also prime myeloid cells for the internalization of immune complexes, IL-12p70 release and the presentation of self peptides to autoreactive T cells enabling them to help B cells with the production of autoantibodies.

The essential role played by intracellular TLRs in the pathogenesis of SLE is also supported by studies demonstrating that lupus-prone mice lacking TLR7 do not produce anti-Sm antibodies and have ameliorated clinical disease, while over expression accelerates autoimmunity [21,22]. The role of TLR9 is less clear from animal models of SLE, as it has opposite functions in different mouse models (reviewed in [23]). Both animal studies and human data show an important role for mainly the intracellular TLRs for immune cell activation in SLE by recognition of DNA or RNA containing immune complexes.

4. Rheumatoid arthritis

RA mostly affects the synovial joints and if left untreated, culminates in cartilage destruction and bone erosion. Autoantibodies are found in most RA patients, with rheumatoid factor (directed against the Fc portion of IgG molecules) and anti-citrullinated protein/peptide antibodies (ACPA) being the most prevalent and also having great diagnostic value. In the last decade, numerous research groups have joined forces in an endeavor to decipher the role of TLRs in the pathogenesis of RA. This has led to several landmark observations, for instance the abundance of TLR2, 3, 4, 5, 7 and 9 expression in RA synovium compared to osteoarthritis patients or healthy controls and the identification of TLR2 stimulation as a strong inducer of chemokine production by synovial fibroblasts [24–28]. The latter is likely to contribute to the accumulation of immune cells in an arthritic joint. In addition, macrophages and DCs from RA patients also show augmented inflammatory responses to TLR ligands [25].

The question that still remains in RA is what triggers these TLRs on both immune and non-immune cells present in an arthritic joint? It is hypothesized that an initial microbial trigger or minor trauma can induce tissue damage, leading to the release of endogenous TLR ligands, thus creating a vicious circle of inflammation. Several microbes that have been implicated in the pathogenesis of RA are mycobacteria, mycoplasma, *Escherichia coli*, *Proteus mirabilis*, Epstein-Barr virus and human parvovirus B19 (reviewed in [29]), although up till now conclusive evidence for any of these is still lacking. Recognition of these bacteria/viruses via TLRs could lead to initial immune activation, which could culminate in chronic inflammation, and could also be involved in triggering disease flares. Recently there is also a renewed interest in oral bacteria, including *porphyromonas gingivalis*, which might be involved in the pathogenesis of RA [30].

Tissue damage and cell stress during synovial inflammation can lead to the production of heat shock proteins (HSPs), altered fibronectin, low molecular weight hyaluronan fragments, RNA release from necrotic cells and increased expression of matrix glycoprotein tenascin-C [31–36]. These are endogenous danger signals that in turn can activate synovial fibroblasts and DCs/macrophages via TLR2, 3 or 4 and thereby stimulate chronic inflammation. HMGB1 is also found in increased levels in RA synovial fluid. Although in SLE the HMGB1 is tightly bound to nucleosomes when released during late apoptosis, it can also be released by necrotic cells and activated immune cells. HMGB1 on its own cannot stimulate cells, but it can form complexes with for example DNA, LPS and IL-1β and thereby enhance their pro-inflammatory potential (reviewed in [37]). Massive cell necrosis can also release intracellular citrullinated proteins and activated citrullinating peptidyl arginine deiminases (PAD). These PAD enzymes can for example citrullinate fibrinogen and α-enolase, present in large amounts in the RA synovium. When these citrullinated proteins are not degraded properly, APCs may recognize them as non-self and present them to T cells, which in turn can trigger autoreactive B cells to produce ACPA. Recently it has been shown by Sokolove et al. that citrullination, besides creating a target of autoantibodies in RA can also be involved in TLR signaling. It has been demonstrated that citrullination of fibrinogen increases the TLR4 stimulating capacity of fibrinogen. The presence of autoantibodies to citrullinated fibrinogen, which are specific for RA patients, resulted in even further enhanced cell activation by interaction with both TLR4 and FcγRs simultaneously [38].

Although most endogenous ligands present in RA activate cells via TLR2 or TLR4, data also points towards a role for TLR8 signaling in RA. Inhibition of TLR4 but also TLR8 was able to inhibit the spontaneous production of TNFα by RA synovial membrane cultures [39,40], suggesting the presence of an unknown ssRNA containing component in the RA synovium. Similar to SLE, autoantibodies aimed at RNA binding proteins may be logical candidates. The RNA binding protein hnRNP-A2 (also known as the RA33 autoantigen) could be a likely candidate in RA; it is highly present in an arthritic joint, autoantibodies have been found in RA and it has been shown to stimulate immune cells via MyD88 [41,42].

Animal models of arthritis support an important role of TLRs in arthritis development (reviewed in [43]). In addition, they also show that TLRs might not only play a role in disease aggravation, but also in control of tolerance. Different TLR knockouts in the IL-1 receptor antagonist knockout model showed opposite effects. While TLR4$^{-/-}$ were protected from severe arthritis, TLR2$^{-/-}$ showed more severe arthritis [40]. The TLR2$^{-/-}$ mice showed a reduced suppressive function of the regulatory T cells and an increase in IFNγ production by T effector cells. Specific blockade of TLR4 was also able to suppress the severity of experimental arthritis [44].

Altogether, we can conclude that TLRs play an intrinsic role in the inflammation seen in RA, although further research is needed to delineate the exact triggers which are crucial to the disease development. Next to TLR triggering itself also the intracellular pathways of TLR signaling are an interesting target for therapy in RA. The first clinical trials have been performed and some more are ongoing at the moment. The near future will tell us if these can be implemented in the therapy of RA.

5. Systemic Sclerosis

In SSc vascular alterations and immunological disturbances are followed by fibrosis of the skin and internal organs, which causes severe disabilities and eventually death. Although the aforementioned pathways are clearly involved, the precise sequel of events is still a matter of intensive research. However, mounting evidence nowadays suggests the role of the immune system in the onset and/or perpetuation of this condition. For example, histopathological studies of unaffected SSc dermis showed rarefaction of capillaries

and the infiltration of mononuclear cells present even before overt fibrosis [45,46]. These cellular infiltrates consist of myeloid APCs and $CD4^+$ T-cells. Second, myeloid DC and monocyte-derived DCs from SSc patients, show a potentiated TLR response especially in the early phase of the disease [47,48]. The increased production of IL-6, TNFα and the anti-inflammatory cytokine IL-10 is specifically found in response to TLR2, TLR3 and TLR4 ligands in DCs, suggesting a role for specific TLRs. The observations that circulating endogenous TLR4 agonists are present in SSc patients [25], combined with the high circulating levels of inflammatory mediators often secreted by TLR stimulated DC/macrophages (TNFα, IL-6 and IL-12p70) [47,49] further substantiates the potential role of TLRs in this condition. Moreover, recent findings have highlighted the potential role of TLRs in inflammatory responses of the skin that quite closely resemble SSc-like disease [49]. More precisely, the TLR3 ligand PolyI:C induced IFN and TGFβ response genes in dermal fibroblast from SSc patients in vitro. Interestingly, although the SSc specific antibodies (anti-topoisomerase and anti-centromere) are present in a substantial part of the patients, the direct involvement in the disease process is lacking. The mechanism through which these antinuclear antibodies are induced is unknown but a process similar to through apoptotic cells like in SLE is a possibility as one report showed an increase of HMGB1 in SSc patients [50]. Concerning the functional consequences of SSc specific antibodies, the recent report from Kim et al. [51] indicating interferogenic activity by anti-topoisomerase I ICs might shed new light on the possible role of autoantibodies in this condition. Interestingly, more and more evidence directs towards the implication of type I IFN in SSc. At first, a role of type I IFN is supported by data on IFN type I signature both in circulating cells as well as SSc skin (reviewed in [52]). This is further supported by a report describing the rapid onset of SSc symptoms in patients treated with intense IFNα therapy [53]. In view of this, pDCs are interesting due to their potential of producing high levels of type I IFNs. Kim et al. showed that interfering with FcγRIIa or RNAse treatment suppresses the IFNα production upon stimulation with anti-topoisomerase I antibodies [51]. This implicates that like anti-Sm antibodies in SLE, anti-topoisomerase I antibodies are taken up by pDCs via FcγRIIa and subsequently activate TLR7 inducing the production of IFNα. In this study, anti-centromere antibodies did not induce the production of IFNα. Next to this, Eloranta et al. demonstrated that sera from both limSSc and difSSc patients contained interferogenic antibodies by activating specifically pDCs [54]. This correlated with the presence of anti-RNP antibodies. In line with these two studies, a recent report showed an association between anti-topoisomerase positivity and the expression of IFN induced genes [55]. Altogether, these observations support the notion that SSc specific antibodies are likely to be implicated in the disease process that might be directed via TLR mediated stimulation. However, this point of view remains to be investigated and warrants further research.

6. Psoriasis and psoriatic arthritis

PsA is an inflammatory disease of the skin, joints and entheses. Approximately 15–25% of psoriasis patients develop arthritis, the incidence of which rises with disease severity [56]. Although the events leading to the development of psoriasis and PsA are unclear the underlying chronic inflammatory immune response is thought to be triggered by unknown environmental factors on a polygenic background with increased susceptibility.

The role played by TLRs in psoriatic disease has been far less extensively studied than in RA and SLE. However, recent findings implicate that innate immunity might play a pivotal role in psoriatic disease. Genetic studies demonstrated associations between inhibitors of TLR and NOD2 signaling (TNFAIP3 (A20), TNIP1) and psoriasis [57]. In addition, it was shown that mutations and polymorphisms in keratinocyte-expressed genes involved in physical barrier function or innate immunity are risk factors for developing psoriasis [58,59]. Immunohistochemistry demonstrated the expression of TLR2, 3 and 4 in keratinocytes and psoriatic skin showed a strong over-expression of TLR2 in the epidermis. Furthermore, dermal DCs were demonstrated to express TLR2 and TLR4 but not TLR9, while epidermal DC (Langerhans cells) expressed TLR4 but not TLR2 or TLR9.

Activation via TLRs of infiltrating pDCs has been proposed to be an important pathogenic event in psoriasis/psoriatic disease. The aggravation and spreading of a psoriatic plaque was described upon the topical treatment with the TLR7 agonist imiquimod. This exacerbation was accompanied by a massive type I IFN production by infiltrating pDCs [60]. Especially pre-psoriatic skin was demonstrated to contain high numbers of pDCs and its chemotactic factor chemerin. Skin from chronic plaques showed low chemerin expression and few pDC in the dermis [61]. The role played by the activation of intracellular TLRs is also apparent from the use of chloroquine, hydroxychloroquine and quinacrine in the treatment of psoriatic disease for over 50 years [62]. These drugs, which were originally used as antimalarials, have an inhibitory effect on the signaling of the endocytic TLRs 3, 7, 8 and 9. A question that remained was how the shielded endocytic TLRs are activated in psoriatic disease. Recently it was demonstrated that the antimicrobial peptide LL37 (cathelicidin), which is highly expressed in psoriatic skin, converts inert self-DNA and self-RNA released by dying cells into a potent trigger of type 1 IFN production by pDCs, by binding the DNA/RNA to form complexes that are delivered to endocytic compartments in pDCs and myeloid DCs. In pDC, self-DNA-LL37 and self-RNA-LL37 complexes activate TLR9 and TLR7, respectively, and trigger the secretion of IFNα without inducing maturation or the production of IL-6 and TNFα. In addition, in contrast to self-DNA-LL37 complexes, self-RNA-LL37 complexes activate myeloid DCs via TLR8 which leads to the maturation of the DCs and the production of TNFα and IL-6. Self-RNA-LL37 complexes were demonstrated in psoriatic skin lesions and were associated with mature myeloid DCs in vivo [63,64].

Other endogenous TLR ligands that have been described in psoriatic disease are the heat shock proteins 27, 60 and 70 which were present in psoriatic skin but not in healthy skin [65]. In addition, exogenous TLR ligands might also play an important role. Recently, in Crohn's disease a primary innate immunodeficiency with hampered TLR responses by macrophages led to an impaired innate immune response and bacterial clearance possibly underlying chronic inflammation [66]. Since psoriatic disease and Crohn's disease share considerable features, both are associated with HLA-B27 positivity, (sub)clinical eye and gut inflammation and the formation of tortuous vessels at inflammation sites [56], an immunodeficiency by macrophages/DCs might be an underlying factor in psoriatic disease as well. Microbial products are profoundly present in psoriatic skin lesions and along the skin basement membrane of unaffected skin from psoriasis patients, while no microbial products are found in skin from non-psoriatic individuals [67]. PsA patients have higher antibody levels against various bacteria and bacterial constituents than psoriasis patients without articular involvement, RA patients or healthy controls (reviewed in [68]). Synovial tissue and enthesis organs are avascular, but tissue microdamage is common and appears to be associated with repair responses with vessel ingrowth. This makes the joint and enthesis organ a site where (components of) bacteria may preferentially localize. In the context of susceptibility-increasing genetic factors, this might lead to the characteristic inflammatory responses of PsA. Altogether, these findings appear indicative of at least a perpetuating role for bacteria and thus for TLR activation in psoriatic disease, especially PsA.

7. Gout

Gout is the most common inflammatory arthritis with increasing incidence over the past decades. The recurrent attacks of inflammation are thought to be caused by intra-articular monosodium urate (MSU) crystal deposition. This can happen whenever there are fluctuations in the urate levels and the synovial fluid becomes supersaturated with urate. In contrast to this there seems to be a continuous deposition of MSU in the joint or in tophi even though the attacks are sporadic. Moreover, only a part of people with hyperuricemia and deposits of MSU in the joints develop gout.

The stimulating capacity of MSU crystals to achieve monocytes/macrophages to produce IL-1β was already recognized 20 years ago, but to date controversy remains about the precise mechanisms through which urate crystals drive inflammation [69]. The first articles showed that MSU crystals are potentially recognized by immune cells through TLR 2 (and probably TLR4) [70,71] and FcγRIIIB [72], subsequently leading to NF-κB activation and downstream signaling culminating in the production of pro-inflammatory cytokines such as IL-6, TNFα, IL-1β and IL-8. Recent studies have led to significant advances in the understanding of the basic biology of crystal-mediated inflammation. In line with the clinical picture, the combination of MSU crystals and fatty acids [73] or LPS [74] is actually causing the attacks of inflammation. In this sequence the signaling from the fatty acid through TLR2 or LPS through TLR4 is important to fully induce IL-1β production. These recent findings change the proposed mechanism in gout dramatically. Even though uric acid has been identified as a danger signal that triggers a cytosolic sensor called the inflammasome, a signaling platform which is required for the activation of IL-1β, these recent findings show that uric acid as a danger signal is not enough. This could well be the first hit to activate the inflammasome but a second hit (like LPS, a fatty acid or maybe even cold) is needed to fully activate IL-1β and start the inflammation. The critical role of IL-1β in the initiation of acute inflammation in gout is reviewed in [75]. In light of the recent findings the presence of S100A8 proteins in gout synovial fluid [76] gains importance as this endogenous TLR4 ligand can create a self-perpetuating loop inducing prolonged (sub)clinical inflammation.

So far, gout is considered a local immune mediated disease. However, the clearly increased risk for cardiovascular diseases would suggest otherwise. Recent data from our group showed that multiple inflammatory molecules, among which TLR binding proteins, are highly expressed even in periods between gouty arthritis attacks (unpublished data). This observation together with the findings described above, is likely to spark the research into the role of the innate immune system in this condition that is also likely to broaden our therapeutic armamentarium for this condition.

8. Conclusions and future perspectives

In this overview we demonstrated that the vulnerable host could become a victim of its own innate immune system. In multiple rheumatic diseases it is shown that cells involved in the disease process have an increased expression of certain TLRs or an increased responsiveness to TLR ligands. This, together with the excessive presence of inflammatory molecules or complexes that can bind to TLRs, stresses the importance of TLR signaling in the pathogenesis of these diseases. In autoimmune diseases like RA, SLE and SSc the adaptive and innate immune system are both involved, whereas in a disease like gout the inflammation is mainly caused by aberrant activation of the innate immune system. In PsA both the innate and the adaptive immune system seem to play an important role although autoantibodies are mostly absent. This would classify PsA more in between autoimmune diseases and autoinflammatory diseases like gout. As knowledge on the contribution of specific parts of the immune system is still evolving, classification of these inflammatory diseases will stay a matter of ongoing debate.

During steady state conditions, host nucleic acids will not trigger TLR activation because they are physically separated from the endosomal compartment containing TLR3, 7, 8 and 9. However,

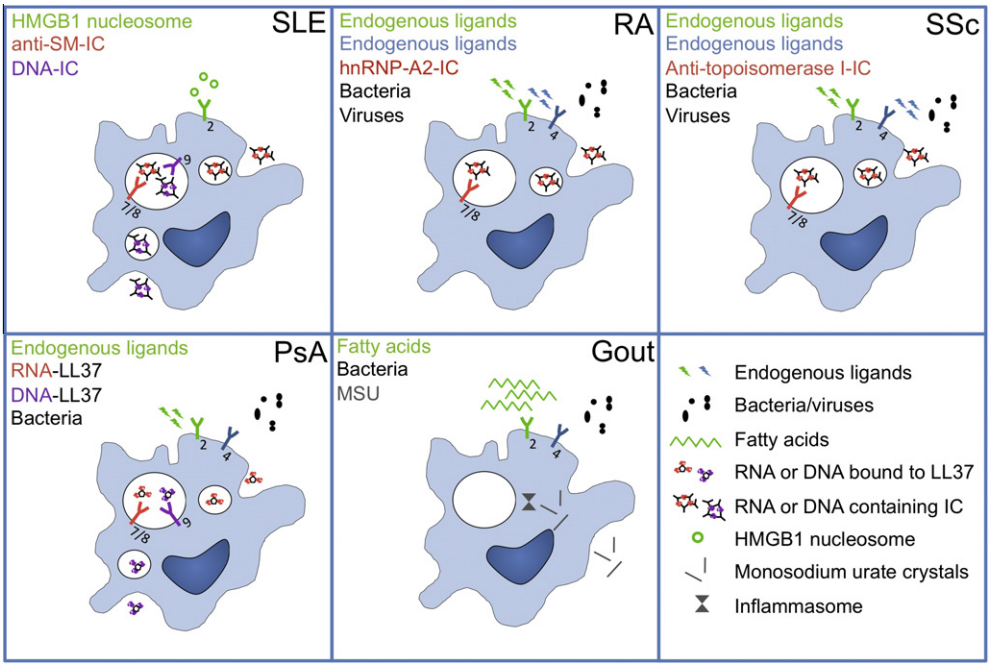

Fig. 1. The involvement of TLRs in SLE, RA, SSc, PsA and gout. The TLRs involved are depicted in a general APC. The colors of the ligands correspond with the colors of the TLRs they bind to: TLR2 (green), TLR4 (blue), TLR7 or TLR8 (red) and TLR9 (purple). TLR7 or TLR8 are shown together as they both bind single-stranded RNA. Bacteria/viruses are shown in black because they can bind to multiple TLRs. RNA/DNA can be transported into the endosome in immune complexes, via binding to Fc gamma receptors on the cell membrane (SLE, RA and SSc) or as a complex with LL37 which also facilitates endosomal entry (PsA).

both in autoimmune and inflammatory rheumatic conditions those regulatory mechanisms may be aberrant, leading to the breakdown of this barrier facilitating RNA/DNA transport into the endosomal compartment, possibly culminating in TLR7, 8 or 9 binding and downstream signaling. For instance, in SLE the presence of autoantibodies directed towards DNA or RNA containing protein complexes facilitates transport to the endosomes via FcγRs. Autoantibody induced uptake of DNA or RNA and subsequent triggering of intracellular TLRs is also suggested to be involved in RA and SSc. In psoriasis/PsA, this transport function is fulfilled by the antimicrobial peptide LL37, which can bind to DNA and RNA and facilitate endosomal entry of LL37-DNA or LL37-RNA complexes. Breakdown of this barrier function thus seems to be a recurrent defect in multiple rheumatic conditions (Fig. 1). Cell-stress and tissue damage can also result in the presence of other endogenous ligands, including multiple heat-shock proteins, which trigger immune cells mainly via TLR2 and TLR4 (Fig. 1). In all the diseases described here it is not a single TLR or a single cell type which is responsible for the development or perpetuation of the disease, but it is a complex network of interacting immune and tissue cells which are in an inflammatory state, at least partially mediated via TLRs.

Future efforts to battle autoimmune/autoinflammatory conditions via restoring TLR mediated response should either target the TLR itself or the effector cell activated by TLR mediated signaling. The latter is nicely exemplified by harnessing tolerogenic DCs in the treatment of arthritis. Tolerogenic DCs are able to re-balance the immune system that has gone awry. Experimental models of arthritis have shown the promise of this theory. Currently, this new means of therapeutic approach is clinically being evaluated in at least two centers (Prof. Thomas, University of Queensland, Australia and Prof. Isaacs, Newcastle University, United Kingdom). The direct inhibition of TLRs or their downstream cascades is somewhat further away from clinics but will certainly enter preclinical evaluation not too long from now. However, the primary question here would be; what price will we pay for the inhibition of TLR pathways? In other words, would such therapies dampen our immune system in such a way that infectious diseases will gain terrain? Such questions were posed in the rheumatology community during the introduction of TNFα neutralizing antibodies, and after that the other biological agents administered to treat many of the conditions discussed here. Surprisingly, side effects, both short-term and long-term are acceptable. Altogether, the rheumatology community seems ready to take the next step; testing therapeutic agents which interfere with TLR signaling pathways and only time will tell how high the toll for bugs is in the treatment of rheumatic conditions.

Acknowledgements

We apologize to colleagues whose work is cited via review rather than original work due to space restraints.

References

[1] Kastner, D.L., Aksentijevich, I. and Goldbach-Mansky, R. (2010) Autoinflammatory disease reloaded: a clinical perspective. Cell 140, 784–790.
[2] Marshak-Rothstein, A. (2006) Toll-like receptors in systemic autoimmune disease. Nat. Rev. Immunol. 6, 823–835.
[3] Takeda, K. and Akira, S. (2005) Toll-like receptors in innate immunity. Int. Immunol. 17, 1–14.
[4] Roelofs, M.F., Abdollahi-Roodsaz, S., Joosten, L.A., van den Berg, W.B. and Radstake, T. (2008) The orchestra of Toll-like receptors (TLRs) and their potential role in frequently occurring rheumatic conditions. Arthritis Rheum. 58, 338–348.
[5] O'Neill, L.A. and Bowie, A.G. (2007) The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat. Rev. Immunol. 7, 353–364.
[6] Re, F. and Strominger, J.L. (2004) IL-10 released by concomitant TLR2 stimulation blocks the induction of a subset of Th1 cytokines that are specifically induced by TLR4 or TLR3 in human dendritic cells. J. Immunol. 173, 7548–7555.
[7] Wenink, M.H. et al. (2009) TLR2 promotes Th2/Th17 responses via TLR4 and TLR7/8 by abrogating the type I IFN amplification loop. J. Immunol. 183, 6960–6970.
[8] Sutmuller, R.P. et al. (2006) Toll-like receptor 2 controls expansion and function of regulatory T cells. J. Clin. Invest. 116, 485–494.
[9] Zanin-Zhorov, A., Cahalon, L., Tal, G., Margalit, R., Lider, O. and Cohen, I.R. (2006) Heat shock protein 60 enhances $CD4^+$ $CD25^+$ regulatory T cell function via innate TLR2 signaling. J. Clin. Invest. 116, 2022–2032.
[10] Neogi, T., Gladman, D.D., Ibanez, D. and Urowitz, M. (2006) Anti-dsDNA antibody testing by Farr and ELISA techniques is not equivalent. J. Rheumatol. 33, 1785–1788.
[11] Kruse, K. et al. (2010) Inefficient clearance of dying cells in patients with SLE: anti-dsDNA autoantibodies, MFG-E8, HMGB-1 and other players. Apoptosis 15, 1098–1113.
[12] Blanco, P., Palucka, A.K., Gill, M., Pascual, V. and Banchereau, J. (2001) Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. Science 294, 1540–1543.
[13] Bengtsson, A.A., Sturfelt, G., Truedsson, L., Blomberg, J., Alm, G., Vallin, H. and Ronnblom, L. (2000) Activation of type I interferon system in systemic lupus erythematosus correlates with disease activity but not with antiretroviral antibodies. Lupus 9, 664–671.
[14] Baechler, E.C. et al. (2003) Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc. Natl. Acad. Sci. USA 100, 2610–2615.
[15] Boule, M.W., Broughton, C., Mackay, F., Akira, S., Marshak-Rothstein, A. and Rifkin, I.R. (2004) Toll-like receptor 9-dependent and -independent dendritic cell activation by chromatin-immunoglobulin G complexes. J. Exp. Med. 199, 1631–1640.
[16] Leadbetter, E.A., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J. and Marshak-Rothstein, A. (2002) Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 416, 603–607.
[17] Means, T.K., Latz, E., Hayashi, F., Murali, M.R., Golenbock, D.T. and Luster, A.D. (2005) Human lupus autoantibody–DNA complexes activate DCs through cooperation of CD32 and TLR9. J. Clin. Invest. 115, 407–417.
[18] Savarese, E. et al. (2006) U1 small nuclear ribonucleoprotein immune complexes induce type I interferon in plasmacytoid dendritic cells through TLR7. Blood 107, 3229–3234.
[19] Napolitani, G., Rinaldi, A., Bertoni, F., Sallusto, F. and Lanzavecchia, A. (2005) Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. Nat. Immunol. 6, 769–776.
[20] Roelofs, M.F. et al. (2009) Type I interferons might form the link between Toll-like receptor (TLR) 3/7 and TLR4-mediated synovial inflammation in rheumatoid arthritis (RA). Ann. Rheum. Dis. 68, 1486–1493.
[21] Christensen, S.R., Shupe, J., Nickerson, K., Kashgarian, M., Flavell, R.A. and Shlomchik, M.J. (2006) Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity 25, 417–428.
[22] Subramanian, S. et al. (2006) A Tlr7 translocation accelerates systemic autoimmunity in murine lupus. Proc. Natl. Acad. Sci. USA 103, 9970–9975.
[23] Kim, W.U., Sreih, A. and Bucala, R. (2009) Toll-like receptors in systemic lupus erythematosus; prospects for therapeutic intervention. Autoimmun. Rev. 8, 204–208.
[24] Radstake, T.R., Roelofs, M.F., Jenniskens, Y.M., Oppers-Walgreen, B., van Riel, P.L., Barrera, P., Joosten, L.A. and van den Berg, W.B. (2004) Expression of Toll-like receptors 2 and 4 in rheumatoid synovial tissue and regulation by proinflammatory cytokines interleukin-12 and interleukin-18 via interferon-gamma. Arthritis Rheum. 50, 3856–3865.
[25] Roelofs, M.F., Joosten, L.A., Abdollahi-Roodsaz, S., van Lieshout, A.W., Sprong, T., van den Hoogen, F.H., van den Berg, W.B. and Radstake, T.R. (2005) The expression of Toll-like receptors 3 and 7 in rheumatoid arthritis synovium is increased and costimulation of Toll-like receptors 3, 4, and 7/8 results in synergistic cytokine production by dendritic cells. Arthritis Rheum. 52, 2313–2322.
[26] Pierer, M. et al. (2004) Chemokine secretion of rheumatoid arthritis synovial fibroblasts stimulated by Toll-like receptor 2 ligands. J. Immunol. 172, 1256–1265.
[27] Seibl, R. et al. (2003) Expression and regulation of Toll-like receptor 2 in rheumatoid arthritis synovium. Am. J. Pathol. 162, 1221–1227.
[28] Tamaki, Y., Takakubo, Y., Hirayama, T., Konttinen, Y.T., Goodman, S.B., Yamakawa, M. and Takagi, M. (2011) Expression of Toll-like receptors and their signaling pathways in rheumatoid synovitis. J. Rheumatol. [Epub ahead of print]
[29] Rashid, T. and Ebringer, A. (2007) Rheumatoid arthritis is linked to Proteus—the evidence. Clin. Rheumatol. 26, 1036–1043.
[30] Lundberg, K., Wegner, N., Yucel-Lindberg, T. and Venables, P.J. (2010) Periodontitis in RA—the citrullinated enolase connection. Nat. Rev. Rheumatol. 6, 727–730.
[31] Brentano, F., Schorr, O., Gay, R.E., Gay, S. and Kyburz, D. (2005) RNA released from necrotic synovial fluid cells activates rheumatoid arthritis synovial fibroblasts via Toll-like receptor 3. Arthritis Rheum. 52, 2656–2665.
[32] Roelofs, M.F. et al. (2006) Identification of small heat shock protein B8 (HSP22) as a novel TLR4 ligand and potential involvement in the pathogenesis of rheumatoid arthritis. J. Immunol. 176, 7021–7027.

[33] Vabulas, R.M., Ahmad-Nejad, P., da Costa, C., Miethke, T., Kirschning, C.J., Hacker, H. and Wagner, H. (2001) Endocytosed HSP60s use Toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. J. Biol. Chem. 276, 31332–31339.
[34] Termeer, C. et al. (2002) Oligosaccharides of Hyaluronan activate dendritic cells via Toll-like receptor 4. J. Exp. Med. 195, 99–111.
[35] Okamura, Y., Watari, M., Jerud, E.S., Young, D.W., Ishizaka, S.T., Rose, J., Chow, J.C. and Strauss III, J.F. (2001) The extra domain A of fibronectin activates Toll-like receptor 4. J. Biol. Chem. 276, 10229–10233.
[36] Midwood, K. et al. (2009) Tenascin-C is an endogenous activator of Toll-like receptor 4 that is essential for maintaining inflammation in arthritic joint disease. Nat. Med. 15, 774–780.
[37] Bianchi, M.E. (2009) HMGB1 loves company. J. Leukoc. Biol. 86, 573–576.
[38] Sokolove, J., Zhao, X., Chandra, P.E. and Robinson, W.H. (2011) Immune complexes containing citrullinated fibrinogen costimulate macrophages via Toll-like receptor 4 and Fcgamma receptor. Arthritis Rheum. 63, 53–62.
[39] Sacre, S.M., Lo, A., Gregory, B., Simmonds, R.E., Williams, L., Feldmann, M., Brennan, F.M. and Foxwell, B.M. (2008) Inhibitors of TLR8 reduce TNF production from human rheumatoid synovial membrane cultures. J. Immunol. 181, 8002–8009.
[40] Abdollahi-Roodsaz, S. et al. (2008) Stimulation of TLR2 and TLR4 differentially skews the balance of T cells in a mouse model of arthritis. J. Clin. Invest. 118, 205–216.
[41] Hoffmann, M.H. et al. (2007) The rheumatoid arthritis-associated autoantigen hnRNP-A2 (RA33) is a major stimulator of autoimmunity in rats with pristane-induced arthritis. J. Immunol. 179, 7568–7576.
[42] Fritsch, R. et al. (2002) Characterization of autoreactive T cells to the autoantigens heterogeneous nuclear ribonucleoprotein A2 (RA33) and filaggrin in patients with rheumatoid arthritis. J. Immunol. 169, 1068–1076.
[43] Huang, Q.Q. and Pope, R.M. (2009) The role of Toll-like receptors in rheumatoid arthritis. Curr. Rheumatol. Rep. 11, 357–364.
[44] Abdollahi-Roodsaz, S. et al. (2007) Inhibition of Toll-like receptor 4 breaks the inflammatory loop in autoimmune destructive arthritis. Arthritis Rheum. 56, 2957–2967.
[45] Ishikawa, O. and Ishikawa, H. (1992) Macrophage infiltration in the skin of patients with systemic sclerosis. J. Rheumatol. 19, 1202–1206.
[46] Kraling, B.M., Maul, G.G. and Jimenez, S.A. (1995) Mononuclear cellular infiltrates in clinically involved skin from patients with systemic sclerosis of recent onset predominantly consist of monocytes/macrophages. Pathobiology 63, 48–56.
[47] van Bon, L. et al. (2010) Distinct evolution of TLR-mediated dendritic cell cytokine secretion in patients with limited and diffuse cutaneous systemic sclerosis. Ann. Rheum. Dis. 69, 1539–1547.
[48] van Lieshout, A.W. et al. (2009) Enhanced interleukin-10 production by dendritic cells upon stimulation with Toll-like receptor 4 agonists in systemic sclerosis that is possibly implicated in CCL18 secretion. Scand. J. Rheumatol. 38, 282–290.
[49] Farina, G.A. et al. (2010) Poly(I:C) drives type I IFN- and TGFbeta-mediated inflammation and dermal fibrosis simulating altered gene expression in systemic sclerosis. J. Invest. Dermatol. 130, 2583–2593.
[50] Yoshizaki, A. et al. (2009) Clinical significance of serum HMGB-1 and sRAGE levels in systemic sclerosis: association with disease severity. J. Clin. Immunol. 29, 180–189.
[51] Kim, D., Peck, A., Santer, D., Patole, P., Schwartz, S.M., Molitor, J.A., Arnett, F.C. and Elkon, K.B. (2008) Induction of interferon-alpha by scleroderma sera containing autoantibodies to topoisomerase I: association of higher interferon-alpha activity with lung fibrosis. Arthritis Rheum. 58, 2163–2173.
[52] Lafyatis, R. and York, M. (2009) Innate immunity and inflammation in systemic sclerosis. Curr. Opin. Rheumatol. 21, 617–622.
[53] Coelho, L.F., de Oliveira, J.G. and Kroon, E.G. (2008) Interferons and scleroderma—a new clue to understanding the pathogenesis of scleroderma? Immunol. Lett. 118, 110–115.
[54] Eloranta, M.L., Lovgren, T., Finke, D., Mathsson, L., Ronnelid, J., Kastner, B., Alm, G.V. and Ronnblom, L. (2009) Regulation of the interferon-alpha production induced by RNA-containing immune complexes in plasmacytoid dendritic cells. Arthritis Rheum. 60, 2418–2427.
[55] Assassi, S. et al. (2010) Systemic sclerosis and lupus: points in an interferon-mediated continuum. Arthritis Rheum. 62, 589–598.
[56] Ritchlin, C.T. (2008) From skin to bone: translational perspectives on psoriatic disease. J. Rheumatol. 35, 1434–1437.
[57] Nair, R.P. et al. (2009) Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. Nat. Genet. 41, 199–204.
[58] Zeeuwen, P.L., de Jongh, G.J., Rodijk-Olthuis, D., Kamsteeg, M., Verhoosel, R.M., van Rossum, M.M., Hiemstra, P.S. and Schalkwijk, J. (2008) Genetically programmed differences in epidermal host defense between psoriasis and atopic dermatitis patients. PLoS ONE 3, e2301.
[59] de Cid, R. et al. (2009) Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis. Nat. Genet. 41, 211–215.
[60] Gilliet, M., Conrad, C., Geiges, M., Cozzio, A., Thurlimann, W., Burg, G., Nestle, F.O. and Dummer, R. (2004) Psoriasis triggered by Toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. Arch. Dermatol. 140, 1490–1495.
[61] Albanesi, C. et al. (2009) Chemerin expression marks early psoriatic skin lesions and correlates with plasmacytoid dendritic cell recruitment. J. Exp. Med. 206, 249–258.
[62] Kalia, S. and Dutz, J.P. (2007) New concepts in antimalarial use and mode of action in dermatology. Dermatol. Ther. 20, 160–174.
[63] Ganguly, D. et al. (2009) Self-RNA–antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. J. Exp. Med. 206, 1983–1994.
[64] Lande, R. et al. (2007) Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature 449, 564–569.
[65] Curry, J.L., Qin, J.Z., Bonish, B., Carrick, R., Bacon, P., Panella, J., Robinson, J. and Nickoloff, B.J. (2003) Innate immune-related receptors in normal and psoriatic skin. Arch. Pathol. Lab. Med. 127, 178–186.
[66] Smith, A.M. et al. (2009) Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease. J. Exp. Med. 206, 1883–1897.
[67] Moen, K., Brun, J.G., Valen, M., Skartveit, L., Eribe, E.K., Olsen, I. and Jonsson, R. (2006) Synovial inflammation in active rheumatoid arthritis and psoriatic arthritis facilitates trapping of a variety of oral bacterial DNAs. Clin. Exp. Rheumatol. 24, 656–663.
[68] Espinoza, L.R., van Solingen, R., Cuellar, M.L. and Angulo, J. (1998) Insights into the pathogenesis of psoriasis and psoriatic arthritis. Am. J. Med. Sci. 316, 271–276.
[69] Di Giovine, F.S., Malawista, S.E., Nuki, G. and Duff, G.W. (1987) Interleukin 1 (IL 1) as a mediator of crystal arthritis. Stimulation of T cell and synovial fibroblast mitogenesis by urate crystal-induced IL 1. J. Immunol. 138, 3213–3218.
[70] Liu-Bryan, R. and Liote, F. (2005) Monosodium urate and calcium pyrophosphate dihydrate (CPPD) crystals, inflammation, and cellular signaling. Joint Bone Spine 72, 295–302.
[71] Liu-Bryan, R., Pritzker, K., Firestein, G.S. and Terkeltaub, R. (2005) TLR2 signaling in chondrocytes drives calcium pyrophosphate dihydrate and monosodium urate crystal-induced nitric oxide generation. J. Immunol. 174, 5016–5023.
[72] Barabe, F., Gilbert, C., Liao, N., Bourgoin, S.G. and Naccache, P.H. (1998) Crystal-induced neutrophil activation VI. Involvement of FcgammaRIIIB (CD16) and CD11b in response to inflammatory microcrystals. FASEB J. 12, 209–220.
[73] Joosten, L.A. et al. (2010) Engagement of fatty acids with Toll-like receptor 2 drives interleukin-1beta production via the ASC/caspase 1 pathway in monosodium urate monohydrate crystal-induced gouty arthritis. Arthritis Rheum. 62, 3237–3248.
[74] Giamarellos-Bourboulis, E.J., Mouktaroudi, M., Bodar, E., van der Ven, J., Kullberg, B.J., Netea, M.G. and van der Meer, J.W. (2009) Crystals of monosodium urate monohydrate enhance lipopolysaccharide-induced release of interleukin 1 beta by mononuclear cells through a caspase 1-mediated process. Ann. Rheum. Dis. 68, 273–278.
[75] Martinon, F. (2010) Mechanisms of uric acid crystal-mediated autoinflammation. Immunol. Rev. 233, 218–232.
[76] Ryckman, C., Gilbert, C., de Medicis, R., Lussier, A., Vandal, K. and Tessier, P.A. (2004) Monosodium urate monohydrate crystals induce the release of the proinflammatory protein S100A8/A9 from neutrophils. J. Leukoc. Biol. 76, 433–440.