Summary

Osteoarthritis (OA) is a multifactorial, painful and disabling disease that affects millions of people globally, with a largely unknown aetiology. OA remains undiagnosed until it becomes symptomatic with advanced structural alterations evident, thus joint replacement may be required. OA is now considered a whole-joint inflammatory disease, associated with synovitis of the fibroblast-like synoviocytes (FLS). FLS are sentinel cells that contribute to OA pathogenesis, through secretion of various catabolic and pro-inflammatory mediators, though the downstream stimuli which initiate and propagate the inflammatory pathway remain poorly defined. Activation of the innate immune Toll-Like Receptors (TLRs) leads to the induction of inflammatory mediators and cellular infiltration seen in most of the joint arthropathies, though the role of TLRs in OA is poorly understood. The aim of this research work was to characterise the role and functionality of TLRs in OA and to identify the key TLRs that modulate OA pathology. Interestingly, we found that TLR3, activated by dsRNA and endogenous alarm signals contained in the OA synovial fluid (SF), plays a key role in OA and this was confirmed by neutralisation of TLR3 expression which shifted the balance from pro-inflammatory to an anti-inflammatory cytokine milieu. Next using a proteomic approach, we found that prohibitin 1 (PHB1), an anti-proliferative molecule, was drastically down-regulated in FLS upon Poly(I:C) stimulation and this was validated through confocal and immunoblot analysis. Thus, PHB1 may be considered as a potential biomarker for tracing RNA borne synovial hyperplasia, indicative of synovitis which directly implies for OA severity and progression. Following proteomic analysis of grade-specific whole synovial tissue, suppression of key complement C3b in grade-2 OA, was evident. Furthermore, grade-specific OA-SF showed an ability to predominantly induce IFNβ in FLS and in HEK293-TLR3 cells in a TLR3 dependent manner. Neutralisation of TLR3 significantly inhibited IFNβ production, probably through regulation/blockade of downstream signalling cascades of OA-SF-induced persistent TLR3 activation. Further, luciferase reporter gene assays have suggested that, this effect may be mediated through the transcription factors IRF3 and IRF9, leading to sustained activation of IFNβ genes. Therefore, TLR3 blockade in FLS may inhibit OA-SF-induced activation of TLR3 and concomitant induction of IFNβ. Likewise, TLR3 blockade also inhibited RANTES production, primarily through blocking of NF-κB. Together, these data indicate that TLR3, expressed on the plasma membrane of FLS, may be a critical target for OA disease intervention. In conclusion, our data suggests, for the first

time that, TLR3 hyper-activation plays a key role in perpetuating synovial inflammation in OA and suggests that therapeutic intervention of OA may be achieved through TLR3 blockade. Despite the significant advances in the understanding and management of OA, significant research must still be undertaken before clinicians can guarantee a quality of life for OA patients, which is free of the debilitating pain. We hope that our efforts would, at least in part, contribute to a better understanding of the pathogenic molecular mechanisms that drive this chronic inflammatory disease. The provision of better treatments will thereby improve the quality of life for patients whose lives are marred by OA and related inflammatory diseases.