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The Role of p38 MAPK in Rheumatic Diseases

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***Abstract*— p38 mitogen activated protein kinase (p38 MAPK) signaling appears to play a significant role in the regulation of immune-mediated inflammatory responses and therefore has been linked with several autoimmune diseases. This review discusses the current data regarding the involvement of p38 MAP in rheumatic diseases characterized by arthritis, with special attention in psoriatic arthritis, an arthritis with no apparent autoimmune features, and rheumatoid arthritis, an arthritis with apparent autoimmune features.**

***Keywords* — autoimmunity, inflammation, psoriatic arthritis signaling, rheumatoid arthritis.**

I. Introduction

Inflammation epitomizes an ordered sequence of events that establish and resolve a successful protective innate immune response against pathogens. Auto-inflammatory processes are sustained hyperactive immune response to self-antigens causing damage to tissues and organs [1-3]. Autoimmune rheumatic diseases (ARD) are heterogeneous, chronic auto-aggressive inflammatory disorders of the skin, connective tissue, and joints. ARD include among others rheumatoid arthritis, systemic sclerosis, and systemic lupus erythematosus [4]. Understanding the mechanisms responsible for the regulation of inflammation is of paramount importance to better design targeted treatment strategies [5-8].

The innate immune system being quite sophisticated, can orchestrate the initiation, continuation, and cessation of inflammatory responses. Antigen presenting cells (APCs) engulf pathogens, present antigenic peptides to T cells and secrete monokines in order to regulate other innate immune cells such as neutrophils, dendritic cells, macrophages, monocytes and Vγ9/Vδ2 T cells [9, 10]. Yet, the role of certain innate participators, such as NK and NKT cells, is still largely undermined in ARD [11-14]. This occurs mainly due to their low-numbers in peripheral blood and tissues, which imposes a significant difficulty in their study. APC function has been recently attributed to NKs themselves and to certain hybrid subsets, such as natural killer dendritic cells (NKDCs), identified in mouse models and humans [15-17].

One of the most significant intracellular proteins that can initiate, perpetuate and resolve inflammation are mitogen-activated protein kinases (MAPKs) p38 primarily studied in macrophages [18, 19]. Accumulating evidence however suggest that p38 members are also activated within several innate cell subsets and may function as a critical players in ARD [20-23]. In this review we focus on two rheumatic diseases characterized by arthritis, psoriatic arthritis (PsA), which has no apparent autoimmune features, and rheumatoid arthritis (RA), which has apparent autoimmune features (rheumatoid factor, anti-citrullinated proteins antibodies [ACPAs]). ACPAs are considered pathogenic for RA [24].

P38 MAPK

Identification

p38 MAPK was originally described in 1994 as the mammalian homologue of the yeast Hog1 gene encoded kinase. In the same year it was also independently described as a kinase activated (phosphorylated) in response to an endotoxin (liposacharide, LPS) and interleukin (IL-1) challenge, phosphorylating downstream targets, such as heat shock protein (hsp)27 and regulating inflammatory gene expression [25-27]. p38 is phosphorylated in response to inflammatory and stress stimuli, such as cytokines, ultraviolet irradiation, osmotic

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shock, and heat shock, and is involved in cytokine regulation, cell differentiation and apoptosis [28].

Isoforms

Four subtypes of p38 proteins have been identified: α (MAPK14), β (MAPK11), γ (MAPK12/ERK6), and δ (MAPK13/SAPK4) [29-31]. p38α and p38β share approximately 75% gene sequence homology, whereas γ and δ are more distantly related, sharing just over 60% of their gene sequence. p38α and p38β are ubiquitously expressed whereas p38γ and p38δ show tissue-specific expression patterns. p38α and p38δ are abundantly expressed in macrophages, neutrophils and T cells, p38γ is highly expressed in skeletal muscle and p38β is abundant in endothelial cells [31, 32].

Phosphorylation

The structure of p38 kinases consists of a 135 amino acid N-terminal domain and a 225 amino acid C-terminal domain with the catalytic site located in the region linking the two domains [33]. p38 isoforms are shaped into different three-dimensional structures according to the precise orientation of the N- and C-terminal domains giving rise to variably sized ATP-binding pockets [34]. p38 members are phosphorylated within the ATP binding cleft on a single threonine (Thr-180) and a single tyrosine residue (Tyr-182). p38 kinases are phosphorylated (activated) after treatment with physiological (tumor necrosis factor [TNF]-α, IL-12 and IL-18, Toll-like receptor [TLR]-9, and TLR-4 ligands) and chemical stimuli, such as phorbol 12-myristate 13-acetate (PMA) plus ionomycin, sodium arsenite and anisomycin. Activation in different cellular compartments is dependent on the specificity of each stimulus.

Regulation

Since p38 phosphorylation can be induced by several agonists, the receptors and downstream converging signaling pathways diversify. Hence, studies have confirmed the existence of a classical activation pattern and two alternative ones [35, 36]. The classical MAPK pathway is evolutionarily conserved and encompasses a sequential phosphorylation of MAPK kinase kinases (MKKK) which activate a dual-specificity MAPK kinases (MKK), which in turn induce p38 MAPK by phosphorylating both threonine and tyrosine residues in a Thr-Xxx-Tyr motif [37, 38]. In the classical p38 MAPK cascade MEKK4 serves as an upstream MKKK and activates MKK3, MKK4, and then p38. MTK1, mixed lineage kinase (MLK) 2/3, apoptosis signal-regulating kinase (ASK) 1, and transforming growth factor β-activated kinase (TAK) 1 are other MKKK kinases capable of activating MKK-6 or p38 signaling [39]. Further upstream activators of MKKKs include the growth arrest and DNA damage-inducible genes 45 (GADD45) proteins, that have been studied in detail and described to be of fundamental importance in leukocytes [40].

An increasing number of important substrates occur also downstream of p38. MAP kinase-activated protein kinase 2 (MK2) and MK3 are phosphorylated and can activate a variety of mediators, such as small HSP27, cAMP-response element-binding protein (CREB), and activating transcription factor (ATF) 1 [41, 42]. So far, various proteins have been identified as downstream substrates of p38, such as mitogen- and stress-activated kinase (MSK), p38-regulated/activated kinase (PRAK), and MAP kinase interaction protein kinase (MNK1) [43, 44]. Several novel proteins have also been identified in whole lysates of myoblasts as direct targets of p38α, including Ahnak, Iws1, Grp78, Pgrmc, Prdx6, and Ranbp2 [45]. Moreover, TPL2/ERK1/2 kinases can also be regulated by p38 γ and δ isoforms [46].

Post-transcriptional regulation of gene expression by p38 MAPK is very important in the regulation of inflammatory responses [47]. Genome-wide analyses have demonstrated that only half of the alterations in gene expression during the immune response can be accounted for by transcriptional regulation. The rest are dependent on changes in mRNA stability [22, 48]. The modulation of mRNA stability is a powerful mechanism for bringing about rapid changes of gene expression, such as those that occur when the innate immune system first encounters a pathogen. Determinants of mRNA stability are usually located within the 3' untranslated region (UTR), and are tandem repeats of the sequence AUUUA termed adenosine/uridine-rich elements (AREs) [49, 50]. AREs contain, and are recognized by mRNA destabilizing proteins including tristetraprolin (TTP) and several others [51].

Phosphatases also exist to deactivate p38 so that p38 is not *ad infinitum* activated. Mitogen-activated protein kinase phosphatases (MKPs) can dephosphorylate MAPKs by binding to the TXY amino acid motif [52]. MKP-1, MKP-4, MKP-5, and MKP7 have been identified to effectively dephosphorylate p38α and p38β [53, 54]. MKPs however, are unable to dephosphorylate p38γ or p38δ [55].

P38 knock-outs

p38α deficiency results in embryonic death due to defects in placental development and erythropoietin expression [56]. However, p38β−/− mice are viable and exhibit no obvious defects in neither gene expression nor lymphocyte development [57]. Single knockouts of either

p38γ or p38δ, and the double knockout are also viable. Importantly, diminished expression of TNF-α, IL-1β, and IL-10 was reported in stimulated macrophages isolated from p38 γ/δ null mice, which suggests that p38 γ/δ can be crucial regulatory constituents of the innate immune response [46].

Inhibitors

Dissection of the p38 MAPK signaling cascade is made possible with the development of specific p38 inhibitors [58, 59]. An array of pyridinyl imidazole anti-inflammatory compounds, such as SKF-86002, SB203580, and SB202190 were among the first available p38 inhibitors acting through competition for the ATP-binding site [60-63]. Pharmaceutical industries have invested heavily in developing competitive and specific compounds and a number of second and third generation of inhibitors, such as SC-79659, SC-80036, VXs, AMG-548, ML3403, pamapimod and AS1940477 [64, 65] . Most of these inhibitors show specific and strong activity against p38 and inhibit the production of proinflammatory cytokines, and, therefore, hold promise as therapeutic agents for chronic inflammatory diseases. For example pamapimod reduced clinical signs of arthritis, bone loss and inhibited TNF-α production in RA synovial explants [66]. Other novel compounds, such as GSK-681323, have been used to treat RA, SCIO-469 to treat multiple myeloma and dental pain, and RWJ67657 has been used as a broad anti-inflammatory agent [67]. Currently, efficacy and safety of p38 inhibitors are under evaluation in clinical trials [68].

p38 MAPK in psoriasis and psoriatic arthritis

The p38 MAPK pathway has been implicated in the pathogenesis of psoriasis, as it is detected by immunohistochemistry and Western blotting in psoriatic skin lesions [69, 70]. Kinase assays also confirmed the increased activity of p38α, p38β and p38δ isoforms in lesional compared to non-lesional psoriatic skin. Phosphorylated p38 in lesional psoriatic epidermis, exhibited a distinct nuclear localization indicative of the kinase participation in the induction of active gene expression [70]. Dual specificity phosphatase (DUSP)1 that can dephosphorylate p38 MAPK and cease its function, is also impaired in psoriasis since its mRNA expression was significantly down-regulated in lesional compared to non-lesional psoriatic skin [71, 72].

Further downstream targets of p38 MAPK signaling, such as MK2, are also activated in the psoriatic epidermis [73]. Keratinocytes transfected with MK2-specific small interfering RNAs showed diminished MK2 expression and significant reduction in the expression of IFN-γ, TNF-α, IL-6, and IL-8 proteins. The mechanism by which p38 MAPK mediates its regulatory effects through downstream kinases has been dissected in mice with deleted MK2 [74]. These mice are deficient in the LPS-induced biosynthesis of several pro-inflammatory cytokines regulated by p38, including TNF-α, IFN-γ, IL-6, and IL-1. They survive LPS-induced endotoxic shock due to a reduction of the secretion of TNF-α by almost 90% [75]. MK2 has been considered as a key molecule participating in host defense against intracellular bacteria through regulation of both TNF-α and IFN-γ production [76, 77].

Mitogen- and stress-activated protein kinase 1 (MSK1) is another downstream target of p38 which regulates the expression of pro-inflammatory cytokine genes through activation of transcription factors. Western blotting analysis revealed a consistent and significant increase in phosphorylated MSK1 (Ser376) in lesional psoriatic skin [78, 79]. Cultured human keratinocytes incubated with anisomycin or IL-1β resulted in the phosphorylation of both p38 MAPK and MSK1 (Ser376) whereas MSK1 (Ser376) phosphorylation was inhibited by pre-incubation with p38 inhibitors or dimethylfumarate [80]. In addition, transcription factors, such as cAMP/calcium responsive element binding protein (CREB) associated with cellular proliferation gene expression, are also phosphorylated in psoriatic skin [81]. Activation of CREB through ERK1/2 is directly linked with the expression of TNF-α, IL-6 and IL-8 [82]. These cytokines are also under direct regulation of the p38 pathway as well [83, 84]. p38 MAPK-induced phosphorylation of STAT-3 and of STAT-1 at serine 727 has also been demonstrated in lesional psoriatic skin [85, 86]. STAT-3, in particular, has been described as the crucial link between activated keratinocytes and immunocytes required for the development of psoriasis in a novel transgenic mouse model [87].

Thus, keratinocytes in the psoriatic epidermis are characterized not only by abnormal proliferation and apoptosis, but also by increased expression of inflammatory cytokines through interaction with immunocytes [84]. This seems to be regulated by the same signals arising from the activation of MAPK signaling cascades of p38 in immune cells [88]. Apart from the classical Th1 mediated response, Th17 cells have recently been demonstrated to sustain inflammation in psoriasis and psoriatic arthritis [89, 90]. The antimalarial drug and autophagy/lysosome inhibitor chloroquine (CHQ) is suggested as potential trigger of drug-induced psoriasis, in which Th17 cell mediated cytokine expression occurs in an p38-dependend IL-23 expression manner [91]. Dendritic cells treated with LPS increase the secretion of both IL-1β and IL-23, and stimulate the secretion of IL-17, IFN-γ, and IL-22 by innate γδ T cells [92]. IL-12, IL-23 and IL-27

production form a activation loop involving cells of innate and adaptive immune response [93]. In bone marrow-derived dendritic cells IL-23 and IL-27 expression is in part regulated by p38 MAPK [94].

Apart from inflammatory gene expression, antimicrobial peptide S100A8, known to be up-regulated in lesional psoriatic skin, was found also to be regulated by a p38 MAPK-dependent mechanism [95]. Similarly, p38-dependent expression was demonstrated for the antimicrobial peptides cathelicidin, human β-defensin-2, human β-defensin-3, and S100A7 in human keratinocytes [96] .

Information on the involvement of MAPK signaling in the pathogenesis of PsA is very scarce, whereas activation of MAPKs, specifically p38 and downstream MK2, has been described in RA synovium and in the collagen-induced arthritis model of RA [64, 75]. Recently, in a mouse model it has been shown that psoriatic skin inflammation facilitated the development of arthritis and enthesitis, both features of PsA [97]. Th1 and Th17 Inflammatory cytokines are up-regulated in psoriasis, PsA and other spondyloarthritides [98-100] and TNF-α inhibitors are the mainstay treatment for psoriasis and PsA [101-103]. Back in 2000, Danning et al have linked elevated pro-inflammatory cytokines with NFκB activation in PsA synovium [104]. More recent findings underlined the participation of both MAPK signaling and NFκB activation in PsA synovium before and after treatment with TNFα inhibitor (etanercept) [105]. Activated p38 was present in both lining and sub-lining area of the synovial membrane and p38 positive cells were detected in inflammatory infiltrates and perivascular areas. In addition, IL-36α is up-regulated in PsA and RA synovium and leads to IL-6 and IL-8 production by synovial fibroblasts through p38/NFκB activation [106].

p38 MAPK pathway in rheumatoid arthritis

The p38 MAPK signaling pathway has been implicated in the pathogenesis of RA. In fact, for several years it has been regarded as a potential therapeutic target for RA and other chronic immune-mediated inflammatory diseases [28, 64].

RA is considered an autoimmune disease. Recent findings suggest that ACPAs are autoantigens in RA recognized by T cells and B cells and most importantly are arthritogenic [24]. Early studies have confirmed the heavy infiltration with T cells carrying activation markers in inflamed RA synovial membrane [107-109]. Infiltrating T cells preferentially expressed IFN-γ, thus aggravating chronic inflammation. Local synthesis of IFN-γ in RA joints is largely induced by the synergistic effect of IL-12 and IL-18 that are produced by activated antigen presenting cells (APCs) [110, 111] . IL-12 and IL-18 induce IFN-γ through the p38 MAPK pathway in T and NK cells [22, 112]. Autoreactive T cells also maintain a spontaneous TNF-α production in rheumatoid synovial tissues via cell conduct and cytokine activation [113]. Activation of p38 MAPK is a critical step for the acquisition of effector function in T cells [114, 115]. TNF-α is one of the major pro-inflammatory mediators in RA and recently, prolactin receptor (PRLR) which regulates the expression and release of TNF-α from CD14(+) monocytes through p38 MAPK was found to be markedly increased in RA patients [116]. This TNF-α release from CD14(+) monocytes can be abolished by PRLR gene silencing or treating with MAPK inhibitor.

Over the last few years, the importance of IL-17 and Th17 cells in the pathogenesis of RA has become apparent [117, 118]. Recent data suggest that engagement of TLR2 enhances IL-17(+) autoreactive T cell responses via p38 MAPK signaling in dendritic cells [119]. Inhibition of p38 MAPK activity dramatically decreased IL-17 gene expression and antigen-specific Th17 responses. In addition, increased salt solutions, found locally under physiological conditions, have been shown to activate the p38/MAPK pathway involving nuclear factor of activated T cells 5 (NFAT5); and serum/glucocorticoid-regulated kinase 1 (SGK1) during cytokine-induced Th17 polarization. In addition, increased dietary salt aggravated experimental autoimmune encephalomyelitis through Th17 cells [120].

Of the p38 MAPK isoforms p38α is the one that strongly expressed in RA synovial tissue and mostly implicated in the pathogenesis of RA [66, 121]. Preferential activation of upstream MKK3 or MKK6 leading to p38MAPK activation in synovial fibroblasts has also been described [122, 123]. Deletion of p38β does not affect experimental arthritis, whereas deletion of the Mapk14 (p38α) confers protection against inflammatory bone loss. In the rat streptococcal cell wall arthritis model, inhibition of p38 MAPK leads to significant reduction of inflammation and cartilage breakdown [124]. Similar findings are also reported in other murine arthritic models, such as adjuvant-induced arthritis and collagen-induced arthritis [125, 126]. Animal models enable the prediction and testing of therapeutic targets, but the pathogenetic mechanisms involved may not be quite similar to those in human RA. Still, studies in animal models suggest that p38 MAPK and upstream kinase inhibition controls well-established arthritis [127]. In these models, several

early and new generation p38 MAPK inhibitors have been proven to be effective in reducing the disease severity.

Disappointingly enough, none of p38 inhibitors have successfully passed late clinical trials for the treatment of RA thus far [68]. Untoward effects that limited their use was significant liver toxicity, and their undesirable ability to cross blood-brain barrier [128]. Therefore, certain early inhibitors such as VX-745 (with promising results in phase II trials for RA) were soon discontinued [129]. Newer inhibitors, such as SCIO469, BIRB796, pamapimod, KR-00348 and AS1940477, are more selective at targeting p38, but show transient inhibition of pro-inflammatory markers [130, 131].

In order to explain the apparent failure of p38 inhibitors in RA, it is important to better understand the complexities of the p38 pathway and its communication with other cellular signaling pathways [28]. Targeting the upstream or MAP kinase kinases, may provide a viable alternative [127]. For example, MKK3 and MKK6, has been shown to partially maintain p38-mediated anti-inflammatory responses in bone marrow-derived macrophages (BMDM) [132]. Conventional p38α inhibitors have limited efficacy in RA, possibly because p38 blockade suppresses the counter-regulatory mechanisms that limit inflammation. For instance, recent data revealed the existence of CD8+FoxP3+ Treg cells in peripheral blood of patients with RA [133]. Unlike their CD4+ counterparts, CD8+FoxP3+ Treg cells inhibited Th17 inflammatory responses thereby limit a wider range of inflammatory pathways. CD8+FoxP3+ Treg cell induction was supported both by p38 phosphorylation intrinsic to naive CD8+ T cells and by monocytes via CD86 and membrane TNFα. In contrast to conventional CD4+ T cells, freshly isolated natural Tregs exhibit marked activation of p38 MAPK [134]. The p38 MAPK pathway is also involved in the conversion of naive T cells into induced Tregs [135]. Inhibition of p38 MAPK activity prevents the TGFβ-dependent conversion of CD4+CD25- T cells into Foxp3+ iTreg *in vitro* [136]. It follows that, p38 MAPK targeting (such as that through pharmacological inhibitors) potentially prevents the induction of iTreg *in vivo* and this may account for the inability of these antagonists *per se* to control the exacerbation of autoimmune disease.

All these data collectively define a largely unknown p38-dependent mechanism of FoxP3+ Treg cell induction. It seems that inhibition of p38 MAPK through pharmacological antagonists exerts two distinct functions. First, they act as potent anti-inflammatory agents by diminishing p38 MAPK mediated IFN-γ, IL-6, and IL-17 expression and; second they lead to functional impairment of suppressor cells, such as regulatory T and probably B cells. While the former function of p38 MAPK antagonists is undisputed, the latter is largely unexplored but certainly unwanted.

Peripheral blood signature studies are of great importance and can provide a lot of information regarding new immune regulatory molecules [137]. Meticulous assessment of cellular populations their signaling pathways and associated gene expressions is necessary in order to advance our knowledge on the pathogenesis of rheumatic diseases and to successfully identify novel molecular therapeutic targets. Application of optimized protocols based on sensitive phospho flow cytometry have been recognized as promising alternatives for the investigation of the phosphorylation of p38 MAPK within different peripheral blood mononuclear cell (PBMC) populations [138-140]. For instance, we have optimized a flow cytometry-based assay that details cellular phenotypic status, signaling status and gene expression analysis, all combined [141, 142]. The availability of multiplexing technologies capable of simultaneously quantifying multiple biomarkers has been particularly helpful in dissecting changes in soluble cytokine and chemokine networks in clinical samples. (Fig. 1). Flow cytometry has been so far useful in revealing the phenotype of rare cells infiltrating psoriatic lesion and their secreted cytokines [143]. One such study in PBMC of patients with PsA identified a unique gene expression signature of MAPK signaling members [144]. These observations could be carefully projected and analyzed in relation to infiltrating lymphocytes from psoriatic skin biopsies [145, 146].

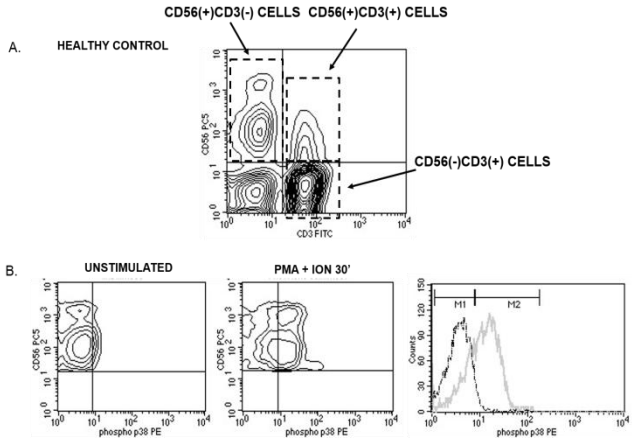

Fig.1 Flow cytometric analysis of P38 MAPK. Representative flow cytometric analysis of p38 MAPK phosphorylation in NK (CD56 positive) cells from a healthy donor following stimulation with PMA and Ionomycin

Yet, there is very little information available on the kinases and signaling pathways activated in the rare NK and NKT cells of PsA patients (Fig. 2). As shown in figure we are currently able to successfully detect several surface epitopes together with phosphorylated kinases such as p38 and Stats and subsequent intracellular gene expression. We have previously shown that p38 MAPK regulates post-transcriptional IFN-γ gene expression in human NK and NKT cells [22]. This possibly occurs via an MKK6/p38/MK2-dependent mechanism for the stabilization of IFN-γ mRNA in NK and NKT cells, and may play an important role in host defense as well. We have previously applied the optimized methodology for the successful application of phospho-specific flow cytometry in order to detect phosphorylated p38 MAPK within peripheral and intrahepatic innate immune cells, such as NK and NKT [147]. Because of the critical and bi-directional role of the p38 MAPK / IFN-γ axis in inflammation and autoimmunity, the elucidation of the molecular mechanisms controlling its expression is the focus of ongoing research by our team [142].

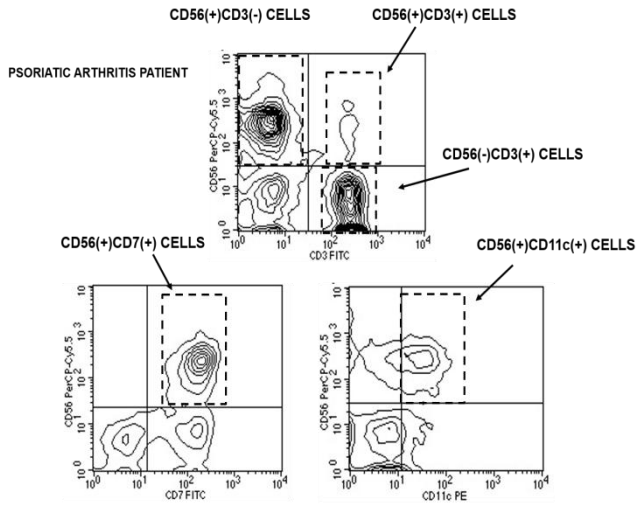

Fig 2. Representative histograms of experiments showing activation of p38 MAPK in a patients with psoriatic arthritis. Surface markers staining includes CD3 (pan-T marker), CD56 and CD7 (NK markers).

II. Conclusions

Current data suggest that p38 MAPK plays an important role in the pathogenesis of RA and possibly PsA. However, more studies are needed to further advance our knowledge on p38MAPK expression in inflammatory as well in suppressor cells during the course of arthritis in order to better define therapeutic strategies.

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Conflict of Interest: None.

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References and Footnotes

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