Deregulation of apoptosis in arthritis by altered signal transduction

An ever increasing body of experimental evidence links the deregulation of signal transduction pathways in cells of the immune system, synovial tissue, articular cartilage and subchondral bone to the pathogenesis and progression of adult rheumatoid arthritis. In this regard, elevated levels, as well as a changing repertoire, of proinflammatory cytokines in the sera and synovial fluids of rheumatoid arthritis patients appear to deregulate activation of three signal transduction pathways, JAK/STAT, SAPK/MAPK and the PI3K/tensin homolog/Akt/mTOR pathways, which are critical to the homeostatic mechanism regulating cell survival and apoptosis.

KEYWORDS: apoptosis articular cartilage autoimmunity cytokines protein kinase rheumatoid arthritis signal transduction small-molecule inhibitors subchondral bone synovial tissue

Adult rheumatoid arthritis

Adult rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology characterized by defective innate and adaptive immune responses resulting in chronic and progressive synovial joint inflammation [1–3]. Extra-articular manifestations of RA include pathology in the lung, eyes, kidney and blood vessels. Anemia and renal amyloidosis may also occur. From an adaptive immune system perspective RA can be characterized by the skewing of T cells of the T-helper (CD4⁺) subset, which preferentially express proinflammatory cytokine genes (i.e., TH, cytokines) at the expense of anti-inflammatory cytokines (i.e., TH, cytokines). However, RA is more than just a TH₁/TH₂ mismatch. Thus, in RA, T cells expressing IL-17 (i.e., Th17 cells) whose differentiation is dependent on IL-1, IL-6, IL-23 and TGF-B contribute to progressive inflammatory responses, as well as B-cell hyperactivity, the latter exemplified by abnormal autoantibody production of IgG and IgM rheumatoid factor, anticyclic-citrullinated peptide antibodies and anti-DNA antibodies [3-5]. Of note, deficient Treg function in the peripheral circulation is also a characteristic feature of RA, although the number of Tregs found in inflamed RA synovial tissue has been found to be increased compared with normal synovial tissue [6,7]. A polymorphism in the gene encoding TLR-4 is only one example of how aberrant innate immune dysfunction could contribute to the pathology of RA [8].

Role of signal transduction in cell survival & apoptosis

The relationship between constitutive and/or deregulated activation of signal transduction

pathways by proinflammatory cytokines depend on cytokines interacting with either type I transmembrane receptors characterized by four α -helical strands and the common amino acid motif, WSXWS in their extracellular domain or type II cytokine transmembrane receptors, which lack the WSXWS sequence [9]. These interactions occur primarily in those signaling pathways that promote aberrant cell survival and 'apoptosis resistance' in RA tissues [10–13].

Besides regulating cell survival and apoptosis in the progression of RA to joint failure, JAK/ STAT, SAPK/MAPK and PI3K/Akt also control the extent to which genes encoding proinflammatory cytokines, matrix metalloproteinases (MMPs) and soluble mediators of inflammation are regulated. In addition, continuous activation of these signaling pathways can occur if upstream and downstream kinase activation is also deregulated. This is likely to be a critical factor for maintaining immune-mediated inflammatory responses in RA. For example, constitutively activated upstream kinases could play a critical role in activating downstream kinases. These upstream kinases include activated kinase-1 (TAK1) also known as MAP3K7 [14,15], IKKβ [16] and MK2/MK3 [17], as well as downstream targets of Toll-like receptor activation of p38 kinase and ERK1/2, such as MSK1/MSK2 [18].

Negative regulation of JAK/STAT

Negative regulators of cell signaling include suppressor of cytokine signaling (SOCS), protein inhibitor of activated STAT (PIAS) proteins and protein tyrosine phosphatases. Experimental manipulation of these proteins in RA inflamed



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synovium could potentially be employed to alter cytokine-mediated signal transduction, proinflammatory cytokine gene expression and apoptosis [19]. In fact, El Kasmi *et al.* found that eliminating the binding of SOCS-3 to JAK3 permitted the IL-6/IL-6 receptor/gp130 pathway to generate an anti-inflammatory response in cultured macrophages [20]. SOCS have also been shown to influence T-helper cell differentiation. In that regard, Li *et al.* showed that bone marrow-derived dendritic cells that were transduced with SOCS-3 significantly inhibited IL-12-induced STAT-4 activation and IL-23-induced STAT-3 activation, while also driving oligodendrocyte glycoprotein-specific T cells to differentiate into the TH, subset [21].

Shuai cited four potential mechanisms involving PIAS proteins that might be exploited to reduce the influence of activated STAT proteins on proinflammatory cytokine gene expression, cell survival and apoptosis [22]. These included PIAS1 and PIAS2 inhibition of STAT1 and STAT3, respectively, preventing the binding of activated STAT proteins to STAT-responsive genes; PIAS proteins blocking the binding of NF-κB to NF-κB binding sites in DNA; PIAS proteins recruiting transcriptional corepressor proteins to block gene expression; and PIAS proteins promoting sumolyation of proteins involved in the inflammatory response. With respect to the role of protein phosphatases in altering immune cell signaling, Zhang et al. showed that SHP-1 and SHP-2 inhibited T- and B-cell receptor signaling [23]. SHP-1 and SHP-2 were also shown to block cytokinereceptor signaling [23] and to alter the activation of CD45 required for T- and B-cell antigen-receptor signaling [24], the latter resulting in inhibition of STAT1-mediated gene expression.

JAK inhibition

Recently there have been several reports demonstrating the clinical efficacy in treating autoimmune diseases with two orally administered small-molecule inhibitors (SMIs) of JAKs namely, tofacitinib, formally known as CP690, 550 [25-27], and ruxolitinib, otherwise known as INCB18424 [27-29]. Tofacitinib is more specific for JAK3 (IC₅₀: 1 nM) with less inhibitory activity exhibited towards the more ubiquitous JAK1 (IC₅₀: 112 nM) and JAK2 (IC₅₀: 20 nM) [25,29]. Ruxolitinib has greater inhibitory specificity for JAK1 and JAK2, (IC₅₀: 3.3 and 2.8 nM), respectively, in contrast to Tyk2 (IC₅₀: 19 nM) and JAK3 (IC₅₀: 323 nM) [28].

The JAK inhibitory profile exhibited by tofacitinib indicated that this SMI should effectively block JAK/STAT activation initiated by γ -chain cytokines, such as IL-2, IL-4, IL-15 and IL-21 via JAK3, whereas IFN- γ , IL-6 and, perhaps, IL-12-/IL-23-activated JAK/STAT would be expected to be blocked to a lesser extent [19].

Baricitinib, an inhibitor of JAK1/JAK2, which is structurally related to ruxolitinib is also currently being evaluated in an RA clinical trial [101]. A selective JAK1 inhibitor, GLPG0634, a JAK2-selective inhibitor, CEP-33779, and VX-509, selective for JAK3 are also in various stages of evaluation for clinical efficacy. At present, a selective Tyk2 inhibitor has not been developed although GLPG-0634 has some inhibitory activity towards Tyk2.

The results of in vitro studies also demonstrated that these JAK-specific SMIs suppressed intracellular signaling through the JAK/STAT1 pathway in TNF-α-stimulated macrophages from healthy donors, as well as in macrophages recovered from the synovial fluid of RA patients [27]. Importantly, RA macrophages are known to play an important role in the pathogenesis and progression of RA because they primarily produce the elevated levels of the proinflammatory cytokines, TNF-a, IL-1 and IL-6. These cells also express high levels of STAT1. However, the positive response by investigators to the potential for significant therapeutic effects of these JAKspecific SMIs must be tempered by the finding that they also stimulated osteoclast formation by increasing the nuclear level of NFATc1 and c-Jun [27], although LaBranche et al. recently showed that tofacitinib suppressed osteoclast mediated bone resorption via decreased T-cell production of RANKL in experimental adjuvant-induced arthritis in rats [30]. Overall, these JAK-specific SMIs are also likely to be relevant in suppressing the progression of RA because they will be expected to regulate the TNF-α/IFN-β/JAK/STAT autocrine loop, which plays a role in macrophage-mediated bone resorption.

The PI3K/Akt/mTOR pathway

In addition to its involvement in regulating innate and adaptive immunity, PI3K/Akt activity is also critical for osteoclast differentiation, as well as for their survival. As osteoclasts significantly contribute to the degradation of subchondral bone in RA, the PI3K/Akt/mTOR pathway is a suitable target for pharmacologic intervention [11]. Indeed, using the mTOR inhibitor everolimus at a dose level (6 mg once daily) that was comparable to that employed in transplantation studies in combination with methotrexate have shown modest clinical benefit in RA and only at the ACR-20 criteria level [31]. At present the safety of everolimus may be inadequate with gastrointestinal, skin and nervous system disorders significantly greater in the everolimus plus methotrexate arm. The most troubling were changes in hematologic parameters, liver function tests and lipid levels, which were more frequent in the everolimus arm compared with the placebo (i.e., methotrexate) arm. However, these adverse events were considered mild and reversible.

Interestingly, rapamycin, another mTOR inhibitor, also was shown to inhibit fibroblast proliferation and IL-17-induced RA fibroblast proliferation was dependent on mTOR activity [32,33]. In the canonical RA pathway, activated fibroblast-like synoviocytes (FLS) and immune cells comprise pannus, the tissue that initiates cartilage destruction [34,35]. Therefore, inhibitors of mTOR activity may show some promise for preventing cartilage degradation in RA in addition to protecting against subchondral bone and matrix degradation.

Proinflammatory cytokines

The recognition that alterations in intracellular signal transduction is orchestrated by the elevated levels of proinflammatory cytokines that play a crucial role in synovial joint destruction in RA [36-38] has also refocused attention on those signaling pathways that perpetuate either the survival of T-cell subsets that regulate RA progression (e.g., Th17 cells and Tregs) or cell death [39], both of which regulate immune responses, as well as establishing and perpetuating chronic inflammation. Future advances in understanding the mechanisms that regulate the balance between cell survival and apoptosis may arise from elucidating how the increased expression of antiapoptosis proteins, the so-called apoptosis resistance molecules found in RA synovial tissue, are regulated [11]. Thus, constructing a more precise list of which molecules are involved in RA progression should aid in directly targeting those signaling pathways that are activated by them.

TNF-related weak inducer of apoptosis: regulator of apoptosis

TNF-related weak inducer of apoptosis (TWEAK) is one such molecule that is a potential target for investigation because it is suspected of playing a role in RA pathogenesis by regulating cell proliferation and angiogenesis. The contribution of TWEAK to deregulated synovial proliferation and immune cell-mediated inflammation is noted. Moreover, TWEAK was found to be elevated in sera of RA patients [11]. However, the results of a recent study also showed that TWEAK can have paradoxical effects on cells. Thus, TWEAK, as expected, induced the production of IL-6 and IL-8 in FLS. However, TWEAK also inhibited the production of these cytokines in TNF- α -activated synoviocytes [40]. The results from another recent study not only found that there were elevated levels of TWEAK in RA synovial fluid when compared with synovial fluid obtained from osteoarthritis patients, but that TWEAK also induced the plasma membrane expression of RANKL by osteoblasts [41]. Thus, these findings represent a potential indirect pathway for preventing bone erosion in RA by using anti-TWEAK strategies to potentially upregulate osteoblast-mediated production of new bone and suppression of osteoclast-mediated bone destruction.

Finally, TWEAK was found to induce IL-17 in murine collagen-induced arthritis where TWEAK was shown to promote the differentiation of Th17 cells [42]. IL-17 is also a known activator of the JAK/STAT pathway [19] .The results from previous studies had also provided experimental evidence that implicated IL-17 as promoting aberrant synovial proliferation [11]. In clinical studies, preliminary evidence has since confirmed an inverse relationship between the number of Th17 cells and circulating levels of IL-17 as well as the response of RA patients to TNF- α blockade [43,44]. Therefore, anti-TWEAK protocols may be capable of decreasing circulating Th17 cells while increasing the response of RA patients to anti-TNF- α therapy.

TNF-related apoptosis inducing ligand: another regulator of apoptosis

TNF-related apoptosis inducing ligand (TRAIL) is a proapoptotic molecule that has been proposed as a way to induce apoptosis in RA synovium [13]. In that regard, the results of a recent study demonstrated that human RASF was resistant to TRAIL-induced apoptosis [45]. However, TRAIL in combination with the anthracycline chemotherapeutic drug, epirubicin, enhanced the sensitivity RASF to TRAIL [45]. This strategy might produce a successful clinical result by directly treating the hyperplastic RA synovium with a combination of TRAIL and epirubicin.

The fact that TRAIL was also shown to have dual functions, however, only adds to the complexity of using TRAIL in RA therapy. Thus, in those RASF that survive the initial treatment with TRAIL, TRAIL was then shown to promote their further survival through the MAPK and PI3-K/Akt pathways [46]. To address this issue, Sun *et al.* found that apigenin, a bioflavonoid, when employed in combination with TRAIL increased TRAIL-induced FLS apoptosis, while also reducing TRAIL-dependent proliferation, possibly by inactivating PI3-K/Akt signaling [47]. Interestingly, the differential response of RA patients to TNF- α blockade therapy was also recently shown to be associated with the TRAILR1 gene polymorphism, which might dictate a clinical response to TRAIL [48]. Despite prior evidence to the contrary that did not detect TRAILR1/TRAILR2 on RA synovial fibroblasts (RASFs), it was subsequently shown that the antihuman TRAIL death receptor 5 (anti-DR5) antibody, TRA-8, induced apoptosis in RA synovial fibroblasts in vitro [49]. Moreover, TRA-8 was shown to promote amelioration of arthritis in human/mouse-chimeric DR5transgenic mice [50]. The mechanism of action of TRA-8 was proposed to involve depleting the M1-type macrophages, possibly by inducing apoptosis in inflamed synovial tissue. Previously, Ohtsuka et al. had shown the anti-DR5 in combination with adriamycin and cisplatin improved the apoptosis-inducing activity of anti-DR5 antibodies against several different types of tumor cell lines by enhancing the activation of caspases, as well as by increasing cytochrome C and Smac/DIABLO release from mitochondria in parallel with a drop in mitochondrial membrane potential [51]. Of note, combining anti-DR5 with these chemotherapeutic agents resulted in a synergistic activation of JNK and p38 kinase which was also dependent on MKK4 activation.

DCR3

DCR3 is a newly identified secreted protein that may ultimately have novel use as a therapeutic target for RA. The expression of DCR3 is elevated in RA synovium and DCR3 was found to be associated with an increase in inflammatory cell infiltrate [52]. However, the results of a recent study showed that DCR3 also had a paradoxical effect in vivo as when it was administered to mice with collagen-induced arthritis, DCR3 attenuated the severity of arthritis characterized by significant modulation of specific immune responses [53]. This modulation of immune cell responses induced by DCR3 in vivo included reduced numbers of CD19⁺ B cells, as well as lower levels of IFN-y, IL-4, IL-17A and Foxp3 (a transcription factor marker for Treg cells)-positive CD4⁺ T cells. DCR3 also reduced levels of IL-6, total IgG2a, and CII-specific IgG2a antibody in serum, as well as inhibiting Pam3CSK4 (i.e., Toll-like receptor 1/2 ligand)-induced B220+

B-cell proliferation *in vitro*. Therefore, by implication, reducing the level of IL-6 via DCR3 may also mean that DCR3 will alter JAK/STAT activation since *IL-6* gene promoter activity is responsive to activated STAT proteins [54].

The results of another recent study with DCR3 may prove to have particular relevance for specifically treating the bone erosions of RA. Thus, Cheng et al. found that DCR3 inhibited osteoclastogenesis by downregulating the expression of the RANKL-induced transcription factor, NFATc1 [55]. DCR3 also inhibited the bone-resorption activity of mature osteoclasts, as well as enhancing RANKL-induced osteoclast apoptosis and Fas-ligand expression. In this regard, DCR3 inhibited RANKL-induced tartrate-resistant acid phosphatase positive multinucleated cells and inhibited RANKLinduced nuclear factor κ-light-chain-enhancer of activated B-cells activation, NFATc1, and cytoplasmic calcineurin-dependent-1 NFATc1 nuclear translocation in the RAW264.7 cell line.

Small ubiquitin-like modifier

Small ubiquitin-like modifier (SUMO) is another molecule of potential interest in the future therapy of RA. Recent experimental evidence showed that TNF-a activated several signaling pathways in human chondrocytes including the MAPK and JAK/STAT pathways [56]. Frank et al. found that TNF- α regulated the expression of SUMO-2, but not SUMO-3 [57]. In order to explore the potential role that SUMO-2/3 may play in experimental RA the expression of SUMO-2/3 was knocked down specifically in synovial tissue from human TNFtransgenic mice. Knockdown of SUMO-2/3 expression resulted in increased TNF- α and IL-1β-mediated expression of stromelysin-1 (MMP-3) and collagenase-3 (MMP-13), but not collagenase-1 (MMP-1). These results also suggested that upregulation of SUMO-2 expression may constitute a protective response with respect to MMP-3/MMP-13-mediated degradation of articular cartilage [57], which is likely to depend on 'cross-talk' between activation of the MAPK and JAK/STAT signaling pathways.

SUMO was also shown to play a seminal role in regulating apoptosis in RA [58]. In that regard, downstream modification of Fas-mediated signaling under the control of modifiers such as sentrin/SUMO-1 and Fas-associated death domainlike IL-1 β -converting inhibitory protein, as well as activation of transcription factors, NF- κ B, STAT3 and p53 via the MAPK and JAK/STAT pathways have all been reported [58–60].

Sumoylation is likely to emerge as a critical transient posttranslational protein modification event resulting in suppression of synovial cell apoptosis in RA [61]. In that regard, Yan et al. showed that SUMO also regulated the effects of several critical anabolic growth factors that play a role in RA, including FGF-2, TGF-β and IGF-1 [61]. These may act by regulating the activation of transcription factors, including, Smad, Elk-1 and HIF-1, which are important for the transcription of these growth factor genes [54]. Thus, activation of these transcription factors would be expected to significantly modify chondrocyte and synovial cell survival, inflammation, aggrecan and collagen degradation as well as responses to hypoxic conditions in the RA milieu.

Forkhead box O: antiapoptosis transcription factor

Finally, in RA synovial tissue, phosphorylation of the anti-apoptotic forkhead box O transcription factor, FOXO1 was observed in both FLS and macrophages, phosphorylation of FOXO3a in T lymphocytes, and phosphorylation of FOXO4 in macrophages alone, with FOXO4 levels particularly enhanced in these cells [62]. In contrast, FOXO3 was implicated as a primary modulator of apoptosis in RA which could alter the severity of experimentally-induced arthritis [63]. Thus, results of a recent study that showed that in rats with collagen-induced arthritis, intra-articular administration of simvastatin alleviated arthritis and suppressed Cyr61 (CCN1) expression [64] may be very relevant to future therapies for RA because the simvastatin effect was characterized in vitro by suppression of FOXO3a phosphorylation. In addition, simvastatin suppressed TNF-\alpha-induced Cyr61 and CCL20 production, as well as preventing the nuclear export, phosphorylation and acetylation of FOXO3a, while maintaining its capacity to bind to the Cyr61 promoter. In vitro studies with RASF suggested a putative mechanism for the effects of simvastatin on FOXO3 activity, as well as its capacity to reduce the severity of arthritis in collagen-induced arthritis. In vitro simvastatin activated SIRT1 induction of FOXO3a in RASF although the precise array of kinase activities involved in SIRT1/FoxO3 signaling in RASF remains to be completely elucidated. However, it was previously shown that FOXO activity correlated with PI3K/Akt/mTOR pathway activity [62]; mammalian SIRT1 deacetylated FOXO3 and/or FOXO4, which dampened FOXO-induced apoptosis, while also promoting

FOXO-induced cell cycle arrest [65]; and that inhibition of FOXO activity by SIRT1 paralleled the effect of its deacetylase activity on the tumor suppressor protein, p53 [66], the latter identified as a contributor to hyperplasia in chronically inflamed RA synovial tissue [10,11].

Conclusion

In RA, altered intracellular signal transduction involving the JAK/STAT, SAPK/MAPK and PI3K/tensin homolog/Akt/mTOR pathways play a critical role in determining whether T and B cells, dendiritic cells, macrophages, synoviocytes, chondrocytes and osteoclasts survive and undergo apoptosis. The recognition that proteins, such as proinflammatory cytokines, TWEAK, TRAIL, DCR3 and SUMO, which are all involved to some extent in fostering aberrant cell survival or induction of apoptosis, can be experimentally manipulated to result in apoptosis has focused attention on whether they may constitute an area for the development of future therapies for RA. Recognizing that activation of the JAK/STAT pathway results in an inflammatory response characteristic of RA has led to the development of SMIs, which are relatively specific for each of the three JAK species. These SMIs are now either available for the medical therapy of RA or in the process of being further studied for their efficacy, safety and tolerability in RA clinical trials.

Future perspective

All the available evidence to date strongly indicates that protein kinases involved in the regulation of intracellular signal transduction are critical to immune-mediated inflammation in RA. SMIs of at least two of these, namely, JAK (i.e., tofacitinib) and Syk (i.e., fostamatinib) have already been tested in RA clinical trials with tofacitinib approved for treating patients with moderate-to-severe RA who have demonstrated an inadequate response to conventional or biologic antirheumatic drugs. Several other smallmolecule inhibitors with activity against JAKs, including baricitinib, GLPG0634 and VX-509, are in various stages of development to assess their safety and tolerability in RA clinical trials. The results of recent studies have also focused attention on the PI3K/Akt/mTOR pathway owing to its role in regulating cell survival and apoptosis. Thus, everolimus and rapamycin, inhibitors of mTOR activity, may be considered for development for the future therapy of RA. Finally, a number of molecules that activate various signal transduction pathways, including TWEAK and

TRAIL, both regulators of apoptosis, as well as DCR3, SUMO and the FOXO family of transcription factors should also be considered for additional analysis since they appear to be integral to the restoration of the balance between cell survival and apoptosis in inflammatory arthritis. Western Reserve University School of Medicine is supported by an Investigator-Initiated Project Grant from Genentech/Roche Group (CA, USA) to CJ Malemud. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Executive summary

Defective intracellular signal transduction deregulates cell survival & apoptosis in adult rheumatoid arthritis

- Continuous activation of JAK/STAT, SAPK/MAPK and PI3-K/Akt intracellular signaling pathways mediated by elevated levels of proinflammatory cytokines have been implicated in the initiation and perpetuation of immune-mediated chronic inflammation and the deregulation of cell survival and apoptosis in adult rheumatoid arthritis (RA).
- In the pathological states, these activated signaling pathways may also occur in response to upstream and downstream kinase deregulation, which, in turn, perpetuate the inflammatory response by deregulating proinflammatory cytokines and matrix metalloproteinase gene expression.

Altered signaling in model cell culture systems correlates with defective regulation of signal transduction in rodent models of RA

- Persuasive evidence from *in vitro* studies of T and B cell, synoviocytes, chondrocytes and osteoclasts from animals and humans showed strong correlation between the finding of defective regulation of intracellular signaling and precocious cell death, aberrant survival or 'apoptosis resistance'.
- Canonical proapoptotic molecules may exhibit dual functions under experimental conditions associated with chronic inflammation, thereby producing unexpected or paradoxical effects, such as proliferation and resistance to apoptosis.
- Newly identified prosurvival and/or proapoptosis molecules may have therapeutic potential once their interaction with multiple signaling pathways, their regulation of immune responses and activation of transcription factors are elucidated.
- Small-molecule inhibitors (SMIs) of JAK ameliorate arthritis severity in rodent models of RA.
- Testing of JAK-specific SMIs in cell culture studies have been extended to their use in rodent models of adult RA.
- Experimental SMIs inhibit the activity of phosphorylated kinases or transcription factors with amelioration of the severity of arthritis.
- The finding that JAK-specific SMIs also stimulate osteoclast formation must be taken into consideration when considering their potential clinical and therapeutic efficacy for reducing bone erosions.

The JAK-specific SMI, tofacitinib, showed clinical efficacy in human RA clinical trials

- Several recently completed human clinical trials demonstrated the clinical efficacy, safety and tolerability of the JAK3-specific SMI, tofacitinib and resulted in its approval by the US FDA.
- Tofacitinib, in combination with methotrexate, can now be employed for the treatment of moderate-to-severe RA in individuals who
 have become intolerant or refractory to previous drug therapy with biological agents or other disease-modifying antirheumatic drugs.
- Inhibitors of mTor have also been evaluated in human RA clinical trials.
- mTOR inhibitors have shown experimental promise for reducing arthritis severity, but an unacceptable safety profile will possibly limit their use in the clinic.
- Future targets for RA may include already defined proapoptosis and antiapoptosis molecules.
- Several molecules of interest include TNF-related weak inducer of apoptosis, TNF-related apoptosis inducing ligand, DRC3 and small ubiquitin-like modifier, which may be future targets for promoting either immune-cell, synoviocyte and chondrocyte survival or apoptosis.

Conclusion

- Results of these clinical investigations should provide the impetus for future drug discovery programs focused on designing novel JAK, SAPK/MAPK and PI3K/Akt-specific SMIs.
- Theoretical constructs linking fundamental immunological responses to clinical immunology will be emphasized in research that will focus on restoring balance within and between B- and T-cell subsets.
- The quest for obtaining therapeutic efficacy when treating RA will unite seemingly disparate elements of synovial joint physiology by focusing on interactions that occur between signaling pathways responsible for osteoclast differentiation and fibroblast proliferation.
- The ultimate goal of developing kinase-specific SMIs would be to inhibit apoptosis of articular chondrocytes in RA joints, while and at the same time inducing apoptosis in activated immune cells, synoviocytes and osteoclasts in chronically inflamed RA synovial tissue.
- Innovative drug delivery technologies could deliver specific drugs to affected RA joints, while sparing other organs, thus minimizing potential drug side effects.

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