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Apoptosis Resistance in Rheumatoid Arthritis Synovial Tissue

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Abstract

The pathogenesis of rheumatoid arthritis (RA) evolves from deregulated cellular and humoral immunity resulting in a chronic and systemic inflammatory response. Perpetuating the sustained inflammation in RA synovial joints requires the migration and retention of activated T-lymphocytes, B-lymphocytes, mast cells, neutrophils and antigen presenting cells. The synovial tissue becomes hyperplastic as a result of unrestrained synoviocyte proliferation and the resistance of synoviocytes, immune and inflammatory cells to apoptosis. Synoviocyte proliferation is mainly sustained by the elevated levels of pro-inflammatory cytokines in the RA synovial joint milieu. Thus, pro-inflammatory cytokines, including tumor necrosis factor-α, interleukin-(IL)-1β and IL-6, IL-17, interferon-γ, among others, predominantly activate the stress-activated protein kinase/mitogen-activated protein kinase (SAPK/MAPK) and the Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathways which are known to cause the induction of apoptosis. However, activation of SAPK/MAPK and/or JAK/STAT pathways can also cause 'cross-talk' and activation of the phosphatidylinositol-3-kinase/Akt pathway which generally results in aberrant cell survival. The synovial tissue of RA synovial joints is also characterized by elevated levels of anti-apoptosis proteins which suppress the apoptotic response. One of the main clinical responses of RA patients to therapy with methotrexate, sulfasalzine and leflunomide, or disease-modifying anti-rheumatic biological drugs, such as antagonists of tumor necrosis factor-α and the IL-6 receptor is actually to suppress the activation of signal transduction that inhibit apoptosis thereby reducing the survival of T- and B-cells, macrophages and inflammatory cells. In addition, several novel experimental strategies are also being considered with the view towards neutralizing those molecules held responsible for the resistance of synovial tissue to apoptosis. Thus, stimulating apoptosis may ameliorate arthritis. These targets include a group of tumor necrosis factor-related proteins, the BH3-only bcl-2 proteins, Fas ligand, cytokines such as IL-17 and IL-19, p53 up-regulated modulator of apoptosis and survivin.

Keywords: Autoimmunity, Apoptosis, Cytokines, Rheumatoid arthritis, Signal transduction, Synovium

Abbreviations: ACR20, 50, 70: American College of Rheumatology-20, 50, 70 response criteria; Ad.TRAIL: Adenoviral-expressing Tumor necrosis factor-related apoptosis protein ligand; APRIL: A proliferation-inducing ligand; ASK-1: Apoptosis signal-regulating kinase-1; BBC3: Bcl-2-binding component-3; Bcl-x(L): B-cell lymphoma-extra large; BH3: Bcl-2 homology domain 3; BNIP-3: Bcl-2/adenovirus E1B 19kDa protein-interacting protein-3; CDK-2: Cyclin D kinase-2; CIA: Collagen-induced arthritis; CTLA-4Ig: Cytotoxic T-lymphocyte antigen-4 immunoglobulin; DAS-28: Disease activity score-28; DAXX: Death-associated protein-6; DC: Dendritic cell; DcR3: Decoy receptor-3; DD: Death domain; DED: Death effector domain; DISC; Death-inducing-signaling complex; DMARDs: Disease-modifying anti-rheumatic drugs; DMARBDs: Disease-modifying anti-rheumatic biologic drugs; FADD: Fas-associated death domain; FasL: Fas(CD95)ligand; FGF-2: Fibroblast growth factor-2; FLICE: FADD-like IL-1-β-converting enzyme; FLIP, FLICE inhibitory protein; Fn14: Fibroblast growth factor inducible 14kDa protein; Foxo-3a: Forkhead box-3a; GADD45β: Growth arrest and DNA-damage-inducible45β protein; HMGB1: High mobility group box-1; ICAM-1: Intracellular adhesion molecule-1; INFs: Interferons; IFN-γ: Interferon-γ; IL: Interleukin; JAK/STAT: Janus kinase/Signal Transducers and Activators of Transcription; LIGHT: Lymphotoxin, exhibiting Inducible expression and competes with herpes simplex virus Glycoprotein D for HVEM, a receptor expressed by T lymphocytes; MCP-1: Macrophage chemotactic protein-1; MIP-1: Macrophage inhibitory protein-1; MLN-51: Metastatic lymph node-51; MMP: Matrix metalloproteinase; MTX: Methotrexate; NF-AT5: Nuclear factor activator of transcription-5; NF-κB: Nuclear factor-κB; NK: Natural killer; OASF: Osteoarthritis synovial fibroblasts; PAR-2: Protease-activated receptor-2; PDL-1: Programmed death ligand-1; PG: Prostaglandin; PARP: Poly-(ADP-ribose)-polymerase-1; PML: Promyelocytic leukemia; PUMA: p53 up-regulated modulator of apoptosis; SIRT-1: Sirtuin-1; SNP: Sodium nitroprusside; SPHK2: Sphingosine kinase-2; SUMO-1: Small ubiquitin-like modifier-1; TIM-3: T-cell immunoglobulin mucin-3; TNF-α: Tumor necrosis factor-α; TNFRP: Tumor necrosis factor-related protein; TNFR-I: Tumor necrosis factor receptor-I; TNFR-II: Tumor necrosis factor receptor-II; T_{reg}: T regulatory ; TRADD: TNF receptor-associated death domain; TRAF-2: TNF receptor-associated factor-2; TRAIL: Tumor necrosis factor-related apoptosis inducing ligand; TSA: Trichostatin; TWEAK: Tumor necrosis factor-related weak (inducer of apoptosis); RANTES: Regulated upon activation, Normal T-cell Expressed and Secreted; TRAIL, Tumor necrosis factor-related apoptosis inducible ligand; rTRAIL: Recombinant Tumor necrosis factor-related apoptosis inducible ligand; RAFLS: Rheumatoid arthritis fibroblast-like synoviocytes; RASF: Rheumatoid arthritis synovial fibroblasts; RF: Rheumatoid factor; RIP: Receptor interacting protein; SAPK/MAPK: Stress-activated protein kinase/mitogen-activated protein kinase; SODD: Silencer of death domain; VEGF: Vascular endothelial growth factor; ZAP-70: ζ-chain-associated protein kinase 70

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Introduction

Dysfunctional cellular and humoral immunity are two of the signature features of rheumatoid arthritis (RA) [1-3]. Aberrant proliferation and survival of activated T- and B-lymphocytes, mast cells, neutrophils, macrophages and accessory antigen-presenting cells, (i.e. dendritic cells; DC) is a key component of RA pathophysiology [4,5]. In part, our understanding of this scenario includes the fact that these cells are attracted to, and retained within synovial joint tissues, as a result of the over-expression of chemokines and adhesion molecules [6]. Additionally, under these conditions, the normally quiescent synovial tissue fibroblasts, also known as synoviocytes, become activated by pro-inflammatory cytokines synthesized and secreted by these cells. Most prominent among these pro-inflammatory cytokines are tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) as well as IL-6, IL-7, IL-8, IL-12/IL-23, IL-15, IL-17, IL-18, IL-32, interferon-γ and growth factors, including, fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF), the latter produced principally by T- and B-lymphocytes and macrophages. The combination of these cytokines and growth factors cause a dysregulation of synoviocyte proliferation that lead to the development of synovial tissue hyperplasia [4,6-9].

Programmed cell death or apoptosis is a major contributor to the maintenance of organ homeostasis. However, in certain pathologic conditions such as RA, 'apoptosis resistance' is a cardinal characteristic of RA synovial tissue [10]. Indeed, many of the commonly employed drug therapies in use for treating RA clinical activity are based, in part, on restoring a balance between synoviocyte proliferation and synoviocyte apoptosis [10-13] as well as diminishing the negative effects of autoreactive T- and B-lymphocytes and antigen-presenting cells to the RA process [14].

This paper will focus on the most recent developments since 2004 in our understanding of the fundamental mechanisms underlying defective apoptosis among the cells of RA synovial tissue with the inclusion of selected papers prior to 2004 to address the field from an historical perspective. The results of these studies have provided evidence for increasing our knowledge base of the underlying cellular, molecular and biochemical mechanisms that could account for 'apoptosis resistance' in various inflammation-based conditions, but focusing on RA synovial tissue.

Current advances in the medical therapy of RA with the incorporation of disease-modifying anti-rheumatic biologic drugs (DMARBDs) into clinical practice have revolutionized the treatment of RA. There is evidence of retarded radiographic progression of joint destruction and a lessening of clinical activity by DMARBDs [15]. However, many of the proteins that play a role in driving the RA process forward, and in promoting 'apoptosis resistance' in particular, cannot be totally neutralized by the commercially-available DMARBDs now in clinical use. Thus, additional studies must be undertaken to provide evidence that correcting 'apoptosis resistance' in RA synovial tissue will totally inhibit the progression of irreversible cartilage and bone destruction in RA. These advances must arise through the discovery and development of novel agents that target the appropriate dysfunctional apoptosis-related pathways characteristic of RA synovium [14,16-23].

Tumor Necrosis Factor-Related Proteins

Tumor necrosis factor-related proteins (TNFRPs), including tumor necrosis factor receptor-I (TNFRI), tumor necrosis factor receptor-II (TNFRII), tumor necrosis factor-related weak inducer of apoptosis (TWEAK), tumor necrosis factor-related apoptosis inducing ligand (TRAIL), decoy receptor-3 (DcR3) and TNFRP-adaptor molecules, such as TNF receptor-associated death domain protein (TRADD), Fas-associated death domain protein (FADD), receptor interacting protein (RIP) and TNF receptor-associated factor 2 (TRAF-2) have been shown to be critical in the regulation of apoptosis in a variety of cells [24-26]. However, during the past 6 years much of the research focus has been devoted to gaining a firmer understanding of the role that TWEAK, TRAIL and DcR3 play in regulating synovial tissue and T- and B-cell apoptosis.

TWEAK

Persuasive evidence has shown that TWEAK and its cognate receptor, FGF-inducible 14kDa protein (Fn14), play an important role in normal physiological and pathological tissue remodeling [27]. However, the role of TWEAK in regulating apoptosis has often only been surmised from its capacity to interfere with the activity of NF-κB [28].

TWEAK exists primarily as a type II transmembrane protein although it may also fuse with a proliferation-inducing ligand (APRIL; TNFSF13) to form the TWEAK/APRIL fusion protein. The TWEAK/APRIL fusion protein is processed in an intracellular compartment where it is then becomes secreted. This soluble form of TWEAK (sTWEAK) was shown to regulate TNF-α-mediated apoptosis [29].

To begin, Kamijo et al. [30] showed that TWEAK was highly expressed on the $CD45^+$-cells of RA synovium. By contrast, Fn14 was found on both $CD45^+$ and $CD45^-$ cells. Further, recombinant TWEAK (rTWEAK) increased the level of IL-6 and IL-8 as well as macrophage chemotactic protein-1 (MCP-1) in cultured RA synovial fibroblasts (RASF) and osteoarthritis synovial fibroblasts (OASF). More importantly, rTWEAK increased the proliferation of freshly isolated RA synovial cells as well as causing an elevated production of cytokines both of which were suppressed by the combined treatment of these cells with anti-TWEAK and anti-Fn14 monoclonal antibodies. Of note, intracellular adhesion molecule-1 (ICAM-1) on RASF, but not on OASF, was up-regulated by rTWEAK. More recently, van Kuijk et al. [31] showed that TWEAK and Fn14 was expressed on RASF and macrophages, but not on T-cells. Interestingly, van Kuijk et al. [31] did not address the apparent discrepancy between their results which failed to find TWEAK on T-cells and those results previously reported by Kamijo et al. [30]. It should be noted, however, that CD45 is not a biomarker for T-cells. Rather CD45 is a protein tyrosine kinase biomarker present on the precursor cell that may eventually differentiate into granulocytes, T- and B-cells, monocytes and thrombocytes. Another study showed that TWEAK levels were substantially elevated in the serum of RA patients and that serum TWEAK levels correlated with RA disease activity [32].

Finally, experimental anti-TWEAK therapy of collagen-induced arthritis (CIA) in the mouse ameliorated disease activity when anti-TWEAK was administered before disease onset but not during the antigen-priming phase [33]. Although inhibition of TWEAK did not alter either cellular or humoral immune responses, the serum level of inflammatory biomarkers such as macrophage inhibitory protein-1 (MIP-1), lymphotactin, interferon (IFN)-γ-inducible protein 10, MCP-1, *Regulated upon Activation, Normal T cell Expressed and Secreted* (RANTES) protein and matrix metalloproteinase-9 (MMP-9) were significantly lower. However, the effect of experimental anti-

TWEAK therapy on inducing synoviocyte apoptosis in CIA was not studied. In another study in murine CIA, anti-TWEAK monoclonal antibody suppressed the development of small blood vessels in arthritic synovial tissue [34]. This finding indicated that TWEAK may also play an important role in stimulating blood vessel development which is a critical component of RA synovial tissue pathology [35].

Based on the results of the results of these studies it is likely that TWEAK plays a prominent role in RA pathogenesis and disease progression. In that respect, TWEAK has been shown to promote synovitis through its capacity to induce aberrant synoviocyte proliferation and to up-regulate the production of biomarkers of inflammation [36].

TRAIL

Apo2L/TRAIL, also known as CD253, is a 281 amino acid type II transmembrane protein that can bind to either of its receptors, TRAIL-RI (i.e. DR4) or TRAIL-II (i.e. DR5). Interestingly, the interaction between TRAIL/DR4/DR5 can trigger either proliferation or apoptosis [37]. TRAIL has also been shown to interact with DcR1 and DcR2 which function as the decoy receptors for TRAIL. Thus, when DcR1 acts as a TRAIL-decoy receptor the interaction between TRAIL/DcR1 is neutralized which may dampen TRAIL downstream effects. Of note, activation of Apo2/TRAIL transcription by INFs was shown to occur by the interaction of INFs with regulatory elements in the Apo2/TRAIL promoter. This shed light on the involvement of Apo2/TRAIL in regulating the balance between survival and apoptosis of NK cells, cytotoxic T-cells and DCs [38].

The impetus for the recent focus on exploiting anti-TRAIL-strategies to induce apoptosis in RA stems mainly from the results of TRAIL-based experimental gene therapy in the murine CIA model which showed that anti-TRAIL suppressed synoviocyte proliferation [39]. In other gene therapy studies, Yao et al. [40] showed that adenoviral-expressing TRAIL (Ad.TRAIL) induced apoptosis in the proliferating synovium in a rabbit model of arthritis while also reducing markers of synovial tissue inflammation. Most critically the Ad.TRAIL-mediated reduction in inflammation occurred without altering cartilage metabolism or the structure of articular cartilage extracellular matrix.

Yang et al. [41] also studied one of several potential mechanisms whereby TRAIL could be exploited to induce apoptosis in apoptosis-resistance RASF *in vitro*. They showed that the proteasome inhibitor, lactacystin caused p53 to accumulate in the cytosol of RASF and also improved the susceptibility of RASF to TRAIL/DR5-induced apoptosis. Interestingly, accumulation of p53 in the cytoplasm did not enhance RASF apoptosis following incubation with FasL, suggesting specificity of TRAIL/DR5-mediated apoptosis. However, the specific role played by p53 in TRAIL/DR5-mediated apoptosis was also better defined. For example, the data from that study [41] showed that silencing p53 with siRNA reduced the RASF apoptotic response to TRAIL/DR5. Further, apoptosis in RASF to response to TRAIL/DR5 was mediated by a vimentin-p53 complex which was caspase-4-sensitive because caspase-4 cleavage of vimentin blunted the RASF apoptosis response to TRAIL/DR5.

Apoptosis can also be initiated by treating normal fibroblast-like synoviocytes (FLS) with recombinant TRAIL (rTRAIL) [42]. However, pre-treatment of FLS with IFN-γ blunted rTRAIL-mediated apoptosis. Of note, none of the pathways that could reasonably be considered as being associated with downstream TRAIL-mediated events associated with apoptosis, including modulation of the TRAIL/DR4/DR5 interaction, a change in pro-caspase-3-/-8/-9 activation or activity, modulation of FADD, TRADD, silencer of death domain (SODD), FLICE inhibitory protein (FLIP) or Bcl-2/Bcl-xL/Bax expression were affected by pre-treatment with IFN-γ. However, activation (i.e. phosphorylation) of STAT1/STAT3/STAT6 was shown to precede the IFN-γ-mediated apoptotic response [43] suggesting that phosphoryation of JAKs was a mechanism likely to be responsible for TRAIL/DR5-induced apoptosis in FLS sensitized by IFN-γ. It should be obvious from the preceding section that although TRAIL/DR5 appears to play an important role in the suppression of synovial tissue apoptosis in experimental arthritis, the real significance of TRAIL in human RA lies in demonstrating that TRAIL levels are elevated in human RA, and furthermore, that neutralization of TRAIL induces apoptosis. Thus, the results from 4 recent studies which analyzed the levels of TRAIL in human RA serum, peripheral blood mononuclear cells, synoviocytes and synovial fluid are particularly relevant to this consideration. First, Secchiero et al. [44] showed that baseline TRAIL levels were higher in the serum of rheumatoid factor-negative RA patients than in the serum of rheumatoid factor-positive RA patients. Also, serum TRAIL levels increased after therapy of RA patients with DMARDs and the increase in TRAIL in serum mirrored clinical responsiveness to DMARD therapy as measured by the Disease Activity Score-28 (DAS-28). Second, TRAIL and DR4/DR5 levels were higher on both $CD4^+$ and $CD8^+$ T-cell subsets from RA patients compared to non-RA patients. However, DR4 and DcR1/DcR2 on $CD8^+$ cells, but not on $CD4^+$ cells were positively correlated with DAS-28 [45], suggesting that elevated TRAIL/DR4/DcR1/DcR2 on $CD4^+$ cells/$CD8^+$ cells may be responsible for dysregulated T-cell proliferation in RA. In the third study, Pundt et al. [46] showed that highly proliferating human RASF were less sensitive to exogenous TRAIL (as well as FasL) than RASF with decreased proliferation, suggesting that TRAIL-mediated stimulation of RASF proliferation could be dependent on other cell cycle factors that regulate the sensitivity of RASF to TRAIL, and even FasL for that matter. Fourth, TRAIL levels in T-cells recovered from RA synovial fluid were compared to TRAIL levels from T-cells recovered from the synovial fluid of patients with traumatic arthritis [47]. Although the synovial fluid T-cells from the RA patients were resistant to FasL-induced apoptosis, they were more sensitive to rTRAIL-induced apoptosis than synovial fluid T-cells from the traumatic arthritis patients. Thus, bioactive TRAIL could potentially be employed as a T-cell cytotoxic agent in RA.

Maintenance of the appropriate levels and biological activity of circulating T-regulatory (T_{reg}) cells are required for maintaining immune tolerance [48]. In that regard, Xiao et al. [49] showed that T_{reg} cells recovered from RA patients exhibited an impaired capacity to limit the proliferation and cytokine production of autologous T-effector cells which appeared to be due to an intrinsic defect in RA T_{reg} cells. Thus, RA T-effector cells had an elevated expression of membrane-associated TRAIL. These cells also released sTRAIL which was considered important because sTRAIL could induce apoptosis in T_{reg} cells. Furthermore, inhibition of TRAIL restored T-effector cell function in response to T_{reg} cells. Thus, TRAIL may be an underlying mechanism responsible for impairment of T-effector cell function in regulating the activity of T_{reg} cells.

Finally, histone acetylation [50] and deacetylation [51] are known to be mechanisms that alter the transcriptional regulation of genes by changing their chromatin structure. With regard to the role of histone acetylation in apoptosis, Jüngel et al. [52] exploited

trichostatin A (TSA), a *Streptomyces* metabolite and inhibitor of mammalian histone acetylases [53], to show that TSA when combined with TRAIL led to an induction of RASF apoptosis, whereas either TSA or TRAIL alone sparingly induced apoptosis in these cells. The mechanism of action of TSA on TRAIL-induced apoptosis was further explored. In that regard, TSA did not alter DR5 expression but did induce cell cycle arrest by up-regulating p21Waf1/Cip1. Thus, TSA appeared to sensitize RASF to TRAIL-mediated apoptosis most likely by "unmasking" specific components in TRAIL-mediated signaling that are regulated by histone acetylases.

DcR3

DcR3 is another member of the TNFR protein superfamily that binds and competitively inhibits FasL, LIGHT (a protein homologous to lymphotoxins, exhibits inducible expression, competes with herpes simplex virus glycoprotein D for HVEM, a receptor expressed by T lymphocytes) and TL1A (encoded by the TNFSF15 gene) [54]. Thus, an anti-DcR3-F_C protein was shown to inhibit Fas-induced apoptosis in FLS and DcR3 siRNA increased the susceptibility of FLS to Fas-induced apoptosis [26]. TNF-α was also shown to increase the expression of DcR3 and to suppress Fas-induced apoptosis [26]. Takahashi et al. [55] recently showed that DcR3 bound to RASF TL1A. It is noteworthy that the interaction between DcR3/TL1A resulted in reduced RASF proliferation in response to pro-inflammatory cytokines.

BH3-Only Proteins/BIM/Apoptosis-Regulating Kinases

BH3-Only Proteins

The BH3-only Bcl-2 proteins are the effector proteins of the canonical or mitochondrial-specific apoptosis pathway [56]. Although the BH3-only group is principally pro-apoptotic, the anti-apoptotic activity of the BH3-only protein group is mitigated by their interaction with BH1-4 Bcl-2 proteins [57]. It was the BH-3-only proteins, particularly, Bim and Bid that were shown to be critical in regulating the progression of experimental arthritis. Thus the activity of Bim and Bid were essential for induction of apoptosis as well as for dampening the inflammatory response [58]. For example, Bim null mice showed an increased severity of arthritis as well as length of time with arthritis in comparison to Bak or Bax null mice [59]. The severity of arthritis correlated with elevated levels of pro-inflammatory cytokines, reduced numbers of TUNEL-positive cells and a lower level of caspase-3. In addition, macrophages isolated from Bim null mice produced more IL-1β in response to lipopolysaccharide or thioglycolate *in vitro* than the wild-type Bim counterpart [60]. In a similar fashion Bid null mice showed increased arthritis severity, bone destruction and pannus formation compared to wild-type mice and Bid null mice resolved K/BxN murine arthritis slower than wild-type mice [61]. In addition to the effects of deleting Bim on murine arthritis, down-regulation of Bim activity has been implicated as the mechanism accounting for the loss of B-cell anergy [62] and aberrant survival of auto-reactive B-cells [63] thus potentially contributing to B-cell survival and synovial tissue hyperplasia in RA.

A BH3-only mimetic peptide has been proposed as a potential RA therapeutic agent [58]. Thus, a mimetic peptide corresponding to the BH3 domain of Bim ameliorated the development of K/BxN arthritis and reduced the number of synovial joint myeloid cells through enhanced apoptosis without inducing a generalized cytotoxic effect [64].

Defective regulation of other BH3-related apoptosis proteins has also been shown to drive human RA synovial tissue hyperplasia. For example, Busteed et al. [65] showed that the anti-apoptosis protein, B-cell lymphoma-extra large [(Bcl-x(L)] protein which is activated by Fas was localized to the synovial tissue lining, endothelium and inflammatory cells from both RA and OA patients, but the level of Bcl-x(L) in RA was significantly greater in RA synovium than in OA synovium, where in the former, the majority of the Bcl-x(L)-positive cells were plasma cells. However, neither age nor duration of disease correlated with the level of Bcl-x(L).

Bcl-2 is another of the anti-apoptosis proteins [10-12]. Bcl-2 forms heterodimers with Bak, Bax and Bcl-(x)L. These proteins and their interactions with one another are so important to an understanding of apoptosis resistance that studies have been undertaken to determine the extent to which inhibitors of Bcl-2, and Bcl-x(L) [66] may have therapeutic efficacy in RA among other autoimmune disorders.

There have been other novel approaches designed to limit the anti-apoptotic effects of Bcl-2. Thus, Kim et al. [67] showed that down-regulation of neuropilin-1, one of the receptors for $VEGF^{165}$ [7,35], using neuropilin-1 siRNA, was associated with both a decrease in Bcl-2 expression as well as the increased translocation of mitochondrial-derived Bax which resulted in spontaneous apoptosis of RA synovium *ex vivo*.

Finally, Bcl-2/adenovirus E1B 19kDa protein-interacting protein-3 (BNIP-3) [68] is a pro-apoptotic protein that can be induced under hypoxic conditions in human FLS *in vitro*. BNIP-3 is also highly expressed in RA synovium. However, Kammouni et al. [69] showed that the pro-apoptotic effects of BNIP-3 were blunted by TNF-α and IL-1β and over-expression of BNIP-3 in RA FLS *ex vivo* induced apoptosis under hypoxic conditions. Taken together, the results of these studies [63-68] suggested novel ways for potentially inducing apoptosis by manipulating apoptosis-related proteins in human hyperplastic RA synovium *in vitro*.

Two other apoptosis-related proteins are worthy of future studies because the activity of these proteins may be essential for limiting T-cell survival in RA at the level of synovial tissue. The programmed death 1 (PD-1)/programmed death ligand 1 (PDL-1) pathway is important with respect to maintaining peripheral tolerance through inhibition of T-cell survival [70,71] and PD-1/PDL-1, among other co-stimulatory molecules (e.g. CTLA-1) were found to be over-expressed in synovial T-cells and macrophages from RA patients [72]. More recently, RA synovial fluids were found to be enriched in $PD\text{-}1^+$-T-cells and $PDL\text{-}1^+$-monocytes/macrophages [73]. In addition, PD-1 null mice showed an increased incidence and greater severity of CIA which was associated with elevated levels of T-cell proliferation and increased IFN-γ and IL-17 production. Of note, anti-PDL-1 antibody treatment ameliorated the severity of arthritis and blunted T-cell proliferation [73].

T-cell immunoglobulin mucin-3 (TIM-3) [74] is another pro-apoptosis-related protein that may become a target for intervention in human RA. In that regard, Lee et al. [75] showed that TIM-3 expression was lower in RA $CD4^+$ T-cells compared to $CD4^+$ T-cells from normal subjects. TIM-3 expression could be increased in healthy T-cells, but not in RA-derived T-cells, by treating the cells with the TIM-3 ligand, galectin-9 [76]. These results have even greater significance in view of previous findings which showed that a stable form of galectin-9, resistant to proteolysis unlike the other galectin isoforms, galactin-1, galactin-3, and galactin-8 stimulated RAFLS apoptosis and suppressed proliferation *in vitro* [77]. Thus, galectin-9-mediated $CD4^+$ cell

apoptosis appears to be deficient in RA. Moreover, TIM-3 blunted T-cell apoptosis in response to galactin-9 which may result from the reduced expression of TIM-3 in RA T-cells, whereas increasing galactin-9 levels by experimentally over-expressing galectin-9 may stimulate synoviocyte apoptosis.

Apoptosis-Regulating Kinases

TNF-α can have an anti-apoptosis effect on cultured RASF [25]. Chen et al. [78] provided evidence that TNF-α blocked sodium nitroprusside-(SNP)-induced apoptosis in cultured RASF. The anti-apoptosis response was dependent on phosphorylating PI3K/Akt and Bad. Further, apoptosis was blocked when Akt was blocked by LY294002 and activity of NF-κB inhibited by pyrrolidine-dithiocarbamate. More recently, Garcia et al. [79] showed that phosphorylation of Akt protected RASF from Fas-induced apoptosis. Phosphorylation of Akt inhibited the cleavage of Bid which blocked the induction of apoptosis. Moreover, over-expression of Bid significantly increased RASF apoptosis which occurred in association with caspase-9 cleavage.

The nuclear protein, sphingosine kinase-2 (SPHK2) is highly expressed in RA synovial tissue [80]. In a recent study, SPHK2 was shown to be abundantly present in and around the nucleus of RASF and SPHK2 was successfully transferred from the nucleus to the cytoplasm after treating RASF with epidermal growth factor [81]. In addition, the sphingosine analogue, FTY720 was activated by SPHK2 and this activation step induced apoptosis in RASF. The results of these studies suggested that in RA, SPHK2, may, in part, be involved in the spontaneous and unregulated proliferation of synoviocytes.

Over-expression of apoptosis signal-regulating kinase 1 (ASK1) using an adenoviral vector containing a constitutive ASK1 gene (i.e. ASK1ΔN) induced apoptosis in cultured human RA synoviocytes [82]. However, in rats with CIA, transfer of ASK1ΔN increased, rather than decreased swelling in the ankle joints which was associated with elevated levels of inflammatory cell infiltrates in the synovial membrane. Thus, ASK1ΔN induced apoptosis in cultured RA synoviocytes, but did not increase apoptosis in the synovium of rats with CIA. More recently, Mnich et al. [83] showed that ASK1 null mice were resistant to the development of arthritis in the K/BxN serum-transfer model but a panel of pro-inflammatory cytokines, chemokines and matrix degrading enzymes were not altered in ASK1 null arthritic mice. Further, ablation of ASK1 with ASK1-siRNA in cultured RASF inhibited TNF-α-induced IL-6 and PGE_2 production, suggesting that ASK1 was mainly involved in the development of general inflammatory responses in this animal model of arthritis.

Fas (CD95)/Fas ligand (CD178)

Fas (CD95)/Fas ligand (FasL; CD178)-induced signaling is a prominent apoptosis pathway in many cell types [10-12]. FasL synthesized predominately by activated T-cells is a homotrimeric membrane-bound molecule with the capacity to bind 3 Fas receptor molecules on the surface of target cells. This interaction results in the formation of death domain (DD) clusters which lead to the recruitment of the cytosolic adaptor protein, FADD which not only contains a DD but also a death effector domain (DED) as well. The DED functions by interacting with a homologous domain in pro-caspase-8. Thus, the Fas trimer/FADD/pro-caspase-8 complex, also called the death-inducing-signaling complex (DISC) eventually drives pro-caspase-8 activation that results in activation of other caspases and apoptosis.

In RA, synovial inflammation is, in part, characterized by resistance of RASF and other inflammatory cells, such as neutrophils to Fas/FasL-induced apoptosis [84]. Restoring Fas/FasL responsiveness of these cells either by directly altering the defective sequence of cellular events resulting in correcting Fas/FasL-deficient apoptosis or by experimental manipulation of other pathways involved in Fas/FasL activation may ultimately prove to be beneficial as a novel therapeutic target for suppressing RA synovial tissue hyperplasia. For example, although TNF-α is a known inducer of apoptosis [85], elevated levels of TNF-α in synovial fluid of RA patients suppress Fas/FasL-induced apoptosis by RASF *ex vivo*. Dyndra et al. [86] showed that the transfer of a tissue inhibitor of metalloproteinases-3 (TIMP-3) gene construct into RASF or into the MRC-5 human lung fetal fibroblast cell line completely reversed the dampening effect of TNF-α on Fas/FasL-induced apoptosis, inhibited TNF-α-induced NF-κB activation and suppressed TNF-α-mediated up-regulation of soluble Fas.

The transcription factor, forkhead box-3a (Foxo-3a) modulates cell survival by suppressing the expression of FasL via the capacity of Foxo-3a to bind to the FasL gene promoter region [87]. In that regard, neutrophils recovered from Foxo-3a-deficient mice had elevated levels of FasL and increased apoptosis in response to TNF-α and IL-1 compared to Foxo-3a-sufficent mice [88]. Moreover, blockade of FasL made Foxo-3a-deficient mice arthritic. In addition, both the phosphorylated and un-phosphorylated forms of Foxo-3a proteins were detected in RA synovium [89]. Thus, targeting Foxo-3a may prove to be beneficial in overcoming Fas/FasL-deficient apoptosis of neutrophils in RA inflamed synovium.

TSA was also shown to act synergistically with Fas to induce RASF apoptosis [90]. TSA reduced the level of FLIP, but not Bcl-2, Bcl-(x) L or Fas which suggested that FLIP was the preferential target for the TSA-mediated effect on Fas.

Another mechanism that may explain the resistance of RASF to Fas-induced apoptosis involves the small-ubiquitin-like modifier (SUMO-1) protein [43]. SUMO-1 levels were shown to be increased in RASF [91]. Further, increased SUMOylation of the promyelocytic leukemia (PML) protein nuclear bodies was identified as the causative event which resulted in an increase in the recruitment of the transcriptional repressor, death-associated protein-6 (DAXX) to the PML-containing nuclear bodies. Interestingly, the nuclear SUMO-protease, SENP1 which was found at lower levels in RASF than in control cells reversed the effect of DAXX. Thus, over-expression of SENP1 may also contribute to resistance of RASF to Fas-induced apoptosis.

Finally, Garcia et al. [92] showed that the frequency of apoptotic cells in poly-(ADP-ribose) polymerase-1 (PARP-1)-deficient FLS was lower than in non-PARP-1-transfected FLS or control siRNA-transfected FLS. However, defective apoptosis did not involve Fas, FADD or pro-caspase-8. Rather PARP-deficient FLS showed elevated PI3K/Akt activation as well as increased c-FLIP-s after treatment with Fas. Of note, chemical inhibition of PI3K/Akt failed to ablate the difference in the frequency of apoptotic cells between PARP-1-deficient and PARP-1-sufficient cells with respect to Fas-mediated apoptosis or c-FLIP-s levels. Thus, experimentally-induced PARP-1 deficiency in RASF increased RASF resistance to Fas-induced apoptosis.

IL-17 and IL-19

IL-17

The discovery of a T-cell subset that expressed the IL-17 gene (i.e. Th17) was key to improving our understanding of how pro-

inflammatory cytokine networking promotes the pathogenesis and progression of RA (2, 6, 48, 93, 94). The Th17 T-cell subset arises from $CD4^+$ T-cells and the progression of this cell lineage is driven by combinations of IL-1, IL-6, IL-7, IL-21 and IL-23 [1, 2, 95-97]. Recently, IL-17 has been implicated in the aberrant survival of synoviocytes in RA [98,99]. In that regard, Toh et al. [98] showed that the capacity of IL-17 to induce the synthesis of synoviolin, an E3 ubiquitin ligase localized to the endoplasmic reticulum serves as an integral component of the endoplasmic reticulum-associated degradation system [100,101] which promoted the survival of RA-FLS and immune cells found in the germinal centers of RA synovium. However, SNP-induced RA-FLS apoptosis was associated with reduced synthesis of synoviolin and this response could be rescued by treatment of the cells with IL-17. Further, silencing RNA directed against IL-17RC or IL17RA increased SNP-induced apoptosis with a concomitant decrease in synoviolin. Of note, the combination of IL-17 and TNF-α was additive and increased synoviolin expression that protected RA-FLS from apoptosis induced by knockdown of synoviolin. Finally, IL-17R-deficient mice with streptococcal cell wall-induced arthritis showed a reduction in the severity of arthritis with significant synovial tissue apoptosis and reduced synoviocyte proliferation and decreased synoviolin gene expression.

IL-19

IL-19 is a cytokine belonging to the IL-10 protein superfamily. In their original report, Liao et al. [102] showed that mouse monocytes treated with IL-19 induced the synthesis of IL-6 and TNF-α as well as apoptosis together with production of reactive oxygen species. However, more recently, Sakurai et al. [103] showed that synovial tissue biopsies from RA patients contained IL-19 and its receptors, IL-20R1 and IL-20R2 which were expressed in both synovial lining and the subsynovial lining layer. The majority of IL-19-positive cells were also vimentin-positive and CD68-positive. More importantly, IL-19 induced phosphorylation of STAT3, increased IL-6 production and significantly reduced apoptosis in synovial cell cultures established from RA synovium.

p53 Upregulated Modulator of Apoptosis (PUMA)

The p53 up-regulated modulator of apoptosis (PUMA) also known as Bcl-2-binding component 3 (BBC3), is pro-apoptotic protein and a member of the Bcl-2 family [104,105]. The results of several studies have demonstrated that p21 levels were reduced in RA whereas deficient synoviocyte apoptosis occurs in RA synovial tissue despite the over-expression of p53 and reduced levels of p21, the latter being associated with erosive synovial joint disease [106-108]. Based on these findings it was hypothesized that inadequate levels of PUMA or deficient PUMA transactivation drove excessive synoviocyte proliferation in RA synovial tissue which could also account for the low level of apoptosis in RA synoviocytes. To address this possibility, Cha et al. [109] first showed that PUMA was adequately expressed in RA synovium. However, the PUMA immunoreactive product was preferentially localized to the synovial sublining cells as distinct from intimal sublining cells. In addition, PUMA mRNA was also detected in RA (as well as OA synovium), suggesting that a potential defect in PUMA activity or its induction by p53 may be responsible. However, PUMA levels did not change after FLS were transduced with adenoviral. p53 (Ad.p53), although p21 levels were enhanced. As expected, Ad.p53 failed to induce FLS apoptosis but hemagglutinin-tagged, full-length PUMA expression vector (HA-PUMA)-transfected FLS became apoptotic and was associated with activation of pro-caspase-3.

The results of another study showed that PUMA could induce apoptosis in both p-53-sufficient- as well as p-53-deficient FLS [110]. Similar studies in FLS derived from p53 null mice or in human FLS transfected with a dominant-negative mutant p53 indicated that PUMA-induced apoptosis did not require p53. More recently, Cha et al. [111] showed that 'slug' protein, encoded by the *SNAI2* gene a highly conserved zinc transcriptional repressor belonging to the snail family of developmental proteins [112] was over-expressed in RA synovial tissue. Further, treatment of RAFLS with hydrogen peroxide and suppression of 'slug' gene expression improved FLS apoptosis which was characterized by increased PUMA-mediated transactivation. Taken together, the results of these studies indicated that maintenance of adequate PUMA levels combined with normal PUMA transactivation was required to effectively induce apoptosis in RA FLS.

Additional Targets That May Be Relevant to Induction of Apoptosis in RA Synovium

Many additional targets have been identified which may eventually be exploited for altering deficient RA synovial tissue apoptosis. These include c-kit kinase, ZAP-70, telomerase, NF-κB-inducible cytokines, SIRT1, Rho kinase, NF-AT5, XIAP [113-115], inhibitors of caspase-3 [113] and Mcl-1 [113] (Table 1).

Recent studies have also shown that the apoptosis inhibitor protein, *survivin*, [114,115], *High Mobility Group Box 1* (HMGB1) protein [139], extruded membrane-bound microparticles [106,139] and *Metastatic Lymph Node 51* (MLN51) protein [140] may also play important roles in preventing synoviocyte, T-cell, B-cell, neutrophil and mast cell apoptosis. The results of two other studies [114, 141] specifically showed imbalanced expression of several pro-apoptosis proteins such as TWEAK, TRAIL and the TWEAK cognate receptor, Fn14 expression and the anti-apoptosis proteins, including, XIAP which likely limits the activity of caspase-3 in RA synovial tissue. Importantly, elevated levels of XIAP and survivin in RA synovium were found to be positively correlated with the low level of apoptosis seen in this tissue [114].

Silencing RNA technology and microRNA studies [142] have also been useful in identifying potential novel targets for intervention with dysregulated synoviocyte and immune cell survival in RA (Table 2).

Apoptotic Responses to Anti-RA Therapies

By far the most significant aspect of this research is the extent to which resistance to apoptosis by synoviocyte, neutrophil and immune cells (e.g. T- and B-cells, DCs) can be overcome by DMARDs such as methotrexate (MTX), sulfasalazine and leflunomide, DMARBDs, such as chimeric and fully humanized anti-TNF-α monoclonal antibodies and anti-TNF-α fusion protein, anti-IL-1 receptor antagonist protein, anti-IL-1 monoclonal antibody or dimeric fusion protein CTLA-4Ig, anti-CD20 monoclonal antibody and anti-IL-6R monoclonal antibody that are now employed in the clinical treatment of RA [97,149-165]. Two elements from the results of these studies stand out. The first piece of objective evidence comes from using various DMARDs and non-steroidal anti-inflammatory drugs (NSAIDs) [166] as probes to study their effects *in vitro* on the induction of apoptosis in RASF, T- and B-cells and macrophages. These results are summarized in Table 3 [167-173].

The majority of these studies showed that methotrexate (MTX) and leflunomide were able to induce apoptosis *in vitro* in a variety

Target	Target Cell	Intervention	Main Result(s)	Reference
c-kit tyrosine kinase[1]	Mast cells	Imatinib mesylate	↑ Apoptosis ↑ caspase-8,-9 ↓ TNF-α	**116**
ZAP-70[2]	B-cells	---	↑ Apoptosis in ZAP-70- B-cells	**117**
Telomerase	T-cells	**---**	↑ Apoptosis in T-cells with Telomerase insufficiency	**118**
NF-κB-inducible cytokines	T-cells	Bortezomib	↑ Apoptosis ↓ TNF-α, IL-1β, IL-6, IL-10	**119**
SIRT1[3]	RASF	TNF-α	↑ SIRT1 ↓ Apoptosis	**120**
PAR-2[4]	RASF	Tryptase	↑ Fas-mediated Apoptosis ↑ Rho kinase	**121**
Survivin	Monocytes	Survivin anti-sense oligonucleotides	↓ IL-6	**122**
NF-AT5[5]	RASF HUVEC[6]	NF-AT5 knock-down	↓proliferation/survival ↓angiogenic processes	**123**
Mcl-1[7]	FLS	Mcl-2 anti-sense adenoviral vector	↑ Apoptosis ↑ bax, bak, bim	**124**
	RASF	EGCG[8]	↑ caspase-3 ↓Akt/NF-κB ↓survival/↑apoptosis	**125**
FasL	Synovial Fluid (SF) T-cells	CD7 fusion protein	↑ Apoptosis/RA and JIA SF T-cells; ↑ Fas-signaling in Th1, but not Th2 cells	**126**
Proinflammatory cytokines, E-selectin genes, adiponectin	MDC/PDC dendritic cells[9]	Berberine	↑ Apoptosis in MDC/PDC but not peritoneal macrophages, RAW 264.7 cells or Jurkat T-cells	**127**
STAT3, NF-κB (p65), bcl-2 in response to activation by IL-6/sIL6R	FLS	Melittin	↑ caspase-3,-9; ↑Apaf-1[10], ↑ ↑cytosolic cytochrome c; ↓ p-STAT3, ↓p65 translocation, ↓ bcl-2, ↓ mitochondrial cytochrome c	**128**
FasL	JIA Monocytes	Staurosporine	↓ Apoptosis; ↓FasL Activation; ↓ Bid cleavage; ↓ Bcl-w	**129**
Geranylgeranyl phosphate	RA Synoviocyte	Fluvastatin/ Pravastatin; GGTI-298[11] Y-27632[12]	↑ Apoptosis by Fluvastatin and GGTI-298 ↔ Apoptosis by Pravastatin; ↑ Apoptosis by Y-27632	**130**

[1]c-kit tyrosine kinase is a mast/stem cell growth factor receptor also known as CD117 [131]
[2]ZAP-70, ζ-chain-associated protein-70 and a member of the tyrosine kinase family that is normally expressed by T-cells and natural killer cells [132]
[3]SIRT1 is silent mating type information regulation 2 homolog (sirtuin-1) and a deacetylating enzyme [133]
[4]PAR-2, Protease-activated receptor-2 and a subfamily member related to G-protein coupled receptors that may be activated by cleavage through their extracellular domain [134]
[5]NF-AT5, Nuclear factor of activated T-cells 5 belonging to the NFAT family of transcription factors [135]
[6]Human umbilical vascular endothelial cells
[7]Mcl-1, Induced myeloid leukemia cell differentiation protein [136]
[8] Epigallocatechin-3-gallate
[9] MDC, myeloid-derived cells; PDC, plasmacytoid-derived cells
[10]Apaf-1, Apoptotic protease activating factor-1
[11]An inhibitor of geranylgeranyl transferase [137]
[12]An inhibitor of RhoA kinase [138]

Table 1: Targets Relevant to Induction of Apoptosis in RA.

Target	Target Cell	Intervention	Main Result(s)	Reference
PLK-1	RA synoviocytes	Si-PKL-1	↓ PKL-1 ↓ proliferation in response to IL1β	**143**
NF-κB	RA-FLS	Si-NF-κB (p65 or p50) ± REL1096[1]	↑ Apoptosis	**144**
GADD45β[2]	RASF	Si-GADD45β	↑ Apoptosis ↓ bcl-2, ↑ bax	**145**
CDK-2,MCP-1	RASF	miR-124a[3]	↓proliferation G1 phase arrest	**146**
Fas-associated factor-1	Jurkat T- cells	miR-146a[4]	↓ Apoptosis	**147**

[1]REL1096 is the p65 (Rel A) subunit of NF-κB
[2] GADD45β is the growth arrest and DNA-damage-inducible45β protein [148]
[3] Putative consensus sites for the binding of miR-124a and miR-146a to the 3'-untranslated regions of cyclin-dependent kinase-2 (CDK-2) and MCP-1, respectively
[4] Putative consensus site for the binding of miR-146a to the 3' untranslated region of Fas-associated factor-1

Table 2: Use of Silencing RNA or microRNA (miR) Technology to Probe Potential Targets for Inducing Apoptosis in RA Synovium or T-cells.

Target	Target Cell	DMARD	Main Result(s)	Reference
ROS[1]	Jurkat T-cells, EL4 Thymoma, Raji B cells	MTX	↓ proliferation; ↑ Apoptosis; ↑ROS	**167**
ICAM-1[2] CLA[3]	T-cells	MTX	↓ ICAM-1, CLA; ↓ T-cell activation	**168**
CD11b, CD64,CD86,CD69	VERA[4] Neutrophils	MTX	Restoration of delayed apoptosis	**169**
Dihydroorotic dehydrogenase	Mast cells	Leflunomide	↓ p-AKT[5] ↓ PDK-1 ↑ Apoptosis	**170**
Caspase-3	RA-FLS	Celecoxib	↔[6] Caspase-3; ↔ Apoptosis	**171**
NF-κB	1° Mφ[7], RAW264.7 Cells	MTX	↑ Apoptosis in 1°Mφ; TNF-α suppressed MTX-induced apoptosis	**172**
Caspases	Jurkat T-cells, THP-1[8]	Infliximab	↑ Apoptosis[9]; ↑ Caspase activity[9] ↓ IL-10[9] ↓ IL-12[9]	**173**

[1]Reactive oxygen species
[2]Intercellular adhesion molecule-1
[3]Cutaneous lymphocyte-associated antigen
[4]Very early rheumatoid arthritis patients
[5]phosphorylated-AKT
[6]No change
[7]Primary macrophages
[8]THP-1 is a human monocyte cell line
[9]*Ex vivo* measurements from cells recovered from human-mouse chimera treated with infliximab

Table 3: Induction of Apoptosis by DMARDs or DMARBDs.

of cells pertinent to RA pathology as well as cells involved in generalized inflammation, including, T-cells, neutrophils, mast cells and macrophages. By contrast, although NSAIDS were proposed as potential apoptosis inducers [166], the NSAID, celecoxib, failed to induce apoptosis in RAFLS *in vitro* [171].

The second body of evidence can be gleaned from the results of *ex vivo* studies where synovial tissue biopsies were obtained from RA patients who had been treated with DMARDS, DMARBDs or NSAIDs. For the most part, these studies were generally designed to determine whether these agents induced apoptosis *in vivo*. In one study, RA patients treated with DMARDs showed increased levels of apoptotic cells in synovial tissue which was accompanied by lower levels of several inhibitors of activated caspases, including FLIP, survivin and XIAP [174]. In another study, MTX did not appear to directly induce apoptosis. Instead, MTX appeared to prime cells in the synovium to become apoptotic which was mediated by the extrinsic and the intrinsic pathway and was JNK-dependent mechanism [175]. Other studies reported on the direct effect of sulphasalazine on neutrophil apoptosis [176] or the role that monocytes play in the inhibition of glucocorticoid-mediated apoptosis in RA [177].

Finally, it should be noted that the results of the aforementioned *ex vivo* studies showing the effect of DMARBDs on immune cell and

synoviocyte apoptosis have focused on those investigations in which RA patients have responded to these drugs in a clinically meaningful way [178-182].

However, two recently completed clinical trials have assessed the efficacy, safety and biological activity of atacicept in anti-TNF antagonist-naïve RA patients and in RA patients who inadequately responded to anti-TNF therapy. The results of these studies stress an important conundrum in evaluating the extent to which this DMARBD will be fully developed for RA therapy.

Atacicept is a soluble fully human recombinant fusion protein comprising the extracellular domain of the *Transmembrane Activator and Calcium modulator and cyclophilin ligand Interactor* (TACI) receptor and the F_C portion of human IgG [183]. Atacicept was previously shown to inhibit B cell maturation/survival factors B lymphocyte stimulator (BlyS) and APRIL both of which are elevated in RA patients and both of which have been postulated to be potential relevant targets for suppressing B-cell survival and B-cell hyperactivity in RA [80].

In one study [184], atacicept reduced IgG, IgA and IgM rheumatoid factor (RF) levels as well as the number of circulating mature B-cells and plasma cells. However, patients receiving atacicept did not meet the clinical primary end-point which was a positive American College of Rheumatology-20 (ACR20)/C-reactive protein (CRP) response. In other words, atacicept at the dosage employed in this clinical trial reduced the level of several biomarkers that are known to drive the progression of RA without showing clinical efficacy at the lowest ACR criteria for an effective clinical response to an experimental therapy. In the other clinical investigation [185], atacicept also was found to reduce total immunoglobulin and RF levels, but failed to alter anti-citrullinated protein antibody levels. The reduction in the levels of IgG- and IgA-RF in response to atacicept was more significant than the response of total IgG and IgA levels to the drug. However, treatment with atacicept also failed to produce a clinically meaningful response.

Conclusions

Experimental RA therapies targeting the activity of specific molecules that limit the induction of apoptosis in RA synovium may be considered for future drug development. The successful use of these interventions designed to increase the frequency of apoptosis in RA synovium may prove to be effective. However, if these pro-apoptosis strategies do not produce a meaningful clinical response it is unlikely that the development of such drugs will have a future in the therapy of RA going forward.

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Disclosure Statement

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