Abnormal proliferation and/or persistence of synoviocytes and inflammatory cells has long been described in inflammatory arthritis conditions, but only relatively recently has substantial attention been drawn to the relevance of abnormal apoptotic processes in disease pathogenesis and treatment. This review summarizes a current understanding of the Fas (CD95)–Fas ligand (CD178) apoptotic system, which has most predominantly been examined in rheumatoid arthritis. There, synovial inflammation is often characterized by a unique resistance to Fas-related apoptosis, and agonistic therapeutic interventions upon Fas have consistently been found beneficial in both animal and human disease models. Therefore, modulation of the Fas pathway will hopefully be of both pathogenic and therapeutic interest in the study of inflammatory arthritis conditions in general.

Inflammatory arthritides, such as rheumatoid arthritis (RA), reflect persistent, synovial inflammation consisting of synoviocytes and leucocytes, particularly macrophages, lymphocytes and neutrophils. The mechanisms that elicit and/or propagate chronic inflammation remain relatively poorly characterized, but accumulating evidence indicates that insufficient apoptosis represents at least one fundamental underlying process [1]. Although several receptor and/or biochemical pathways are known to regulate cellular apoptosis, the Fas (CD95)–Fas ligand (FasL, CD178) death receptor pathway has received the most attention in inflammatory arthritides, particularly RA, and is reviewed here. For discussions on the role of apoptosis in RA and other autoimmune diseases in general, the reader is directed to one of several recent reviews [e.g. 1, 2].

A simplified view of Fas-mediated apoptosis

Fas and FasL both exist in membrane (mFas, mFasL) and soluble (sFas, sFasL) forms, but only engagement of mFas leads to the activation of caspase-8 via the Fas-associated death domain protein (FADD; Fig. 1) [3]. Activated caspase-8 may lead to apoptosis via at least two well-described pathways: (i) direct activation of caspase-3; and (ii) alteration of mitochondrial transmembrane potentials via Bcl-2 homology 3 (BH3)-interacting death-domain agonist (BID), leading to the cytoplasmic translocation of cytochrome c, which leads to activation of caspase-9, which in turn activates caspase-3. Both pathways are regulated at the level of caspase-8 activation by the endogenous inhibitor FADD-like IL-1β-converting enzyme (FLICE)-inhibitory protein (FLIP), which may also be recruited by FADD. Interestingly, FLIP may also participate in an alternate signalling pathway, recruiting tumour necrosis factor-associated factor (TRAF) 1, TRAF2, the MAP kinase kinase kinase Raf1 and receptor-interacting protein (RIP) to activate extracellular signal-regulated kinase (ERK) and nuclear factor κB (NF-κB) pathways, leading to proliferation and/or inflammation. This differential activity of FLIP, which appears to reflect activity of short vs long FLIP isoforms that promote the death vs proliferation pathways, respectively, mediates a major decision in response to Fas signalling: death vs proliferation/inflammation [reviewed in 4, 5].

Fas in the pathogenesis of rheumatoid arthritis

The potential importance of the Fas pathway in the pathogenesis of arthritis has been studied most extensively in RA, where the long-observed transformed appearance of synoviocytes and/or other synovium-associated cells has been proposed to result from a perturbed ‘oncogene pathway’ [6]. Apoptotic cells are uncommonly observed in RA tissues in vivo, but synoviocytes, synovial T cells and macrophages have often been observed to express high levels of Fas and/or FasL, and are highly susceptible to Fas/FasL-induced apoptosis in vitro. This contrasts with osteoarthritis, in which such abnormalities in Fas/FasL expression and susceptibility to Fas-induced apoptosis are generally not observed [e.g. 7–9].

This discrepancy between an absence of apoptotic cells in situ and enhanced susceptibility to Fas-induced apoptosis in vitro probably reflects multiple anti-apoptotic processes and/or phenomena in the rheumatoid synovium (Table 1). For instance, increased intrasyovial and/or serum sFas appears to compete with mFas and prevent apoptosis of synoviocytes [10]. Also, in some studies, invading T cells have been found to be defective in FasL expression, which could account for ineffective clearance of activated (Fas-expressing) cells [11]. Interestingly, such a mechanism may be particularly important in the often RA-associated large granular lymphocyte leukaemia, where high levels of sFasL may account for neutropenia via the induction of apoptosis, while high levels of sFas protect the leukaemic cells themselves from Fas-induced apoptosis [12, 13]. In addition, synoviocyte- and/or stromal cell-derived cytokines including transforming growth factor (TGF) β1 [14], basic fibroblast growth factor (bFGF), tumour necrosis factor (TNF)-α and interleukin (IL)-1 [15–18] protect RA synoviocytes from Fas-induced apoptosis, and such factors may account for the ability of RA T cells to be protected from apoptosis via their close interactions with fibroblast-like synoviocytes [19]. Also, RA synovial macrophages and fibroblasts up-regulate endogenous inhibitors of the Fas pathway, including FLIP [20–22] and the relatively recently described Fas-interacting protein sentrin [23]. Furthermore, rheumatoid synovial fluid contains high levels of nitric oxide (NO), which inhibits caspase-3 [24], as well as stromelysin-1 (matrix metalloproteinase-3, MMP-3), which can...
Fas as a therapeutic target in rheumatoid arthritis

Such observations strongly suggest that modulation of the Fas pathway in vivo may provide therapeutic benefit. Many current RA therapies are in fact known to induce apoptosis in synovial cells, such as methotrexate and TNF-directed therapies, and appear to do so at least in part via Fas, at least in some pathogenic cell populations, such as T cells and/or synovial macrophages [18, 34–36]. Thus, approaches targeted more specifically against Fas/FasL may be of benefit (summarized in Table 2).

Animal models

Several murine models have now been successfully used to support the Fas pathway as a therapeutic target in inflammatory arthritis: in proteoglycan-induced arthritis, aberrant expression of FLIP in CD4⁺ T cells appears to protect against apoptosis and/or activation-induced cell death (AICD) in the joint [37]. Also, mice bearing a homozygous mutation in the src homology 2 domain-bearing protein tyrosine phosphatase (SHIP)-2 binding site of the IL-6 family cytokine receptor gp130 (F759/F759) develop a spontaneous RA-like arthritis associated with defective IL-6-induced Fas expression and AICD in T cells [38], and MRL/lpr or MRL/gld mice, which bear defects in Fas and FasL, respectively, spontaneously develop an intense, symmetrical synovitis, again probably attributable to T cells [39]. Furthermore, bisindolylmaleimide VIII, which potentiates Fas-mediated apoptosis in T cells, prevents disease in an adjuvant arthritis model [40]; and C2-ceramide, which induces apoptosis by Fas, improves synovial hyperplasia in the arthritis of MRL/lpr mice, which bear defects in Fas and FasL, respectively, spontaneously develop an intense, symmetrical synovitis, again probably attributable to T cells [39]. Furthermore, bisindolylmaleimide VIII, which potentiates Fas-mediated apoptosis in T cells, prevents disease in an adjuvant arthritis model [40]; and C2-ceramide, which induces apoptosis by Fas, improves synovial hyperplasia in the arthritis of MRL/lpr mice, which bear defects in Fas and FasL, respectively, spontaneously develop an intense, symmetrical synovitis, again probably attributable to T cells [39]. Additionally, bisindolylmaleimide VIII, which potentiates Fas-mediated apoptosis in T cells, prevents disease in an adjuvant arthritis model [40]; and C2-ceramide, which induces apoptosis by Fas, improves synovial hyperplasia in the arthritis of MRL/lpr mice, which bear defects in Fas and FasL, respectively, spontaneously develop an intense, symmetrical synovitis, again probably attributable to T cells [39]. Furthermore, bisindolylmaleimide VIII, which potentiates Fas-mediated apoptosis in T cells, prevents disease in an adjuvant arthritis model [40]; and C2-ceramide, which induces apoptosis by Fas, improves synovial hyperplasia in the arthritis of MRL/lpr mice, which bear defects in Fas and FasL, respectively, spontaneously develop an intense, symmetrical synovitis, again probably attributable to T cells [39]. Furthermore, bisindolylmaleimide VIII, which potentiates Fas-mediated apoptosis in T cells, prevents disease in an adjuvant arthritis model [40]; and C2-ceramide, which induces apoptosis by Fas, improves synovial hyperplasia in the arthritis of MRL/lpr mice, which bear defects in Fas and FasL, respectively, spontaneously develop an intense, symmetrical synovitis, again probably attributable to T cells [39]. Furthermore, bisindolylmaleimide VIII, which potentiates Fas-mediated apoptosis in T cells, prevents disease in an adjuvant arthritis model [40]; and C2-ceramide, which induces apoptosis by Fas, improves synovial hyperplasia in the arthritis of MRL/lpr mice, which bear defects in Fas and FasL, respectively, spontaneously develop an intense, symmetrical synovitis, again probably attributable to T cells [39].
TABLE 2. Evidence regarding the therapeutic utility of Fas pathway agonism in inflammatory arthritis

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Model(s) used</th>
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<tbody>
<tr>
<td>Support therapeutic utility</td>
<td></td>
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<tr>
<td>Compounds which induce or potentiate Fas-induced apoptosis</td>
<td></td>
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<tr>
<td>Bisindolylmaleimide VIII</td>
<td>Adjuvant arthritis</td>
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<tr>
<td>C2-ceramide</td>
<td>MRL/lpr, RA synoviocytes</td>
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<tr>
<td>NF-κB inhibitors</td>
<td>SCW, Pristane</td>
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<tr>
<td>Anti-sense oligonucleotides against FLIP</td>
<td>RA synoviocytes</td>
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<tr>
<td>Knockout mice with defects in the Fas pathway</td>
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<tr>
<td>FasLox3a-deficient mice</td>
<td>K/BxN serum transfer, CIA</td>
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<tr>
<td>Anti-Fas antibodies</td>
<td>MRL/gld, HTLV-1 transgenic</td>
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<tr>
<td>Gene therapy</td>
<td>RA-scid, RA synoviocytes</td>
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<tr>
<td>FasL</td>
<td>CIA</td>
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<tr>
<td>MMP-3</td>
<td>RA-scid, RA synoviocytes</td>
</tr>
<tr>
<td>Do not support therapeutic utility</td>
<td>DBA/lpr mice, CIA</td>
</tr>
</tbody>
</table>

The table summarizes interventions that were beneficial vs detrimental in the indicated models of arthritis (support and do not support therapeutic utility, respectively, Fas modulation). CIA, collagen-induced arthritis; FLIP, FADD-like IL-1β-converting enzyme (FLICE)-inhibitory protein; gld, generalized lymphoproliferation disease (Fasl) mutation; HTLV-1 Tg, human T-cell lymphotropic virus-1 transgenic mice; lpr, lymphoproliferation (Fas) mutation; Pristane, pristane-induced arthritis; RA-scid, severe combined immunodeficient mice engrafted with human rheumatoid arthritis tissue; RA synoviocytes, in vitro-cultured rheumatoid arthritis synoviocytes; SCW, streptococcal cell wall-induced arthritis.

More direct evidence for the Fas system in arthritis includes the ability of agonist, apoptosis-inducing anti-Fas antibodies to effectively treat arthritis in several arthritis models, including collagen-induced arthritis [44], MRL/gld mice [45] and human T cell leukaemia virus (HTLV)-1 Tax transgenic mice [46], although it remains unclear if such effects relate directly to apoptosis in T cells vs macrophages vs synoviocytes, etc. Still, in the HTLV-1 env-pX transgenic model of arthritis, disease is accordingly exacerbated on an lpr (Fas-mutant) background, but is improved in a Fas transgenic background [47]. Finally, gene transfer of FasL, such as via adenoviral vectors, dendritic cells or Chinese hamster ovary cells, ameliorates collagen-induced arthritis, probably by inducing apoptosis in most if not all pathogenic cell populations, including T cells, macrophages and synoviocytes [48–50]. Thus, direct evidence has demonstrated Fas as a therapeutic target in murine arthritis.

Importantly, though, at least in some murine contexts, Fas may play a more direct pro-inflammatory role. For instance, DBA/lpr mice, which are defective in Fas signalling, are less susceptible to collagen-induced arthritis than DBA/+ counterparts, perhaps relating to a stimulatory role of the Fas pathway in fibroblasts [51]. In addition to signalling via ERK/NF-κB (Fig. 1), this may reflect an ability of the Fas pathway to potentiate signalling via the IL-1 receptor and/or Toll-like receptor 4, which recognizes lipopolysaccharides [52]. The relevance of such observations to the treatment of human inflammatory arthritis remains to be determined.

Human(ized) models

Indeed, many studies with human synovium, generally from RA, uniformly support the utility of agonist intervention upon the Fas pathway. For instance, in vitro, staphylococcal enterotoxin B induces CD4+ synovial T cells to kill synoviocytes via FasL [53]; C2-ceramide induces apoptosis of RA synoviocytes [54]; and gene transfer of tissue inhibitor of metalloproteinases-3 (TIMP-3), which inhibits MMP-3 activity, facilitates apoptosis and blocks the anti-apoptotic effects of TNF-α in RA synoviocytes [55]. Such studies again are largely correlative, with potentially confounding influences of the non-Fas effects of the treatments, but nonetheless support the concept of Fas modulation as a therapeutic strategy.

More direct evidence includes one study demonstrating the ability of anti-sense oligonucleotides against FLIP to sensitize RA synoviocytes strongly to Fas-induced apoptosis [56]. Furthermore, in a model in which severe combined immunodeficient (scid) mice are engrafted with RA synovium, treatment with an apoptosis-inducing anti-Fas antibody, as well as gene therapy with FasL or FADD, induces apoptosis in both synoviocytes and mononuclear cell populations, diminishing cellular infiltrates [57–60]. Thus, if apoptotic strategies are to be used therapeutically in inflammatory arthritis, current evidence altogether strongly supports activation of the apoptotic Fas pathway as a primary objective, at least in RA.

Conclusions

Apoptotic pathways, particularly involving Fas-FasL, are aberrant in expression and function in RA. Overall, they exhibit inappropriately low activity, possibly resulting in or at least contributing to persistent synovial cell proliferation and/or inflammation. Intervention upon the Fas-FasL pathway, by directly or indirectly up-regulating and/or inducing its activity, protects against arthritis in animal models, and reduces and/or resolves inflammation in human RA studies. Indeed, it is interesting to note that although few direct human RA studies have been performed to demonstrate the pathogenic and/or therapeutic relevance of the Fas-FasL pathway in vivo, clinical responses to anti-TNF therapies correlate with the induction of apoptosis in macrophages, but not lymphocytes, suggesting that Fas and/or other apoptosis pathways may represent a common, practical mechanism of therapy [36]. On the other hand, some evidence indicates that, at least in some animal models, the Fas-FasL system may instead have a pro-inflammatory effect. Given the otherwise overwhelming direct evidence to the contrary, though, this secondary Fas pathway seems likely to play a clinically minor role, at least in RA; nonetheless, such observations suggest that caution is warranted in the use of Fas as a therapeutic target, and certain, perhaps uncommon, clinical situations within RA or other inflammatory arthritides may instead benefit from blockade, rather than antagonism, of Fas [61]. Consequently, one ongoing challenge will involve identifying the subset(s) of arthritis patients that could clearly benefit from Fas agonism vs antagonism. In addition, another challenge remains the continuing need to direct therapy towards pathogenic cells (leucocytes, synoviocytes) as specifically as possible, since systemic Fas activation is almost certainly detrimental [62]. Therefore, continued investigation into the biochemical, cellular and clinical contexts of the Fas pathway in the broad spectrum of inflammatory arthritides will hopefully continue to provide insight into both the pathogenic relevance and therapeutic capabilities of Fas pathway modulation.

Key messages

- Resistance to Fas-mediated apoptosis is often seen in rheumatoid arthritis.
- Activation of the Fas pathway via therapeutic intervention successfully treats many animal and human models of arthritis.
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