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BH3-only proteins in rheumatoid arthritis: Potential targets for therapeutic intervention

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Abstract

Rheumatoid arthritis (RA) is a debilitating disease resulting in the destruction of bone and cartilage, and the permanent disfigurement of joints. Although the precise cause of RA is currently unresolved, it has become clear that the damaging effects are a result of the toxic milieu caused by an influx of inflammatory cells and the resulting heightened pro-inflammatory state within the joint. As the amount of literature that suggests that this preponderance of cells is a result of decreased local apoptosis in the joint continues to increase, in this review we describe how Bcl-2 family pro-apoptotic BH3-only proteins, particularly Bim and Bid, could act to protect against the development of the disease. We also suggest a role for BH3-mimetic drugs as potential therapeutics in the treatment of RA.

Keywords

Rheumatoid Arthritis; Bim; Bid; BH3-only; BH3 mimetic; Apoptosis

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease of unknown origin that targets the joints, resulting in inflammation and eventually destruction of cartilage and bone. In RA, this destructive arthropathy is the end result of a milieu of highly inflammatory cytokines and chemokines including tumor necrosis factor-α (TNFα), interleukin-1 (IL-1), IL-8, and monocyte chemoattractant protein-1 (MCP-1) (Pope, 2002). Once the resident cells in the joint have begun to create this toxic inflammatory environment, a positive feedback loop is created. The influx of cytokines results in the recruitment of additional inflammatory cytokine and chemokine producing cells such lymphocytes and monocytes, the induction of angiogenesis, and synovial fibroblast proliferation. This in turn leads to an increased production of more inflammatory cytokines, and thus to further amplified pathogenesis. While the exact cause of disease progression leading to bone destruction is currently unknown, it is clear that the severity of disease is related to the overabundance of inflammatory cells in the joint, and that chronic disease may be a result of a prolonged lifespan of these cells by dysregulation of

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apoptosis. Anti-apoptotic protein Mcl-1 has recently been shown to be upregulated in RA macrophages and synovial fibroblasts (Liu *et al.*, 2005; Liu *et al.*, 2006), meanwhile, Bim and Bid, both BH3-only pro-apoptotic initiator proteins, have been shown to protect against the prolonged persistence of inflammatory arthritis in a mouse model (Scatizzi *et al.*, 2006; Scatizzi *et al.*, 2007). With expanding evidence that multiple apoptotic regulators are responsible for protecting against RA, perhaps by controlling apoptosis it may be possible to shift the balance back to homeostasis by causing controlled cell death in the infiltrating cells in the joint and decrease the severity of disease.

Rheumatoid arthritis disease pathogenesis

Although the exact etiology of RA is currently unclear, several aspects of disease pathogenesis are well characterized. A synovial joint is composed of two bones separated by a synovial membrane, cartilage, and a joint cavity filled with synovial fluid. In a normal joint, the synovial lining is 1 to 2 cell layers thick, and the bone is smooth in appearance. However, in an RA joint, the synovial lining can become hyperplasic, and increase to 10 to 12 cell layers thick. In chronic RA, the synovial tissue will eventually invade cartilage and bone in the joint, forming pannus and directly (through osteoclast absorption of bone) and indirectly (through the secretion of destructive inflammatory proteases) destroy the surrounding bone and tissue by erosion. While the presence of numerous pro-inflammatory cytokines and chemokines including TNFα, IL-1β, IL-8, and MCP-1 (Pope, 2002), as well as other cytokines such as IL-20 (Hsu *et al.*, 2006), IL-22 (Ikeuchi *et al.*, 2005), IL-23 (Brennan and Beech, 2007; Murphy *et al.*, 2003) seem to support the profuse amounts of lymphocytes, macrophages, and synovial fibroblasts in the RA joint (Pope, 2002), this increase in multiple cell types in the RA joint stands in stark contrast to the noxious environment within the diseased joint where reactive oxygen species and death ligand expressing cells are present in high amounts. In an attempt to explain the increased number of cells within the RA joint, dysregulation of apoptosis became a natural theory.

While the first studies to detail the role of apoptosis in RA by investigating the presence of DNA strand breaks proved to be conflicting (Firestein, 1995; Matsumoto *et al.*, 1996; Nakajima *et al.*, 1995; Sugiyama *et al.*, 1996), the idea that decreased apoptosis helps to produce the high number of cells in the RA joint has been supported by further studies focusing on examination of cellular morphology and electron microscopy of RA synovial tissue (Firestein, 1995; Matsumoto *et al.*, 1996; Nakajima *et al.*, 1995; Pope, 2002; Sugiyama *et al.*, 1996). Increasing the amount of apoptosis in rodent models of arthritis has been reported to decrease disease severity (Fujisawa *et al.*, 1996; Miagkov *et al.*, 1998; Zhang *et al.*, 1997). Meanwhile, juvenile pauciarticular arthritis patients were found to have increased rates of myeloid cell apoptosis as compared to juvenile patients with polyarticular arthritis (Harjacek *et al.*, 2001). Taken together, these data suggest that decreased apoptosis may play an important role in the increased cellularity in the RA joint, and that by increasing the amount of apoptosis in the joint, it is possible to decrease the number of cells in the joint and ease the effects of RA progression.

The role of apoptosis in limiting cellularity in the joint

Apoptosis is a highly conserved cell death process by which cells can be eliminated without destroying surrounding cells or tissue. Upon apoptotic death, cellular receptors specific for apoptosis are either upregulated or exposed. These receptors are recognized by macrophages, resulting in clearance of the apoptotic cells by phagocytosis. This is in contrast to cell death by necrosis, where cells rupture and release their contents upon death (Liu and Pope, 2004). There are two central mammalian pathways of apoptosis; the extrinsic pathway mediated by death-receptor, death-ligand interactions and the intrinsic pathway mediated by the loss of mitochondrial membrane potential. Briefly, in the extrinsic pathway recognition of a death receptor by its cognate receptor leads to the recruitment of adaptor proteins and pro-caspases,

resulting in the death-inducing signaling complex (DISC) (Walczak and Sprick, 2001). The DISC, mediated by the close proximity of its component pro-enzymes, leads to the catalysis of caspases 8 and 10, leading to cell death.

The intrinsic apoptosis pathway is mediated by the Bcl-2 protein family. Induction of the intrinsic pathway by a number of cellular stressors results in the disruption of homeostatic balance between the pro- and anti-apoptotic Bcl-2 family members. The increased presence of pro-apoptotic proteins leads to the release of cytochrome C and other apoptogenic molecules (such as Smac/DIABLO) from the mitochondria, resulting in the formation of the apoptosome by cytochrome C, Apaf-1, and pro-caspase 9. The apoptosome acts as a self-propagating signal amplification loop, leading to the autocatalysis of pro-caspase 9 to active caspase 9 and the eventual death of the cell (Haupt *et al.*, 2003).

Within the Bcl-2 family, there are both anti-apoptotic proteins (e.g.: Bcl-2, Bcl-xL, Mcl-1, A1) as well as pro-apoptotic proteins (e.g.: Bak, Bax, Bad, Bid, Bim, Noxa, Puma). While the anti-apoptotic proteins contain 3 or all 4 of the Bcl-2 homology domains (BH domains), the pro-apoptotic proteins are further classified into multi-BH (Bak and Bax) and BH3-only domain proteins (Bim, Bid, Noxa, Puma, and others) (Adams and Cory, 1998; Youle and Strasser, 2008). Furthermore, the BH3-only pro-apoptotic proteins are further subdivided into initiator and sensitizer proteins. The initiator proteins such as Bid and Bim have an affinity for all of the anti-apoptotic proteins, as well as for Bak and Bax, while the sensitizer proteins (Bad, Bik/Blk/Nbk, Bok/Mtd, Hrk/DP5, Bmf, Noxa, and Puma/Bbc3) have a more restricted pattern of binding (Chen *et al.*, 2005; Letai *et al.*, 2002; Marani *et al.*, 2002; Wang *et al.*, 1996; Youle and Strasser, 2008). The BH3-only protein Bid also acts as a connection between the extrinsic and intrinsic pathways. In some cell types, truncation of Bid to tBid by caspase 8 from the extrinsic pathway leads to translocation of tBid to the mitochondria, where it initiates the intrinsic pathway. In this way, apoptosis is amplified by the activation of both apoptosis pathways (Li *et al.*, 1998; Wang *et al.*, 1996).

The RA joint contains an inordinate number of lymphocytes, macrophages, and synovial fibroblasts (Pope, 2002). This increase in cell number corresponds with increased expression of Bcl-2 (Perlman *et al.*, 2000), Mcl-1 (Liu *et al.*, 2005; Liu *et al.*, 2006), and Bcl-xL (Busteed *et al.*, 2006) in RA synovium as compared to normal synovial tissue. Meanwhile, increased presence of cells with higher Bcl-2 expression correlate with increased lining thickness and inflammatory score (Perlman *et al.*, 2000) and mRNA expression levels of Bcl-2 are higher in RA synovial tissue as compared with osteoarthritis (OA) synovial tissue (Salmon *et al.*, 1997). These data are consistent with additional studies in an adjuvant-induced arthritis model in rats, where it has been reported that there are increased levels of Bcl-2 at the sites of erosion and inflammation (Perlman *et al.*, 2001). Taken together, these data strongly suggest that tilting the homeostatic balance towards the anti-apoptotic Bcl-2 family members plays an important role in the increased cellularity of the RA joint and thus the severity of disease.

BH3-only domain proteins are vital in the maintenance of tolerance in RA

While there is a preponderance of data suggesting that the apoptotic balance is shifted towards the anti-apoptotic Bcl-2 family members in the RA joint, specific studies focusing on the pro-apoptotic components in the relationship are somewhat less well investigated. The multi-domain pro-apoptotic protein Bax has been reported to be upregulated in RA synovial tissue as compared to control tissue, however this expression seems to be insufficient to induce apoptosis, and it is unclear whether this represents the active form of Bax (Hilbers *et al.*, 2003). These data are supported by studies in the K/BxN serum transfer-induced model of the effector phase RA in mice in which both Bak- and Bax-deficient (Bak−/− or Bax−/−) mice on the C57Bl/6 (B6) background displayed no increase in ankle circumference as compared to control mice (Scatizzi *et al.*, 2006). Furthermore, the BH3-only protein Puma has been detected

in the sublining, but not the synovial lining, region of the joint (Cha *et al.*, 2006), but this expression appears to not significantly increase systemic apoptosis within the joint.

While Bax and Puma expression are increased in the RA joint despite the overall decrease in apoptosis, the BH3-only protein Bim seems to play an important role in the maintenance of tolerance in RA. Synovial fibroblasts from RA patients which were treated with Bim-RNAi failed to undergo apoptosis induction by antisense Mcl-1. Moreover, these Bim-RNAi treated fibroblasts also displayed a level of apoptosis similar to a more complete blockade of intrinsic apoptosis by treatment with Bak-Bax-RNAi (Liu *et al.*, 2005). These data suggest that the BH3-only initiator Bim is as important in the maintenance of tolerance in RA as the combined multi-domain pro-apoptotic executioner proteins Bak and Bax. This data is corroborated by mouse studies in the K/BxN model of arthritis in which Bim−/− mice displayed a sustained inflammatory response as compared to B6 control, Bak−/−, or Bax−/− mice. This included an increase in ankle swelling by 2 days post-serum transfer which did not resolve by day 7 as in the control mice. Furthermore, histologic scoring of Bim−/− sections at day 7 suggested a decrease in pannus formation, osteoclast function, bone erosion, and inflammation (Scatizzi *et al.*, 2006). Pro-inflammatory cytokine levels were increased in Bim−/− over the course of the study while anti-inflammatory cytokines were decreased, and fewer apoptotic cells were detected by TUNEL staining in the joints of the Bim−/− mice after K/BxN serum transfer as compared to B6 mice. Bim−/−mice also display an increase in the number of circulating peripheral blood inflammatory monocytes (Hutcheson *et al.*, 2005), a population which is likely become synovial macrophages during the progression of RA, suggesting a potential role for apoptosis in generally, and Bim specifically, in myeloid cell apoptosis and maintenance of tolerance in RA.

The loss of BH3-only protein Bid has also been reported to result in an increase in K/BxN serum transfer induced arthritis as displayed by a delay in the onset of the resolution phase of disease (Scatizzi *et al.*, 2007). While the end result at day 7 following K/BxN serum transfer is similar to Bim−/− mice (Scatizzi *et al.*, 2006), including increased ankle swelling, increased numbers of neutrophils and macrophages, and a decrease in the number of apoptotic cells, there is a lack of pro-inflammatory cytokines, and no significant difference in disease development at day 2 or 4 after serum transfer (Scatizzi *et al.*, 2007). These data indicate the BH3-only protein Bid in limiting the effector phase of inflammatory arthritis, however the effect seems to be less prominent, potentially as a result of the unique position of Bid at the intersection of the intrinsic and extrinsic apoptosis pathways.

Function and regulation of Bim

The pro-apoptotic BH3-only protein is a potent initiator of apoptosis responding to a number of apoptotic stimuli in a wide range of cell types including hematopoietic cells, neurons, epithelial cells, endothelial cells, osteoclasts, mast cells, and germ cells (Akiyama *et al.*, 2003; Alfredsson *et al.*, 2005; Hughes *et al.*, 2006; Ley *et al.*, 2005; O'Reilly *et al.*, 2000; Youle and Strasser, 2008). At homeostasis, Bim can be bound to the dynein motor complex via the dynein light chain (Puthalakath *et al.*, 1999), thus keeping Bim separate from the mitochondria and other pro-apoptotic proteins, preserving the mitochondial membrane. However, in the presence of apoptotic stimuli, Bim is allowed to translocate to the mitochondria, where it is theorized it interacts with anti-apoptotic proteins, to which Bim has global affinity (Chen *et al.*, 2005), neutralizing their pro-survival effects. It is also clear that Bim is upstream in the intrinsic apoptosis pathway from multi-domain proteins Bak and Bax, as concomitant loss of Bim and Bak or Bax results in more profound phenotypes than the loss of Bim, Bak, or Bax singularly (Hutcheson *et al.*, 2005).

Since Bim has such a wide range of pro-apoptotic targets, there would be the potential for massive amounts of apoptosis without sufficient checks and balances. As such, Bim can be

modified both on a transcriptional and a post-translational level. While there are varying reports on how transcription of Bim is increased by JNK family members (Putcha *et al.*, 2001; Whitfield *et al.*, 2001), Bim mRNA and protein expression have been shown to be increased following cytokine withdrawal as a result of the forkhead-like transcription factor FOXO3A (Dijkers *et al.*, 2000). Following translation, Bim is regulated primarily by its ubiquitination state. Phosphorylation of Bim increases ubiquitination and proteosomal degradation of Bim leading to an increase in cell survival, while growth factor withdrawal in osteoclasts results in a decrease in ubiquitination, a decrease in proteosomal degradation of Bim, and a predilection for cell survival (Hughes *et al.*, 2006).

Since Bim is expressed in such a wide range of cell types, including hematopoietic cells, and since it is capable of targeting all of the known anti-apoptotic proteins, it is not surprising that Bim is vital for maintaining homeostasis in a number of cell types, with global Bim deficiency (Bim−/−) resulting in increased numbers of lymphocytes as well as myeloid cells (Hutcheson *et al.*, 2005). Furthermore, Bim acts to protect against the development of autoimmunity. As mice deficient for Bim age, they develop hypergammaglobulinemia and high titers of autoantibodies, symptoms similar to human systemic lupus erythematosus (SLE). Although this phenotype is somewhat muted on the generally autoimmunity resistant C57Bl/6 background, further loss of Fas in addition to Bim-deficiency leads to the development of splenomegaly, lymphadenopathy, increased autoantibodies, and significantly increased kidney pathology by 4 months of age in female mice (Hutcheson *et al.*, 2008). Furthermore, on the C57Bl/6 ×129 mixed background, approximately half of Bim−/− mice succumb to a fatal autoimmune disease characterized by proliferative glomerulonephritis with immune complex deposition by one year of age (Bouillet *et al.*, 1999).

Bim as a regulator of autoimmunity

Perhaps unsurprisingly, considering the wide distribution of Bim and the vital role it plays in the initiation of apoptosis, multiple cell types are affected by dysregulation of Bim expression resulting in the aforementioned autoimmune phenotypes. In fact, Bim has been shown to be required for the deletion of autoreactive thymocytes (Bouillet *et al.*, 1999; Villunger *et al.*, 2004), with an abnormal accumulation of mature CD4, CD8 double negative thymocytes in Bim-deficient mice (Hutcheson and Perlman, 2007). Bim has also been found to be required for deletion of mature T cells (Davey *et al.*, 2002). Beyond its role in the development of T cells, Bim also plays a critical role in the shutdown of the T cell response, as evidenced by the increased survival of Bim−/− CD4+ and CD8+ T cells following *Staphylococcus aureus* enterotoxin B (SEB) activation as compared to wild-type cells (Hildeman *et al.*, 2002) and prolonged survival of activated CD8+ T cells in Bim-deficient mice infected with herpes simplex virus (Pellegrini *et al.*, 2003). Taken together, these data suggest that Bim is critical for the proper development, shutdown, and deletion of T cells, which are found in large numbers in the synovium. Thus, dysregulation of Bim could lead to unchecked T cell proliferation, leading to the production of pro-inflammatory cytokines found in the RA joint (including IL-17 and IFN-γ (Brennan and Beech, 2007; Firestein, 2003)) and recruitment of additional immune effector cells to the RA joint.

Bim is also necessary for the proper regulation of B cell deletion both during development in the bone marrow as well as in the periphery (Enders *et al.*, 2003) as well as for germinal center-derived memory B cells and antibody forming cells (Fischer *et al.*, 2007). Furthermore, BAFF (B cell activating factor belonging to the TNF family) regulates B cell survival by down-regulating Bim via the ERK signaling pathway (Fischer *et al.*, 2007), resulting in a loss of B cell anergy (Oliver *et al.*, 2006). This relationship between Bim and B cell deletion is important with regards to the pathogenesis of RA because B cell depletion therapy is efficacious to some degree in the treatment of the disease (Bugatti *et al.*, 2007; Edwards *et al.*, 2004). Aside from

the production of autoantibodies including RF factor and anti-citrullinated antibodies, B cells can play a role in the pathogenesis of RA by mediating B-T cell interactions as well as by producing cytokines which can influence the pro-inflammatory environment, including alteration of Th1/Th2 T cell populations (Bugatti *et al.*, 2007).

Macrophages are central to the pathogenesis of RA. Monocytes from the peripheral blood differentiate into synovial macrophages in the joint. These macrophages come in two varieties – type A synoviocytes in the synovial lining and interstitial macrophages distributed throughout the synovium. These cells are key producers of a number of pro-inflammatory cytokines including IL-1, IL-6, IL-8, IL-12, IL-15, and IL-18. Furthermore, additional inflammation is mediated by macrophages secreting chemoattractant chemokines such as MCP-1, MIP-1a, RANTES, PARC, and fractalkine (Szekanecz and Koch, 2007). Clearly, monocytes and their derivative macrophages can play a sizable role in shifting the balance of the RA joint towards pro-inflammatory conditions. Furthermore, it has previously been shown that expression of anti-apoptotic protein Mcl-1 is increased in RA macrophages as compared to normal cells (Liu *et al.*, 2006). This suggests a potential direct link between macrophages and apoptosis in RA. The development of osteoclasts from monocyte-lineage cells further increases the importance of myeloid cells in the pathogenesis of RA, as osteoclasts are responsible for the destruction of subchondral bone. Bim has been shown to play a critical role in the maintenance of both osteoclast survival and activity (Akiyama *et al.*, 2003; Wakeyama *et al.*, 2007). Furthermore, loss of Bim has been shown to increase the number of circulating peripheral blood monocytes (Hutcheson *et al.*, 2005) amount of pro-inflammatory cytokines in the joints of mice following the induction of K/BxN serum transfer-induced arthritis (Scatizzi *et al.*, 2006). T cells, B cells, myeloid cells including macrophages, synovial fibroblasts, and osteoclasts are all important cell populations with regards to the pathogenesis of RA. Meanwhile, Bim plays an important role in maintenance of tolerance in all of these cell types. Taken together, these data suggest that Bim is a viable target for therapeutic intervention in RA.

Function and regulation of Bid and its effect on autoimmunity

While Bid is similar to Bim in that it is also a BH3-only pro-apoptotic initiator protein, its role at the crossroads of the extrinsic and intrinsic pathways makes it more complicated to completely define the function of Bid. Bid is widely expressed, with the highest level of expression being present in the kidney (Wang *et al.*, 1996). While few studies have focused on the transcriptional control of Bid, it has been found that p53 is a positive-regulator, suggesting that Bid plays a critical role in p53 mediated apoptosis, and in fact, Bid−/− fibroblasts are resistant to DNA damage induced apoptosis by p53-mediated agents such as adriamycin (Sax *et al.*, 2002). Full length Bid has relatively poor pro-apoptotic activity, and in fact, it has no amino acid sequence homology with Bak, Bax, or Bcl-2 like proteins (Chou *et al.*, 1999; McDonnell *et al.*, 1999). Thus, Bid must be truncated to tBid by caspase or granzyme mediated proteolysis (Li *et al.*, 1998; Luo *et al.*, 1998). While this often occurs as a result of caspase 8-mediated cleavage, Bid is not required for CD95-induced apoptosis of lymphocytes, suggesting Bid likely functions as an enhancer of the extrinsic pathway (Yin *et al.*, 1999). However, Bid-deficient hepatocytes and fibroblasts did display some resistance to death-receptor mediated apoptosis, suggesting that the role of Bid may be cell type specific (Yin *et al.*, 1999). Despite caspase 8 being the primary activator of Bid proteolysis to tBid, other caspases can induce a similar effect, and granzyme also is capable of Bid truncation, suggesting that Bid may function in general amplification of apoptosis under some apoptotic conditions as well as in perforin-dependent killing by cytotoxic T cells (Sutton *et al.*, 2000; Waterhouse *et al.*, 2005). Furthermore, Bid plays a role in the maintenance of myeloid homeostasis. Bid-deficient mice are viable and display normal homeostasis in all tissues at early ages. However, as the mice age, they develop an increased number of neutrophils around

18-24 months. Myeloid precursor cells from Bid−/− mice are resistant to death-receptor mediated apoptosis and display a competitive advantage in repopulating a marrow compartment (Zinkel *et al.*, 2003). While the exact role it plays in the development of experimental RA is unclear, the loss of Bid resulted in a decrease in apoptosis in the joint, suggesting Bid functions to prolong survival of macrophages, synoviocytes, osteoclasts, and potentially other cell types, perpetuating the development and prolonged regulation of a pro-inflammatory environment (Scatizzi *et al.*, 2007). As such, perhaps targeted tBid could function as a supplementary therapeutic target, though the widespread effects of Bid across both mammalian apoptotic pathways may complicate the effects of any treatment.

BH3-mimetics as potential therapeutics in RA

Recently, several natural and synthetically developed compounds that mimic the function of the BH3 domain of pro-apoptotic Bcl-2 family have been developed, predominantly for potential therapeutic usage in treating various types of cancers. Since it has been reported that some BH3-only proteins play a protective role with regards to RA, these mimetics may also be an effective treatment for autoimmunity, including RA. A variety of these mimetic compounds have been recently classified, and a handful of these compounds have reached preclinical trials, with both encouraging and discouraging data. ABT-737 is one synthetic BH3-mimetic, designed to target the BH3-binding pocket of Bcl-xL (Oltersdorf *et al.*, 2005). It has been shown that ABT-737 action requires the presence of Bak and Bax, and thus is most similar to a sensitizing BH3-only protein (Labi *et al.*, 2008; Oltersdorf *et al.*, 2005). While *in vivo* mouse xenograft tumor models were encouraging (Oltersdorf *et al.*, 2005), ABT-737 may not be an excellent candidate for BH3 mimetic treatment of RA, as it has no effect on cells expressing high levels of Mc1-1 (Chen *et al.*, 2005; Konopleva *et al.*, 2006; van Delft *et al.*, 2006), considering the increased expression of Mcl-1 in RA macrophages. Obatoclax from GerminX Pharmaceuticals demonstrated *in vitro* efficacy in non-SCLC, mantle cell lymphoma as well as multiple myeloma cells, but these data could not be replicated following xenograft transfer of tumors *in vivo* (Labi *et al.*, 2008; Perez-Galan *et al.*, 2007). More recently, obatoclax has been shown to sensitize human cholangiocarcinoma cells my inhibiting Mcl-1 binding to Bak and Bim (Mott *et al.*, 2008). Furthermore, obatoclax has been reported to affect expression levels of Bim (Nguyen *et al.*, 2007; Trudel *et al.*, 2007), and competes with a Bid-BH3 for binding of Mcl-1, Bcl-2, and Bcl-xL in the BH3 binding pocket (Mott *et al.*, 2008; Nguyen *et al.*, 2007). However, obatoclax is believed to function by disrupting the binding of Bak and Mcl-1 (O'Brien *et al.*, 2008). Since Bak has not been shown to play a predominant role in human RA, and since Bak does not play a role in at least some rodent models of inflammatory arthritis (Scatizzi *et al.*, 2007), it is possible that obatoclax may not be a suitable BH3-mimetic for RA treatment, although it certainly invites further study.

One interesting option for potential BH3-mimetic treatment of RA is delivery of a Bim-BH3 peptide. Given that Bim has been shown to play a role in the K/BxN model of inflammatory arthritis (Scatizzi *et al.*, 2006), and given the large role for Bim in maintaining homeostasis in the immune system (Hughes *et al.*, 2006; Strasser, 2005; Youle and Strasser, 2008), a mimetic which contains the BH3 region from the Bim protein could be a promising therapeutic agent in RA. Previous studies have shown that conjugation of the TAT sequence from HIV to the BH3 domain is sufficient to induce apoptotic cell death in multiple cancer cell lines in a dose dependent fashion (Kashiwagi *et al.*, 2007). Furthermore, BH3 peptide to Bim has been shown to have an affinity for Mcl-1 (Chen *et al.*, 2005; Letai *et al.*, 2002). Taken together these data suggest that TAT-Bim may provide an excellent therapeutic opportunity by allowing for the re-establishment of apoptotic homeostasis, resulting in cell death in the RA joint and the potential alleviation of RA.

Conclusion

A decrease in apoptosis in the joint results in an increase in joint cellularity and local inflammation, leading to the destruction of bone, ligament, and cartilage in chronic cases of RA. While reports have indicated that anti-apoptotic Bcl-2 protein levels are increased in the RA joint, there has been little indication that these expression levels are indicative of disease progression, synovial lining thickness, or outcome. Meanwhile, in a rodent inflammatory arthritis model that resembles the effector phase of RA, loss of the pro-apoptotic BH3-only domain proteins Bim and Bid were shown to prolong the course of disease, while singular loss of either the multi-domain proteins Bak and Bax had no effect on the outcome of disease. These data suggest that an initiator BH3-only protein such as Bim may play an important role in protecting against RA. In fact, Bim is critical in the maintenance of tolerance and homeostasis in a wide range of cell types, including immature and mature T, B, and myeloid cells, which incidentally all play a role in the establishment or extension of inflammation in RA. With the rise in popularity of BH3-mimetic drugs, it seems reasonable to consider that these drugs may be able to have an effect on the course of disease in RA, potentially re-establishing apoptotic homeostasis and preventing further damage in the joint.

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