Published in final edited form as:

Nat Rev Rheumatol. 2016 August 23; 12(9): 543–558. doi:10.1038/nrrheum.2016.132.

The inflammatory role of phagocyte apoptotic pathways in rheumatic diseases

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Abstract

Rheumatoid arthritis affects nearly 1% of the world's population and is a debilitating autoimmune condition that can result in joint destruction. During the past decade, inflammatory functions have been described for signalling molecules classically involved in apoptotic and non-apoptotic death pathways, including but not limited to toll-like receptor signalling, inflammasome activation, cytokine production, macrophage polarization and antigen citrullination. In light of these remarkable advances in the understanding of inflammatory mechanisms of the death machinery, this review provides a snapshot of the available evidence implicating death pathways, especially within the phagocyte populations of the innate immune system, in the perpetuation of rheumatoid arthritis. Elevated levels of signalling mediators of both the extrinsic and intrinsic apoptotic as well as the autophagy death pathways are observed in the joints of patients with rheumatoid arthritis. Furthermore, in rheumatoid arthritis patients, risk polymorphisms are present in signalling molecules of the extrinsic apoptotic and autophagy death pathways. Although research into the mechanisms underlying these death pathways has made considerable progress, this review highlights areas where further investigation is particularly needed. This exploration is critical, as new discoveries in this field could lead to the development of novel therapeutic targets for rheumatoid arthritis and other rheumatic diseases.

Rheumatoid arthritis (RA) is a multifactorial disease that can be initiated by the combination of environmental and genetic factors. RA is one of the most highly prevalent autoimmune diseases; nearly 1% of the world's population suffer from this condition¹. During RA, dysregulation of the immune system leads to persistent synovial inflammation, cellular infiltration, proinflammatory cytokine production as well as progressive deformation and/or destruction of cartilage and bone in the joint. Ultimately, these events can result in chronic pain, severe disability and increased mortality. Whereas lymphocytes are necessary for the initiation of RA², professional [G] phagocytic cells of the innate immune system, including macrophages and dendritic cells (DCs), are also emerging as important cell populations in the pathogenesis of this debilitating disease.

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Author contribution

C.C. researched data for the article. All authors substantially contributed to discussion of content, and reviewed/edited the manuscript before submission. C.C. and H.P. wrote the article.

Competing interests

The authors declare no competing interests.

Although macrophages and DCs can arise from a common progenitor and share a variety of roles, including antigen presentation, participation in T cell homeostasis and maintenance of gut immunity, they also maintain distinct functionality within the immune system and in the context of disease³⁻⁵. Over the past twenty years, synovial macrophages have gained the reputation of being crucial for the persistence of RA⁶. Circulating monocytes in peripheral blood — classically activated monocytes in particular — are increased in patients with RA⁷. These classically activated monocytes migrate to the joint and differentiate into macrophages, thereby leading to an increased macrophage presence that correlates with articular destruction^{8,9}. In RA, macrophages are highly activated, express increased levels of toll-like receptors (TLRs), such as TLR2, TLR3, TLR4 and TLR7, and contribute to synovial inflammation and destruction of cartilage and bone through the production of degradative enzymes, cytokines and chemokines^{6,10-13}. Approved therapeutics, including anakinra and etanercept that target IL-1 β and TNF α , respectively, decrease inflammation, bone destruction and macrophage numbers in the synovial sublining¹⁴⁻¹⁶. Furthermore, a reduced macrophage number in the synovial sublining correlates with better outcomes in RA and serves as a biomarker to determine the efficacy of a particular therapy^{17,18}.

Phagocytes respond to molecules released by and/or exposed on [G] apoptotic cells to either suppress or elicit an inflammatory response¹⁹. Under homeostatic conditions, professional phagocytes (macrophages and immature DCs), non-professional phagocytes (epithelial cells and fibroblasts) and specialized phagocytes (Sertoli cells and retinal pigment epithelial cells) mediate the removal of apoptotic cells depending on the environmental context¹⁹. Engulfment of apoptotic cells by phagocytes occurs in a step-wise fashion. First, the apoptotic cell releases ‘find-me’ signals that attract, and potentially activate, phagocytes¹⁹. The phagocyte must then distinguish the apoptotic cell from surrounding healthy cells through engulfment receptors, including scavenger receptors (CD36) and tyrosine kinases (tyrosine-protein kinase Mer; MerTK), that recognize ‘eat-me’ signals (for example, plasma membrane lipid phosphatidylserine) on the apoptotic cell¹⁹. Subsequently, extensive rearrangement of the phagocyte's cytoskeleton enables the internalization of the apoptotic cell¹⁹. The last step involves the processing of the ingested material, which causes the phagocyte to release anti-inflammatory mediators that assist in the dampening of local inflammation¹⁹.

The central mechanism for cell clearance is controlled by the programmed cell death machinery. Failure to clear apoptotic cells can induce tissue inflammation caused by the release of cellular contents and/or exposure to sequestered components, which subsequently engage receptors for damage-associated molecular patterns (DAMPs). The activation of DAMPs might lead to immune responses to self-antigens or autoimmune conditions such as RA¹⁹. Interestingly, administration of early-stage dying cells reduces disease severity in murine models of inflammation, probably through elicitation of anti-inflammatory mediators²⁰.

Professional phagocyte populations in RA pathogenesis

As we will show in this review, macrophages and DCs are vital to the pathogenesis of RA, thus underscoring the necessity of delving further into their functionality within the arthritic joint.

Macrophages

Depending on the microenvironment, activated monocytes can differentiate into two main macrophage subtypes: classically activated (M1 macrophages) and alternatively activated (M2 macrophages). M2 macrophages can be further categorized into subsets according to the applied stimuli and the consequential transcriptional alterations^{21,22}. Studies within the past decade suggest that murine macrophages stimulated with IFN γ , LPS and granulocyte-macrophage colony stimulating factor (GM-CSF) become classically activated (M1 macrophages). M1 activation results in the induction of proinflammatory cytokines, including TNF, IL-1, IL-12, IL-18 and IL-23; production of nitric oxide (NO) and reactive oxygen species; antigen presentation; expression of CC chemokine receptors CCR1 and CCR5; promotion of type 1 T helper (T_H1) and type 17 T helper (T_H17) cell responses; and resulting in an effective mechanism of pathogen killing²². Murine macrophages skewed towards an M2 phenotype by fungal cells, immune complexes, parasites, complement components, apoptotic cells, macrophage colony stimulating factor (M-CSF), IL-4, IL-13, IL-10, and TGF β can be subdivided into four groups (M2a-d) depending on the stimuli²². Murine M2 macrophages produce IL-10, arginase 1, chitinase-like protein 3 (also known as Ym1), RELM α , and express CD206 and CCR3, which in combination promote pathogen defense, phagocytosis, extracellular matrix production, apoptotic cell clearance, wound healing and/or anti-inflammatory activities²². However, macrophages have been found to exhibit phenotypic plasticity^{23,24}. Changes in the environmental milieu can promote crossover between each group, allowing for an extensive variation in their phenotype^{23,24}. Only within the past three years has the M1–M2 paradigm been recognized as a set of well-defined molecular events that are highly reproducible under controlled conditions *in vitro*, but have little to no relevance *in vivo*^{25–27}. Thus, *in vivo*, populations of macrophages can share both M1-like and M2-like characteristics.

The origin of synovial macrophages is still enigmatic. Bone marrow-derived monocytes were originally identified as precursors of tissue-resident macrophages, including synovial macrophages^{28–30}. However, later studies have shown that many tissue-resident macrophages, such as microglia in the brain, alveolar macrophages in the lung and Kupffer cells in the liver, originate from yolk sac or fetal liver progenitors^{31–40}. Importantly, these tissue-resident macrophages are long-lived and able to maintain homeostasis via *in situ* proliferation, without the recruitment of circulating monocytes^{41–43}. However, some tissues, such as skin and gut, contain monocyte-derived cells under steady-state conditions, while recruitment of monocyte-derived macrophages to other organs (for instance, the lungs, the peritoneal cavity or the spleen) is a hallmark of inflammation^{31,44}. Nonetheless, the role of the apoptotic pathway in the homeostasis of macrophages remains unknown.

We have shown that the murine and human synovium contains a heterogeneous population of macrophages⁴⁵. More specifically, the murine joint contains two major populations of

macrophages that can be distinguished on the basis of the expression of MHC II. In the context of the inflamed murine joint, tissue-resident MHC II-negative synovial macrophages possess a high phagocytic capacity and function in part to maintain joint integrity⁴⁵. This suggests that inefficient repair of the damaged joint by this macrophage population potentially plays a vital role in propagating chronic arthritis. In a murine model of acute inflammatory arthritis, the serum transfer-induced arthritis model, classically activated, proinflammatory M1-like macrophages derived from monocytes and recruited to the joint switch their phenotype towards a more wound-healing M2-like phenotype, independent of further monocyte recruitment⁴⁵. Moreover, this switch in macrophage identity during the resolution phase of experimental arthritis coincides with upregulation of genes known to be involved in lipid processing and [G] efferocytosis, such as *Mertk*, *CD36*, *Pparg* and *Tgm2*⁴⁵. In conjunction with the switch in macrophage identity, the number of neutrophils rapidly diminishes during the resolution phase of experimental arthritis⁴⁵. This evidence suggests that dying neutrophils, through an unknown apoptotic pathway, stimulate recruited macrophages to adopt an M2-like wound-healing phenotype⁴⁵. Consistent with this theory, classically activated M1-like macrophages recruited during the early stages of experimental arthritis-mediated inflammation can efficiently clear apoptotic neutrophils⁴⁶. Therefore, defective clearance of neutrophils by synovial macrophages and/or defective macrophage polarization towards the wound-healing phenotype might be key mechanisms leading to propagation of RA, and might potentially be controlled by apoptotic pathways.

Dendritic cells

In RA, DCs contribute to the marked increase of leukocyte infiltration to synovial tissue⁴⁷. DCs might also have a role in the initiation of the disease by producing cytokines and presenting arthritogenic antigens, which together activate autoreactive T cells^{48,49}. Analyses of human RA synovial tissue and murine models of RA indicate that DCs drive the formation of the ectopic lymphoid organs commonly observed in synovial biopsies⁵⁰. Altered expression of Fc- γ -RII as well as heightened responses to TLR2 and TLR4 activation are observed in DCs from patients with high levels of RA disease activity^{51,52}. A clear type I interferon signature is detected in a substantial portion of patients with RA, implicating plasmacytoid DCs in RA pathogenesis, as this population is a major producer of type I interferon^{53,54}. Furthermore, decreased circulating DCs in patients with RA suggests that plasmacytoid and myeloid DCs might selectively home to the inflamed joint⁵⁵⁻⁵⁹. This decrease correlates with an enriched population of DCs possessing a high T cell stimulatory capacity in inflamed synovial tissue⁵⁵⁻⁵⁹.

Phagocytosis of neutrophils in RA

Short-lived cells that infiltrate inflamed tissue, such as neutrophils, must also be removed during the resolution of inflammation¹⁹. Neutrophils recruited to the site of bacterial infection can die through the formation of neutrophil extracellular traps (NETs)⁶⁰, which consist of nuclear chromatin and histones that facilitate trapping and killing of bacteria. NETs are thought to initiate inflammation, although certain types of NETs are also capable of contributing to the resolution of inflammation⁶¹. These data suggest a delicate balance between the proinflammatory and anti-inflammatory capacity of apoptotic cells, which is dependent on the stage of cell death¹⁹. As neutrophils are abundant in the RA synovial fluid,

NETs might contribute to the initiation or the perpetuation of inflammatory arthritis through the constant activation of macrophages in the joint. Whereas inefficient clearance of dying cells by phagocytes might contribute to the break of tolerance, leading to the pathogenesis of RA, defects in cell death machinery within phagocytes could also have deleterious downstream effects. Furthermore, inefficient clearance might occur in response to an efficacious therapy that results in synovial cell death. Although the mechanisms underlying RA-associated autoimmunity are not fully elucidated, insufficient apoptosis is known to have a fundamental role in RA pathogenesis^{62,63}.

Cell death pathways in phagocytes

During the past decade, inflammatory functions have been described for molecules that are classically involved in apoptotic and non-apoptotic cell death pathways through the study of both human samples and murine models (see Box 1 for descriptions of murine models of inflammatory arthritis). Below is a discussion of these newly discovered mechanisms associated with cell death signalling mediators with a focus on the phagocytic cell populations of innate immunity in the development of inflammatory arthritis and other rheumatic diseases (see TABLE 1).

Extrinsic pathway of apoptosis in RA

The apoptotic machinery has long been associated with cell fate. Whereas ligation of death receptors triggers cell death pathways, their activation by cognate ligands can also trigger death-independent functions, including cellular proliferation and inflammatory pathways⁶⁴. Apoptosis can occur via two distinct pathways. The extrinsic pathway involves transduction of the apoptotic signal after the binding of death receptors, such as Fas, TNF receptor 1 (TNFR1) or TNF-related apoptosis inducing ligand (TRAIL) receptor R1 and R2, to their ligands FasL, TNF and TRAIL. In contrast, the intrinsic pathway signals through the mitochondria and is regulated by the Bcl2 protein family (described in detail below). In an example of the extrinsic pathway, binding of the homotrimeric death ligand FasL to its cognate death receptor Fas facilitates the recruitment of the Fas-associated death domain protein (FADD). Subsequently, FADD recruits the inactive form of caspase 8, the aggregation and homodimerization of which result in its autocatalysis and/or activation. This step induces the degradative phase of apoptosis through the activation of caspase 3 and caspase 7, which are coordinators of downstream apoptotic cellular processes⁶⁵. CASP8 and FADD-like apoptosis regulator (also known as FLIP), a catalytically inactive homolog of caspase 8, acts as an endogenous regulator of the Fas pathway at the level of caspase 8 activation. FLIP is recruited by FADD upon Fas activation and can either inhibit or potentiate the binding of FADD and caspase 8, depending on its concentration^{66,67}. An alternative pathway of Fas-induced cell death involves crosstalk between the extrinsic and the intrinsic apoptotic pathway. This crosstalk is mediated by the activation of the Bcl2 pro-apoptotic protein BH3-interacting domain death agonist (also known as BID)^{68,69}, which is cleaved by caspase 8 following Fas ligation. The cleaved form of BID is then targeted to the mitochondria and ultimately induces the intrinsic apoptotic pathway⁷⁰.

In vivo, very few apoptotic cells are generally observed in RA tissues, despite high expression levels of Fas and/or FasL⁷¹⁻⁷⁴. This evidence indicates that in RA the function of the death receptor Fas is independent of cell death. Other studies have shown that reduced expression of FasL on invading T cells might account for the ineffective clearance of activated Fas-expressing cells⁷⁵. In addition, synovial cell production of proinflammatory cytokines protects RA synoviocytes and invading T cells from Fas-induced apoptosis⁷⁶⁻⁷⁸. RA synovial fluid contains high levels of NO that can potentially block apoptosis, as NO inhibits the action of caspase 3, a coordinator of the degradative phase of the apoptotic pathway⁷⁹. Taken together, these studies imply that defective Fas signalling contributes to the pathogenesis of RA (TABLE 1).

Evidence from studies in experimental models implicates the extrinsic pathway of apoptosis in arthritis. Below, we discuss the death receptor Fas and its downstream signalling components in phagocytic cell populations of the innate immune system in these models.

Fas

Mice homozygous for the *lpr* mutation of the *Fas* gene display normal postnatal development, but with age manifest [G] splenomegaly, [G] lymphadenopathy, [G] hypergammaglobulinemia, autoantibody production, [G] glomerulonephritis and presence of double negative T cells (CD3⁺ CD4⁻ CD8⁻ B220⁺). The development of this advanced systemic lupus erythematosus (SLE)-like disease, which can include inflammatory arthritis, is highly dependent on strain background⁸⁰. For example, the autoimmune-prone MRL mouse strain with the *lpr* mutation (MRL.*Fas*^{*lpr*}) not only presents with the most severe SLE-like disease, but also spontaneously develops chronic synovitis⁸¹.

The advent of conditional gene deletion has enabled cell-specific dissection of the functions of Fas and its downstream signalling components in haematopoietic cell populations (TABLE 1). Mice with conditional deletion of *Fas* in the myeloid cell compartment, which includes monocytes, macrophages and neutrophils, develop an SLE-like disease characterized by splenomegaly, hypergammaglobulinemia, glomerulonephritis and production of autoantibodies as well as proinflammatory cytokines⁸². In these mouse mutants, the accumulation of peripheral monocytes, macrophages and granulocytes increases the activation of and disrupts the normal distribution of both DCs and T cells, which indicates that, in myeloid cells, Fas has an indirect role in the activation of other immune cells⁸². TLR activation is a critical step in the activation and functional differentiation of macrophages; therefore, it has a crucial role in the development and persistence of SLE-like disease in mice⁸³. Mortality of myeloid cell-specific *Fas*-knockout mice following a high-dose lipopolysaccharide LPS injection is higher than that of *Fas*-intact mice⁸². Thus, loss of Fas in myeloid cells results in a heightened systemic reaction to TLR ligation, suggesting that this death receptor not only initiates the extrinsic apoptotic pathway, but also maintains TLR activation.

The C57BL/6 mouse strain carrying the *lpr* mutation (C57BL/6.*Fas*^{*lpr*}) present an exacerbated K/B×N serum transfer-induced arthritis that is characterized by increased inflammation, pannus formation and destruction of bone and cartilage compared with control C57BL/6 mice⁸⁴. Further, macrophages from C57BL/6.*Fas*^{*lpr*} mice are primed for

increased cytokine production. However, mice with the same Fas mutation on the DBA/1 background present reduced severity of CIA⁸⁵, which indicates that strain background and choice of murine arthritis model are critical when functional defects and their effect on arthritis pathogenesis are examined. In contrast to C57BL/6.*Fas^{lpr}* mice, the onset of K/B×N serum transfer-induced arthritis in young myeloid cell-specific *Fas*-knockout mice is comparable to that of control mice, although arthritis resolution is accelerated in the chronic phase, as evidenced by the reduction of inflammation and neutrophil infiltration⁸⁶. Consistent with a more rapid resolution of disease, the joints of myeloid cell-specific *Fas*-knockout mice have higher levels of IL-10, as well as reduced expression of CXCL5 (a neutrophil chemotactic chemokine) and the TLR2 ligand endoplasmic reticulum protein (also known as GRP94)⁸⁶. Furthermore, *Fas*-deficient macrophages produce higher levels of IL-10 when stimulated with IL-1 β and endoplasmic reticulum protein *in vitro*⁸⁶. These data indicate that Fas signalling in mouse macrophages propagates joint inflammation by decreasing expression of IL-10 and increasing expression of endoplasmic reticulum protein⁸⁶. This mechanism might also be relevant for inflammation in human RA joints. Similar to myeloid cell-specific *Fas*-knockout, Fas deficiency in DCs results in systemic autoimmunity⁸⁷. However, whether DC-specific Fas signalling promotes joint inflammation as observed in macrophages remains to be determined.

FADD

In the sequence of signaling events, binding of FasL to the death receptor Fas facilitates recruitment of FADD. In severe combined immunodeficient (SCID) mice engrafted with human RA synovium, the local injection of a replication-defective adenovirus expressing FADD induces apoptosis in the synovium and suppresses synoviocyte proliferation⁸⁸. In addition, FADD can sequester MyD88 (an adapter protein essential for most TLR signalling), thereby hindering the activation of the transcription factor NF- κ B. Therefore, the reduction of FADD, or reduced recruitment of FADD to the Fas receptor as occurs in RA, might account for the enhanced NF- κ B signalling and the production of proinflammatory cytokines⁸⁵. Furthermore, in an *in vitro* cultured mouse organ, FADD expression can be regulated by adenosine receptors, which can control FADD secretion through the process of microvesicle shedding⁸⁹. In the synovial fluid of patients with RA, in addition to a high concentration of adenosine, the presence of microvesicles that originate from monocytes, neutrophils and lymphocytes represent another potential source of FADD⁹⁰. These microvesicles can trigger the release of chemokines, cytokines and other inflammatory mediators from synovial fibroblasts⁹⁰. This evidence suggests a link between the regulation of FADD by microvesicle shedding and the perpetuation of RA, as the reduction of intracellular FADD due to adenosine-dependent microvesicle shedding could lead to defective apoptosis and excess proliferation of synovial fibroblasts, and the resulting downstream consequences⁹¹. Thus, *FADD* gene transfer or activation of FADD might be effective therapeutic strategies for RA⁸⁸.

Mice with a targeted *Fadd* deletion in neutrophil and monocyte/macrophage populations have chronic inflammation associated with elevated production of proinflammatory cytokines and increased populations of macrophages, neutrophils and B cells⁹². Global deletion of *Myd88* has been found to rescue these phenotypes, indicating that the

inflammation caused by the loss of FADD is attributable to MyD88 signalling, rather than defects in cell death⁹². Another study by the same group also demonstrated that, similarly to myeloid cell-specific *Fas*-knockout mice, DC-specific FADD deficiency results in systemic inflammation, higher levels of proinflammatory cytokines and increased numbers of macrophages, neutrophils and B cells compared to FADD-intact mice. These phenotypes are attributed to the FADD deficiency-induced depletion of CD103⁺ DCs within gut-associated lymphoid tissues⁹³. Chronic inflammation in DC-specific *FADD*-knockout mice is prevented by global, but not DC-specific, deletion of *Myd88* as well as oral antibiotic treatment⁹³. Although arthritis has never been examined in the context of myeloid or DC-specific FADD deficiency, this aspect warrants further investigation owing to the potential role of FADD in RA, and the contributions of innate immune cell populations to disease pathogenesis.

FLIP

The best-characterized mediator of cell death pathways in RA is perhaps FLIP. This protein is recruited by FADD upon Fas activation and can either inhibit or potentiate binding of FADD and caspase 8, according to its concentration⁶⁵. FLIP is highly expressed in synovial macrophages of patients with RA, and forced reduction via chemical inhibition with bisindolylmaleimide I of FLIP within macrophages sensitizes this cell population to Fas-mediated apoptosis⁹⁴⁻⁹⁷.

Mice deficient in FLIP in the myeloid cell population present with reduced body weight and die by 3–5 weeks of age, which demonstrates that FLIP expression in myeloid cells is critical for postnatal survival⁹⁸. Furthermore, in an alternate model of myeloid cell-specific FLIP deficiency, mice show complete deletion of FLIP in neutrophils but only partial deletion of FLIP in monocyte precursors and peripheral monocytes and display an increase number of circulating neutrophils accompanied by extensive multiorgan neutrophil infiltration⁹⁸. Similar results were obtained by another group that used a different model of myeloid cell-specific FLIP deficiency in which FLIP is specifically deleted in monocyte populations⁹⁹. The inability to differentiate *ex vivo* cultures of FLIP-deficient c-Kit⁺ hematopoietic stem cells to macrophages and the fact that a model of myeloid cell-specific FLIP deficiency present with elevated levels of circulating monocytes, yet had greatly reduced macrophage populations in the spleen, lymph nodes, and peritoneum indicate that FLIP is crucial for macrophage differentiation⁹⁸.

Although arthritis has not been examined in myeloid cell-specific FLIP-deficient mice, a study published in 2015 reported that targeted deletion of FLIP in both the CD8⁺ and CD11b⁺ DC compartments of mice promotes the spontaneous development of an erosive inflammatory arthritis that resembles RA and is accompanied by the production of autoantibodies to joint antigens¹⁰⁰. Prior to the onset of arthritis, DC populations (in particular CD8⁺ DCs) are substantially reduced in the thymus, spleen and lymph nodes¹⁰⁰. Although DC-specific *FLIP*-knockout mice are lymphopenic, autoreactive CD4⁺ T cells are detected, suggesting that these cells might participate in the propagation of arthritis¹⁰⁰. Furthermore, in these mutant mice, reduced numbers of regulatory T (T_{REG}) cells correlate with the severity of arthritis, and arthritis is ameliorated by the adoptive transfer of T_{REG}

cells¹⁰⁰. In a report by a different research group, mice lacking FLIP in DCs were found to develop neutrophilia (caused by excessive production of granulocyte colony-stimulating factor receptor, (G-CSF)) and splenomegaly. In contrast to the previous study, peripheral CD11b⁺, CD8⁺ and plasmacytoid DC populations were not numerically affected; nonetheless, they were hyperactivated as evidenced by increased expression of MHC II, CD80 and CD86. These peripheral DC populations, together with FLIP-deficient bone marrow-derived DCs, are hyper-responsive to stimulation by TLR2, TLR4 and dectin 1¹⁰¹. However, this murine model of DC-specific FLIP deficiency does not develop spontaneous arthritis, which might be due to either differences in the efficiency of *Flip* deletion or variability in colony environment, which can dictate the composition of the gut microbiome¹⁰¹⁻¹⁰⁴. Taken together, these data demonstrate that the role of FLIP in DCs is to reduce the inflammatory response induced by DCs; nonetheless, the underlying mechanism is still unclear.

Caspase 8

FADD also recruits pro-caspase 8, and aggregation and homodimerization of pro-caspase 8 results in its autocatalysis and/or activation⁶⁵. A genome-wide association study meta-analysis in >100,000 individuals of European and Asian ancestry (29,880 of whom had RA), evaluated almost 10 million single-nucleotide polymorphisms (SNPs) and revealed a SNP associated with RA risk within the locus that contains the gene encoding for both caspase 8 and FLIP¹⁰⁵. Whereas the prospective link between RA risk and FLIP action in DCs has been described, the association between caspase 8 and RA remains a mystery. Despite the role of caspase 8 in cell death being well-established, mounting evidence implicates this protein in death-independent activities, including motility¹⁰⁶, metastasis¹⁰⁷, suppression of inflammation^{108,109}, NF- κ B activation¹¹⁰ and interferon regulatory factor (IRF) 3 degradation^{108,111}.

Specific deletion of caspase 8 in myeloid cells leads to the development of a mild systemic inflammation, which is characterized by splenomegaly, lymphadenopathy, deposition of immune complexes in the kidney, [G] proteinuria, hypergammaglobulinemia and elevated amounts of serum cytokines¹¹². Although Ly6C^{high} and Ly6C^{low} CD11b⁺F4/80⁺ splenic populations are increased in myeloid cell-specific *Casp8*-knockout mice, these cells do not sufficiently induce antigen-specific T cell proliferation¹¹². *In vitro* studies revealed that caspase 8-deficient macrophages are prone to caspase-independent death in response to death receptor ligation, yet caspase 8-deficient myeloid cell populations are not predisposed to unchecked survival¹¹². Indeed, analysis of mixed bone marrow chimeric mice demonstrates that caspase 8 deficiency does not enable myeloid populations to preferentially expand¹¹². Despite the relatively mild inflammatory phenotype observed in myeloid cell-specific *Casp8*-knockout mice, caspase 8-deficient bone marrow-derived macrophages are hyper-responsive to TLR activation (FIG. 1)¹¹². Furthermore, myeloid cell-specific caspase 8 deficiency seems to enhance the response to TLR activation *in vivo*, as injection of TLR ligands into mutant mice upregulates the expression of co-stimulatory CD86 on the Ly6C^{high}CD11b⁺F4/80⁺ splenic population¹¹². In addition, oral antibiotic treatment to remove a significant portion of the gut microbiota prevents inflammatory disease phenotypes in young myeloid cell-specific *Casp8*-knockout mice (FIG. 1)¹¹². These data suggest that

gut microbiota contribute to this phenotype, potentially by providing endogenous TLR ligands to stimulate already hyper-activated myeloid cell populations. Moreover, caspase 8 controls the polarization of macrophages in response to M1-skewing media (IFN γ and LPS, as demonstrated by the prevented formation of true M1 macrophages in *Casp8*-deficient mice (FIG. 2)¹¹². Another group evaluated caspase-8 deficiency in myeloid cells (*LysM^{cre}/Casp8^{del/flox}*), yet these mice fail to generate caspase-8 deficiency in all myeloid cell populations or *in vivo* dysregulation of macrophage populations, presumably due to the cell-specific caspase 8 deletion construct¹¹³. Taken together, these studies implicate caspase 8 in the regulation of macrophage response to TLR activation and macrophage polarization. Furthermore, requirement of caspase 8 for macrophage polarization indicates that disrupted caspase 8 signalling in synovial macrophages might be a fundamental defect at the root of RA pathogenesis.

DC-specific deletion of caspase 8 in mice is sufficient to induce an aggressive SLE-like disease^{114,115}. In these mutant mice, DCs do not display a survival advantage, indicating that defective DC apoptosis is not the underlying cause of the observed inflammation¹¹⁴. Furthermore, caspase 8-deficient bone marrow-derived DCs are hyper-responsive to TLR activation, and caspase 8-deficient splenic DCs upregulate co-stimulatory molecules in response to administration of TLR agonists *in vivo* (FIG. 3)¹¹⁴. Similar to patients with SLE, DC-specific *Casp8*-deficient mice present an interferon signature (FIG. 3) that might reflect a higher activity of IRFs — a family of transcription factors known to have a role in the production of type 1 interferons¹¹⁴. Although caspase 8 has been shown to process IRF-3 for degradation¹¹¹, and that *Casp8*-deficient bone marrow-derived DCs constitutively express elevated IRF3¹¹⁴, deletion of *Irf3* in DC-specific *Casp8*-knockout mice did not prevent, but instead exacerbated, SLE-like disease¹¹⁴. Moreover, deletion of a redundant homolog of IRF3, IRF7, neither prevented nor exacerbated disease in these mutant mice¹¹⁴. As in DC-specific FADD-deficient mice, deletion of *MyD88* prevents SLE-like disease in DC-specific caspase 8-deficient mice (FIG. 3), although oral antibiotic treatment is ineffective at diminishing inflammation in these mutants¹¹⁴. These data demonstrate that caspase 8 in DCs maintains tolerance through MyD88 signalling, independently of cell death and IRF-3 or IRF-7¹¹⁴. Although caspase 8 can regulate multiple functions in macrophages and DCs, future studies are needed to determine the direct impact of these functions on the onset and progression of arthritis as well as RA susceptibility.

Intrinsic pathway of apoptosis

The intrinsic pathway of apoptosis is regulated by the Bcl2 protein family, which is divided into anti-apoptotic and pro-apoptotic members¹¹⁶ (Box 2). The pro-apoptotic family is divided into two additional categories: multi-Bcl-2 homology (BH) domain proteins and BH3-only domain proteins. The current prevailing view is that the BH3-only proteins either activate the multi-BH pro-apoptotic proteins or sequester Bcl2 anti-apoptotic proteins to induce apoptosis¹¹⁶. Alternatively, Bcl2 pro-apoptotic proteins might bind and prevent the BH3-only domain proteins from activating the apoptotic cascade¹¹⁶. The fact that not all BH3-only domain proteins bind to all Bcl2 anti-apoptotic members adds a further level of complexity. Bim, BID and PUMA are the most promiscuous, as they equally bind to all Bcl2 anti-apoptotic proteins, whereas BAD and Noxa only bind to some Bcl2 anti-apoptotic

members¹¹⁷. This specificity correlates with their ability to induce apoptosis¹¹⁷. Peptides containing the BH3 domains of Bim, BID or PUMA are sufficient to induce apoptosis in whole cells and can disrupt the membrane potential in isolated mitochondria¹¹⁷. By contrast, BH3-only proteins are unable to induce apoptosis and require additional unknown signals to activate the apoptotic cascade; thus, they are considered 'sensitizers' of apoptosis¹¹⁷. Once the apoptotic signalling is initiated, BAK and BAX form homodimers and heterodimers at the mitochondrial outer membrane, causing the release of inner mitochondrial proteins such as Cytochrome c^{117,118}. Subsequently, Cytochrome c binds to Apoptotic protease-activating factor 1 (APAF-1) and caspase 9 forming the apoptosome, which activates downstream caspases (such as caspase 3 and caspase 7) that cleave numerous proteins and degrade DNA^{117,118}.

Intrinsic pathway of apoptosis in RA—The Bcl2 protein family has been extensively studied over the past decade in synovial tissue from patients with RA. The vast majority of the work has focused on whole-tissue expression rather than a particular cell type. For example, individual cell populations expressing Bcl2, Bcl-xL or Mcl1 has not been examined in a systematic and/or quantitative manner. Bcl2 expression is increased in patients with RA compared with patients with osteoarthritis, and its concentration correlates with disease activity¹¹⁹. Similarly, Bcl-xL is highly expressed in the RA synovium and correlates with the number of inflammatory cells in the synovial sublining¹²⁰. Mcl1 expression is increased in RA synovial fluid macrophages compared with normal macrophages differentiated from peripheral blood monocytes *in vitro*¹²¹, although its expression in the synovial tissue is enhanced in the synovial lining and sublining, particularly in the synovial fibroblast population¹²². Similarly, PUMA is detected in the synovial sublining and is expressed at high levels in cultured RA synovial fibroblasts¹²³. Further, BAX expression is reduced in RA synovial tissue as compared with normal synovial tissue¹²⁴, although individual synovial cell types expressing BAX are unknown. In contrast, only the expression of Bim has been shown to be reduced in the synovial lining, particularly within the macrophage population¹²⁵. Taken together, these data suggest that in the RA synovium, anti-apoptotic Bcl2 proteins are highly expressed, whereas expression of pro-apoptotic proteins is reduced. Future studies will be required to identify the individual populations of cells expressing Bcl2 proteins in the RA synovium.

Intrinsic pathway of apoptosis in experimental arthritis—Genetically modified mice have been utilized to understand the role of Bcl2 protein family in the development and sustainment of RA-like disease (TABLE 1). Initial studies in CIA mouse models focused on the overexpression of Bcl2 and Bcl-xL in lymphocytes. Results showed that although overexpression of Bcl-xL in B cells exacerbate CIA¹²⁶, in T cells it attenuates the development of arthritis, potentially through their resistance to apoptosis, delayed cell cycle progression and inhibition of IFN- γ production in response to type II collagen challenge¹²⁷. Similarly, overexpression of Bcl2 in all hematopoietic cells reduces the development of CIA, potentially by resulting in decreased production of antibodies against type II collagen¹²⁸. Although treatment with the Bcl2 antagonist ABT-737, which acts as a BAD mimetic, has proved effective at inhibiting RA-like disease in CIA, other studies have questioned the therapeutic value of this inhibitor because of its toxicity^{129,130}. Loss of BID or Bim, but not

Bak1 or Bax, results in increased K/BxN serum transfer-induced arthritis severity^{131,132}. In arthritic mice lacking BID, secretion of CXC chemokine and IL-1 β , as well as the number of macrophages, are increased, whereas the number of apoptotic cells in the synovium is decreased compared with wild-type mice¹³². Similarly, arthritic mice lacking Bim exhibit increased osteoclast activity, which is associated with enhanced bone destruction¹³¹. Furthermore, these mice have elevated pannus formation, which might be due to the lower numbers of apoptotic cells¹³¹. Additionally, in *Bim*^{-/-} mice, expression of CXC chemokine and IL-1 β in the arthritic joints is increased, whereas serum TGF- β is decreased¹³¹. Treatment with a peptide mimetic corresponding to the BH3 domain of Bim was sufficient to prevent the onset of RA-like disease and to suppress established disease in mice¹²⁵. The transactivator of transcription (TAT)-Bim-BH3 peptide preferentially affects myeloid cells and prevents their infiltration into the joint¹²⁵. Moreover, treatment with the TAT-Bim-BH3 peptide does not result in negative systemic effects, as demonstrated by normal liver function and platelet numbers¹²⁵. Overexpression of Bcl2 in hematopoietic cells reduces the development and severity of K/BxN serum transfer-induced arthritis in mice, potentially owing to an expansion of Ly6C^{low} monocytes and increased expression of the inhibitory IgG receptor Fc γ RIIb on leukocytes¹²⁸. Thus, these data demonstrate that the Bcl2 protein family might be an appropriate target for novel RA therapeutics. However, as only one study directly examined the innate immune system in murine models of RA-like disease, cell-specific apoptotic roles for Bcl2 family members need to be further investigated in the context of experimental inflammatory arthritis.

Similar to members of the extrinsic apoptotic pathway, members of the intrinsic apoptotic pathway also possess non-apoptotic functions. None of the transgenic mice overexpressing Bcl2 proteins develop SLE-like disease. Moreover, mice globally deficient in Bax, Bak or BH3-only proteins also fail to develop SLE-like disease. In contrast, *Bim*^{-/-} mice develop systemic autoimmunity that is associated with increased numbers of monocytes and macrophages^{133,134}. Thus, these data suggest that although Bim is considered promiscuous and capable of sequestering all anti-apoptotic proteins¹³⁵, its role in autoimmunity does not match that of Bcl-2 and Bcl-xL, as demonstrated by the fact that overexpression of anti-apoptotic proteins fails to induce autoimmunity¹³⁶⁻¹³⁸. These data also indicate that, in contrast to other BH3-only proteins, Bim has a substantial role in autoimmunity. *Bim*^{-/-} macrophages display increased markers of activation and secrete elevated levels of IL-1 β following thioglycollate elicitation or TLR ligation¹²⁵. Although these macrophages have not been examined for their ability to process and present antigens to T cells, Bim-deficient DCs induce strong T cell responses in OT-I and OT-II TCR transgenic mice (C57BL/6 mice transgenic for a TCR specific for an ovalbumin peptide)¹³⁹. Similar results were observed in mice that overexpress Bcl2 in DCs¹⁴⁰⁻¹⁴²; however, unlike those cells¹⁴⁰, *Bim*^{-/-} DCs cause autoantibody production following adoptive cell transfer and LPS stimulation in control mice¹³⁹. Furthermore, a 2011 study reported that loss of BID in macrophages has an inhibitory effect on the production of proinflammatory cytokines in response to ligands of nucleotide-binding oligomerization domain (NOD) proteins, but not those of TLR; this effect was independent of the BH3 domain¹⁴³. These data suggest that, unlike other BH3-only proteins, Bim might have other functions beyond its role apoptosis.

Necroptotic pathway

Pathways of programmed cell death other than apoptosis have been identified in the past 5 years, including [G] necroptosis¹⁴⁴. The apoptotic pathway (described above) and the newly described necroptotic pathway are both essential for cell turnover, yet the underlying mechanisms differ and depend on the cytosolic milieu. Necroptosis is promoted by the receptor interacting serine/threonine-protein kinases 1 and 3 (RIPK1 and RIPK3), as well as the mixed lineage kinase domain-like protein (also known as MLKL). When apoptosis is blocked, necroptosis can be triggered by TNF (a cytokine abundantly present at the sites of inflammation) or by other stimuli¹⁹, involving components of the extrinsic apoptotic pathway. The binding of TNF to its receptor, TNF-R1, at the cell surface initiates the signal for recruitment of FADD. The aggregation of FADD then facilitates the recruitment of pro-caspase 8, which becomes active after dimerization. Active caspase 8 can either initiate the degradative phase of apoptosis or block necroptosis, depending on the availability of FLIP^{135,145}. Low levels of FLIP enable the formation of caspase 8 homodimers, and apoptosis ensues as described above¹⁴⁵. Conversely, when present at sufficient levels, FLIP can be recruited by FADD to form a heterodimer with caspase 8; this complex not only prevents apoptosis, but also limits necroptosis by suppressing RIPK1–RIPK3 signaling¹⁴⁵. Furthermore, in the absence of FADD or caspase 8, apoptosis is blocked; however, RIPK1–RIPK3 signalling would proceed unchecked, thus leading to necroptosis¹⁴⁵. Because necroptotic cells can promote inflammation resulting from the release of cytokines, DAMPs and pathogen-associated molecular patterns (PAMPs), necroptosis signalling mediators warrant examination in the context of the RA joint. Other caspase 8 death-independent functions have also been directly linked to RIPK activity. For example, TLR engagement provokes RIPK signalling independent of death receptor activation, leading to the formation of the ripoptosome — a complex containing similar proteins involved in necroptosis — or the necrosome (composed of FADD, caspase 8, FLIP and RIPK1)¹⁴⁶. Ripoptosome/RIPK3 activity induces IL-1 β production¹⁴⁷, whereas caspase 8/RIPK signalling can regulate the NLRP3 inflammasome^{115,148,149}. Due to the mounting death-independent functions assigned to signalling molecules involved in the activation of the necrosome or ripoptosome pathways, these signalling mediators remain under intense investigation.

Whereas *Ripk1*^{−/−} mice die perinatally¹⁵⁰, *Ripk3*^{−/−} mice show no gross defects in development¹⁵¹. Deletion of either *Ripk1* or *Ripk3* rescues embryonic lethality associated with the global knockout of *FADD* and *Casp8* in mice, but not *FLIP*^{145,152,153}. Similarly, conditional loss of RIPK3 in myeloid cells and DCs rescues the phenotypes associated with FADD deficiency in knockout mice^{92,93}. In addition, symptoms of systemic inflammation in myeloid cell-specific caspase 8-deficient mice are ameliorated by deletion of *Ripk3* (FIG. 1)¹¹². However, RIPK3 is not involved in the aggressive SLE-like disease in DC-specific caspase 8-deficient mice^{114,115}. *In vitro* studies implicate RIPK1 also in the hyper-activation of bone marrow-derived macrophages and bone marrow-derived DCs from myeloid cell-specific and DC-specific *Casp8*-deficient mice following TLR activation^{112,114} (FIG. 1 and FIG. 3). These data suggest that uncontrolled RIPK1 activity might contribute to the SLE-like disease of mutant mice, although the role of this protein in inflammatory arthritis remains unresolved. A 2015 report shows that *Ripk3*^{−/−} mice display a more rapid resolution of K/BxN arthritis compared with control mice, whereas mice deficient for *MLKL* responded

normally to the induction of serum-induced arthritis¹⁵⁴. These results indicate that the action of necroptotic mediators in macrophage and DC populations should be examined in the context of RA.

Inhibitors of apoptosis

Inhibitors of apoptosis proteins (IAPs) are also linked to RA. IAPs can regulate apoptosis by directly binding to and preventing downstream effector functions of caspase 3, caspase 7 and caspase 9, thereby blocking the assembly of caspase 8 and caspase 10^{155,156}. In response to a pro-apoptotic stimulus, the diablo homolog, mitochondrial (also known as second mitochondria-derived activator of caspases (Smac)) can be liberated from the mitochondria and translocate into the cytoplasm to block the anti-apoptotic activity of IAPs^{157,158}. A number of compounds have been designed to mimic the structure of these molecules. Smac mimetics bind specifically to three IAPs, including E3 ubiquitin-protein ligase XIAP, Baculoviral IAP repeat-containing protein 2 (also known as C-IAP1) and Baculoviral IAP repeat-containing protein 3 (also known as C-IAP2)¹⁵⁵. One such Smac mimetic downregulates XIAP, C-IAP1 and C-IAP2 and can induce cell death in apoptosis-resistant RA fibroblast-like synoviocytes, thereby implicating this molecule as a local therapeutic for RA¹⁵⁵.

Murine studies show that mice with myeloid cell-specific deletion of cIAP-1 and either global knockout of cIAP-2 or global knockout of both cIAP-2 and XIAP develop splenomegaly as well as a spontaneous inflammatory arthritis resulting from myeloid cell dysfunction and mediated by TNF and IL-1 β ¹⁵⁴. These mutant mice also develop a more exacerbated K/BxN serum transfer-induced arthritis than IAP-intact mice, as demonstrated by the use of mixed bone marrow chimeras that reduced the systemic inflammation associated with global loss of IAPs¹⁵⁴. These data indicate that IAPs tightly control the apoptotic machinery. Although the function of IAPs in macrophages is implicated in the regulation of the arthritis development, their role in joint DCs has not yet been examined.

Autophagy

Autophagy is a survival strategy whereby a cell, when deprived of nutrients or subjected to other stressors, can degrade dysfunctional and/or unneeded organelles using its own lysosomal machinery. Studies completed within the past five years suggest that the impaired apoptosis observed in RA synovial tissues might be the result of increased autophagy¹⁵⁹⁻¹⁶¹. In cultured RA synovial fibroblasts, induction of autophagy and low expression of DNA damage-inducible transcript 3 protein (also known as CHOP) protects these cells from death by limiting the endoplasmic reticulum (ER) stress response¹⁵⁹. Furthermore, whole RA synovial tissue is characterized by higher expression of Beclin-1 (a protein that stimulates the initiation of autophagy) and microtubule-associated proteins 1A/1B light chain 3B (also known as LC3 B; a component of autophagosomes or double-membrane vesicles responsible for delivering cytoplasmic material to lysosomes), which coincides with a deficiency in the Beclin-1-targeting microRNA miR-30a¹⁶⁰. Autophagy induction by proteasome inhibition or ER stress is more pronounced in RA synovial fibroblasts than in those found in osteoarthritis joints¹⁶¹. Further, the autophagy pathway not only promotes cell death in the context of ER stress, but can also protect against cell death caused by

proteasome inhibition¹⁶¹. Thus, autophagy has both cytoprotective and cytotoxic roles in RA synovial fibroblasts, and can therefore function as an adaptor between cell death and survival¹⁶¹⁻¹⁶³. Methotrexate, the most widely used DMARD, suppresses proliferation of lymphocytes and macrophages, but has a limited pro-apoptotic effect in RA synovial fibroblasts¹⁶⁴⁻¹⁶⁶. Resistance to methotrexate treatment is caused, in part, by the induction of autophagy¹⁶⁷. In contrast to RA synovial fibroblasts, RA T cells are characterized by reduced autophagy, and therefore are prone to undergo apoptosis¹⁶⁸. Exaggerated T cell apoptosis leads to chronic T cell loss and development of lymphopenia, which has been proposed as a risk factor for autoimmunity¹⁶⁸.

Studies in mice also suggest that autophagy is linked to RA pathogenesis. In antigen-presenting cells such as macrophages and DCs, autophagy is a key cellular process responsible for the [G] citrullination of antigens to be presented at the cell surface in the context of MHC¹⁶⁹. Autoantibodies to citrullinated proteins are found in patients with RA and correlate with disease severity¹⁷⁰. Members of the autophagy signalling pathways, including Ubiquitin-like modifier-activating enzyme ATG7 (a protein essential for the elongation of the phagophore — also known as isolation membrane — during autophagy) and Beclin-1, are upregulated in osteoclasts of patients with RA¹⁷¹. In mice overexpressing human TNF, RA-like phenotypes, including osteoclastogenesis and destructive arthritis, occur spontaneously. Transplantation of bone marrow cells from myeloid cell-specific *Atg7*-knockout mice into mice overexpressing human TNF reduces osteoclast numbers and protects mice from TNF-induced bone erosion, proteoglycan loss and chondrocyte death, but not inflammation^{171,172}. Data obtained from these mutant mice implicate osteoclast autophagy in the pathogenesis of RA; nonetheless, since myeloid cell-specific *Atg7* deletion will not only occur in osteoclasts but also in circulating neutrophils and monocytes, autophagy of monocyte-derived macrophages that accumulate in the RA joint might also be involved. A 2015 study showed that the anti-diabetic drug metformin, previously reported to stimulate autophagy, can mitigate inflammation in K/BxN arthritis¹⁷³. *In vitro*, metformin stimulates AMP-activated protein kinase (AMPK) activity in macrophages during arthritis. This stimulation inhibits Serine/threonine-protein kinase mTOR signalling, thereby correcting the impaired autophagic flux associated with experimental arthritis and suppressing NF κ B-mediated signalling through degradation of I κ B kinase (IKK)¹⁷³. These data, in addition to the risk polymorphisms in autophagy signalling mediators identified in patients with RA and SLE, implicate autophagy in the pathogenesis of autoimmune rheumatic diseases¹⁷⁴⁻¹⁷⁶. Therefore, the role of autophagy, especially in RA synovial macrophage and DC populations, merits further investigation.

Conclusions

Great strides have been made in the past decade in the attempt to dissect the role of cell death-associated signalling molecules in the pathogenesis of RA. Phagocytosis of dying cells by professional phagocytes, such as macrophages and DCs, is crucial to maintain tissue homeostasis; however, when this process goes awry, inflammation and exposure to self-antigens might occur, leading to the onset of autoimmunity. In the context of the joint, phagocytosis of infiltrating short-lived cells, such as neutrophils, might signal macrophages to adopt a wound healing phenotype, thus implicating synovial macrophages in the control

of inflammation. However, should synovial macrophages, or DCs, present with defects in signalling mediators and/or inhibitors of cell death pathways, detrimental outcomes are predicted to occur within both the homeostatic and RA joint. As these signalling mediators and/or inhibitors of cell death pathways can also maintain functions beyond their involvement in cell death, further investigation is warranted to fully characterize their roles within synovial phagocyte populations for discovery of potentially novel therapeutic targets.

Acknowledgements

This work was supported by grants from the NIH to C.M.C. (K01AR064313), R.M.P. (AR065076, AR067687) and H.P. (AR064546, AR050250, AR054796, AI092490, HL108795), and funds provided to R.M.P. from the Pfizer Aspire award and to H.P. by the Solovy-Arthritis Research Society Chair in Medicine.

Biography

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Glossary

phagocytic

the capacity of a cell to take in particles such as bacteria, parasites, dead host cells and cellular and foreign debris

apoptotic

a cell undergoing the process of programmed cell death, apoptosis

efferocytosis

a process similar to, yet distinct from, phagocytosis, whereby phagocytes engulf and eliminate apoptotic cells to promote the resolution of inflammation

splenomegaly

abnormal enlargement of the spleen

lymphadenopathy

disease of the lymph nodes, in which they are abnormal in size, number, or consistency

proteinuria

the presence of abnormal quantities of protein in the urine, which may indicate damage to the kidneys

hypergammaglobulinemia

a condition that is characterized by the increased levels of a certain immunoglobulin in the blood serum

glomerulonephritis

acute inflammation of the kidney, typically caused by an immune response

necroptosis

a programmed form of necrosis, or inflammatory cell death

citrullination

the conversion of the amino acid arginine in a protein into the amino acid citrulline

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Key points

Macrophages and dendritic cells are essential for the pathogenesis of RA.

Phagocytosis of dying cells is necessary for the resolution of inflammation, and defects in this process can lead to rheumatic disease

Macrophage-specific and dendritic-cell-specific defects in mediators of the extrinsic apoptotic pathway induce altered responses in experimental arthritis, and overexpression and/or deletion of components of the intrinsic apoptotic pathway influence the development of experimental arthritis

Global deletion of necroptotic signalling mediators results in a more rapid resolution of experimental arthritis, whereas macrophage-specific deletion of inhibitors of apoptosis exacerbates experimental arthritis

Autophagy might have a cell-specific inhibitory and proinflammatory role in RA and experimental arthritis

Risk polymorphisms in mediators of the extrinsic apoptotic pathway and autophagy have been identified in patients with RA

Box 1. Murine models of inflammatory arthritis

Murine models of inflammatory arthritis share many characteristics of RA and are vital for understanding the pathogenesis of disease. Two such murine models are the collagen-induced arthritis (CIA) and the K/BxN serum transfer-induced arthritis. (CIA) is a multifaceted immunologically-mediated disease involving lymphocytes (especially type 17 T helper cells) and inflammatory cells of innate immunity, which infiltrate joint tissue and induce pathology¹⁷⁷⁻¹⁷⁹. CIA is initiated by immunization with bovine type II collagen emulsified in complete Freund's adjuvant¹⁸⁰. DCs present type II collagen to T cells, leading to the activation of T helper (T_H) cells¹⁸⁰. T_H cells specific for type II collagen provide help to B cells to produce antibodies against type II collagen, and this culminates in the activation of the complement cascade within the joint¹⁸⁰. Subsequently, the complement system activates endothelial cells, which release chemo-attractants that stimulate inflammatory cell migration into the joint¹⁸⁰. These inflammatory cells are activated through Fcγ receptors to produce cytokines such as TNF and IL-1, thereby leading to tissue destruction¹⁸⁰. IL-17 is crucial for the development of CIA^{181,182}. However, since clinical trials have shown that suppression of IL-17 is not as beneficial as treatment with anti-TNF or IL-6¹⁸³, the clinical relevance of the CIA model is now in question.

In contrast to CIA, the K/BxN serum transfer-induced arthritis murine model mimics the effector phase of RA. K/BxN mice, which are the transgene-positive offspring of KRN and NOD mice, develop a severe, spontaneous, symmetric, erosive and chronic arthritis that mimics RA¹⁸⁴. The K/BxN serum transfer-induced arthritis model relies on the ability of the KRN-derived transgenic T cell receptor to recognize a ubiquitous self-peptide (glucose-6-phosphoisomerase; G6PI) when presented in the context of the NOD-derived MHC II allele H-2k (A^g7), which is followed by B cell activation and production of anti-G6PI antibodies¹⁸⁵. Arthritis is passively transferred via a single intravenous injection of K/BxN serum. The K/BxN serum transfer-induced arthritis model includes initiation, propagation and resolution phases and is advantageous, not only because it is easily transferred to most mouse strains, but also because it is not dependent on T cells and B cells; instead, the model relies on innate immune cells to mediate disease¹⁸⁶⁻¹⁹⁰. The K/BxN serum transfer-induced arthritis model can be useful in determining the impact of signalling mediators, specifically in cell populations of the innate immune system.

Box 2 Bcl2 protein family			
Box 2			
Bcl2 protein family			
• Anti-apoptotic members			
Protein name	Alternative protein name	Gene name	Genetic association to human rheumatic disease
Apoptosis regulator Bcl2	Bcl2	<i>BCL2</i>	-increased expression in RA synovium ^{78,119} -increased expression in primary Sjogren syndrome salivary glands ¹⁹¹ -polymorphism associated with increased risk of RA ¹⁹²
Bcl-2-like protein 1	BclxL	<i>BCL2L1</i>	-increased expression in RA synovium ¹²⁰ -increased expression in primary Sjogren syndrome salivary glands ¹⁹¹
Induced myeloid leukemia cell differentiation protein Mcl1	Mcl1	<i>MCL1</i>	-up-regulated in RA macrophages and synovial fibroblasts ^{121,122}
• Pro-apoptotic members			
Protein name	Alternative protein name	Gene name	Genetic association to human rheumatic disease
<i>multi-Bcl-2 homology (BH) domain proteins</i>			
apoptosis regulator BAX	<i>BAX</i>	BAX	-increased expression in RA synovial tissue ¹²⁴
Bcl2 homologous antagonist/killer	<i>BAK1</i>	BAK	-overexpressed in autoimmune lesions of patients with Sjogren syndrome ¹⁹³ - polymorphisms associated with Sjogren syndrome ¹⁹⁴
<i>BH3-only domain proteins</i>			
Bcl-2-like protein 11	Bim	<i>BCL2L11</i>	-reduced expression in macrophages in RA synovial tissue ¹²⁵
Bcl2-associated agonist of cell death	BAD	<i>BAD</i>	n/a
Bcl2-related ovarian killer protein	Bok	<i>BOK</i>	n/a
Bcl2-interacting killer	Bik	<i>BIK</i>	n/a
BH3-interacting domain death agonist	BID	<i>BID</i>	n/a

• Pro-apoptotic members				
Protein name	Alternative protein name	Gene name	Genetic association to human rheumatic disease	
Activator of apoptosis hara-kiri	DP5	<i>HRK</i>	n/a	
Bcl2-modifying factor	BMF	<i>BMF</i>	n/a	
Phorbol-12-myristate-13-acetate-induced protein 1	Noxa	<i>PMAIP1</i>	XX	
Bcl2-binding component 3	PUMA	<i>BBC3</i>	-increased expression in sublining of the RA joint ¹²³

Figure 1. Extrinsic and Intrinsic Pathways of Apoptosis

a | An example of the extrinsic pathway is depicted, wherein binding of FasL to its cognate death receptor Fas facilitates the recruitment of FADD. FADD then recruits the inactive form of caspase 8, the aggregation and homodimerization of which result in its autocatalysis and/or activation. This step induces the degradative phase of apoptosis through the activation of caspase 3 and caspase 7, which are coordinators of downstream apoptotic cellular processes. FLIP, a catalytically inactive homolog of caspase 8, acts as an endogenous regulator of the Fas pathway at the level of caspase 8 activation. FLIP is recruited by FADD upon Fas activation and can either inhibit or potentiate the binding of FADD and caspase 8, depending on its concentration. b | An alternative pathway of Fas-induced cell death involves crosstalk between the extrinsic and the intrinsic apoptotic pathway. This crosstalk is mediated by the activation of the Bcl2 pro-apoptotic protein BID, which is cleaved by caspase 8 following Fas ligation. c | The intrinsic pathway of apoptosis is regulated by the Bcl2 protein family, which comprise both anti-apoptotic and pro-apoptotic members. The pro-apoptotic family includes multi-Bcl-2 homology (BH) domain proteins and BH3-only domain proteins. One view is that the BH3-only proteins either activate the multi-BH pro-apoptotic proteins or sequester Bcl2 anti-apoptotic proteins to induce apoptosis. Alternatively, Bcl2 pro-apoptotic proteins bind and prevent the BH3-only domain proteins from activating the apoptotic cascade. Nevertheless, once the apoptotic signalling is initiated, BAX and BAK form homodimers and heterodimers at the mitochondrial outer membrane, causing the release of inner mitochondrial proteins such as Cytochrome c. Subsequently, Cytochrome c binds to APAF-1 and caspase 9 forming the apoptosome, which activates caspase 3 and caspase 7.

Figure 2. Caspase 8-deficient macrophages are hyper-responsive to toll-like receptor (TLR) activation

a | In wild-type macrophages, caspase 8 blocks receptor interacting serine/threonine protein kinase (RIPK) signalling, and a normal transcriptional response follows activation of surface toll-like receptor (TLR) 4 and endosomal TLR7 and TLR9 in both *in vitro* and *in vivo* settings. b | In the absence of caspase 8, RIPK signalling proceeds unchecked, and signalling through surface and endosomal TLRs is augmented, leading to upregulation of proinflammatory mediators. c | Blockade of RIPK signalling, via deletion of RIPK3 or chemical inhibition of RIPK1 by necrostatin-1, or removal of endogenous TLR ligands in the gut through oral antibiotic treatment, restores the normal activity of caspase 8-deficient macrophages.

Figure 3. Caspase 8 is required for normal M1 macrophage polarization *in vitro*

a | Following treatment with IFN- γ and lipopolysaccharide (LPS) to induce M1 macrophage polarization *in vitro*, caspase 8 can block receptor interacting serine/threonine protein kinase 1 (RIPK1) signalling. b | In the absence of caspase 8, RIPK1 signalling proceeds unchecked, leading to an abnormal response in M1 macrophage polarization. c | Blockade of RIPK1 signalling, via chemical inhibition with necrostatin-1, restores normal M1 polarization of caspase 8-deficient macrophages.

Figure 4. Caspase 8-deficient dendritic cells (DCs) are hyper-responsive to toll-like receptor (TLR) activation

a | Caspase 8 can block receptor interacting serine/threonine protein kinase 1 (RIPK) signalling during TLR activation of dendritic cells (DCs) in both *in vitro* and *in vivo* settings. b | In the absence of caspase 8, unchecked RIPK signalling increases both surface and endosomal TLR signalling, leading to upregulation of interferon response genes and subsequent onset of systemic autoimmunity. c | Blockade of RIPK signalling, via either RIPK3 deletion or RIPK1 chemical inhibition by necrostatin-1, restores the normal activity of caspase 8-deficient DCs *in vitro*, whereas targeted deletion of Myeloid differentiation primary response protein MyD88 in these cells prevents onset of systemic autoimmunity.

Table 1

SLE-like and RA-like disease in murine models with defects in cell death pathways

Signalling pathway	Protein	Mouse model	Cell types	Presence of SLE-like disease *	RA-like disease
Extrinsic apoptotic pathway	Fas	MRL. <i>Fas</i> ^{lpr}	All cells	++++ ⁸¹	Spontaneous arthritis ⁸¹
		C57BL/6. <i>Fas</i> ^{lpr}	All cells	+++ ⁸⁰	Exacerbated K/BxN serum-transfer-induced arthritis ⁸⁴
		DBA. <i>Fas</i> ^{lpr/lpr}	All cells	?	Reduced CIA ⁸⁵
		<i>LysM</i> ^{cre/cre} <i>Fas</i> ^{flox/flox}	Monocytes, macrophages, neutrophils	++ ⁸²	More rapid resolution of K/BxN serum-transfer-induced arthritis ⁸⁶
	FADD	<i>CD11c</i> ^{cre} <i>Fas</i> ^{flox/flox}	Dendritic cells	++ ⁸⁷	?
		<i>LysM</i> ^{cre/cre} <i>FADD</i> ^{lox/flox}	Monocytes/ macrophages/ neutrophils	++ ⁹²	?
		<i>CD11c</i> ^{cre} <i>FADD</i> ^{flox/flox}	Dendritic cells	++ ⁹³	?
	FLIP	<i>LysM</i> ^{cre/cre} <i>Flip</i> ^{del/flox}	Monocytes/ macrophages/ neutrophils	NA ⁹⁸	NA ⁹⁸
		<i>LysM</i> ^{cre/+} <i>Flip</i> ^{del/flox}	Monocytes/ macrophages/ neutrophils	- ⁹⁸	?
		<i>CD11c</i> ^{cre} <i>Flip</i> ^{flox/flox}	Dendritic cells	- ¹⁰⁰	Spontaneous arthritis ¹⁰⁰
	Caspase 8	<i>LysM</i> ^{cre/cre} <i>Casp8</i> ^{flox/flox}	Monocytes/ macrophages/ neutrophils	++ ¹¹²	?
		<i>LysM</i> ^{cre/cre} <i>Casp8</i> ^{del/flox}	Monocytes/ macrophages/ neutrophils	- ¹¹³	?
		<i>CD11c</i> ^{cre} <i>Casp8</i> ^{flox/flox}	Dendritic cells	+++ ^{114,115}	?
Necroptotic pathway	RIPK3	<i>RIPK3</i> ^{-/-}	All cells	- ¹⁵¹	More rapid resolution of K/BxN serum-transfer-induced arthritis ¹⁵⁴
Intrinsic apoptotic pathway	Bak	<i>Bak</i> ^{-/-}	All cells	- ^{135,195}	No effect in K/BxN serum-transfer-induced arthritis ¹³¹
	Bax	<i>Bax</i> ^{-/-}	All cells	- ^{135,195}	No effect in K/BxN serum-transfer-induced arthritis ¹³¹
	Bid	<i>Bid</i> ^{-/-}	All cells	- ^{135,195}	Exacerbated K/BxN serum-transfer-induced arthritis ¹³²
	Bim	<i>Bim</i> ^{-/-}	All cells	+++ ^{133,134}	Exacerbated K/BxN serum-transfer-induced arthritis ¹³¹
	Vav-Bcl2	Vav-Bcl2	All hematopoietic cells	++ ¹⁹⁶	Reduced K/BxN serum-transfer-induced arthritis and CIA ¹²⁸
	Bcl-xL	Ep-Bcl-xL	B cells	- ¹⁹⁷	Exacerbated CIA ¹²⁶
Inhibitors of apoptosis	IAP protein family	Lck-Bcl-xL	T cells	- ¹⁹⁸	Reduced CIA ¹²⁷
		<i>LysM</i> ^{cre/cre} <i>cIAP1</i> ^{flox/flox} <i>cIAP2</i> ^{-/-}	monocytes/ macrophages/ neutrophils	+ ¹⁵⁴	Spontaneous arthritis ¹⁵⁴
		<i>LysM</i> ^{cre/cre} <i>cIAP1</i> ^{flox/flox} <i>cIAP2</i> ^{-/-} <i>XIAP</i> ^{-/-}	monocytes/ macrophages/ neutrophils	+ ¹⁵⁴	Spontaneous arthritis ¹⁵⁴
					Exacerbated K/BxN serum-transfer-induced arthritis

Signalling pathway	Protein	Mouse model	Cell types	Presence of SLE-like disease*	RA-like disease
Autophagy pathway	Atg7	<i>CD11c^{cre} cIAP1^{flox/flox} cIAP2^{-/-}</i>	dendritic cells	?	?
		<i>CD11c^{cre} cIAP1^{flox/flox} cIAP2^{-/-} XIAP^{-/-}</i>	dendritic cells	?	?
		Human TNF transgene with adoptive transfer of <i>LysM^{cre/cre} Atg7^{flox/flox}</i> bone marrow cells	osteoclasts/monocytes/macrophages/neutrophils	- 171,199	Protection from human TNF transgene-induced arthritis ¹⁷¹
		<i>CD11c^{cre} Atg7^{flox/flox}</i>	dendritic cells	?	?

* increasing plus signs denote increasing disease severity