Chapter 2

Mast cells in rheumatic disease

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Abstract

Rheumatoid Arthritis is a chronic autoimmune disease with a complex disease pathogenesis leading to inflammation and destruction of synovial tissue in the joint.

Several molecules lead to activation of immune pathways, including autoantibodies, Toll-Like Receptor ligands and cytokines. These pathways can cooperate to create the pro-inflammatory environment that results in tissue destruction. Each of these pathways can activate mast cells, inducing the release of a variety of inflammatory mediators, and in combination can markedly enhance mast cell responses.

Mast cell-derived cytokines, chemokines, and proteases have the potential to induce recruitment of other leukocytes able to evoke tissue remodeling or destruction. Likewise, mast cells can secrete a plethora of factors that can contribute to tissue remodeling and fibroblast activation.

Although the functional role of mast cells in arthritis pathogenesis in mice is not yet elucidated, the increased numbers of mast cells and mast cell-specific mediators in synovial tissue of rheumatoid arthritis patients suggest that mast cell activation in rheumatoid arthritis may contribute to its pathogenesis.

Pathogenic pathways in rheumatoid arthritis

Rheumatoid arthritis is a systemic autoimmune disease characterized by chronic inflammation of the synovial lining of the joint, and is one of the most common autoimmune diseases affecting approximately 1% of the general population (Gabriel, 2001). Synovitis, inflammation of the synovial tissue, is mediated through leukocyte infiltration of the tissue, and leads to hyperplasia of fibroblast-like synoviocytes and tissue remodeling. Likewise, synovitis can induce cartilage destruction and bone erosion, ultimately leading to destruction of the joint. Clinically, synovitis induces pain and swelling of the involved joints, and the tissue destruction evoked can lead to disabilities if left untreated.

It is currently believed that different cells of the immune system play a role in the pathogenesis of rheumatoid arthritis. However, the exact cause of rheumatoid arthritis is not known. Genetic risk factors (such as HLA) underlying disease susceptibility are often involved in T and B cell responses and the presence of activated B cells and T cells in the inflamed synovium of rheumatoid arthritis patients indicate that adaptive immunity plays a prominent role. Furthermore, the presence of autoantibodies in the majority of patients points towards an important role for B cells in rheumatoid arthritis. However, besides the role of adaptive immune cells in initiation of autoreactive responses, innate immune cells are thought to play an important role during the effector phase by sustaining inflammation.

Treatment is usually aimed at lowering disease activity via immunosuppression, which can be achieved in various ways including through the interference with B cell-mediated immunity, co-stimulatory pathways, and inhibition of proinflammatory cytokines, suggesting that these pathways play an important role in disease pathogenesis.

Autoantibodies

A major effector function thought to contribute to pathogenesis in rheumatoid arthritis is mediated by autoantibodies. The classical autoantibody system associated with rheumatoid arthritis is rheumatoid factor, which recognizes the Fc portion of IgG. However, rheumatoid factor is not specific for rheumatoid arthritis patients, as it is also produced in a number of other inflammatory conditions, therefore its role in disease pathogenesis is often questioned. An important group of autoantibodies in rheumatoid arthritis targets modified proteins, with anti-citrullinated protein antibodies (ACPA) being the most well-characterized. These antibodies recognize a variety of proteins or peptides in which the amino acid arginine is modified into a citrulline through a posttranslational modification process mediated by Peptidyl Arginine Deiminase (PAD) enzymes. PAD enzymes are normally present inside cells and can be activated by high calcium levels when cells, such as neutrophils, undergo apoptosis, an event readily occurring during inflammation (Gyorgy et al., 2006). PAD enzymes that are transported to the outside of cells can citrullinate the extracellular matrix and in doing so can create targets for ACPA. Citrullinated proteins can be found in a variety of inflamed tissues, including the

synovial tissue of rheumatoid arthritis patients (Baeten et al., 2001; Makrygiannakis et al., 2006). ACPA can recognize many citrullinated proteins such as vimentin, filaggrin, and fibrinogen. Because fibrinogen and vimentin are also present in the extracellular matrix of the synovium, these proteins are often considered as important target antigens for ACPA (Klareskog et al., 2008).

ACPA show a very high specificity for rheumatoid arthritis, and are present in the majority (~70%) of rheumatoid arthritis patients (Nishimura et al., 2007; Schellekens et al., 2000). Since their discovery ACPA are mainly used as diagnostic marker. However, it is now becoming increasingly clear that ACPA might also play a functional role in the pathology of rheumatoid arthritis. Several observations underlie this notion. ACPA can be observed already years before the onset of symptoms, and rarely develop after onset of clinical manifestation of rheumatoid arthritis (Rantapaa-Dahlqvist et al., 2003; Ronnelid et al., 2005). The latter indicates that it is not likely that ACPA are a consequence of the inflammation present in rheumatoid arthritis patients. $ACPA^+$ and $ACPA^-$ patients differ considerably with respect to the underlying genetic and environmental risk factors, suggesting that rheumatoid arthritis consists of two different disease entities: ACPA+ and $ACPA^-$ rheumatoid arthritis (Huizinga et al., 2005; Klareskog et al., 2006; Pedersen et al., 2007; van der Helm-van Mil et al., 2006). Furthermore, $ACPA^+$ and $ACPA^-$ rheumatoid arthritis patients have a different disease course with $ACPA^+$ patients having a more progressive disease, characterized by increased radiological joint damage and worse disease activity scores (Meyer et al., 2003; Ronnelid et al., 2005). These findings suggest that ACPA contribute to disease pathogenesis.

When ACPA antibodies are adoptively transferred into mice with a low-level synovial inflammation caused by anti-collagen antibodies, ACPA (reactive with citrullinated fibrinogen or collagen II) could enhance arthritis, implicating their direct involvement in the inflammatory process (Kuhn et al., 2006; Uysal et al., 2009).

Other autoantibodies present in rheumatoid arthritis patients include antibodies directed against carbamylated proteins, or anti-Carbamylated Protein Antibodies (anti-CarP), another autoantibody directed towards modified proteins. Like ACPA, Anti-CarP are present before disease onset and associate with disease severity in (ACPA-negative) rheumatoid arthritis patients, and could potentially contribute to disease pathogenesis (Shi et al., 2011).

Toll Like Receptor ligands

Toll Like Receptor (TLR) activation is another important pathway for immune activation in rheumatoid arthritis. Although TLR are particularly known for their role in protection against pathogens, through their recognition of pathogen associated molecular patterns, endogenous ligands have been reported to trigger these receptors as well. Such endogenous ligands are present in conditions of stress or tissue damage, and often are intracellular molecules that can be either passively or actively released upon cell death. As

rheumatoid arthritis, like other inflammatory conditions, is related to tissue destruction, cell death and the associated presence of endogenous TLR ligands is a common feature in synovium of patients. Several examples have been described of damage associated endogenous TLR ligands present in synovium, including HMGB1, heat shock proteins, tenascin c, and fibronectin (Gondokaryono et al., 2007; Martin et al., 2003; Midwood et al., 2009; Pullerits et al., 2003; Taniguchi et al., 2003).

These endogenous ligands are thought to contribute to the chronicity of inflammation, as they can activate TLRs, inducing an inflammatory response, further tissue and cellular damage, and thereby the sustained release of damage associated TLR ligands.

Next to damage-associated TLR ligands, cell death can also lead to release of PAD enzymes into the extracellular environment, leading to generation of citrullinated proteins, including fibrinogen. Citrullinated fibrinogen, one of the antigens recognized by ACPA, was shown to trigger TLR4 (Sokolove et al., 2011). Therefore, chronic inflammation is often related to release or generation of TLR ligands, leading to a self-amplifying inflammatory loop (Fig. 1).

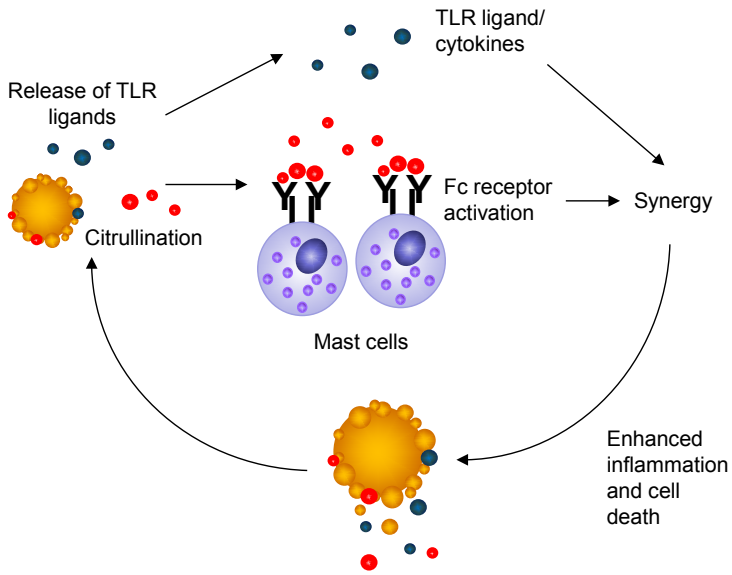

Figure 1. Damage associated molecular patterns (DAMPs), cytokines and citrullinated proteins are all implicated in rheumatoid arthritis pathogenesis and are released upon inflammation, in particular in association with cell death. Both have been shown to activate mast cells: citrullinated proteins can form immune complexes with ACPA autoantibodies, and activate mast cells through Fc γ receptors; DAMPs can activate mast cells through Toll Like Receptors (TLRs); various cytokines can activate mast cells. In the environment of the inflamed joint, all of these triggers are present at the same time, and together lead to synergy in mast cell activation. This synergy leads to enhanced tissue inflammation, in particular neutrophil influx, leading to cell death in the tissue. This cell death can lead to an amplification loop by generating more endogenous ligands and citrullinated proteins.

T helper cells

The strong genetic association of the HLA region with disease susceptibility suggests the involvement of T helper cells in the etiology of rheumatoid arthritis. The association to HLA-DR alleles is not completely understood, but is specifically related to the ACPA response and could therefore be attributed to the helper function of T cells by which they can drive autoantibody responses by B cells (van der Helm-van Mil et al., 2006).

However, T cells themselves may also exert pathogenic effects, for example through their production of cytokines. Initially, T_h1 cells, producing IFNγ and TNFα were thought to drive the immune response in rheumatoid arthritis. Since discovery of a wide variety of T helper cell subsets, T_h17 cells (producing IL-17) have been proposed as the most relevant subset of T cells in relation to arthritis, although their putative role in- or contribution to the pathogenesis of rheumatoid arthritis in humans is unclear (Benedetti and Miossec, 2014).

Cytokines & chemokines: inflammatory mediators

The importance of proinflammatory cytokines in the pathogenesis of rheumatoid arthritis is well established. The development of biologic agents that target various immune mediators has dramatically improved the patient prognosis in the past decades, and most of these biologicals target cytokines or cytokine receptors. Established and approved therapies for rheumatoid arthritis block cytokine responses to TNFα and IL-6 (Smolen et al., 2007). Cytokines are produced in response to immune cell activation, and can activate cells in an autocrine, paracrine or systemic manner, leading to gene transcription of other cytokines, MMPs and other proinflammatory molecules. Thereby they contribute to the self-amplifying loop of immune activation. The cytokines mentioned above have a variety of target cells and functions, thereby able to trigger tissue inflammation, cartilage destruction, bone erosion and angiogenesis.

Activation of mast cells in rheumatoid arthritis

The mast cell is a potent immune cell from the myeloid lineage and is well-known for its granules containing inflammatory mediators which can be rapidly released upon activation. Mast cells reside at interfaces with the external environment, where they act as first line of defense against invading pathogens, such as parasites and bacteria. In addition, mast cells play an important role in allergic diseases (Sayed et al., 2008). As there is overlap in the mechanisms involved in hypersensitivity in allergy and autoimmune diseases, a role for mast cells in autoimmune disease has long been postulated. Several clinical findings support an active role of mast cells in rheumatoid arthritis pathogenesis, and suggest that mast cells are activated in the synovium of rheumatoid arthritis patients.

Mast cell hyperplasia in synovium

It has been shown that increased numbers of mast cells are present in synovium of rheumatoid arthritis patients, with numbers up to 5% of the total cell number in synovium

(Crisp et al., 1984; Malone et al., 1986). Increased mast cell numbers, or so-called mast cell hyperplasia, is a hallmark of multiple autoimmune diseases.

Growth factors and cytokines in synovial tissue, such as stem cell factor, the critical growth factor for mast cell survival, as well as IL-3 and IL-4 are present in synovial tissue of rheumatoid arthritis patients. These mediators can induce proliferation of mast cells, whereas in addition, stem cell factor and TGFβ have been shown to induce recruitment of mast cells, (Olsson et al., 2001) suggesting that the accumulation of mast cells in synovium may be the consequence of an ongoing inflammatory response mediating mast cell expansion through increased recruitment and proliferation.

In addition to the accumulation of mast cells, it has been reported that the proportion of different mast cell subsets is changed in the synovium of rheumatoid arthritis patients. Two main subsets of mast cells exist based on the expression of proteases, divided in tryptase-only positive cells (MC_T) and tryptase-chymase double-positive cells (MC_{TC}). Whereas normal synovium mainly contains MC_{TC} cells, early inflammation in rheumatoid arthritis is associated with a selective expansion of MC_T, followed by increases of MC_{TC} in established or chronic disease (Gotis-Graham and McNeil, 1997; McNeil and Gotis-Graham, 2000; Olsson et al., 2001). These changes are often correlated with clinical characteristics; MC_T numbers in early disease associate with inflammation, whereas the MC_{TC} numbers in chronic disease associate with tissue remodeling features, which may underlie active involvement of both subsets in different pathological processes.

Mast cell mediators in synovial tissue or fluid

Mast cells produce a range of mediators, through three major pathways of secretion. First of all, they are characterized by presence of intracellular granules, containing preformed mediators such as histamine, proteases, proteoglycans, and heparin, which are rapidly released upon degranulation. Certain activation pathways can induce the release of lipid-derived mediators, produced from arachidonic acid, such as leukotrienes and prostaglandins. Finally, mast cell activation induces gene transcription, leading to de novo synthesis of cytokines, chemokines and growth factors, which can be released within several hours of activation.

Although most of de novo-produced cytokines are not mast cell-specific, several preformed granule proteins are more or less specifically expressed by mast cells, including the mast cell specific proteases tryptase and chymase. Both histamine and tryptase are elevated in synovial fluid of rheumatoid arthritis patients likely reflecting local mast cell activation (Buckley et al., 1997; Frewin et al., 1986; Lavery and Lisse, 1994; Malone et al., 1986). Furthermore, mast cells have been reported to be the main IL-17-positive cells in the inflammatory joint of rheumatoid arthritis and spondyloarthropathy patients (Hueber et al., 2010). As discussed below, several of these mediators can contribute significantly to inflammation in the joint.

Mast cell activation pathways in rheumatoid arthritis

Mast cells are most well-known because of their role in IgE-mediated immune responses as they express the high affinity FcεRI, and therefore have originally mainly been considered for their role in allergic diseases. However, the importance of mast cells in IgE-independent responses has been appreciated in the last decades, and has led to increased understanding of mast cell function in a variety of immune responses, including autoimmune disease.

Mast cell activation by autoantibodies

Depending on their specific isotype, antibodies can exert immune activation by binding to cellular Fc receptors and activation of complement. Because various isotypes of ACPA (IgG, IgA, IgM) have been previously demonstrated, ACPA are, in principle, able to activate the immune system via both pathways (Verpoort et al., 2006).

The potential of ACPA to activate complement has been shown in vitro. ACPA bound to immobilized antigen activated the complement system, via both the classical and alternative pathways (Trouw et al., 2009). These pathways can activate mast cells, for examples through the cleavage product C5a. It has been shown in mice that C5aR activation of synovial mast cells is essential for the induction of arthritis (Nigrovic et al., 2010). However, in humans, it is not clear whether this pathway contributes to autoantibody-mediated mast cell activation.

Besides indirect activation of immune cells via complement activation, autoantibodies can also directly activate cells upon crosslinking of Fc receptors, in particular Fcγ receptors (binding IgG), Fcε receptors (IgE), and Fcα receptors (IgA). As ACPA are mainly present as IgM and IgG isotypes, the binding of IgG-ACPA to Fcγ receptors is thought to play a major role in autoantibody-mediated pathogenesis.

Activating Fc receptors are predominantly expressed by myeloid immune cells, including mast cells. In mice, certain mast cell subsets, including synovial mast cells, express the activating FcγRIIIa, (Benhamou et al., 1990; Fang et al., 2013; Latour et al., 1992) the receptor involved in arthritis induced by anti-collagen autoantibodies (Díaz de Ståhl et al., 2002). Human mast cells have been shown to express FcγRIIA, whereas there is some controversy regarding expression of FcγRI (Jonsson et al., 2012; Lee et al., 2013; Suurmond et al., 2014a). We have recently shown that human cultured mast cells could be activated by ACPA immune complexes in a citrulline-dependent manner (Suurmond et al., 2014a). This activation was mediated through crosslinking FcγRIIA. As this receptor was expressed by synovial mast cells from all patients analysed, we propose that this receptor is a major player in autoantibody-mediated mast cell activation.

Mast cell activation by Toll like receptor ligands

Toll Like receptors (TLRs) are expressed by a variety of immune cells, and are considered to act as sentinels of the immune system. As mast cells are thought to play an important

role in protection against pathogens, their expression of TLRs has been studied in different cell subsets and species. Although some variation is present in expression of these receptors, mast cells generally express a wide variety of TLRs, and triggering of TLR by pathogen associated molecular patterns induces activation of mast cells (Kulka et al., 2004; Matsushima et al., 2004; McCurdy et al., 2003; Varadaradjalou et al., 2003).
Importantly, mast cells also express those TLRs that are thought to mediate responses to endogenous ligands released in inflammatory conditions. The main receptors involved in such responses are TLR2, TLR4 and endosomal TLRs which sense nucleic acids (Midwood et al., 2009; Piccinini and Midwood, 2010).
We have recently shown that human mast cells indeed respond to HSP70, an endogenous ligand for TLR4, which is present in rheumatoid arthritis synovium (Suurmond et al., 2014a). Another endogenous TLR ligand, the extra domain A of fibronectin, can induce joint inflammation in mice in a mast cell- and TLR4-dependent manner, (Gondokaryono et al., 2007) suggesting that this pathway of mast cell activation can contribute to pathogenic responses in RA.

Mast cell activation by cytokines
As described above, several cytokines or growth factors are involved in survival and expansion of mast cells in synovium. In addition, cytokines can activate mast cells directly. Such cytokines include IL-3, IL-4, IL-5, and IL-33, each of which are increased in synovial tissue or fluid of rheumatoid arthritis patients. However, stimulation of mast cells with cytokines alone usually mediates mainly proliferation with only a low level of activation. Importantly, the cytokine environment can play an important role in priming of mast cell responses to other triggers (Junttila et al., 2013). IL-33 has been shown to enhance arthritis in a mast cell-dependent manner, (Xu et al., 2008) suggesting that activation or priming of mast cells by cytokines can significantly alter inflammatory responses in the joint.

Mast cell - T cell interactions
The interaction between mast cells and T helper cells has been explored in recent years. In both human and mouse, mast cells have been shown to present antigens to $CD4^+$ T cells, thereby enhancing T cell responses with the possibility of skewing specific T helper subsets as well (Gaudenzio et al., 2013; Kambayashi et al., 2009; Suurmond et al., 2013). Besides antigen presentation, mast cell-derived cytokines can also induce T cell activation (Nakae et al., 2005). Although we have recently shown that the interaction between T helper cells and mast cells does not only activate T cells, but can also change mast cell phenotype, the exact influence of T cells on mast cell function has been studied sparsely (Baram et al., 2001). Whereas regulatory T cells can inhibit mast cell activation, the effect of T cells involved in rheumatoid arthritis, such as T_h17 cells, is not known (Gri et al., 2008; Kashyap et al., 2008). However, these cell types are likely to interact and it is tempting to speculate that such an interaction contributes to pathogenesis of rheumatoid arthritis. A

recent study indeed suggested that mast cells can regulate T cell responses in an arthritis mouse model, by inducing $CD4^+$ T cell expansion and T_h1 and T_h17 cytokine secretion (Schubert et al., 2014).

Chronic inflammation mediated by a complex interplay of multiple pathways

As rheumatoid arthritis is characterized by the activation of multiple immune pathways, these pathways are likely to interact. For example, it has been shown for different types of myeloid cells that activation through TLRs synergizes with triggering of Fc receptors (Suurmond et al., 2014a; Suurmond et al., 2014b; Vogelpoel et al., 2014). As mast cells can be activated by different cytokines, several studies have investigated the interaction between cytokine- and FcεRI-mediated activation. These studies have shown increased degranulation and cytokine production when mast cells are exposed to combined triggers of e.g. IL-3, IL-4 and IL-33 with FcεRI crosslinking (Gebhardt et al., 2002; Lorentz et al., 2005; Ochi et al., 2000; Rivellese et al., 2014). Whereas these studies are important for understanding of the role of cytokines in allergic responses, Fcγ receptors, as compared to FcεRI, are probably more important for mast cell activation in rheumatoid arthritis.

In this context, IL-33 was shown to enhance immune complex mediated mast cell responses through Fc γ receptors (Kashiwakura et al., 2013). In addition, we have studied the interaction of Toll Like receptor triggering on Fcγ receptor mediated mast cell activation, and shown that this greatly enhanced cytokine production by human mast cells (Suurmond et al., 2014a). Importantly, we also showed this interaction was present in an antigen-specific system using ACPA autoantibodies and endogenous TLR ligands present in synovium.

Such a synergy between TLR or cytokines and Fc receptor responsiveness likely represents a physiological function of the immune system to mount an enhanced response when antibodies are produced after the first encounter of a pathogen (Abraham and St John, 2010). Whereas this is conceivably highly beneficial when a pathogen needs to be eliminated, such responses in an autoimmune setting can drive chronic inflammation, because it can lead to further release of modified self-antigens and TLR ligands (Fig. 1). Therefore, synergy in mast cell responses may contribute to chronicity of rheumatoid arthritis.

Mast cell effector functions in rheumatoid arthritis

Mast cells are well-known for their potent and quick effector functions, such as present during allergic reactions. However, as tissue-resident cells, their physiological role is thought to be protection against pathogens, as well as to contribute to wound healing (Abraham and Malaviya, 1997). Therefore, it is not surprising that they also contribute to these processes during autoimmune responses.

Mast cell-mediated tissue inflammation

During certain bacterial infections, mast cells can orchestrate a local inflammatory response by rapidly increasing vascular permeability and releasing chemokines. Thereby they contribute to the recruitment of neutrophils and other immune cells, ultimately resulting in amplification of the local inflammatory response (Malaviya et al., 1996). Rheumatoid arthritis is also characterized by accumulation of immune cells. Whereas the synovial lining mainly contains monocytes/macrophages and T cells, synovial fluid is the site to which neutrophils are recruited. In humans, it has been shown that neutrophil chemoattraction to the synovial fluid is mainly mediated by IL-8, a cytokine produced (although not exclusively) by mast cells in response to ACPA autoantibodies and TLR ligands (Chen et al., 2001; Koch et al., 1991; Suurmond et al., 2014a). In mice, mast cell-derived TNFα and leukotriene B4 can both mediate neutrophil recruitment as well (Biedermann et al., 2000; Nigrovic et al., 2007; Zhang et al., 1995; Zhang et al., 1992). In addition, histamine can increase vascular permeability, thereby augmenting neutrophil recruitment (Fig. 2A) (Binstadt et al., 2006).

These and other mast cell-derived chemokines can also induce recruitment of T cells and monocytes, although evidence indicating that this also occurs in the context of autoimmunity is scarce. Growth factors for neutrophils and macrophages, such as GM-CSF and G-CSF are also produced by mast cells, suggesting that besides inducing cellular infiltration, mast cells may also contribute to survival of these cell subsets.

Crosstalk between synovial fibroblasts and mast cells

An important consequence of the chronic tissue inflammation present in rheumatoid arthritis is activation of synovial fibroblasts, also called fibroblast-like synoviocytes, the main stromal cell type of the synovium. Activation of synoviocytes in rheumatoid arthritis leads to their proliferation and reduced apoptosis, secretion of cytokines and chemokines and invasiveness, whereby synoviocytes invade the underlying cartilage/collagen tissue (Lafyatis et al., 1989).

Synovial fibroblasts can be activated by multiple pathways, including TLR activation, and cytokines (Pierer et al., 2004). Cytokines implicated in this process are TNFα, IL-1, and IL-17 (Granet et al., 2004; Hot et al., 2012). Mast cells can produce each of these cytokines, thereby potentially contributing to activation of synovial fibroblasts (Fig. 2B). In addition, other mast cell mediators, such as histamine and tryptase have been shown to induce activation and inhibition of apoptosis in synovial fibroblasts cells as well (Sawamukai et al., 2010; Zenmyo et al., 1995).

Likewise, interaction between synovial fibroblasts can also lead to bi-directional crosstalk, whereby fibroblasts recruit and activate mast cells, for example through stem cell factor and IL-33 (Xu et al., 2008).

Tissue remodeling sustained by mast cells

Tissue inflammation and activation of fibroblasts goes hand-in-hand with various tissue remodeling processes, characterized by angiogenesis, breakdown of cartilage and bone erosion.

Angiogenesis occurs mainly in the synovial lining of the joint, where rapid-growing fibroblasts and infiltrating immune cells require increased amounts of nutrients and oxygen supplied through the blood. Angiogenesis is mediated by growth factors such as VEGF and FGF, and angiogenic cytokines such as IL-8, TNFα and GM-CSF, but can also be mediated by mast cell granule-derived mediators such as heparin, tryptase and chymase (Fig. 2C) (Azizkhan et al., 1980; Blair et al., 1997; Muramatsu et al., 2000; Paleolog, 2002). Mast cells are often found in close proximity to blood vessels, and their numbers are often associated with angiogenesis, especially in the context of tumors and wound healing (Ribatti, 2013; Wulff and Wilgus, 2013). Although no functional data are available on the direct role of mast cells in synovial angiogenesis, their secretion profile suggests that they may contribute this process.

The two main destructive processes in rheumatoid arthritis are cartilage breakdown and bone erosion. Synovial fibroblasts, next to chondrocytes have been implicated in cartilage breakdown. Both cell types secrete matrix degrading enzymes such as matrix metalloproteinases (MMPs) (Tolboom et al., 2002). These enzymes can break down extracellular matrix proteins such as collagen, aggrecan and fibrinogen. An important feature of MMPs is their secretion as inactive pro-enzymes which need to be cleaved by other MMPs or other proteinases to become activated (Van Wart and Birkedal-Hansen, 1990). As this cleavage occurs in the extracellular space, the proteases required for cleavage can be derived from different cellular origins. In this respect, mast cell tryptase could play a prominent role as it is known for its ability to activate MMPs (Gruber et al., 1988; Magarinos et al., 2013). In doing so, mast cells can contribute to loss of cartilage through activation of MMPs via secretion of tryptase (Fig. 2C).

Osteoclast activation is the main mechanism leading to bone erosions. Although mast cells are not known to release RANKL, a major factor involved in osteoclast activation, mast cells may contribute to setting the balance in bone homeostasis. For example, patients with mastocytosis (systemic mast cell hyperplasia) exhibit features of accelerated bone turnover, possibly through a direct effect of histamine on osteoclasts (Nakamura et al., 1996; Seitz et al., 2013).

In summary, mast cells can secrete a variety of mediators which are implicated in many of the basic pathogenic hallmarks of rheumatoid arthritis.

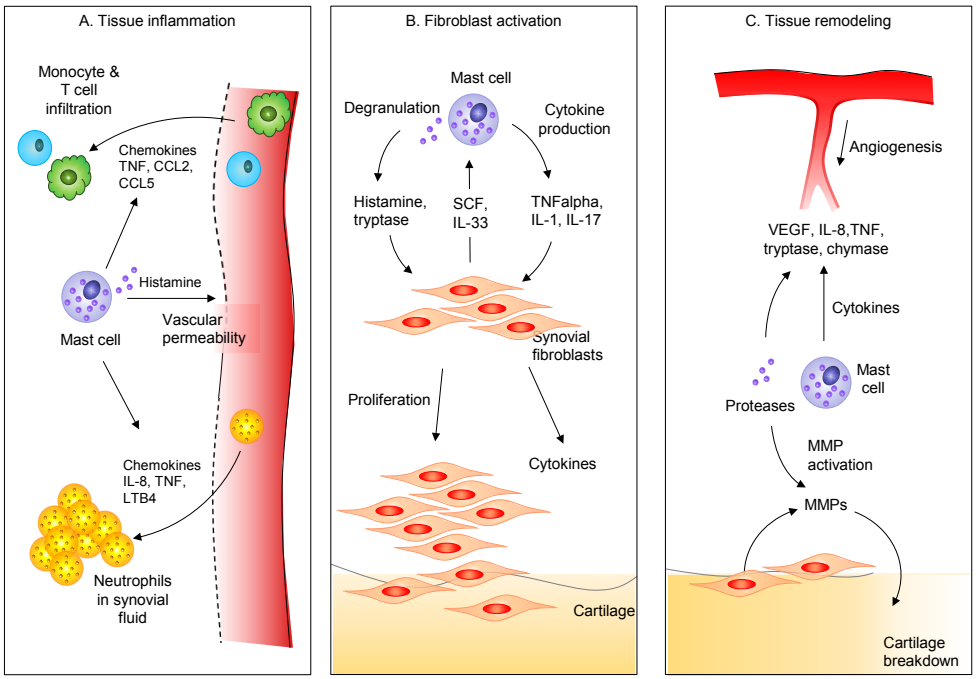

Figure 2. Mast cell contribution to pathogenic processes in rheumatoid arthritis.
(A) Activated mast cells can amplify tissue inflammation through several mechanisms. They increase vascular permeability through release of histamine, leading to increased recruitment of immune cells. In particular, neutrophils are recruited into synovial fluid by chemokines such as IL-8, TNFα, and leukotrienes, whereas monocytes and T cells are recruited to the synovial tissue through chemokines such as TNFα, CCL2, and CCL5.
(B) Mast cells in synovium have a bidirectional interaction with fibroblasts, whereby fibroblasts can activate mast cells through growth factors and cytokines (SCF, IL-33), and activated mast cells in turn can activate synovial fibroblasts. Mast cell degranulation can induce proliferation of fibroblasts by histamine and tryptase, and cytokine production by mast cells (TNFα, IL-1, IL-17) can led to activation of synovial fibroblasts. Together, this crosstalk can induce fibroblasts invading into the underlying cartilage tissue.
(C) Mast cell-derived cytokines and proteases can contribute to increased angiogenesis,a process required for the increased metabolic demand in inflamed tissue. Furthermore, various mast cell proteases can lead to extracellular MMP cleavage, leading to their activation, a crucial process in the breakdown of cartilage.

Mouse models for arthritis and mast cell involvement

Arthritis mouse models

Insight in the contribution of mast cells to pathogenesis of rheumatoid arthritis has also been obtained using models of experimental arthritis.

The first study to show an important role for mast cells in arthritis was performed in mice deficient in kit signaling, $Kit^{W}Kit^{W\text{-}v}$ mice. In this study, experimental arthritis, induced by K/BxN serum transfer, was completely abolished in the absence of mast cells. Transfer of cultured bone marrow derived wild-type mast cells to mast cell deficient mice restored the incidence of arthritis after K/BxN serum transfer, indicating a direct effector function of mast cells in the development of arthritis (Lee et al., 2002). The critical role of mast cells for development of arthritis in this model has boosted the recognition of mast cells as a non-redundant cell in the development of autoimmune disease.

However, the findings from this study have been recently challenged in different models (Table 1). First of all, $Kit^{W\text{-}Sh}/Kit^{W\text{-}Sh}$ mice, another mast cell deficient mouse due to defect kit signaling, were able to develop arthritis after passive transfer of anti-collagen type II antibodies (Zhou et al., 2007). In addition, $Kit^{W}Kit^{W\text{-}v}$ mice had normal arthritis development in the collagen induced arthritis model (Pitman et al., 2011). Unlike neutropenic $Kit^{W}Kit^{W\text{-}v}$ mice, $Kit^{W-sh}/^{W-sh}$ mice have a baseline pro-inflammatory phenotype, including neutrophilia (Michel et al., 2013; Nigrovic et al., 2008). Therefore, these confounding results have sometimes been attributed to the neutrophilia in $Kit^{W-sh}/^{W-sh}$ mice, which renders them insensitive to mast cell-mediated neutrophil recruitment, a critical event in early arthritis development (Brown and Hatfield, 2012).

Of the mast cell deficient mouse models independent of kit, two models have been used to study arthritis. In one study, the $Cpa3^{Cre/+}$ mice, which are mast cell deficient, were fully susceptible to the induction of serum-induced arthritis and clinical scores, histology and gene expression analysis were comparable to wild-type mice (Feyerabend et al., 2011). Therefore, it was concluded that the role of mast cells in arthritis is limited. Whereas mast cell deficiency using Mcpt5-Cre iDTR mice did not affect serum-induced arthritis either, these mice experienced reduced arthritis upon immunization with collagen, (Schubert et al., 2014) suggesting that further research is needed to increase our understanding of these discrepancies.

Despite these contradictory findings using mice with a complete mast cell deficiency, additional evidence for mast cell-mediated pathogenesis in arthritis comes from studies using mice deficient in mast cell-specific proteases, such as chymase or tryptase. Mice deficient in mMCP4, the homologue of human chymase, develop less severe arthritis upon collagen induced arthritis (Magnusson et al., 2009). Mice which are deficient in either tryptase mMCP6 and/or -7, especially in combination with heparin-deficiency, display a reduced severity of adjuvant-induced arthritis and K/BxN induced arthritis (for mMCP6 deficiency) (McNeil et al., 2008; Shin et al., 2009). In addition, mast cell-specific

(Mcpt5-Cre-mediated) deficiency in A20, a regulatory molecule, leads to increased mast cell activation, thereby exacerbating collagen induced arthritis (Heger et al., 2014).
As most of these mouse models contain a single deficiency in a mast cell-specific mediator, and are therefore not associated with any other defects such as the kit mutant mice, these studies provide compelling evidence for mast cell involvement in arthritis, despite the contrasting data obtained with mast cell deficient mouse models. Therefore, more research is needed to increase our understanding of the role of mast cells in rheumatoid arthritis.

Pharmacological inhibition of mast cells

As several lines of evidence suggest a role for mast cells in rheumatoid arthritis, intervention with mast cell activation could potentially form novel therapies. The drug cromolyn is clinically used as a treatment for asthma patients. The exact mechanism of cromolyn in not completely understood, but it is described to prevent the release of mast cell specific mediators like histamine from rat peritoneal cells (Cox, 1967). Cromolyn is described as a mast cell stabilizing agent and is used frequently in mouse studies. The effect of cromolyn as a prolactive on CIA was investigated in DBA/1 mice. A lower clinical score and radiographic score were observed compared to non-treated mice, when cromolyn was administered when first symptoms of clinical arthritis became evident (Kobayashi et al., 1999). In addition, it was shown that intra-articular treatment of cromolyn or salbutamol prevented angiogenesis, pannus formation and joint destruction in mice.(Kneilling et al., 2007) Recently however, the specificity of cromolyn and the sensitivity of different types of mast cells to cromolyn in mice is under debate (Oka et al., 2012). Also, the specificity of salbutamol can be questioned since it has also inhibits the secretion of pro-inflammatory cytokines by macrophages and T cells (Kneilling et al., 2007). Therefore, development of mast cell-specific therapeutics is needed to establish the exact role of mast cells in rheumatoid arthritis.

Table 1. Overview of experimental arthritis in mast cell-deficient or mast cell protease-deficient mice

Mouse strain	Deficiency	Arthritis model	Outcome	References
Mast cell deficiency				
$Kit^{W}Kit^{W-v}$ (W/Wv)	Mast cell deficient through SCF-receptor mutation	K/BxN	Mast cell deficient mice resistant to develop arthritis. Restored with systemic or local engraftment of BMMCs	Kneilling et al., 2007; Lee et al., 2002
		CIA	No effect of mast cell deficiency	Pitman et al., 2011
Kit^{W-Sh}/Kit^{W-Sh}	Mast cell deficient through defects in SCF	α-collagen type II antibody transfer	No effect of mast cell deficiency	Zhou et al., 2007
Cpa3-Cre (cre-master)	Mast cell deficient through Cre-mediated toxicity	K/BxN	No effect of mast cell deficiency	Feyerabend et al., 2011
Mcpt5-Cre iDTR	Mast cell deficient upon injection of DT (only connective tissue-like MC)	K/BxN	No effect of mast cell deficiency	(Schubert et al., 2014)
		CIA	Reduced arthritis in mast cell deficient mice	
Mast cell protease-deficiency				
Chymase	$mMCP4^{-/-}$	CIA	Reduced arthritis	Magnusson et al., 2009
Tryptase Heparin complexes	$mMCP6^{-/-}$ $mMCP7^{-/-}$	K/BxN	Reduced arthritis	Shin et al., 2009
	$NDST\text{-}2^{-/-}$ & Combinations	mBSA/IL-1β	Reduced arthritis	McNeil et al., 2008
Mast cell-conditional knockout				
A20-deficiency	Mcpt5-Cre A20Fl/Fl	CIA	Exacerbated arthritis	Heger et al., 2014
Pharmacological mast cell inhibition				
Cromolyn	(not mast cell specific)	CIA	Reduced arthritis	Kneilling et al., 2007; Kobayashi et al., 1999

Conclusions

Rheumatoid arthritis is a complex autoimmune disease caused by environmental and genetic interactions leading to a chronic activation of many (immune) cells in the synovial tissue. The pathology of rheumatoid arthritis involves multiple activation pathways and interactions between a variety of cell types with arthritogenic functions leading to the progression of joint destruction.

Mast cells can also be found in rheumatoid arthritis tissue, which indicates a possible role for this potent cell in the disease pathology. Many *in vivo* arthritis studies in mice have aimed to clarify the precise role of mast cells. However, since mouse models do not fully reflect the disease process and as some models for mast cell deficiency have additional non-mast cell defects, it is difficult to assess the specific role of mast cells on disease pathogenesis *in vivo*.

Nevertheless, mast cells have the capacity to respond to a wide range of activating ligands in synovium and their effector functions likely reflect their potential role in pathogenesis of rheumatoid arthritis.

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