



# OSTEOARTHRITIS



# Synovial mast cells in Osteoarthritis

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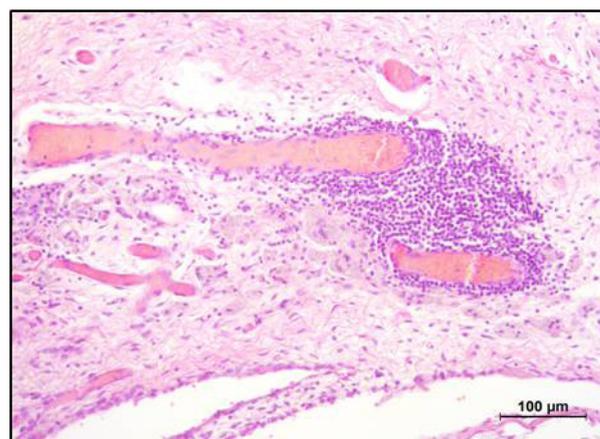
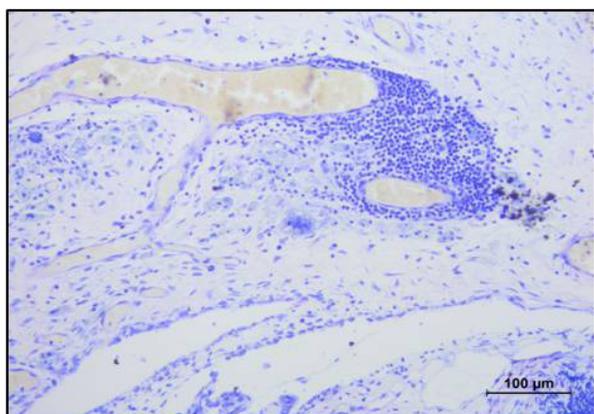
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## Introduction

Osteoarthritis (OA) has traditionally been considered as a non-inflammatory disease, because synovial fluid analysed from OA joints presents fewer leukocytes compared with that of rheumatoid arthritis (RA), reactive arthritis and even septic arthritis [1]. However, over the last decades, the gap between inflammatory and degenerative arthritis is becoming less defined. It has been well established that the traditional view of OA as only cartilage disease is obsolete. OA should be considered a whole joint disease, where the inflammation of synovial membrane plays a key role in the pathogenesis and clinic of the disease [2]. Clinical symptoms as palpable joint swelling, night pain and morning stiffness are strong indicators of synovitis. In addition, several researches through ultrasonography and Magnetic Resonance Imaging (MRI) have demonstrated the presence of inflammation also in joints where synovitis was not clinically detectable (**Figure 1**) [3].



**Figure 1:** Sample of synovial membrane obtained from knee OA. On the left, specimen was stained with Blue Toluidine (20X), On the right with hematoxylin eosin (EE) (20X). it is worth of note the perivascular inflammatory infiltrate and macrophages.

Most frequent types of immune cells found in OA are macrophages, T lymphocytes and mast cells (MCs). Current researches pointed out that the number of infiltrating immune cells as well as the expression of cytokines were higher in RA than in OA. However, the detection of enriched MCs in OA compared to RA indicates that inflammation in OA may be quantitatively less but qualitatively different from RA [4].

Moreover, MCs differ from other immune cells in the ability to be stained by basic dyes following formalin fixation [5]. These deficiencies have certainly ensured that the study of MCs in joint diseases has been neglected for long time. Therefore,



their role in synovial inflammation and in the pathophysiology of OA remains poorly understood.

Here we present an overview of the current knowledge on MCs involvement in OA, including the intriguing hypothesis of MCs acting as subtle immunomodulatory cells and the emerging concept of synovial MCs as initiator of synovitis.

### Mast cells

Mast cell is a type of granulocyte derived from the myeloid stem cell. Although best known for their role in allergy and anaphylaxis, MCs are characterized by pleiotropic functions that relies on their capacity to secrete a plethora of soluble mediators. MCs have the unique ability to release pre-formed substances stored in intracellular granules, as well as the ability to synthesize several mediators “de novo” [6]. It has been shown that the regulated release of peptides, amines, lipids, and even some gases relies on through several molecular pathways that include constitutive and regulated exocytosis (degranulation), diffusion and membrane transporters [7].

MCs have the ability to release various mediators from different compartments, following different stimuli (i.e. IgE mediated or not IgE mediated) and through different pathways. Whereas, constitutive exocytosis relies on secretory vesicles, two types of degranulation have been described for MCs: piecemeal and anaphylactic degranulation [8]. The former is characterized by a selective release of portions of the granule contents, without granule-to-granule and/or granule-to-plasma membrane fusions. This way of degranulation has been observed in numerous settings, ranging from chronic psychosocial stress [9] to TLR stimulation [10]. The latter is the explosive release of granule contents or entire granules to the outside of cells after granule-to-granule and/or granule-to-plasma membrane fusions. In contrast, this type of degranulation has been detected in allergic phenomena following FcεRI-crosslinking [11]. Little is known about the molecular machinery involved in these processes.

MCs are morphologically characterized by numerous, electron dense cytoplasmic granules which contain biogenic amines, several serine and other proteases, lysosomal enzymes, cytokines, and proteoglycans [12]. MCs are widely distributed at sites of contact with the outside world (i.e. mucosal and epithelial tissues). Echtenacher et al. [13] and Malaviya et al. [14] reported their presence even in the peritoneum that represents the last structure delimiting the peritoneal cavity. Authors proposed that MCs possess a primary role in the defense against septic peritonitis in the mouse. Furthermore, studies reported their presence even in human synovium [15]. Even though, over the last decades our understanding about the immuno-pathological role of MCs has been enriched, several interesting questions remain, however, to be addressed.

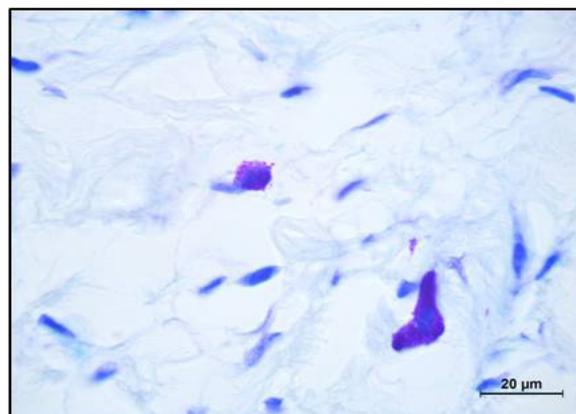
Several studies proposed that the net contribution of MCs can be either pro and anti-inflammatory, depending on context [16]. Surely a role pro-inflammatory of MCs is well recognized in allergic phenomena, where the inflammatory consequences of MCs activation can be life-threatening as in asthma or anaphylaxis [17]. However, it is clear that most of contradictory opinions arise from MCs heterogeneity. This heterogeneity may occur as differences in histochemical, biochemical, and functional characteristics [6]. In this context, it has been shown that MCs from mouse, can be distinguished from the T cell-independent connective tissue-type MCs and the T cell-dependent mucosal type MCs. The former is typically present in connective tissue

and located predominantly around nerves and vessels. In addition, their maturation does not depend from T-cell. The latter is predominant within epithelium and lamina propria and they remain as committed progenitors until acted on by T cell–derived cytokines [18].

In humans, the strict classification into mucosal and connective tissue-type MCs is not possible and the characterization of MCs proteases is the main criterion to differentiate MCs subtypes [19]. Their granules could contain both tryptase and chymase (MCTC), or staining for tryptase (MCT) or chymase alone (MCC). Expression of chymases and tryptases varies between tissues and in addition, modifications occurs in physiological and pathological states [16]. The MCTC subset is typically present within connective tissue such as skin, bowel submucosa, and breast parenchyma, located in proximity to vessels and nerves; the MCT subset, predominates at mucosal and epithelial surfaces [20]. For these evidences, it reasonable to postulate that MCTC and MT subsets of human, correspond respectively to connective- and mucosal-type MCs of mouse. Indeed, it has been shown that patients with acquired immunodeficiency syndrome or combined immunodeficiency diseases are characterized by deficit of mucosal type MCs probably due to T-cell deficiency [21]. On the other hand, inflammatory conditions seem to be correlated with a higher number of MCT cells into mucosa [22–24]. Furthermore, it is worth of note that MCs heterogeneity is not limited to proteases of secretory granules or T-cell dependency, but MCs subtypes reflects even functional heterogeneity. Indeed, studies demonstrated that MCTC and MCT differ in the ability of generate and release a wide range pattern of cytokines. [25].

### Mast cells in normal human synovium

The presence of MCs in normal human joint has been recognized for a long time [26]. Albeit, they are not observed within cartilage or periarticular bone, it has been well established their presence in normal synovium [27]. MCs are predominately located in the sub lining, residing within 70 mm of the synovial surface, clustered around vessels and nerves and forming up to 3% of all cells within the synovium [15]. These cells exhibit a typical mast cell morphology and range in diameter from 10 to 60 μm [28]. They can be recognized by its content of metachromatic granules when appropriately fixed and stained with metachromatic dyes such as toluidine blue (**Figure 2**), or in addition, they can be detected by immunohistochemistry analysis throughstaining with polyclonal rabbit anti-human CD117 antibody (**Figure 3**).



**Figure 2:** Isolated MCs presents in the sub lining of knee synovium, stained with Toluidine Blue. Original magnification: 100x



**Figure 3:** Isolated MCs presents in the sublining of knee synovium, stained with anti-CD117. Original magnification: 40x

The role of MCs in the normal synovium still remains unclear, but current researches suggest that they act as sentinels, monitoring the vulnerable acellular joint cavity regarding early evidence of infection [29]. Furthermore, several authors attributed to synovial MCs a key role in the synthesis of hyaluronic acid (HA) [30]. According to this theory, the granules of synovial MCs contain heparin that could represent the preliminary stage in the synthesis of HA [30].

As mentioned above, MCs are phenotypically categorized on the basis of proteases in their cytoplasmic granules and to date, there have been relatively few attempts to characterize MCs populations in normal synovial tissue. Buckley MG et al. performed a detailed investigation of MCs heterogeneity in synovial tissue obtained from subjects without joint diseases. The authors observed that specimens from healthy knee are characterized by the presence of both MCTC and MCT subsets, located respectively around vessels and nerves and in proximity to the superficial lining [31].

### Mast cells in inflammatory arthritis

Nowadays, the primary role of synovial MCs in inflammatory arthritis is well documented. Expansion of the MCs population in synovium has been observed in a wide range of inflammatory conditions (i.e. gout [32], rheumatic fever [33] and psoriatic arthritis [34]). However, most of current researches point out the role of MCs in RA partially due to its notably prevalence in terms of joint pathology [28,29,35,36]. Rheumatoid joints exhibit an important increase of MCs compared with normal synovium [12]. It is important to underline that large variation of MCs is documented in literature within RA synovium. Variability, that seems to be related to degree of inflammation, and not to age, gender or duration of the disease. It was noted that higher synovial MCs counts tend to occur with more active clinical and histologic synovitis [28].

Regarding MCs subtypes, Immunostaining analysis have shown that both MCT and MC<sub>TC</sub> subsets expand within RA synovium but with different regional predominance. Studies reported that the MC<sub>T</sub> subset is mostly located near the leukocytic infiltrate and superficial hypertrophic synovium, whereas MC<sub>TC</sub> prefer the deeper and more fibrotic regions. In addition, in early active synovitis, MCT predominate over MC<sub>TC</sub> [35].

One possible explanation about this regional distribution should be sought in the functional heterogeneity in MCs subsets. It has been demonstrated in the lung that MC<sub>T</sub> subset has

the ability to elaborate potentially proinflammatory mediators such as IL-5 and IL-6 [25], whereas the MC<sub>TC</sub> subset is involved in the stromal response through production of fibrotic mediators as IL-4 [37]. Although, these functional abilities have not been documented in the synovium, it is reasonable to propose to these subsets, two distinct pathophysiological roles in inflammatory arthritis. MC<sub>T</sub> might be involved in acute episodes of pain and inflammation, known as “flare”, whereas, MCTC might have a primary role in chronic stage of disease.

To the best of our knowledge, a growing body of evidences supports the role of MCs in inflammatory arthritis. At the same time, their role has been extensively debated in the past years, because of controversial results obtained in different animal models [38].

Lee et al. reported a reduction in the severity of K/BxN serum-induced arthritis in animals deficient in MCs due to spontaneous mutations affecting c-KIT (Kit<sup>W/W-v</sup>), indicating that MCs are essential for the development of arthritis [39]. On the other hand, several authors observed that the severity of K/BxN serum-induced arthritis in Kit<sup>W-sh/W-sh</sup> was not influenced by MCs deficiency, generating conflicting results [40,41]. Upon further investigations, it has been demonstrated that mutations affecting c-KIT (Kit<sup>W/W-v</sup>) were correlated to MCs and neutrophil deficiency, whereas mice Kit<sup>W-sh/W-sh</sup> were found to be neutrophilic. For these evidences, it seems that MCs could play a key role in the development of arthritis, but their deficiency might be compensated by neutrophils.

In recent years, Schubert et al. [42] studied two models of arthritis namely, K/BxN serum-induced arthritis and collagen-induced arthritis in mice characterized by normal immune system despite the absence of MCs. The authors observed that MCs were redundant in the first model, in which synovitis is induced passively by the injection of an arthritogenic serum, meanwhile the reduction of the severity of arthritis in the second one proposed the key role of MCs in the initiation of synovitis where arthritis depends from active immunization.

Van der Velden and colleagues [43] corroborated this hypothesis. They reported that depletion of MCs in established arthritis did not affect clinical outcome. However, depletion during the preclinical phase resulted in a significant reduction in arthritis. Even though it is mandatory to look for confirmations in humans, these data suggest that MCs play a role in the regulation of the adaptive immune response during the development of arthritis.

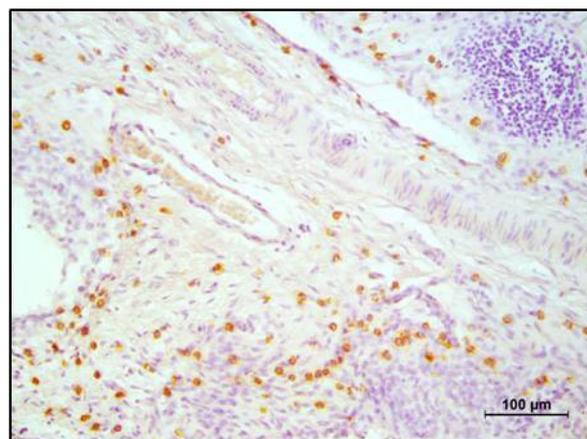
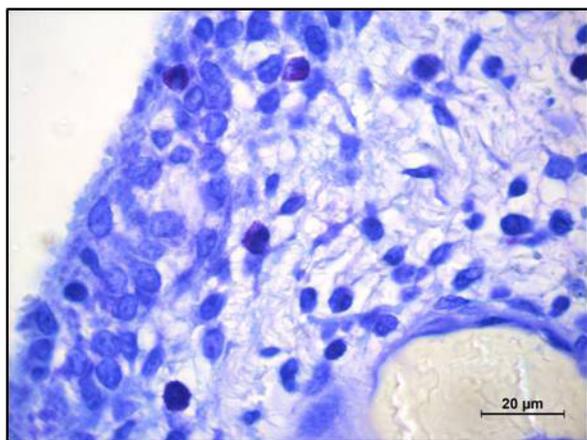
### Mast cells in OA: player or viewer?

While the relationship between MCs and inflammatory arthritis is known, only in the last recent years, several group of research focused on the synovial MCs in OA. Surely the lack of animal models of OA and the old assumption of its non-inflammatory nature have ensured that the current knowledge about this topic is poor and weak.

The presence of greater numbers of MCs in OA synovium compared to control ones has been demonstrated for a long time (Figure 4) [31,44,45], but only recently it has been proposed their active role regarding clinic and pathogenesis of the disease. In addition, the detection in OA synovial fluid of MCs mediators as histamine and tryptase has provided compelling evidence that the MCs may be in an activated state [12].

Regarding MCs subtypes, Buckley and colleagues observed

the numbers of MC<sub>Tc</sub> differed little between OA and control tissue, whereas the increase number of MCs was largely due to a selective expansion of MC<sub>T</sub>, predominately located in the sub lining [31]. Only limited data are available to explain the increase of MCs within OA synovium. Several theories have been proposed. MCs could be recruited from circulating progenitors or stimulated to proliferate in presence of synovitis. On the other hand, they could result from maturation of local MCs precursors. Indeed, a recent study reported that osteoarthritic synovium contained a great number of mesenchymal stem cells (MSCs) that might differentiate into MCs in OA environment. MSCs markers (CD90, CD271) were detected in the intimal and subintimal tissue layers where MCs have been observed [46].



**Figure 4:** Sample of synovial membrane obtained from knee OA. On the left, specimen was stained with anti CD117 (20x), MCs are predominantly located beneath the superficial lining. On the right, MCs with metacromasia (100x).

The role of MCs in OA is still debated, mainly because of controversial results obtained from in vitro studies. Indeed, it has been reported that MCs obtained from osteoarthritic tissue, may be activated by several secretagogues as IgE, calcium ionophores, compound 48/80, substance P as well as stem cell factor (SCF) [47,48]. In contrast to MCs isolated from rheumatoid arthritis, they do not exhibit response to anaphylatoxin C5a for absence of its receptor on the membrane [49].

To date, recent studies suggest that the presence of MCs in OA might play a key role in pain and disability reported by patient [50,51]. In line with this hypothesis, our group recently investigated the effect of anti Ig E therapy in atopic patients affected by knee OA. The authors found an improvement of OA symptoms during follow up period and they hypothesized that such improvement was due to the partial blockage of MCs activation via IgE related [52].

A possible explanation, should be sought in the release of neurotrophines as nerve growth factor (NGF). To date, it has been well established that NGF is expressed not only by neuronal tissues but it is also produced and released as precursor molecule (proNGF) by cells of the immune system as MCs, and articular cartilage [53].

Various roles for NGF have also been proposed, including a chemoattractant for MCs. In addition, it has recognized its ability to promote development and differentiation of immature MCs [54]. In line with these evidences, NGF may play an important role in MCs accumulation in non-allergic inflammatory conditions as OA. Moreover, it is an important mediator of pain and hyperalgesia [55].

Expression and secretion of NGF can be regulated by several factors. TNF- $\alpha$  and IL-1 $\beta$ , both increased in OA environment, can increase synthesis and secretion of NGF by articular chondrocytes [53]. NGF binds two classes of receptors, the common low affinity p75NTR and the more selective high affinity tropomyosin-related kinase (TrkA) receptor. Results of previous studies showed that both receptors were expressed in articular cartilage and reported to be higher in OA [56]. Their presence warrants further functional investigation of their active involvement in synovitis because data about its role in articular cartilage physio-pathology are scant and conflicting [57].

In addition, growing evidences indicate that blocking NGF signaling using anti NGF agents (i.e. tanezumab) provide effective pain relief, confirming the role of NGF-TrkA axis in pain response [58].

Another line of enquiry proposes that MCs could contribute to cartilage damage and/or osteophyte formation in OA but data were obtained in a small cohort and require replication [50]. Additionally, or alternatively, the increase number of MCs might be seen as an arrangement in order to address the reduction of hyaluronic acid as a consequence of cartilage breakdown in OA setting [30].

Summarizing the current literature, it has well established that osteoarthritis results from a complex interplay of multiple factors, intrinsic and extrinsic of the joint. The initiating mechanism of OA is not fully elucidated, but it is most likely represented by damage to articular cartilage that once begun, activates several pathways leading to cartilage breakdown and synovitis [1]. We could consider OA as a type 4 hypersensitivity disease where the synovial MCs plays a central role. Like RA, even in OA, it has recognized that the innate immune system plays a key role in the pathogenesis of the disease.

In the early phase of the disease, physical forces and over use determine the release of breakdown products from damaged extracellular matrix (i.e. biglycan and fibronectin) in synovial fluid [1,59]. Subsequently, these products acting as damage associated molecular patterns (DAMPs) are capable of inciting local inflammatory responses. Indeed, they are recognized by toll-like cell surface receptors (TLRs), localized on the plasma membrane of immune cells.

It has reported the presence of TLRs (i.e. TLR-4) on peripheral blood-derived and lung MCs [60,61] and for that, we hypothesized that synovial MCs express on their surface these or other receptors able to bind DAMPs and induce the beginning of catabolic responses in the joint. At the same time, the research carried out by Van der Velden [43], gives us good reason to assume that proper synovial MCs might act as sentinel of joint damage.

In the setting of several joint diseases, MCs might be activated in different ways. As mentioned above, MCs have been found in synovium of joint affected by gout or pseudogout [32]. It has been demonstrated that inorganic crystals, including basic calcium phosphate and calcium pyrophosphate dehydrate crystals could act as DAMP. Furthermore, the features of flares of acute gout as rapidity of onset, redness, pain and swelling, suggest that the acute attack is determined by a massive degranulation of synovial MCs induced by crystals.

On the other hand, RA is an auto immune disease characterized by potential immune complexes able to induce the release of breakdown products from cartilage, meanwhile in OA setting, we propose that the subsequent and continue use of joint provide the satisfactory mechanical stress capable of releasing DAMP in synovial fluid.

Once activated, MCs might promote synovitis by recruiting inflammatory cells, inducing synovial fibroblast hyperplasia, enhancing vasopermeability and angiogenesis through the release of inflammatory mediators as histamine, proteases, IL-1, IL-6, TNF- $\alpha$  and VEGF [12].

In conclusion, in this review we presented numerous evidences suggesting that MCs are likely to be involved in the pathogenesis and clinic of OA, where they seem to play a complex and possibly multifaceted role.

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