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Review

Fc-gamma receptors and S100A8/A9 cause bone erosion during rheumatoid arthritis. Do they act as partners in crime?

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Abstract

Bone erosion is one of the central hallmarks of RA and is caused by excessive differentiation and activation of osteoclasts. Presence of autoantibodies in seropositive arthritis is associated with radiographic disease progression. ICs, formed by autoantibodies and their antigens, activate Fcγ-receptor signalling in immune cells, and as such stimulate inflammation-mediated bone erosion. Interestingly, ICs can also directly activate osteoclasts by binding to FcγRs on their surface. Next to autoantibodies, high levels of alarmins, among which is S100A8/A9, are typical for RA and they can further activate the immune system but also directly promote osteoclast function. Therefore, IC-activated FcγRs and S100A8/A9 might act as partners in crime to stimulate inflammation and osteoclasts differentiation and function, thereby stimulating bone erosion. This review discusses the separate roles of ICs, FcγRs and alarmins in bone erosion and sheds new light on the possible interplay between them, which could fuel bone erosion.

Key words: rheumatoid arthritis, bone erosion, osteoclasts, osteoimmunology, immune complexes, autoantibodies, FcγRs, DAMPs, alarmins, S100A8/A9

Rheumatology key messages

- Immune complex-activated Fcγ-receptors indirectly regulate bone erosion by increasing inflammation and directly stimulating osteoclastogenesis.
- The alarmin S100A8/A9 promotes inflammation-induced bone erosion but also directly activates osteoclast resorbing activity.
- Immune complex-activated Fcγ-receptors and S100A8/A9 might synergistically fuel bone erosion.

Bone erosion in RA

RA is a chronic inflammatory disease that primarily affects synovial joints and is a major cause of disability in adults, with a prevalence of 0.5% to 1% in European and North American populations [1, 2]. Clinical manifestations include pain, swelling, redness and limitation of movement of the affected joints. Pathologically, RA is characterized by massive synovial inflammation with influx of cells from both the innate and adaptive immune system, resulting in the erosion of cartilage and bone in the affected joint [1, 3]. This predominantly affects the juxta-articular bone, although generalized osteoporosis is also observed [4]. Under physiological conditions, bone homeostasis is maintained by accurately balanced bone formation by osteoblasts and resorption by osteoclasts. During RA, however, decreased bone formation by osteoblasts and increased differentiation and activation of osteoclasts shift this balance towards bone resorption [5–7]. Factors that promote osteoclastogenesis are produced by cells from the innate and adaptive immune system as well as by activated fibroblasts present in the inflamed synovium. Together, this leads to excessive osteoclast differentiation and activity [8].Inflammatory cytokines such as IL17, TNFα, IL6 and IL1β produced by innate immune cells and T cells, especially of the Th17 subtype, enhance osteoclastogenesis via an increased production of RANK ligand (RANKL), a crucial inducer of osteoclastogenesis, by synovial fibroblast [8–10]. In addition, pro-inflammatory

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cytokines directly stimulate osteoclastogenesis [11–14]. Moreover, membrane-bound RANKL on infiltrating Th17 and B cells can serve as additional stimulus to promote osteoclastogenesis [15, 16].

Next to these well-known factors that promote osteoclastogenesis, damage-associated molecular patterns (DAMPs) and alarmins, which are actively secreted by activated cells of the innate immune system or passively released upon cell death, are crucial inducers of bone erosion during RA [17–20].

In seropositive arthritis, the presence of autoantibodies strongly associates with radiographic disease progression including bone erosion, suggesting their crucial involvement in the disease pathology [21–25]. IgG autoantibodies together with their cognate antigen form ICs that stimulate immune cells via their interaction with Fc-gamma receptors (FcγRs), resulting in increased production of pro-inflammatory mediators that can stimulate osteoclasts. However, a growing body of evidence supports the view that ICs can additionally directly activate osteoclasts [26–28]. This is particularly interesting because bone damage is observed in seropositive RA patients before clinical disease onset, highlighting a mechanism of osteoclast activation that is independent of joint inflammation [29].

All of the above-mentioned factors interact with each other, therewith fuelling bone erosion in RA. In this review, we will focus our attention on the role of ICs, FcγRs and alarmins in bone erosion during RA and we will shed new light on their possible interplay in stimulating the resorbing activity of osteoclasts. A better understanding of their individual functions and their cross-talk might offer a new therapeutic target, especially for those RA patients that do not respond to currently available treatments.

Osteoclast differentiation and function

Bone erosion is mediated by mature osteoclasts, which are big, multinucleated cells differentiated from myeloid precursors under the influence of RANKL and M-CSF [30–33]. M-CSF promotes the proliferation and survival of the precursors and stimulates their cell surface expression of RANK [34–36]. Subsequently RANK-RANKL-induced signalling leads to activation of nuclear factor of activated T cell (NFATc1), the master regulator of osteoclastogenesis, resulting in the expression of genes involved in osteoclast differentiation and function [37–39]. Next to RANK signalling, a co-stimulatory signal provided by activating immunoreceptor tyrosine–based activation motif (ITAM)-bearing adaptor molecules is required for the calcium signalling that is needed for NFATc1 activation [40–42]. Known ITAM-bearing adaptor molecules involved in osteoclastogenesis are the γ-chain and DNAX activation protein of 12 kDa (DAP-12), which function via interactions with various receptors, among which triggering receptor expressed on myeloid cells 2 (TREM2), paired immunoglobulin-like receptor (PIR-A), osteoclast-associated immunoglobulin-like receptor (OSCAR) and the FcγRs [41].

Fc-gamma receptors

FcγRs are receptors for IgG-containing ICs. Humans have seven activating FcγRs: FcγRIA/IB/IC, FcγRIIA/IIC and FcγRIIIA/B, and one inhibitory: FcγRIIB. Mice have four FcγRs: the activating FcγRI, FcγRIII and FcγRIV, whereas FcγRIIB is inhibitory [43, 44].

FcγRs are differentially expressed on a broad range of myeloid cells, including monocytes, macrophages, neutrophils, dendritic cells (DCs), mast cells and osteoclasts, but also on NK cells and B cells [43, 44].

The binding of ICs to activating FcγRs induces their cross–linking and the consequent phosphorylation of tyrosines in the ITAM-domain of their associated γ-chain, resulting in the recruitment of spleen tyrosine kinase (SYK). SYK, in turn, phosphorylates multiple downstream molecules involved in the activation of transcription factors, cytoskeleton remodelling and the release of intracellular calcium from the endoplasmic reticulum. Through this complex signalling, activating FcγRs stimulate various cellular functions such as phagocytosis, antigen presentation, antibody–dependent cellular cytotoxicity and regulation of cytokine and chemokine production [44, 45].

The signalling of activating FcγRs is counterbalanced by the inhibitory FcγRIIB. Upon co-ligation with an activating FcγR, the tyrosine of the inhibitory immunoreceptor tyrosine–based activation motif (ITIM) domain contained in FcγRIIB intracellular portion is phosphorylated, resulting in the recruitment of the SH2-containing inositol phosphatase (SHIP) protein that mediates the inhibition of ITAM-mediated signalling of the activating FcγRs [46].

The outcome of IC-induced FcγRs signalling is dependent on various factors. Firstly, activating and inhibitory FcγRs are often co-expressed on the same cell. Their ratio, which is affected by pro and anti-inflammatory mediators, determines the activation/inhibition of the cell [47–49]. T cells, although they do not express FcγRs themselves, are central in the production of factors that promote FcγR expression, e.g. IFNγ and IL10, or decrease their expression, e.g. IL4/IL13. Secondly, the various IgG subclasses have different affinities for the various FcγRs. Consequently, when a certain IgG subclass has a higher affinity for the inhibitory FcγRIIb as compared with the activating FcγRs, a higher expression of the activating receptors will be required to reach the threshold for cell activation. Contrarily, the threshold will be lower when the affinity of the IgG is higher for the activating receptor compared with the inhibitory one. Detailed studies on the interactions of murine and human IgGs and FcγRs can be found elsewhere [50, 51]. Lastly, the glycosylation state of the IgGs influences their binding ability to FcγRs and consequently their efficiency in inducing cell activation [52, 53].

More recently, it has been discovered that under specific conditions the normally activating ITAM domain can surprisingly also trigger an inhibitory signalling. A ligand binding with low valency, such as a monomeric ligand, lacks the ability to induce co-aggregation of activatory receptors and thereby only causes a partial phosphorylation of the ITAM domain. This results in the recruitment of

the inhibitory SHIP, rather than SYK [54, 55]. In a second mechanism, an extensive but unsustained co-aggregation of the receptors causes a non-productive signal and the sequestration of critical signalling molecules from other activating receptors [55].

Fc-gamma receptors are strongly involved in the regulation of bone erosion during RA

Interestingly, the presence of autoantibodies in seropositive RA patients strongly associates with bone erosion [21–25]. Among the autoantibodies present in RA patients are rheumatoid factor, and autoantibodies directed against citrullinated, acetylated and carbamylated proteins [56–59]. ICs containing these autoantibodies have been found in the circulation, synovium and synovial fluid of RA patients [60–62]. IgG-containing ICs can lead to bone erosion either via FcγR stimulation on inflammatory cells, which promotes inflammation, or via direct binding to FcγRs on osteoclasts themselves, thereby directly regulating the differentiation and function of these cells.

Fc-gamma receptors indirectly regulate bone erosion via the recruitment and activation of immune cells during RA

Numerous preclinical and clinical studies suggest the crucial involvement of FcγRs in the regulation of inflammation during RA. Although many of these studies did not include the analysis of bone erosion, it is broadly accepted that the severity of inflammation strongly correlates with bone erosion. The observation that the expression of FcγRs is altered on immune cells in the circulation and synovium of RA patients might serve as a first indication for their involvement in inflammation [63–70]. Moreover, next to altered expression level, several single-nucleotide polymorphisms (SNPs) in the genes encoding FcγRs that alter the affinity of FcγRs for IgGs have been described, whereas some of them even influence the susceptibility to RA development and the response to treatment [71–77].

Despite the clear indications that FcγRs are important in inflammation-driven bone erosion during RA, it is challenging to study the differential roles of the individual FcγRs, because of the complexity and redundancy of the IgG-FcγRs signalling. Induction of arthritis models in knockout animals for one or multiple FcγRs has been of great help in this respect, as reviewed elsewhere [78, 79]. These studies show that the deletion of the γ-chain, which results in the lack of membrane expression of all activating FcγRs, ameliorates the disease severity in a multitude of arthritis models [80–85]. In addition, the individual activating FcγRs appear to have a different relative importance and differential function in the activation of immune cells and during the various disease phases of RA [26, 81, 83, 86–94]. In contrast, the inhibitory FcγRIIb inhibits inflammation both via the inhibition of activating FcγR signalling and providing a negative feedback on the autoantibody production by B-cells [80, 82, 86, 93, 95–98].

Nonetheless, although murine and human FcγRs do show many similarities, they do not completely overlap and therefore the findings of murine studies cannot be directly translated to humans. Some studies performed in transgenic mice that express human FcγRs confirmed their crucial involvement in experimental arthritis [99, 100].

Together, these data suggest a clear involvement of FcγRs in the regulation of inflammation in RA, which indirectly results in the promotion of bone erosion. In addition, however, a direct regulation of osteoclast differentiation and activity by the binding of ICs to the FcγRs on their surface has been described.

Direct effects of Fc-gamma receptor signalling on osteoclast differentiation and function

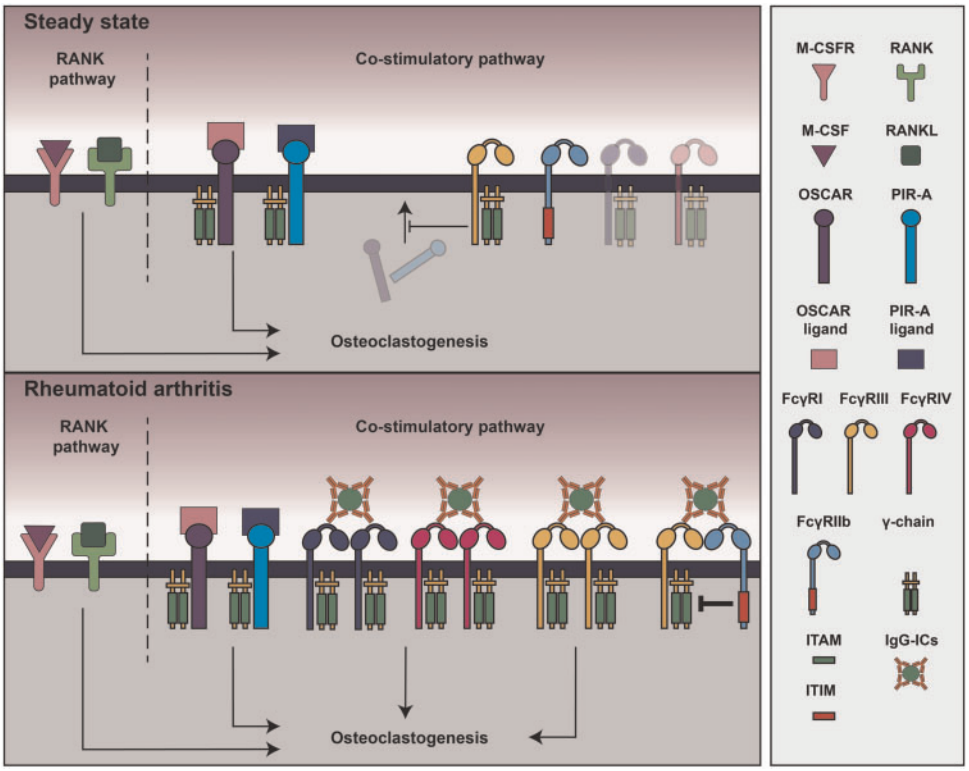
Osteoclast precursors and mature osteoclasts do differentially express FcγRs [26, 28]. The involvement of these receptors in osteoclast differentiation and function has been suggested to run via modulation of the ITAM/ITIM co-stimulatory pathway of osteoclastogenesis, during both steady state conditions and inflammatory conditions, when they can get activated by ICs [27] (Fig. 1).

In the absence of ICs during steady state conditions, FcγRs cannot cross-link and signal. In this situation, the activating FcγRI and IV in mice do not appear to have a major role in the regulation of bone homeostasis. Indeed, the single deletion of FcγRI or IV in naïve mice does not result in major differences in bone characteristics and osteoclast development *in vivo* [26]. Similarly, FcγRIIb$^{-/-}$ precursors do not show alterations in osteoclast differentiation *in vitro* when cultured in the absence of ICs [27, 101]. Surprisingly, instead, naïve FcγRIII$^{-/-}$ mice have increased osteoclast numbers and an osteoporotic phenotype, suggesting that FcγRIII acts as an inhibitor of osteoclastogenesis during steady-state conditions [27]. Possibly explaining this surprising inhibitory finding, *in vitro* experiments proved that FcγRIII sequesters the γ-chain, therewith preventing it from pairing with both OSCAR and PIR-A receptors that use the γ-chain to induce the co-stimulatory signalling that is necessary for osteoclastogenesis [27]. Accordingly, FcγRIII expression decreases during osteoclastogenesis and FcγRIII^{low} precursors differentiate more efficiently into osteoclasts compared with FcγRIII^{high} cells [27]. Interestingly, however, deletion of the γ-chain that results in the impaired function of all activating FcγRs, OSCAR and PIR-A, does not affect the number of osteoclasts and their activity *in vivo* and *in vitro*. This probably results from a compensatory effect of DAP12-bearing receptors that can activate the co-stimulatory pathway independent of the γ-chain [42].

In the inflammatory environment that is present during RA, however, the relative importance of the various FcγRs in the process of osteoclastogenesis is changed. In this situation, ICs are present that can modulate the co-stimulatory signalling for osteoclastogenesis. Moreover, the expression of FcγRs on the osteoclast precursors is changed in favour of the activating FcγRs, rendering the cells more susceptible to their stimulation by ICs. A clear example of this is the fact that the bone marrow cells from mice with CIA express a higher level of FcγRIII and IV, and a decreased level of FcγRIIb [27].

In general, signalling via the activating FcγRs leads to increased osteoclast differentiation whereas an FcγRIIb-

Fig. 1 Role of murine Fc-gamma receptors in osteoclastogenesis in steady state conditions and during RA



In steady state conditions, whereas FcγRI and FcγRIV do not play a major role in osteoclastogenesis, FcγRIII surprisingly inhibits osteoclast differentiation by sequestering the γ-chain from OSCAR and PIR-A. During RA, the expression of activating FcγRs is increased and ICs are present. IgG-ICs can induce osteoclastogenesis by binding to FcγRI, FcγRIII and FcγRIV and stimulating co-stimulatory signalling for osteoclastogenesis. The function of the activating FcγRs can be counteracted by the inhibitory FcγRIIb. FcγR: Fc-gamma receptor; OSCAR: osteoclast associated immunoglobulin-like receptor; PIR-A: paired immunoglobulin-like receptor.

mediated inhibitory signal decreases their differentiation. More in detail, stimulation of osteoclast precursors with IgG2a-ICs or IgG2b-ICs, which bind to FcγRI and FcγRIV with a higher affinity compared with the inhibitory FcγRIIb, increases both osteoclast differentiation and function *in vitro* and *in vivo* [27]. Likewise, artificial cross-linking of FcγRI and FcγRIV leads to increased osteoclast differentiation without affecting their resorbing activity *in vitro* [26]. Further confirming the importance of FcγRIV in osteoclastogenesis, it has been shown that induction of a serum transfer model in osteoclast-specific FcγRIV$^{-/-}$ mice resulted in decreased osteoclast numbers and bone erosion as compared with wild type controls even though the level of inflammation was comparable [26].

In contrast with the clear involvement of FcγRI and IV in IC-induced osteoclastogenesis, IgG1-IC-mediated FcγRIII signalling appears to be strongly compensated, both *in vitro* and *in vivo*, by the inhibitory FcγRIIb for which IgG1-ICs have a higher affinity. Nevertheless, the differentiation of bone marrow cells from mice with CIA, which have increased FcγRIII and decreased FcγRIIb levels, into osteoclasts is promoted by IgG1-ICs. This suggests that FcγRIII is involved in the induction of osteoclastogenesis under inflammatory conditions [27].

In contrast to the activating FcγRs, binding of ICs to FcγRIIb inhibits osteoclast differentiation and function by dampening the γ-chain-activating pathway. FcγRIIb$^{-/-}$ mice with spontaneous increased IgG titres develop osteoporosis, which is reversed by an additional knockout of the activating FcγRs [27].

So whereas it is generally accepted that IC-mediated signalling of activating FcγRs increases osteoclastogenesis, examples are present where a decreased osteoclast differentiation is observed, which possibly runs via an ITAM-mediated inhibitory signal (ITAMi or competition for signalling molecules) [101, 102]. Interestingly, this inhibition of osteoclastogenesis can be overruled by pro-inflammatory mediators, present in the joint during RA [101].

Resembling murine osteoclasts, cross-linking of all individual FcγRs on human osteoclasts induces increased osteoclast differentiation without affecting their resorbing activity *in vitro* [26]. However, the differential function of

the various FcγRs during *in vivo* osteoclastogenesis in humans remains unknown.

Further complicating the picture, the regulation of osteoclastogenesis by ICs depends on the glycosylation state of the IgGs in the ICs. In line with the observation that de-sialylated IgGs bind to FcγRs with higher affinity, these complexes have stronger stimulatory effects on both murine and human osteoclasts [27, 28]. Interestingly, in line with this, RA patients with low Fc sialylation levels of IgGs have significantly higher bone loss, emphasizing its importance in FcγR-mediated bone erosion [28].

Alarmins

Alarmins comprise another group of proteins that are present in the RA environment and associate with bone resorption. These are endogenous molecules that belong to the group of damage-associated molecular patterns (DAMPs), which are rapidly released into the extracellular environment during infection, tissue damage and sterile inflammation. After secretion, they activate the immune system by binding to their pattern recognition receptors (PRRs). The majority of alarmins are present in the cytoplasm of innate immune cells, where they exert physiological functions that are often not directly related to the immune response. Once secreted, they potently induce inflammation via stimulation of innate immune cells or the attraction and activation of antigen presenting cells (APCs) [103, 104].

The contribution of alarmins during RA-associated bone erosion

High levels of alarmins released by cells in the synovium can contribute to bone erosion, both indirectly via the induction of inflammation and directly via stimulation of osteoclasts. The detailed functioning of alarmins in arthritis has been recently reviewed elsewhere [17]. Next to other alarmins such as high-mobility group box 1 (HMGB1), heat-shock proteins (HSPs), tenascin C and other S100 family members, particularly S100A8/A9 appears to have a pivotal role.

S100A8/A9

S100A8/A9, also referred to as calprotectin or MRP8/14, is a small calcium binding protein, that is present in the cytoplasm of neutrophils, monocytes, activated macrophages, keratinocytes and epithelial cells, and osteoclasts [105–109]. Like all alarmins, S100A8/A9 exerts various intracellular functions [110–113], but during inflammation and tissue damage, S100A8/A9 is released into the extracellular environment where it alarms the immune system. S100A8/A9 particularly binds to toll-like receptor 4 (TLR4), although binding to the receptor for advanced glycosylation end product (RAGE) has been described [114–117].

Many studies suggest the importance of S100A8/A9 in both experimental arthritis and human RA patients. S100A8/A9 protein levels are increased in synovium, synovial fluid and serum or plasma of RA patients and correlate with disease activity [118–123]. Moreover, levels of S100A8/A9 have been suggested as a marker to monitor treatment responses and to predict radiographic progression [120, 124–126].

In addition to the data obtained from studies in RA patients, systemic and synovial expression levels of S100A8/A9 correlate with joint inflammation and damage in various murine experimental arthritis models [19, 20, 90, 127]. Moreover, induction of antigen-induced arthritis in S100A9$^{-/-}$ mice results in reduced inflammation and bone erosion compared with control mice [18, 128].

S100A8/A9 can induce bone erosion in arthritis via the induction of inflammation. S100A8/A9 stimulates the production of pro-inflammatory factors by endothelial cells and T cells and increases the production of inflammatory cytokines, chemokines and MMPs by monocytes and macrophages [110, 114, 129–134]. In addition, S100A8/A9 can increase inflammation by attraction and activation of leukocytes and by activation of dendritic cells (DC) [110, 129, 135–138]. Together, this S100A8/A9-induced inflammation in the joint can stimulate osteoclast differentiation and activity.

Next to the promotion of inflammation, a direct effect of S100A8/A9 on osteoclasts has been found. An *in vitro* study with murine osteoclasts showed that S100A8 enhances the further fusion of RANKL-differentiated osteoclasts and increases their resorbing activity via its binding to TLR4(18). Interestingly, the exposure of human monocytes to S100A9 directly promotes their differentiation to osteoclasts in the absence of RANKL [139]. However, it was recently found that stimulation of human monocytes with S100A9 before exposure to RANKL hampers their differentiation into osteoclasts by inhibiting the M-CSF-mediated upregulation of RANK (personal observation), together suggesting a possible dual function of S100A8/A9 on precursors *vs* mature osteoclasts.

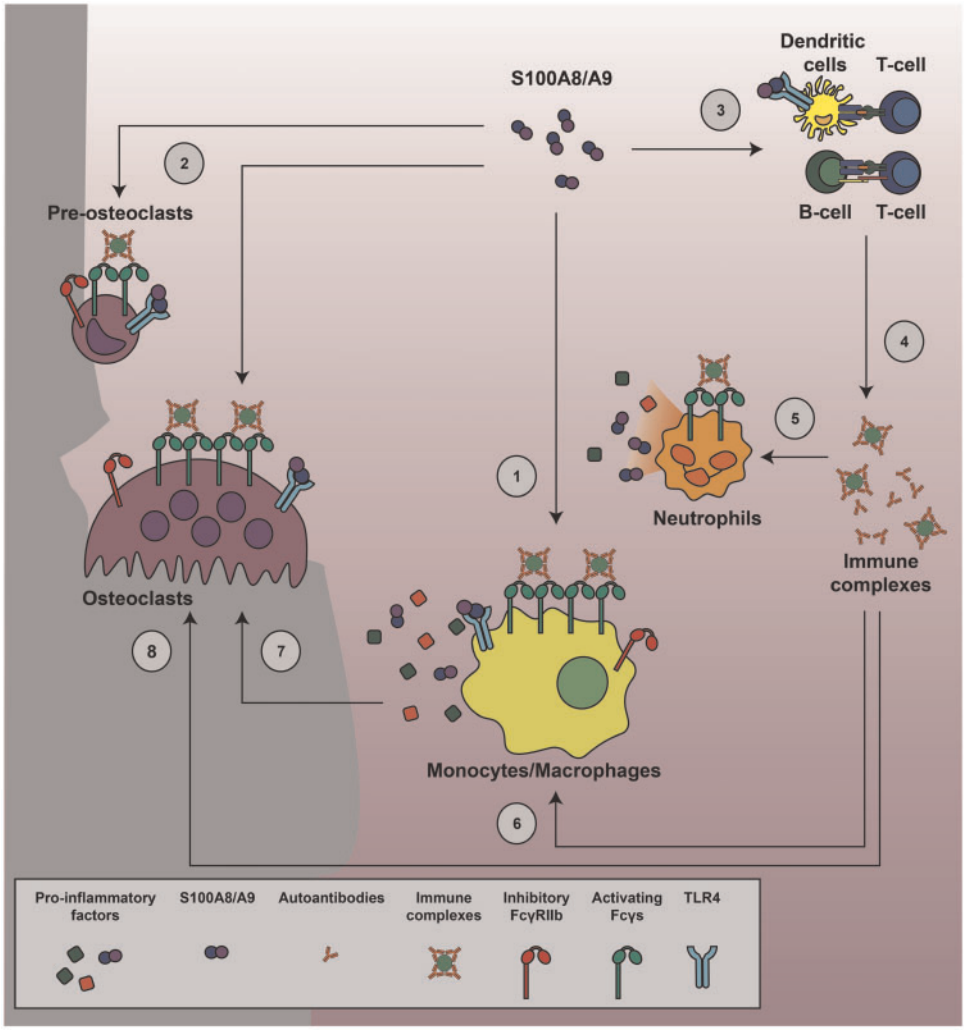
FcγRs and S100A8/A9: do they act as partners in crime, together inducing bone erosion during RA?

As described in this review, IC-activated FcγR signalling and S100A8/A9 both act as independent but crucial players in the process of bone erosion during RA. However, because both are present in high levels during seropositive arthritis, we would like to propose here that they could possibly act in a synergistic manner to fuel bone erosion.

The existence of an interplay between ICs and TLR4-ligands has already been highlighted for a long time in the context of infectious diseases, during which IgG-opsonised pathogens can activate FcγRs and PRRs, including TLR4. Highlighting the existence of this interplay, injection of IgG-coated erythrocytes in mice augments the LPS-induced increase in TNFα serum levels via FcγRs [140] while vice versa the injection of LPS increases FcγR-mediated clearance of ICs and antibody-dependent cellular cytotoxicity [141].

It is likely that during seropositive RA a similar interplay takes place. In this context, ICs would function as activators of FcγRs while DAMPs like S100A8/A9 would function as activators of TLR4. In this section, we will highlight

Fig. 2 The hypothetical interplay between immune-complex-activated Fc-gamma-receptors and S100A8/A9 in fuelling bone erosion during RA

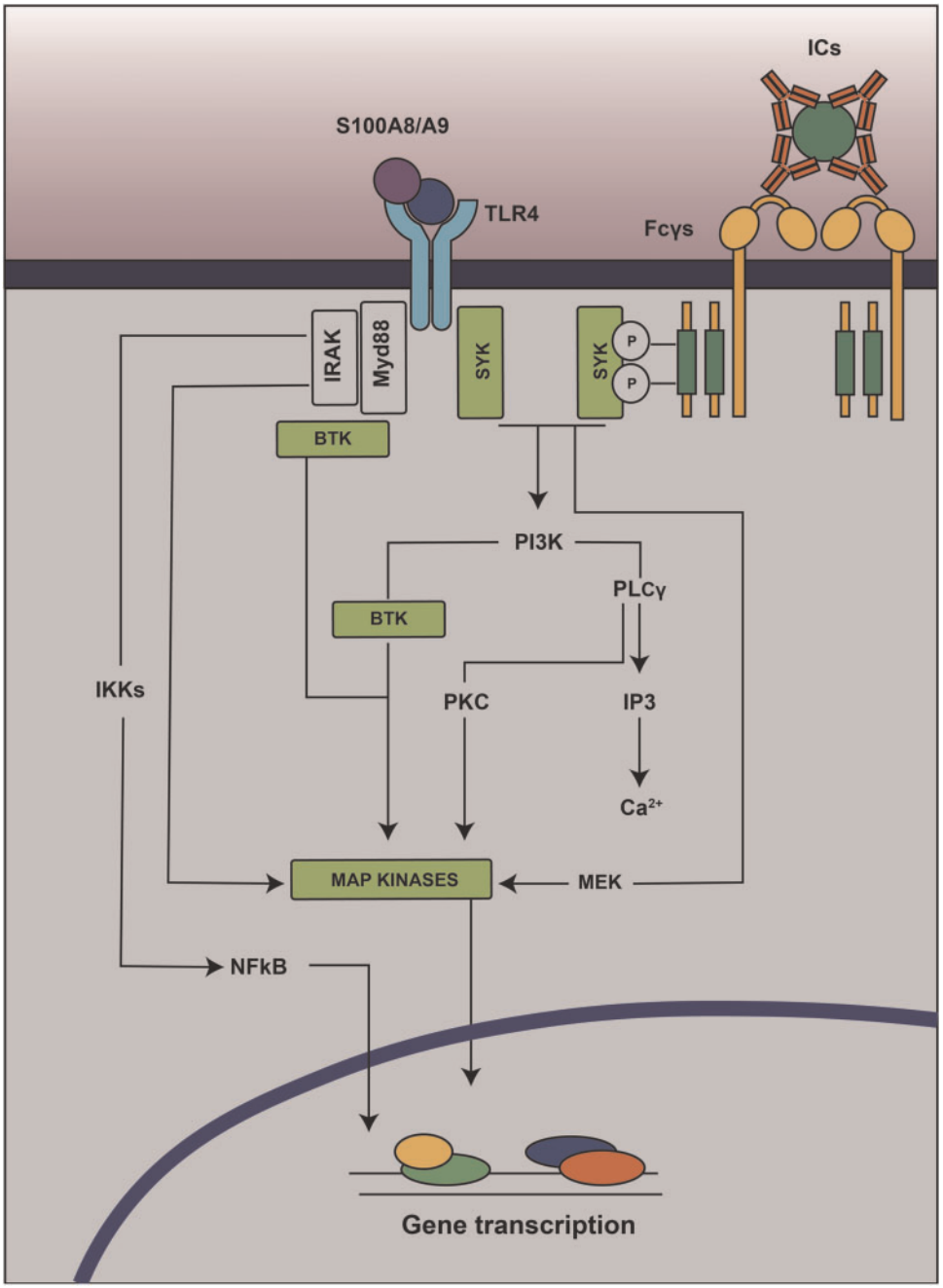


S100A8/A9 induces a shift towards expression of activating FcγRs on innate immune cells [1], osteoclast precursor, and mature osteoclasts [2] thereby rendering them more sensitive to IC stimulation. Moreover, S100A8/A9 can increase the availability of ICs via promotion of antigen presentation [3], resulting in increased production of autoantibodies [4]. In turn, ICs induce NETosis of neutrophils [5] and activation of monocytes/macrophages via the binding to their FcγRs [6] leading to increased production of pro-inflammatory factors, which can directly activate osteoclasts [7]. Moreover, ICs can directly stimulate osteoclasts via binding to the FcγRs [8]. FcγR: Fc-gamma receptors.

various possible levels of interplay between these two inducers of bone resorption. ICs and S100A8/A9 can influence each other's expression and production or, acting on the same cell, they can potentiate each other's molecular signalling pathways either at the receptor level or at the level of shared signalling molecules (Figs 2 and 3). Firstly, it has been shown that S100A8, as important pro-inflammatory factor present in the arthritic joint, enhances the expression of the activating FcγRI and IV, but not of the FcγRIII on murine macrophages via TLR4(47). In contrast, TLR4 agonists downregulate the inhibitory FcγRIIb on monocytes [142]. This shift in expression towards activating FcγRs on immune cells likely results in an enhanced responsiveness to ICs, in increased inflammation and thus indirectly to enhanced bone erosion. A similar shift in FcγR expression can be hypothesized for osteoclast precursors and osteoclasts, potentially leading to a direct increase in their IC-mediated activation.

Next to effects on the FcγR expression, S100A8/A9 could increase the availability of FcγR ligands by increasing the production of autoantibodies via the promotion of antigen presentation [138]. In addition, as already described for macrophages, S100A8/A9 might cause a shift in the balance of FcγRs on DCs promoting their

Fig. 3 Overview of the hypothetical intracellular interplay between immune-complex-activated Fc-gamma-receptors and S100A8/A9-activated toll-like receptor 4



Firstly, FcγRs and TLR4 might physically interact, facilitating the recruitment of shared signalling molecules. Secondly, there can be an intracellular cross-talk between the two signalling pathways, eventually leading to activation of gene transcription. SYK or BTK, that can be activated by both activating FcγRs and TLR4, represent a possible first point of cross-talk. Moreover, more downstream molecules such as MAPK may also be a site of interaction. BTK: Bruton's tyrosine kinases; FcγR: Fc-gamma receptors; MAPK: mitogen-activated protein kinase; SYK: spleen tyrosine kinase; TLR4: toll-like receptor 4.

IC-mediated maturation and activation, leading to increased activation of the adaptive immune system and eventually to increased production of autoantibodies. This increased production of autoantibodies and formation of ICs will probably result in further FcγR-activation either on immune cells or on osteoclasts. Vice versa, binding of ICs

to FcγRs on immune myeloid cells will likely result in an increased release of S100A8/A9. Moreover, FcγR-activation in neutrophils results in the formation of S100A8/A9-containing NETs [143–145], which can stimulate immune cells and osteoclasts.

Secondly, a synergy of FcγRs and TLR4 on the same cell has been shown for various myeloid cells [146–151]. While the stimulation of FcγRs alone results in very little cytokine production and secretion, co-stimulation with LPS is very effective in this regard for human monocytes, macrophages, neutrophils and dendritic cells [147, 148]. Similarly, citrullinated fibrinogen-containing ICs, which can simultaneously activate TLR4 and FcγRs, result in increased production of TNFα [152]. In many of these studies, the main FcγR involved in the cross-talk with TLR4 is FcγRIIA. Although this FcγR does not have an orthologue in mice, a similar cross-talk has been identified between murine FcγRIII and TLR4 in neutrophils and macrophages [153]. S100A8/A9 that like LPS binds to TLR4 might lead to a comparable enhancement of the cytokine secretion by immune cells in response to ICs.

The mechanism through which FcγR-ICs and S100A8/A9-TLR4 can act as partners in crime in individual cells can take place at multiple levels (Fig. 3). A first interaction can be at the receptor level. Activating FcγRs and TLR4 might be in proximity inside lipid rafts of the cell membrane, resulting in their physical interaction or in facilitating the recruitment of shared signalling molecules. It has already been shown that FcγRIII and TLR4 physically interact in murine neutrophils and macrophages and that this interaction is needed for FcγRIII signalling [153]. A second mechanism can take place at the level of intracellular cross-talk between the FcγR and S100A8/A9 signalling pathways. SYK kinase that can be activated as the result of both signalling of the activating FcγRs and TLR4 signalling, represents a possible first point of intracellular cross-talk [154]. Confirming this possibility, FcγR-TLR cross-talk in human M2 macrophages is SYK-dependent [150]. In addition, Bruton's tyrosine kinases-dependent molecular cooperation between FcγRIIa and TLR4 has been shown in neutrophils [149]. However, the two signalling pathways also share more downstream molecules such as mitogen-activated protein kinase pathway molecules, which could be further points of interaction. Together these studies suggest that, although according to our knowledge not yet investigated, it is likely that additive or even synergistic effects of ICs and S100A8/A9 take place in osteoclasts, which could result in increased bone resorption. Mainly the SYK kinase, as crucial stimulator of the co-stimulatory signal of osteoclastogenesis and the central hub of IC and S100A8/A9-mediated osteoclast activation might be interesting in this perspective [155].

Conclusion

As described in this review, both ICs and S100A8/A9 are keys inducers of bone erosion in RA, indirectly via the stimulation of immune cells and via the direct stimulation of osteoclast differentiation and function. In addition, we here propose that they could activate immune cells and osteoclasts in an additive or even synergistic manner, thereby fuelling bone erosion.

Therapeutic targeting of FcγRs or S100A8/A9 separately might offer a good alternative for RA patients that do not respond to current available treatments. In particular, the existence of a really fine regulation of the ICs-FcγRs system offers the possibility for a highly specific therapy. However, the complexity and the redundancy of the system will require additional investigation to reveal the differential function of the individual FcγRs and their function in various phases of RA in order to reach such a specificity. In this regard, taking the possible cross-talk between ICs-FcγRs and S100A8/A9 into account, the targeting of S100A8/A9 might offer an alternative strategy to indirectly target the ICs-FcγRs system, with fewer difficulties.

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