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# Fc gamma receptor mediated modulation of dendritic cells as a potential strategy in the battle against rheumatoid arthritis

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## ABSTRACT

Autoimmune diseases such as rheumatoid arthritis (RA) result from a deregulation of immune responses culminating in immune-mediated tissue injury. In RA, this tissue injury is mainly reflected by synovitis and subsequent joint damage, although involvement of visceral organs (heart, lungs and kidneys) often leads to severe comorbidity. Accumulating evidence points towards dendritic cells (DC) as the principal regulators of the balance between immunity and tolerance. Recently, a large body of evidence has demonstrated that the balance between activating and inhibitory Fc gamma receptor (Fc $\gamma$ R) subtypes is intricately involved in the regulation of DC behaviour. In this overview we summarise recent findings from our group and others that suggest an important role for Fc $\gamma$ R in arthritis. Furthermore, we postulate novel mechanisms of how triggering of Fc $\gamma$ R might be used to manipulate DC function and combat autoimmunity. When DC are envisaged as useful targets in the light of DC immunotherapy in RA, detailed knowledge on the regulatory pathways of Fc $\gamma$ R in RA is of paramount importance.

## KEYWORDS

Dendritic cells, Fc gamma receptors, rheumatoid arthritis

## INTRODUCTION

Dendritic cells (DC) represent a unique set of antigen-presenting cells unrivalled in their capacity to attract and activate naive T cells and are therefore considered the most influential cells in the regulation of the immune response and the orchestration of tolerance.<sup>1</sup> How DC fulfil these

opposing tasks is potentially hidden in the fact that many features of DC are divided in time and place and translated into two developmental stages, namely immature and mature DC.<sup>2,3</sup> Immature DC (iDC) are present in virtually all tissues where they encounter both self and non-self antigens. Triggered by a multitude of signals including selected pathogens and proinflammatory mediators, iDC mature after which they display an extraordinary immune-stimulatory capacity. Compared with iDC, mDC express high levels of co-stimulatory and MHC molecules, *de novo* expression of maturation markers such as DC-LAMP and CD83 and low levels of receptors involved in antigen capture. This phenotype makes mDC perfectly adept for the presentation of antigens to neighbouring T cells that were captured beforehand.<sup>2,4,5</sup>

Autoimmune diseases, such as RA, develop as a result of a loss of immune tolerance culminating in immune-mediated tissue injury. Since DC are believed to be key regulators in directing the fine balance between tolerance and immunity, a major goal in the treatment of autoimmune diseases could be to deflect the presentation of self-antigens by DC to T cells so that anergic T cells or regulatory T cells are induced, thereby inducing tolerance.<sup>1,6,8</sup> Nowadays, an important role has been suggested for DC in synovial inflammation, supporting the theory that targeting DC is likely to be beneficial in RA.<sup>9-13</sup> However, tuning DC function as a therapeutic target in RA has not been tested thus far. In contrast, alteration of DC function has been exploited in other pathological conditions including cancer and transplantation medicine and holds great promise as a future therapy.<sup>14</sup> In the battle against cancer, DC cultured with a proinflammatory phenotype and loaded with antigenic cargo originating from the tumour are generated and administered to patients eliciting antitumour responses. It is reasonable to

postulate that the potency of these 'DC vaccine therapies' might be extrapolated to create tolerogenic DC in case of autoimmunity and transplantation medicine, providing an attractive strategy to break the vicious circle of chronic inflammatory responses in autoimmune diseases such as RA.

For the recognition and processing of antigens and subsequent activation, iDC are equipped with several mechanisms of which receptor-mediated endocytosis is considered the most important. Various receptors mediate antigen uptake; the Fc gamma receptors (FcγR) form the basis of this review. FcγR constitute a group of receptors designed to recognise IgG immunoglobulins or IgG-containing immune complexes (IC) and are abundantly present in serum and synovial fluid of patients with rheumatoid arthritis (RA). In humans three classes can be distinguished, FcγRI, FcγRII and FcγRIII.<sup>15,16</sup> FcγRII is further divided into two subtypes: FcγRIIa and FcγRIIb. FcγRIIa, together with FcγRI and III, activate cellular responses upon triggering, whereas FcγRIIb is a unique inhibitory FcγR.<sup>17,18</sup> Since both activating and inhibitory FcγR are expressed on various immune cells, the concerted action of these opposing signalling systems unequivocally determines the cellular response.<sup>19</sup> In accordance with this, it has been clearly shown that the determination of DC phenotype and behaviour is critically determined by FcγR triggering through IC, stressing the involvement of FcγR in both autoimmunity as well as tumour immunity.<sup>19-21</sup> This review will mainly focus on the involvement of monocyte-derived DC (moDC), and their surface FcγR, in the pathogenesis of RA. In addition we will elaborate on the potential of moDC and FcγR as promising therapeutic targets in the treatment of RA.

## ARE DC IMPLICATED IN SYNOVIAL INFLAMMATION?

Many reports demonstrate the presence of DC in inflamed synovial tissue suggesting a critical role in RA. Recently, Weyand and colleagues categorised synovial inflammation into three main groups based on the appearance of DC and their location in secondary lymphoid organs.<sup>22</sup> Remarkably, also fully matured DC are present in perivascular regions and ectopic lymphoid organs in synovial tissue of RA patients, which is highly suggestive of an altered maturation process during synovitis.<sup>11,22-24</sup> Highly interesting was the finding that synovial DC in RA reflect characteristics of cell activation such as increased expression of inducible heat-shock protein 70,<sup>25</sup> presentation of specific immunepitopes,<sup>23</sup> RANK-RANKL interactions which mirror DC-T cell interaction in the target organ and the expression of transcription factors such as Jak3, STAT1, STAT4 and STAT6.<sup>11,26</sup> Interestingly, alterations in DC phenotype

and behaviour are not confined to the synovium in RA. In fact, our group has demonstrated that moDC obtained from circulating peripheral blood mononuclear cells displayed an increased expression of FcγRII.<sup>12</sup> Further research has led to the observation that this increased expression of FcγRII resulted in an altered production of pro- and anti-inflammatory mediators (TNF-α, IL-6 and chemokines) upon triggering by immune complexes (IC).<sup>27,28</sup> In addition, moDC from RA patients were found to express high levels of Toll-like receptors (TLR) and were found to react more strongly upon TLR ligand stimulation than those from healthy controls. This suggests active involvement of moDC in the inflammatory process.<sup>29</sup> TLR are pattern recognition receptors that bind bacterial fragments and viral RNA (exogenous proteins) on the one hand, but on the other hand also 'endogenous ligands' including heat-shock proteins and cartilage degradation products.<sup>30</sup> Since TLR triggering provides a very potent stimulus for immune activation of DC, the finding that TLR signalling is likely to occur in RA suggests a critical role for moDC during this condition.

More evidence for the involvement of DC in synovitis originates from an elegant study from Leung *et al.* which demonstrates that DC primed with collagen are able to induce inflammatory arthritis after transfer.<sup>9</sup> In line with this finding, DC that were genetically modified to express the anti-inflammatory cytokine IL-4 were capable of fully inhibiting collagen-induced arthritis.<sup>31,32</sup> Since IL-4 is known to be a modulator of FcγR balance, this latter effect might be partially explained by the alteration of FcγR expression locally due to the presence of IL-4 producing cells. Likewise, IL-10-treated DC and exosomes derived from these DC were able to completely block the onset of a collagen-induced arthritis and even reduce the severity of an established arthritis.<sup>33</sup> Altogether, these data clearly indicate that DC are involved in the onset and perpetuation of the inflammatory circle of synovitis and suggest that DC targeting is a challenging approach to treat rheumatoid arthritis.

## THE ROLE OF FcγR IN ARTHRITIS

The use of experimental arthritis models combined with the availability of FcγR knockout mice has led to the appreciation of the role of FcγR in arthritis. For example, during antigen-induced arthritis and immune complex mediated arthritis the presence of activating FcγR (FcγRI and FcγRIII, mice lack FcγRIIa) was associated with chondrocyte death and cartilage erosion.<sup>34-37</sup> Further investigation of the FcγR subtypes revealed that the expression of FcγRIII was required for the development of collagen-induced arthritis since FcγRIII<sup>-/-</sup> mice developed virtually no inflammatory signs and subsequent cartilage

damage.<sup>38</sup> The absence of the inhibitory FcγR subtype IIb, on the other hand, renders mice susceptible to collagen-induced arthritis.<sup>39</sup> In addition, it was recently hypothesised that FcγRIIb reduces both joint inflammation and destruction by inhibition of the activating FcγR and by the efficient clearance of immune complexes.<sup>40</sup> An important role for the inhibitory FcγRIIb in arthritis was further substantiated by the finding that arthritis can be induced by a single injection of IgG anti-collagen type II antibody in mice lacking this receptor.<sup>41</sup> In addition, it was observed that IL-13 mediated upregulation of FcγRIIb prior to immune-complex arthritis inhibited chondrocyte death and cartilage matrix degradation, two key features of joint damage.<sup>42</sup>

The role for FcγR in synovial inflammation in humans is less clear, but accumulating evidence suggests that these receptors are of considerable importance. Until recently a major problem in the study of FcγR on human myeloid cells was the inability to distinguish FcγRII subtypes (FcγRIIa and FcγRIIb) on the cellular surface. In the synovial tissue macrophages and DC are abundantly present and it was demonstrated that these cells express significantly higher levels of FcγRII and III when compared with those present in synovium from healthy controls.<sup>12,43</sup> Similarly, it was found that moDC obtained from patients with RA express FcγRII at significantly higher levels than those from their healthy counterparts.<sup>12</sup> Since these DC display an anti-inflammatory phenotype upon IC stimulation the increased expression of FcγRII was thought to reflect an increase in FcγRIIb. Intriguingly, this increased expression of FcγRII was unique for patients with active RA and still present after full maturation, suggesting that local factors responsible for this phenomenon are present during early moDC life as suggested previously.<sup>44</sup> With the recent development of a unique antibody directed specifically against the inhibitory FcγRIIb (gifted by MacroGenics, Inc), it could be demonstrated that the inhibitory FcγRIIb subtype was largely responsible for the increased expression of FcγRII as observed before (manuscript in preparation). This is conform the hypothesis that FcγRIIb is pivotal in the counter-regulatory response of proinflammatory responses in RA. It was highly interesting to see that the functional polymorphism of the FcγRIIb (I232T) was identified as the strongest prognostic factor for radiological joint damage in RA and was found to modulate moDC function, further substantiating the important role of FcγR in RA.<sup>45</sup>

#### ARE DC AND FcγR CONNECTED IN RA PATHOLOGY?

In order to prevent chronic inflammation and subsequent tissue damage, in time every established immune response

has to be terminated. To this end, the immune system is provided with a multitude of inhibitory receptors that counteract the immune response.<sup>46</sup> The inhibitory FcγRIIb is a perfect example of such. As discussed before, the expression of FcγRIIb on moDC from RA patients is found to be increased compared with those from healthy controls. Intriguingly, this increased expression was exclusively found in patients with an active phase of disease. Therefore, we postulated that FcγRIIb might function as a counteractive mechanism to dampen proinflammatory responses. During inflammation both pro- and anti-inflammatory cytokines are generated that regulate the balance between activating and inhibitory receptors, including FcγR. TNF-α and IFN-γ are known to induce the upregulation of mainly activating FcγR whereas IL-4 and IL-13 were recently identified as factors that induce the opposite.<sup>19,47-49</sup> Bearing these data in mind a conceptual framework can be envisaged in which proinflammatory cytokines will upregulate activating FcγR during the early stages of inflammation. Circulating immune complexes CIC, which are omnipresent in RA, will potentate the proinflammatory reaction by inducing cytokine production and moDC maturation. To counteract this initial proinflammatory response, anti-inflammatory cytokines will be produced through which inhibitory FcγR gain the upper hand, the IC will then provide a negative feedback loop silencing the inflammatory response. In line with this conceptual framework, IL-13 was found to be increased in both serum and synovial fluid from RA patients further supporting this hypothesis.

Why then do RA patients develop chronic disease? Our previous findings along with the observations that intravenously administered immune globulins (IVIG) lack clinical efficacy in RA patients despite their success in many other inflammatory conditions including inflammatory myositis, Guillain Barré syndrome and multiple sclerosis, prompted us to hypothesise that the FcγRIIb pathway is defective in RA.<sup>50-54</sup> The finding that IL-13-mediated upregulation of FcγRIIb is absent in RA strongly supports this hypothesis and warrants further research.

#### CAN FcγRS BE USED AS THERAPEUTIC TARGETS IN RA?

Triggering of Toll-like receptors (TLR) provides a strong stimulus for DC activation. The notion that TLR are involved in the regulation of both innate and adaptive immune responses sparked a revolution of research in the potential role of these receptors in a plethora of inflammatory conditions. In RA, the role of TLR in the disease pathogenesis has only recently become a subject of intense investigation. Recent research has

demonstrated that TLR are expressed at increased levels in RA synovium,<sup>29,55</sup> moDC from RA patients reacted more potently with TLR agonists compared with DC from healthy controls, producing vast amounts of proinflammatory cytokines,<sup>29</sup> and RA synovial fibroblasts are activated by RNA released from necrotic cells.<sup>56</sup> All these data suggest that a role for TLR in RA is likely. Recent observations suggesting an interaction between the inhibitory FcγRIIb and TLR<sub>4</sub> are therefore intriguing since this might provide new insights in how to modulate TLR responses *in vivo*.<sup>49</sup> It is tempting to speculate that local moDC might act as a counter-regulatory mechanism with the purpose to silence the inflammatory response and restore tolerance after eradication of the provocative element. DC fulfil a central role in the organisation of immune responses against pathogenic invaders and in preventing autoimmune responses harmful to the host. Their directive task in shaping the immune response makes DC excellent targets in the battle against rheumatoid arthritis and other autoimmune diseases. To suppress (auto)immune responses several potential strategies can be envisaged in which active modulation of DC function plays a prominent part. One approach would be aimed at stimulating the tolerogenic capacities of DC *in vivo*, preferably at the site of inflammation. Current research is now focussing on whether the inhibitory FcγRIIb could be targeted for this goal.

Although still little is known regarding the signals that can induce the tolerogenic pathway in DC, accumulating evidence indicates that various immunosuppressive pharmacological substances are able to modulate DC phenotype and function and it is conceivable that some might shift the balance between immunity and tolerance by interfering in DC pathways.<sup>57</sup> Another strategy for the use of DC to combat autoimmune diseases might be to manipulate DC *ex vivo*, delivering 'programmed DC'. By means of *in-vitro* culture strategies (Vit-D<sub>3</sub> or dexamethasone or IL-10) or genetic modification the DC's tolerogenic immune suppressive programme can be instigated. The potential effectiveness of such strategies has already been demonstrated in transplantation models in which moDC differentiated in the presence of vasoactive intestinal peptide, a known immunosuppressive neuropeptide, expressed an immune regulatory phenotype able to prevent acute graft-versus-host disease *in vivo*.<sup>58</sup> In addition, DC genetically modified to express IL-4 were able to abrogate synovial inflammation and abolish subsequent cartilage damage in experimental arthritis models.<sup>32</sup> Another possible way by which DC characteristics can be manipulated lies with the Toll-like receptors. Repetitive triggering of DC via TLR was shown to abrogate their proinflammatory capacity whereas combinations of TLR were found to unleash a synergistic effect on DC with respect to DC activation.<sup>29,59,60</sup> This

suggests that stimulation of TLR, the selective blocking of TLR or the modulation of TLR responses might be used to modulate DC behaviour in such a manner that is favourable for the host. The use of such TLR-activated DC has shown promising results in the battle against melanoma.<sup>61,62</sup> In the case of autoimmune diseases the inhibitory FcγRIIb might prove to be a valid target since this inhibitory receptor seems interconnected with TLR signalling and is known to be important in the delicate balance between tolerance and autoimmunity.<sup>49,63</sup>

## FUTURE PROSPECTIVE

Both DC and FcγR are implicated in the inflammatory pathways during synovitis in RA. The modulation of the balance between activating and inhibiting FcγR might provide a means to modulate the behaviour of DC. However, the upregulation and/or function of FcγRIIb seems to be defective in RA; further studies resolving the cause of this potentially altered FcγRII regulation pathway are currently being conducted. For a better understanding of DC biology in autoimmune diseases, more knowledge on this inhibitory pathway is warranted and might provide new clues regarding potential new treatment strategies to battle autoimmune diseases including RA.

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