REVIEW

Dendritic cells, Fc γ receptors, and Toll-like receptors: potential allies in the battle against rheumatoid arthritis

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Recent findings suggest an important role for Fc γ receptors and Toll-like receptors expressed by dendritic cells (DC) in the pathogenesis of rheumatoid arthritis (RA). Possibly, DC behaviour might be tuned to counteract the misbalanced immune system in RA. Understanding the precise mechanisms that determine the skewed immune response in RA may provide new clues for the therapeutic use of DC in RA.

an attractive strategy in the battle against autoimmune diseases such as RA. Before that, however, the role of DC in RA and other autoimmune diseases must be explored in more detail. Fc γ receptors (Fc γ R) and Toll-like receptors (TLR) are highly involved in the modulation of DC biology and may fulfil a critical role in the modulation of autoimmune diseases in the near future. Alterations in DC phenotype and behaviour in RA potentially triggered by these receptors have been reported and may thus represent new targets in the treatment of this condition and are reviewed here.

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by synovial inflammation and subsequent damage of cartilage and underlying bone. Despite extensive research on the immunological mechanisms, which show a massive influx of T cells, B cells, fibroblast-like synoviocytes, macrophages, and dendritic cells (DC) in the synovial tissue, the exact pathophysiological pathways remain unclear.^{1–3} During synovial inflammation, critical events such as neoangiogenesis, cellular hyperplasia, and a massive influx of inflammatory cells take place, which are largely orchestrated by a complex interplay of proinflammatory cytokines, chemokines, and matrix metalloproteinases. Nowadays, a large body of evidence points towards antigen presenting cells (APC) as pivotal regulators of these critical events.^{4–6}

“Dendritic cells are the key regulators of the balance between tolerance and immunity”

As “professional APC”, DC are believed to be key regulators in directing the fine balance between tolerance and immunity.^{7–10} Autoimmune diseases, such as RA, are characterised by a loss of tolerance to the body's own constituents that results in a destructive process directed to a specific organ site. Because DC have a major role in both immunity and tolerance, the major goal in the treatment of autoimmune diseases is therefore to enhance the tolerogenic properties of DC.⁹ As demonstrated recently, exploiting the immune activating abilities of DC holds great promise for the treatment of cancer and transplantation medicine.¹¹ In cancer treatment, DC loaded with antigenic cargo originating from the tumour are generated by various protocols and administered to the patients. The potency of so-called “DC vaccine therapies” might be extrapolated to tolerogenic DC in cases of autoimmunity and transplantation, providing

DC BIOLOGY DURING STEADY STATE CONDITIONS

The biology of DC starts early in the bone marrow, where precursor DC differentiate from stem cells and subsequently migrate into the peripheral blood. Further on in their development, these precursor cells become immature DC and seed the tissues to monitor invading pathogens. The regulation of DC function is complex. It involves multiple DC subsets, is highly dependent on their developmental state, and critically dependent on the external signals a DC receives. DC research in men is focused primarily on monocyte derived DC, for mainly technical reasons. Immature monocyte derived DC (iDC) exhibit an unsurpassed machinery to take up and process antigens. Upon encountering antigens and/or numerous stimuli, including tumour necrosis factor α (TNF α), interleukin (IL) 1, CD40L, and heat shock proteins (HSP), often referred to as “danger signals”, DC undergo major changes such as a rapid internalisation of antigen uptake receptors and up regulation of costimulatory molecules and major histocompatibility complexes (MHC).^{12–13} As a result, fully matured DC (mDC) down regulate their endocytic activity but are highly adept at antigen presentation and subsequent T cell stimulation. This dramatic conformational change of cell surface is accompanied by an increased secretion of proinflammatory mediators such as cytokines (TNF α , IL6, and IL12) and chemokines and expression of chemokine receptors (fig 1).^{14–15}

Abbreviations: APC, antigen presenting cell(s); CTA4Ig, T lymphocyte associated antigen 4 immunoglobulin; DC, dendritic cell(s); Fc γ R, Fc γ receptor(s); HSP, heat shock protein(s); MHC, major histocompatibility complex; IC, immune complex(es); iDC, immature monocyte derived DC; IL, interleukin; IVIg, intravenous immunoglobulin; mDC, fully matured DC; RA, rheumatoid arthritis; TLR, Toll-like receptor(s); TNF α , tumour necrosis factor α

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Figure 1 Phenotype and functional behaviour of DC during development. iDC and mDC express a distinct repertoire of surface molecules and produce a different set of inflammatory mediators. iDC express high levels of antigen uptake receptors (FcγR, TLR, and DC-SIGN) and produce low levels of inflammatory mediators. Expression of the chemokine receptors CCR1, CCR5, and CCR6 results in chemoattraction to inflammatory sites. mDC, in contrast, express low levels of antigen receptors but express molecules which are involved in the presentation of antigen (MHC) and stimulation of T cells (CD80, CD86). Moreover, mDC secrete high quantities of proinflammatory mediators such as TNFα, IL12, and chemokines to attract and activate neighbouring T cells with utmost efficiency. mDC express particularly CCR7 for homing towards the T cell-rich areas in the lymph nodes.

iDC take up antigens by constitutive macropinocytosis, phagocytosis, and receptor mediated endocytosis, including FcγR, and express multiple TLR to sense their environment. In this review we focus on the last two receptor types and their function in DC and RA.⁷

Toll-like receptors (TLR)

TLR constitute a group of receptors involved in the recognition of a multitude of signals that inform the innate immune system about its environment. The stimuli triggering TLR signalling are often called “danger signals” and include exogenous compounds (LPS, LTA, flagellin, CPG motifs), often referred to as pathogen associated molecular patterns.^{12–16} The main function of the immune system is to detect the presence of invading micro-organisms, but it is also intricately involved in sterile inflammation as a response to endogenous ligands released upon cell stress and damage. Several reports imply that endogenous proteins (HSP,^{17–18} fibronectin,¹⁹ hyaluronic acid²⁰) can also activate TLR. Upon triggering, most TLR induce immune activation, including DC maturation and subsequent secretion of proinflammatory mediators such as cytokines and chemokines.^{17–18, 21–23}

All these features aim at one goal: obtaining efficient attraction, binding, and activation of immune cells, and subsequent elimination of the potentially harmful trigger with utmost efficiency. To date, 11 members of the TLR family have been identified in mammals.²⁴ For most of the TLR, ligands have been identified and intracellular pathways are being elucidated. It is now becoming clear that signalling cascades activated upon binding differ between TLR agonists,

which opens the opportunity to modulate both innate and adaptive immune responses.

Fcγ receptors (FcγR)

FcγR are IgG-specific receptors, which in man can be divided into three classes. The FcγRI (CD64) is a high affinity receptor that mainly binds monomeric IgG, whereas FcγRII (CD32) and FcγRIII (CD16) interact preferentially with complexed IgG, often referred to as immune complexes (IC).^{25–27} The FcγRII can be further divided into three subclasses—namely, FcγRIIa, FcγRIIb, and FcγRIIc; the last of these is still without a clear function. After ligand binding, FcγRI, FcγRIIa, and FcγRIII are activating receptors, whereas FcγRIIb is the only receptor known with the opposite effect. All these FcγR subtypes are expressed by DC and mediate endocytic transport of antigen-antibody complexes in these cells.^{28–29} Furthermore, it has been shown that FcγR triggering by IC is critically involved in the determination of APC phenotype and behaviour both in autoimmunity and in tumour immunity.^{30–31}

EVIDENCE FOR THE INVOLVEMENT OF DC IN INFLAMMATORY PROCESSES SUCH AS RA

The availability of innovative molecular techniques and expanding possibilities for experimental arthritis models has sparked a research revolution into the critical pathways of synovial inflammation and subsequent cartilage destruction, two key features of RA. The first finding that pointed towards the involvement of the immune system in RA was the association of HLA-DR with disease susceptibility in the late 1970s.³² The discovery of IC which can contain autoantibodies, including rheumatoid factors, and the recognition of their role in the inflammatory pathway, together with the high specificity of the recently isolated citrullinated peptides in RA, further substantiated the potential role of the immune system both in the initiation and perpetuation of the disease.^{33–35}

“Dendritic cells have a role in the initiation and perpetuation of RA”

The potential involvement of DC in RA was first recognised by Thomas and coworkers, who demonstrated the presence of fully matured and activated DC in synovial tissue from patients with RA.⁵ Nowadays, a substantial body of evidence supports an important role of DC in both the initiation and perpetuation of RA.

Firstly, the presence of matured and activated DC in synovial fluid and tissue from patients with RA was demonstrated by several groups and was put forward as the first real indication of the involvement of DC in the inflammatory cascade of arthritis.^{5, 36–39} Intriguingly, these synovial DC are well organised in lymphoid structures, suggesting a potential role of synovium as an ectopic lymphoid organ. The latter was suggested by Weyand and Goronzy, who characterised different types of synovial inflammation based upon the presence of lymphoid follicles or germinal centres, or both.⁴⁰ However, DC are not only located in these ectopic lymphoid tissues but are also highly present in the perivascular regions, where they are potentially involved in the chemoattraction of other inflammatory cells.³⁸ Of great interest was the finding that not only DC in the synovial joint express a different phenotype but also DC obtained from peripheral blood of patients with RA showed phenotypic and functional differences in comparison with those from healthy controls.^{39–41}

Secondly, T lymphocyte associated antigen 4 immunoglobulin (CTA4Ig) was recently introduced as a new treatment strategy in RA.⁴² CTLA4Ig specifically prevents

the efficient interaction between activated T cells and DC by blocking the interaction between the costimulatory molecules CD80 and CD86 located on DC, and CD28 on T cells, thereby potentially preventing the amplification of the inflammatory reaction. The finding that administration of CTLA4Ig to patients with RA had promising clinical effects supports the notion that DC-T cell interaction is a crucial step in the synovial inflammation seen in RA.

FC γ R AND SYNOVIAL INFLAMMATION

The crucial role for Fc γ R in arthritis has been demonstrated in numerous studies using experimental arthritis models. These studies show the important role of activating Fc γ R in association with chondrocyte death and cartilage erosion^{43–46} and demonstrate the role of inhibitory Fc γ R in the onset and severity of collagen induced arthritis and immune complex arthritis.^{46, 47} In addition, targeted deletion of the Fc γ RIIb gene greatly enhanced the pathological signs of IC mediated inflammatory conditions^{48–50} (reviewed by Pritchard *et al*⁵¹). These findings indicate that the delicate balance between activating and inhibitory Fc γ R is of paramount importance and, at least partly, determines the susceptibility and severity of arthritis.

“The delicate balance between activating and inhibitory Fc γ R determines the severity of arthritis”

In humans, iDC from patients with RA (RA DC) were found to express the Fc γ RII at significantly higher levels than those from their healthy counterparts and did not decrease even after full maturation.³⁸ Further analyses of RA DC showed that the balance of Fc γ RII was skewed towards the inhibitory subtype Fc γ RIIb. The functional consequence of this altered Fc γ R balance became readily clear after Fc γ R mediated triggering of these cells. Whereas DC from healthy controls increased the production of inflammatory mediators upon Fc γ R mediated triggering, the opposite effect was seen upon stimulation of DC from patients with RA.^{39–41} The finding that monocyte derived dendritic cells from patients with RA still express higher levels of Fc γ RII, even after many days of cell culture, is intriguing and suggests that local factors present during early life programme cells towards a specific phenotype. The existence of such instructing signals has been suggested by various groups recently.^{52–53} In line with this finding, Santiago-Schwarz and coworkers demonstrated that increased levels of soluble TNF receptor (p55) present in RA synovial fluid induced the CD14 derived DC pathway in RA.⁵⁴ Similarly, we have found that both RA serum and RA synovial fluid induce an up regulation of Fc γ RII on DC from healthy controls which, in contrast, was absent in DC from patients with RA, suggesting an aberrant Fc γ RII expression regulatory pathway in RA (unpublished data). As a potential consequence of this, the activated immune response becomes uncontrolled owing to a lack of negative control mechanism, which leads to a vicious circle of inflammation ending up in joint destruction.

IL4, IL13, and interferon γ represent two cytokine pathways known to play a part in the regulation of Fc γ R expression. On monocytes, IL4 selectively increased the expression of the inhibitory Fc γ RIIb, whereas stimulation with interferon γ resulted in a balance towards Fc γ RIIa.⁵⁵ However, IL4 is scarce in RA and thus less likely to have a role in the increased expression of Fc γ RIIb on DC from patients with RA.⁵⁶ IL13, on the other hand, is abundantly present in the synovial compartment during inflammatory conditions.^{56–58} Highly interesting, therefore, was the finding that the addition of IL13 to DC from healthy controls resulted

in a clear increased expression of Fc γ RII, whereas DC from patients with RA failed to show this effect.⁵⁹

TLR AND IMMUNE ACTIVATION IN ARTHRITIS

TLR are heavily involved in the detection of “danger signals” and regulation of DC function and are therefore the subject of investigation in autoimmunity. These “danger signals” include both exogenous, often pathogen related signals, as well as endogenous proteins. It was recently demonstrated that these endogenous signals can, in conditions of cell stress and/or structural cell damage, lead to primary immune responses and represent natural initiators of autoimmunity.^{12, 60–61} The critical role of TLR in the initiation of autoimmune disease and orchestration of the simultaneous stimulation of distinct TLR pathways resulted in the breakthrough of tolerance and subsequent onset of experimental autoimmune encephalomyelitis.^{62–63} Iwahashi and colleagues demonstrated that TLR2 was highly expressed on monocytes and synovial macrophages from patients with RA and that TLR2 mediated stimulation resulted in a high production of TNF α , the key inflammatory cytokine in RA.⁶⁴ DC constitutively express most TLR and this expression is tightly tuned by the presence of pro- and anti-inflammatory cytokines. Various groups have demonstrated that the ligation of various TLR resulted in an increased production of chemokines during inflammatory responses.^{65–66} Recently, we found that monocytes and DC from patients with RA are highly responsive to certain TLR agonists and produce higher quantities of proinflammatory cytokines than those from healthy controls.⁶⁷ In line with this finding we and others have demonstrated that TLR are significantly more highly expressed in RA synovial tissue than in synovial tissue of healthy donors, suggesting a role for TLR in synovial inflammation.^{67–69} Moreover, we have shown that the expression of TLR2 and TLR4 was largely orchestrated by cytokines (IL12 and IL18), which are locally present during arthritis. Because endogenous ligands are likely to be released by cells undergoing stress or damage the increased cell turnover in cases of minor trauma or inflammation is likely to initiate a reaction leading to TLR mediated triggering of inflammatory cells in RA.

A direct link between the presence of endogenous ligands and DC activation in RA was provided by Martin *et al*.⁷⁰ This study demonstrated that inducible HSP70 was increased in RA synovial fluid and on DC isolated from RA synovial fluid. Based on these findings it was postulated that iHSP70 may act as a chaperone molecule facilitating the uptake of antigens by DC, resulting in a potentiation of the inflammatory response in the joint. Besides endogenous TLR ligands, several studies have examined the potential role for exogenous TLR ligands in the induction of synovial inflammation. For example, bacterial DNA and/or peptidoglycan (bacterial cell wall constituent), which were both detected in RA synovium, may trigger DC via TLR mediated pathways, thereby amplifying the inflammatory cascade. Evidence for a role for TLR in RA pathogenesis was further substantiated by the finding that the TLR4 (Asp299Gly) functional variant was associated with the disease pathogenesis.⁷¹ Taken together, it is tempting to speculate that TLR have a role in the onset and/or severity of the disease, which was recently underlined by findings which suggested a critical role for TLR in autoimmunity.^{72–73}

IS INHIBITION OF THE IMMUNE RESPONSE HAMPERED IN RA?

Every immune reaction that is initiated has to be terminated. Therefore, the immune system is equipped with a myriad of inhibitory receptors that counteract the immune response.⁷⁴ TLR are often considered to be involved in elicitation of the

Figure 2 Immune response by DC upon TLR mediated pathways in healthy people and patients with RA. (A) TLR are triggered by a multitude of stimuli (1), leading to activation of DC and production of various proinflammatory mediators (TNF α , IL12, chemokines) to eliminate the potentially harmful trigger. The presence of IC in this phase leads to additional activation of DC because the balance between activation and inhibitory FcγR is skewed towards the former (2). During the late phase of the immune response anti-inflammatory cytokines, designed to dampen the immune response, lead to a skewing of the FcγR balance toward the inhibitory subtype (3). Local production of IC now results in abrogation of the proinflammatory response and perhaps even a restoration of tolerance mediated by DC (4). In patients with RA (B), more TLR agonists are present in the synovial compartment, leading to an increased activation state of DC (1). This leads to a higher production of cytokines and chemokines by DC upon triggering both specific and aspecific stimuli (2). Although it was found that DC from patients with RA express a high level of inhibitory FcγRIIb subtype, DC from patients with RA still produce higher amounts of proinflammatory mediators even upon stimulation with IC (2). Levels of IL13 and IL10 are increased during synovial inflammation. However, we have shown that the increase of the inhibitory FcγRIIb is impaired upon stimulation with IL13. Although IC are abundantly present in the patients with RA (3), this leads to an inefficient up regulation of FcγRIIb, and thus dampening of the immune response as seen in healthy subjects (4).

immune response, whereas inhibitory and stimulatory roles for FcγR have been reported.⁷⁵ Triggering of TLR by both endogenous and exogenous ligands, for example, results in a potent stimulus to produce TNF α and IL1, two key inflammatory cytokines in RA. Furthermore, elicitation of activating FcγRs, more specifically FcγRIIIa, was found to result in an increased secretion of TNF α and IL1 by monocytes (Abrahams *et al*^{73a}). In this light it can be envisaged that IC containing autoantibodies exert their detrimental effects by activating FcγR.

Recently, a clear interaction between TLR9 and FcγRIIa was demonstrated in the uptake of DNA-containing IC from patients with systemic lupus erythematosus and subsequent activation of plasmacytoid DC.⁷⁶ Although the exact pathways that orchestrate the inhibitory action of FcγRIIb with regard to activating FcγR and TLR have not been elucidated thus far, recent data from our group suggested that the inhibitory FcRIIb may inhibit TLR response upon triggering by IC.⁴¹ Intriguingly, a recent report indicates that TLR triggering might also enforce the tolerance state of dexamethasone treated APC.⁷⁷ During inflammation both pro- and anti-inflammatory cytokines are generated, which thus determine the balance between activation and inhibitory receptors, including FcγR. In healthy people, IC are produced during an inflammatory reaction and might serve as “down-tuners”

of the immune response by FcγR dependent mechanisms. Normally, and probably initially, the balance of FcγR is skewed towards the activating FcγR subtypes. Activation of these receptors by IC then results in the production of the inflammatory response. Later on in the immune response, the production of IL4, IL10, and IL13 alters the balance of FcγR toward the inhibitory subtypes. IC present than stimulate mainly inhibitory FcγR and the immune response is abrogated or perhaps even restored towards tolerance.

The finding that stimulation of RA DC with IL13 does not increase the expression of FcγRII may suggest that this down regulatory circuit is affected in RA, resulting in a vicious circle (fig 2). This hypothesis is further substantiated by the finding that intravenously administered immunoglobulin (IVIg) lacks clinical efficacy in patients with RA^{78–79} despite its success in many other inflammatory conditions including inflammatory myositis,⁸⁰ Guillain-Barré syndrome,⁸¹ and multiple sclerosis.⁸² The fact that IVIg is not effective in the treatment of RA underlines the possibility of a defect in this pathway in this disease because this is one of the mechanisms considered to underlie the potential therapeutic effects of IVIg. Taken together, we envisage that the immune system in RA is triggered, potentially through TLR dependent pathways by, for example, endogenous ligands, resulting in

the activation of various inflammatory cells and a cascade of proinflammatory events. The inflammation then continues owing to the failure of a feedback mechanism that normally counteracts the proinflammatory immune response. The precise pathways involved in the potential interaction between activation and inhibitory systems of the immune response need to be explored in more detail, including the way in which TLR and inhibitory FcγR participate in this process.

HOW CAN DC BE USED IN THE TREATMENT OF RA?

DC are intricately involved in initiating immunity against pathogenic invaders and in preventing autoimmune responses harmful to the host. To perform this dual task, the immune system has developed unique mechanisms, central and peripheral tolerance, both of which involve DC. The potency of DC in shaping the immune response makes them promising targets in the battle against autoimmune diseases. Several potential strategies can be envisaged to modulate DC function in such a way as to actively suppress immune responses. One approach would aim at stimulating the immune down-modulatory capacities of DC in vivo, preferably at the site of inflammation.

"Dendritic cells are promising targets in the battle against RA"

The oversimplified concept that iDC are tolerogenic and mDC immunogenic has recently been challenged by findings from several studies, which suggested that creating tolerogenic DC or "semimature DC" is an active process. However, the possibility cannot be fully excluded that a specific tolerogenic DC subset exists.^{83, 84} Tolerogenic, semimature DC are still difficult to distinguish from fully matured DC but are low secretors of numerous proinflammatory mediators, which provide the so-called "third" signal during the APC-T cell interaction. The third signal is held responsible for a major part of the efficiency of T cell activation as it has been suggested that low production of proinflammatory mediators by DC is responsible for the induction of anergic T cells or regulatory T cells that actively suppress immune responses.⁸⁵ In corroboration with these observations it was found that the inhibition of TNFα activity during the in vitro development of DC resulted in semimatured DC which might account for at least a part of the therapeutic effect seen during the treatment of RA with TNFα inhibitors in clinical practice.^{86, 87} Though little is still known about the signals that would activate the immune inhibitory pathway in DC, numerous pharmacological substances can affect DC phenotype or function and, conceivably, some might alter the balance between immunity and tolerance.⁸⁸

An alternative way to combat autoimmune diseases successfully might be reached through the ex vivo development of so-called "programmed DC". DC could be generated by in vitro culture strategies (vitamin D3 or dexamethasone or IL10) or genetic modification that uncover the tolerogenic immune suppressive programme of DC. The potential effectiveness of such a strategy has already been demonstrated using experimental arthritis models, in which DC genetically modified to express IL4 abrogated synovial inflammation and abolished subsequent cartilage damage.⁸⁹ In addition, repetitive triggering of DC by TLR has been shown to abrogate the proinflammatory capacity of APC.⁸⁴ This suggests that TLR might also be used to modulate the immune response in a way that is favourable for the host. Programmed DC for the induction of immunity are already used in the battle against various cancers, including melanoma.^{90, 91}

CONCLUDING REMARKS

Although, there is substantial evidence that DC have the potential to silence harmful immune responses, many questions still remain. The most effective way of inducing the DC tolerogenic programme and the phenotype and function of such DC still needs to be defined. Another crucial issue concerns the stability of tolerogenic DC in an inflammatory environment in vivo. A stable immune suppressive DC phenotype is likely to be essential in the battle against autoimmune diseases such as RA. Further exploration of the precise role of TLR and FcγR in synovial inflammation and DC behaviour may provide us with information that enables us successfully to alter DC function in RA and, subsequently, prevent the detrimental effects commonly seen in this disorder.

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