

# Dendritic cells as targets for therapy in rheumatoid arthritis

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**Abstract** | Dendritic cells (DCs) are central in inducing immunity and in mediating immune tolerance in their role as professional antigen-presenting cells. In the absence of DCs, a fatal autoimmunity develops in animal models. Although the role of DCs has been investigated extensively in the pathogenesis of rheumatoid arthritis (RA), it remains unclear whether DCs initiate autoimmunity in this disease. Nevertheless, evidence points towards a significant role for DCs in disease maintenance and progression. Current biologic therapies target cytokine products of antigen-presenting cells, such as tumor necrosis factor, interleukin-1 and interleukin-6. Emerging therapies for RA exploit the tolerogenic capacity of DCs. ‘Tolerogenic’ DCs can be generated from myeloid precursors *ex vivo*, loaded with antigen, and manipulated to suppress autoimmune responses *in vivo*, through the induction of activation-induced cell death, anergy, and/or regulatory T cells. Cells that are primed by DCs, such as B cells, type 1 and type 17 T helper cells, and that have been implicated in certain models of autoimmunity, are also being considered as additional targets for immune-based therapy. Studies to validate these approaches to ameliorate autoimmunity will be necessary before their application in the clinic.

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## Background

Rheumatoid arthritis (RA) is an autoimmune disease marked by the infiltration of synovia and synovial compartments with dendritic cells (DCs), monocytes, T cells, B cells, neutrophils and natural killer (NK) cells.<sup>1</sup> DCs have a central role in the induction of immunity (Box 1; Figure 1). In peripheral tissues, DCs exist as immature cells, and undergo differentiation after exposure to proinflammatory cytokines, immune complexes that contain autoantibodies, or pathogens and endogenous inflammatory factors (for example, heat-shock proteins, high mobility group box-1 protein) that are recognized by Toll-like receptors (TLRs) expressed by DCs. Following migration to lymph nodes, mature DCs process acquired antigens onto major histocompatibility complex (MHC) molecules and present them to naive T cells. DCs also secrete cytokines, directing naive T cells toward different types of T helper (T<sub>H</sub>) cell: T<sub>H</sub>1, T<sub>H</sub>2 or T<sub>H</sub>17 cells.<sup>2</sup> Furthermore, DCs promote the differentiation and maturation of antibody-producing B cells (Figure 1).

DCs are also important for maintaining intrathymic and peripheral tolerance, and their depletion in animal models is associated with the onset of fatal autoimmune-type disease.<sup>3</sup> Under steady-state conditions, immature DCs recognize and phagocytose dying apoptotic cells during physiologic cell turnover,<sup>4,5</sup> rendering DCs tolerogenic:

they produce immunosuppressive cytokines and promote ‘cross-tolerance’. Responding T cells that are specific for the self-antigens contained within apoptotic cells are also rendered cross-tolerant, becoming anergic, acquiring a regulatory immunosuppressive phenotype (T<sub>REG</sub> cells), or undergoing activation-induced cell death.<sup>6</sup> Tolerogenic DCs induce hyporesponsiveness even in memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells.<sup>7</sup> Generally, this process does not result in DC maturation, but aberrations in this pathway—either failed clearance of dead cells and/or exposure of DCs to maturation signals (for example, endogenous inflammatory factors and pathogens)—abrogates their tolerogenic capacity.<sup>6,7</sup>

In this article, we discuss the role of DCs in the pathogenesis of RA in the context of other immune cells and soluble mediators, and evaluate emerging approaches to RA treatment that focus on DCs.

## Preclinical data

The synovium of RA patients is characterized by the perivascular accumulation of immature and mature DC subsets, in close association with T cells and B-cell follicles.<sup>1,8,9</sup> Synovial fluid from RA patients contains higher numbers of myeloid DCs (mDCs)<sup>10</sup> and plasmacytoid DCs (pDCs) (Box 1) compared to the blood, which signifies a role for these antigen-presenting cells in disease perpetuation.<sup>11</sup> Results from *in vitro* studies suggest that DCs migrate into the joint in response to locally produced cytokines and chemokines, or differentiate locally from myeloid progenitors in response to growth factors present in synovial fluid.<sup>12,13</sup>

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## Competing interests

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### Antigen presentation and cytokine production

DCs might contribute to ongoing inflammation through the presentation of autoantigens, as suggested by animal models of autoimmune arthritis,<sup>14</sup> or by producing pro-inflammatory factors (Figure 1). DCs derived from the joint or from monocytes can present human cartilage glycoprotein 39 and epitopes from synovial fluid constituents, respectively, to antigen-specific T cells.<sup>15,16</sup> Indeed, synovial DCs show evidence of activation *in vivo* in RA patients: upregulation of the expression of MHC molecules, co-stimulatory molecules, RelB,<sup>9</sup> and receptor activator of nuclear factor  $\kappa$ B (RANK) and its ligand (RANKL).<sup>17</sup> When stimulated *ex vivo* with immune complexes or TLR agonists, synovial DCs respond with an increased production of proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF).<sup>1</sup> DCs migrating into synovial fluid might undergo activation in response to locally produced cytokines or endogenous factors released from dying cells during inflammation.<sup>18</sup>

### Indirect contribution of DCs to RA pathogenesis

DCs might also indirectly contribute to RA pathogenesis. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a negative regulator of T-cell activation that is also expressed on T<sub>REG</sub> cells.<sup>19</sup> CTLA-4 expression on T<sub>REG</sub> cells suppresses DC activation by downregulating the co-stimulatory molecules CD80 and CD86,<sup>19</sup> but polymorphisms in the gene encoding CTLA-4 that might possibly reflect either a lack of effector T-cell blockade or reduced T<sub>REG</sub> cell activity at the level of DCs are associated with RA.<sup>20</sup>

The RA synovium is characterized by the formation of ectopic lymphoid organs resembling germinal centers. These centers contain plasma cells that express activation-induced cytidine deaminase and produce anti-citrullinated protein antibodies (ACPAs).<sup>21</sup> ACPAs and rheumatoid factor are presumed to bind to Fc receptors on macrophages and DCs, inducing their activation and consequent production of proinflammatory cytokines.<sup>22</sup> pDCs also accumulate in rheumatoid synovium and, through the production of type I interferon (IFN), might enhance autoantibody production.<sup>23</sup> Indeed, a subset of RA patients express a type I IFN signature that is associated with ACPA production.<sup>24</sup>

### DCs and T helper cell differentiation

RA was initially considered to be driven by T<sub>H</sub>1 cells. However, the identification of T<sub>H</sub>17 cells and IL-17 and IL-23 within affected tissues and/or fluids of patients has implicated both T<sub>H</sub>1 and T<sub>H</sub>17 cells in RA pathogenesis,<sup>25,26</sup> particularly as the p40 subunit (common to both IL-12 and IL-23) and the IL-23-specific p19 subunit are essential for joint inflammation in the collagen-induced arthritis mouse model.<sup>27</sup> DCs are required for the differentiation of T<sub>H</sub>1 and T<sub>H</sub>17 subsets from naive precursors (Figure 1). T<sub>H</sub>1 cells produce IFN- $\gamma$ , which activates macrophages and enhances the production of IL-1, IL-6 and TNF. IL-17 stimulates fibroblasts, endothelial and epithelial cells to produce IL-6 and IL-8, recruits neutrophils and

### Key points

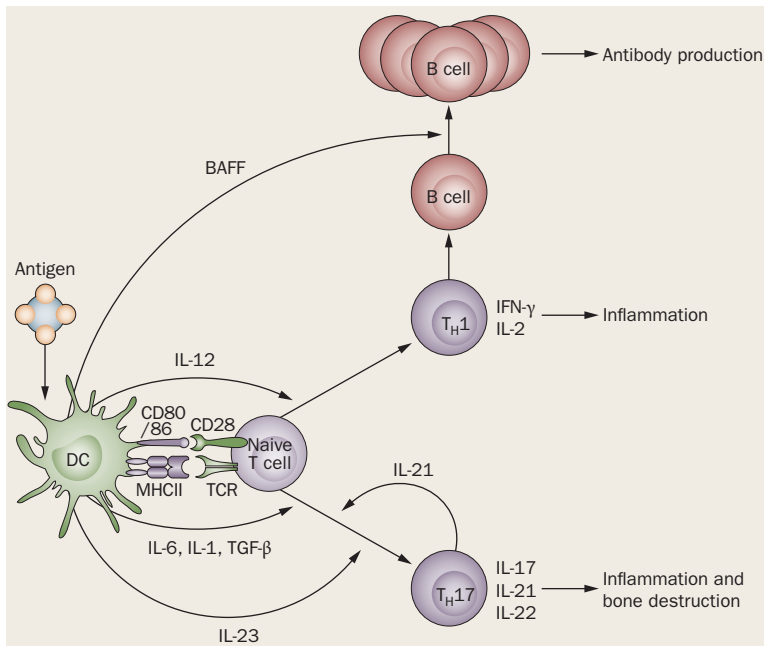
- Dendritic cells (DCs) are implicated in the pathogenesis of rheumatoid arthritis (RA)
- These cells and their products, such as tumor necrosis factor, interleukin-1 and interleukin-6, localize in rheumatoid synovium
- The proinflammatory products of DCs can be targeted to ameliorate RA
- DCs can also be manipulated to induce tolerance in animal models; studies are underway to test their tolerogenic activity in patients with RA

### Box 1 | Dendritic cells

- DCs are professional antigen-presenting cells abundant at body surfaces and within tissues, where they sense microbes and sample the environment for antigens
- On antigen capture, DCs migrate to lymphoid tissues, present processed antigens to naive T cells, and induce immunity or tolerance
- To activate T cells, DCs must undergo 'maturation', involving the upregulation of MHC, co-stimulatory molecules CD80, CD86, activation markers and cytokine production
- Depending on the stimulus, maturation confers on DCs the ability to differentiate naive T cells into T<sub>H</sub>1, T<sub>H</sub>2 or T<sub>H</sub>17 cells. Maturing DCs also express cytokines, such as BAFF, that induce the activation and maintenance of B cells
- For antigen uptake, DCs express several receptors: C-type lectin receptors recognize glycoproteins on microbes, Toll-like receptors recognize an array of molecules expressed by pathogens, and Fc $\gamma$  receptors recognize Ig-containing immune complexes and, consequently, constitute a link between humoral and cell-mediated immunity
- Two major subsets of DCs exist: plasmacytoid (CD123<sup>+</sup>, CD45RA<sup>+</sup>) and myeloid (CD11c<sup>+</sup>, CD45RO<sup>+</sup>), characterized by distinct origins, receptors and functions. Myeloid DCs can be further subdivided based on location and function
- DCs are important for maintaining tolerance in the thymus and periphery. Constitutive DC ablation breaks self-tolerance of CD4<sup>+</sup> T cells, causing autoimmunity
- DCs have multiple receptors for the uptake of apoptotic cells undergoing physiologic turnover, from which they acquire self-antigens, rendering them tolerogenic
- Tolerogenic DCs retain the ability to migrate to lymph nodes and cross-present antigens to T cells, but show resistance to maturation stimuli, decreased expression of CD80, CD86, low production of IL-12, and high production of immunosuppressive mediators, such as TGF- $\beta$ , IL-10 and IDO
- Tolerogenic DCs can induce cell death, anergy or a regulatory T-cell phenotype

Abbreviations: BAFF, B-cell activating factor belonging to the tumor necrosis factor family; DCs, dendritic cells; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; MHC, major histocompatibility complex; TGF- $\beta$ , transforming growth factor  $\beta$ ; T<sub>H</sub>, T helper cells.

monocytes,<sup>28,29</sup> and, in animal models, induces osteoclastic bone resorption.<sup>30,31</sup> Synovial tissue DCs express IL-12 p70 and the IL-23 subunit p19; these cytokines are essential for full differentiation of T<sub>H</sub>1 and T<sub>H</sub>17 cells, respectively, providing a mechanism by which their production is locally facilitated or perpetuated (Figure 1).<sup>23</sup> The presence of IL-17 has been reported in the synovial fluid and membrane of RA patients, with its expression predicting radiologic disease progression;<sup>30,32</sup> however, other reports suggest that this cytokine and T<sub>H</sub>17 cells are not abundant in rheumatoid synovial fluid.<sup>33</sup> Furthermore, variants of *IL12B* (which encodes the p40 subunit of IL-12)



**Figure 1** | DCs: mediators of immunity and tolerance. DCs activate naïve T cells through three signals: antigenic peptide on MHC molecules, co-stimulatory molecules (CD80, CD86) and cytokines. IL-12 from DCs promotes differentiation of  $T_H1$  cells, which produce IFN- $\gamma$  and IL-2, required for cell-mediated immunity.  $T_H1$  cells also induce B-cell differentiation into plasma cells; DCs, by producing BAFF, mediate proliferation of these antibody-producing cells. DC products, such as TGF- $\beta$ , IL-1 and IL-6, induce  $T_H17$ -cell differentiation. IL-23, produced by DCs, and IL-21, produced by  $T_H17$  cells, are necessary for proliferation and further maturation of  $T_H17$  cells.  $T_H17$  cells produce IL-17, which drives inflammation and bone resorption. IL-17 induces RANK expression on precursor osteoclasts and RANKL expression on osteoblasts and mesenchymal cells; TNF and IL-1 induce RANKL expression on synovial fibroblasts. Interaction of RANKL with RANK on osteoclast progenitors leads, ultimately, to bone resorption. In addition, cytokines secreted by DCs,  $T_H1$  and  $T_H17$  cells activate macrophages and induce nonhematopoietic cells to produce inflammatory cytokines (IL-1, IL-6 and TNF) and chemokines, which together lead to tissue destruction and inflammation. Abbreviations: BAFF, B-cell activating factor belonging to TNF family; DCs, dendritic cells; IFN- $\gamma$ , interferon  $\gamma$ ; IL, interleukin; MHC, major histocompatibility complex; RANK, receptor activator of nuclear factor  $\kappa$ B; RANKL, RANK ligand; TCR, T-cell receptor;  $T_H$ , T helper cells; TNF, tumor necrosis factor; TGF- $\beta$ , transforming growth factor  $\beta$ .

and *IL23R*, which have previously been associated with inflammatory diseases, do not seem to confer a significantly increased risk of developing RA.<sup>34</sup> Brentano *et al.*<sup>35</sup> have observed abundant expression of the p19 subunit, but not the p40 subunit, of IL-23 in RA synovial lining, indicating that bioactive IL-23 is not produced by synovio-cytes. Furthermore, levels of bioactive IL-23 did not differ significantly between RA and osteoarthritis synovial fluid.<sup>35</sup> Transgenic expression of p19 in mice leads to systemic inflammation,<sup>36</sup> so perhaps it contributes to RA through p40-independent mechanisms.<sup>35</sup> In summary, further verification for a role of  $T_H17$  cells and related cytokines in RA pathogenesis is required.

### Inducing DC tolerance

The development of animal models in which DCs are selectively depleted should enable the specific role of

these cells in the pathogenesis of autoimmune arthritis to be addressed.<sup>3,37</sup> Nevertheless, DCs can also be exploited to induce tolerance *in vivo*. In animal models, immunomodulation of immature DCs using immunosuppressive cytokines, genetic engineering, proteins and drugs confers tolerance by favoring  $T_H2$ -mediated immune responses over  $T_H1$ -mediated proinflammatory responses, or by inducing IL-10-producing  $T_{REG}$  cells, anergy or activation-induced cell death.<sup>38</sup>

Human immature DCs can be rendered tolerogenic *in vitro* by pre-exposure to autologous apoptotic cells (which express self-antigens), skewing T-cell priming and preventing the activation of memory T cells.<sup>6</sup> Ligation of individual receptors on human DCs that recognize apoptotic cells (such as CR3 and CR4) inhibits IL-12 production, generates transforming growth factor  $\beta$  (TGF- $\beta$ ) and specifically causes T-cell anergy and activation-induced cell death.<sup>7</sup> DC maturation can also be abrogated *in situ* using antibodies to C-type lectin receptors.<sup>39</sup> When complexed to antigens, antibodies to DEC-205 induce  $T_{REG}$  cells in animal models.<sup>40</sup> Similarly, C-type lectin receptors, such as the DC immunoreceptor, negatively regulate the function of DCs.<sup>41</sup> A comparison of these different approaches to inducing tolerogenic DCs is warranted to identify those most efficacious in inducing tolerance.

### Future clinical development

Patients who do not respond to DMARDs such as methotrexate require additional treatment with biologic agents, such as those that target TNF, IL-1 (produced by joint constituents, including DCs), B cells or co-stimulatory molecules on DCs. Anti-TNF therapies ameliorate clinical symptoms and also reduce the number of peripherally activated mDCs and pDCs *in vivo*; *in vitro*, the treatment diminishes DC maturation and their capability of producing proinflammatory cytokines and chemokines.<sup>13,42</sup> These observations reinforce the strategy of targeting mediators of inflammation and bone resorption, particularly at the level of the DC (Supplementary Table 1 online). Below, we review emerging therapies that focus on DCs and related targets to treat RA.

### Tolerogenic DCs

The administration of tolerogenic DCs, or even  $T_{REG}$  cells, might be treatment options for patients with recalcitrant disease. In the first human study to test the effects of tolerogenic DCs, we showed that injection of immature DCs pulsed with influenza matrix peptide resulted in the transient induction of antigen-specific, IL-10-producing, CD8<sup>+</sup>  $T_{REG}$  cells that blocked IFN- $\gamma$  production and cytolytic function by effector CD8<sup>+</sup> T cells.<sup>43</sup> The effect of injecting autologous, monocyte-derived DCs pulsed with a mixture of four citrullinated peptide antigens derived from vimentin, fibrinogen  $\alpha$  chain, fibrinogen  $\beta$  chain and collagen type II into patients with RA who express RA-associated *DRB1* alleles<sup>44</sup> and are ACPA-positive is currently being studied (R. Thomas, personal communication). The DCs are pretreated with

BAY 11-7082, an inhibitor of RelB nuclear translocation, which renders them tolerogenic, and then subsequently pulsed with the peptides.<sup>45</sup> If determined to be safe, it might be possible to advance tolerogenic DC therapy by simultaneously blocking co-stimulation using, for example, abatacept, which maintains DCs in their immature, tolerogenic form; this strategy has shown proven synergistic effects in animal models of transplantation.<sup>46</sup>

### Inhibitors of co-stimulation

T<sub>REG</sub> cells employ several mechanisms to maintain self-tolerance: production of TGF- $\beta$  and IL-10, inhibition of cell metabolism and blockade of DC function via CTLA-4, and sequestration from effector T cells.<sup>19,47</sup> Abatacept, a CTLA4-Ig fusion protein that modulates T-cell co-stimulation, has proven efficacious in RA patients who are resistant to DMARDs and anti-TNF therapy. The fusion protein might also enhance the expression in mDCs and pDCs of indoleamine 2,3-dioxygenase (IDO), an enzyme that metabolizes tryptophan to kynurenine and induces the differentiation of T<sub>REG</sub> cells from naive T cells.<sup>48–50</sup>

### Inflammatory cytokines

#### IL-6

IL-6 is a prototypic inflammatory cytokine found in RA synovial fluid.<sup>51</sup> Tocilizumab, an antibody that binds the IL-6 receptor, has proven efficacious in the treatment of RA in combination with DMARDs, and is currently in phase III trials (Supplementary Table 1 online).<sup>52</sup>

#### IL-23

IL-23 is required for the maturation of T<sub>H</sub>17 cells (Figure 1). Neutralizing antibodies to p40—ustekinumab and ABT-874—have shown promise against psoriasis and inflammatory bowel disease, but await testing in RA patients.<sup>53</sup> Targeting the p19 subunit of IL-23 might provide a more selective way of blocking T<sub>H</sub>17 differentiation without affecting T<sub>H</sub>1 cells, as T<sub>H</sub>1 cells are also required for immunity against pathogens.

#### IL-17

Clinical trials are underway to test agents that target IL-17 as well as IL-22, another cytokine produced by T<sub>H</sub>17 cells. Unlike T<sub>H</sub>1 cells, T<sub>H</sub>17 cells are resistant to suppression by T<sub>REG</sub> cells in animal models of autoimmunity.<sup>54</sup> However, T<sub>H</sub>17 cells directly stimulate the production of IL-6 and TNF at sites of inflammation; these cytokines abrogate T<sub>REG</sub> cell development. Current anti-IL-1 and anti-IL-6 therapeutics might also ameliorate inflammation by inhibiting T<sub>H</sub>17 cell differentiation.

### Inhibitors of osteoclastogenesis

RANKL is expressed by DCs, T cells (for example, T<sub>H</sub>17 cells) and fibroblast-like cells; its interaction with RANK on osteoclast progenitor cells induces osteoclastogenesis. Denosumab, an antibody against RANKL, has been shown to retard radiologic progression in RA.<sup>55</sup>

### Small molecule inhibitors

The intracellular kinases Janus kinase 3 (JAK3) and spleen tyrosine kinase (Syk) are involved in signaling through the common  $\gamma$  chain of cytokine receptors and/or receptors that have immunoreceptor tyrosine-based activation motifs (such as the Fc $\gamma$  receptor). As rheumatoid synovial tissue DCs express JAK3, inhibitors of JAK3 could conceivably block DC activation.<sup>53</sup> JAK3 and Syk inhibitors in RA have shown encouraging results in phase II trials.<sup>53</sup> Mutations in *STAT4*, which encodes a transcription factor that transduces signals from IL-12, IL-23 and type 1 IFN in T cells and monocytes, and *STAT3*, which encodes a transcription factor critical for T<sub>H</sub>17 cell differentiation, have been linked to RA.<sup>20,26</sup> These transcription factors, in addition to those governing T<sub>H</sub>17 differentiation (such as retinoic acid-related orphan receptor [ROR $\gamma$ t] and aryl hydrocarbon receptors), are potential attractive targets for RA therapy. A caveat of inhibiting transcription factors, however, is the potential for adverse effects as the pathways are common across multiple cell types.

### Outlook

DCs have the potential to treat autoimmunity by virtue of their natural ability to induce tolerance *in vivo*. The identification of relevant antigens in RA and optimal approaches to generating tolerogenic DCs will be required to maximize their immunomodulatory activity. Studies linking genetic alterations with RA make it conceivable that therapy might be further tailored to gain maximum benefit. Approaches to targeting inhibitory receptors on DCs (for example, CR3 and DEC-205) with antigen fused to antibodies are another option for inducing DC tolerance and preventing maturation. It is possible that other DC subsets, such as pDCs, could be used to induce tolerance: when activated via TLRs or following engagement of CD80 or CD86 with CTLA-4, pDCs express IDO and induce T<sub>REG</sub> cells.<sup>49,56</sup> Induction of IDO in animal models of arthritis controls the accumulation of pathogenic T cells at the site of inflammation,<sup>57,58</sup> and IDO-expressing DCs can reverse arthritis.<sup>58</sup> The combination of established therapies, along with active immunomodulation through the use of DC-based therapies, might further improve our ability to ameliorate autoimmunity in RA.

#### Review criteria

Our search efforts included the PubMed database and Cochrane Library using the term “rheumatoid arthritis” in association with specific terms, including “dendritic cells and autoimmunity”, “dendritic cells and regulatory T cells”, and “tolerogenic dendritic cells”. We reviewed all types of article, including original papers, Review articles and case reports published between June 1999 and June 2009. Efforts were made to refer to primary papers whenever possible, or to comprehensive Reviews. In addition, we searched clinicaltrials.gov to identify ongoing clinical trials using dendritic cells to treat autoimmune diseases and current therapies in clinical trials for rheumatoid arthritis.

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**Supplementary information**

Supplementary information is linked to the online version of the paper at [www.nature.com/nrrheum](http://www.nature.com/nrrheum)