Published in final edited form as:

Nat Rev Rheumatol. 2009 October ; 5(10): 566–571. doi:10.1038/nrrheum.2009.185.

Dendritic cells as targets for therapy for Rheumatoid Arthritis

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Abstract

Dendritic cells (DC) play a central role in the induction of immunity and also in tolerance in their role as professional antigen-presenting cells (APC). In the absence of DC, a fatal autoimmunity develops in animal models. While the role of DC has been investigated extensively in the pathogenesis of Rheumatoid arthritis (RA), it remains unclear if DC initiate autoimmunity in this disease. Nevertheless, evidence points towards a significant role for DC in its maintenance and progression. Current biological therapies of RA are designed to ameliorate disease by targeting downstream products of APC such as TNF α , IL-1 and IL-6. Emerging therapies for RA are exploiting the tolerogenic capacity of DC. “Tolerogenic” DC 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 (AICD), anergy, and/or regulatory T cells. Cells that are primed by DC such as B cells, T helper (Th)-1(Th1) and Th17 cells, and which 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 they are applied in the clinic.

Background

Rheumatoid arthritis (RA) is an autoimmune disease marked by infiltration of synovia and synovial compartments with dendritic cells (DC), monocytes, T cells, B cells, neutrophils and NK cells¹. In this perspective we discuss the role of DC in RA pathogenesis in the context of other immune cells and soluble mediators, and evaluate emerging approaches to treat RA that focus on DC.

DC play a central role in the induction of immunity (Box 1; Figure 1). In peripheral tissues DC exist as immature cells, and undergo differentiation after exposure to pro-inflammatory cytokines, immune complexes containing autoantibodies, or pathogens and endogenous inflammatory factors (e.g. heat shock proteins, high mobility group box (HMGB)-1 protein,) recognized by Toll like receptors (TLR), a family of pattern recognition receptors expressed by DC. Following migration to lymph nodes, DC process and present acquired antigens onto MHC molecules to naïve T cells and secrete cytokines resulting in skewing of naïve T cells toward T helper (Th) -1(Th1), Th2, or Th17 cells². They also promote differentiation and maturation of antibody-producing B cells (Figure 1A).

Search strategy: Our search efforts included PubMed and Cochran Library using the term “rheumatoid arthritis” and associated specific terms including dendritic cells and autoimmunity, dendritic cells and regulatory T cells, and tolerogenic dendritic cells. We reviewed primary papers, and review articles from June 1999 to June 2009. We reviewed all types of articles including original papers, reviews, case reports, etc. Efforts were made to refer to primary papers whenever possible and 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.

Competing interests: None

Box 1

- DCs are professional antigen presenting cells (APC), abundant at body surfaces and within tissues where they sense microbes and sample the environment for antigens.
- Upon antigen capture DC migrate to lymphoid tissues, where they present processed antigens to naïve T cells, and induce immunity or tolerance.
- DC must undergo a process of “maturation”, exemplified by the up regulation of MHC and costimulatory molecules (CD80/86), activation markers and cytokine production in order to activate T cells.
- Depending upon the stimuli, maturation of DC confers them with the ability to differentiate naïve T cells into Th1, Th2 or Th17 cells. Maturing DC also express cytokines that enable the activation of B cells and NK cells.
- For antigen uptake, DCs express a variety of receptors: C type lectin receptors (CLR) which recognize carbohydrate moieties of glycoproteins on microbes and Toll like receptors (TLR), pattern recognition receptors that recognize an array of molecules expressed by pathogens, Fcγ receptors (FcγR) which recognize Ig-containing immune complexes (IC) and as a result constitute a link between humoral and cell-mediated immunity.
- There are two major subsets of DCs, plasmacytoid DC (pDC, CD123+, CD45RA+) and myeloid DC (mDC, CD11c+, CD45RO+) characterized by distinct origins, receptors and functions. mDC can be subdivided into further subsets based on their location and function (e.g. Langerhans cells).
- DCs play a critical role in the maintenance of tolerance in the thymus and in the periphery. Constitutive ablation of DC breaks self-tolerance of CD4+ T cells and results in autoimmunity.
- DCs acquire self-antigens in the form of apoptotic cells undergoing physiologic turnover. DCs have multiple receptors for the uptake of apoptotic cells, the ligation of which renders them tolerogenic.
- Tolerogenic DC retain the ability to migrate to lymph nodes and cross present antigens to T cells, however, there is a decrease in co-stimulatory molecules (CD80/CD86), resistance to maturation stimuli, low production of IL-12, and high production of immunosuppressive mediators such as TGFβ, IL-10 and Indoleamine 2,3-dioxygenase (IDO).
- Tolerogenic DC can induce cell death, anergy or regulatory T cells.

DC also maintain intrathymic and peripheral tolerance, and in animal models their depletion is associated with the onset of fatal autoimmune-type disease³. Under steady state conditions, immature DC recognize and phagocytose dying apoptotic cells (AC) during physiologic cell turnover^{4, 5}, rendering DC tolerogenic; they produce immunosuppressive cytokines and promote “cross-tolerance”. Responding T cells specific for self-antigens contained within AC are rendered “cross-tolerant” becoming anergic, acquiring a regulatory immunosuppressive phenotype (Treg) or undergoing activation induced cell death (AICD; Figure 1B)⁶. These tolerogenic DC (TDC) induce hyporesponsiveness even in memory CD4+ and CD8+ T cells⁷. Generally this process does not mature DC, but aberrations in this pathway, either failed clearance of dead cells and/or exposure of DC to maturation signals (e.g. endogenous inflammatory factors, pathogens) abrogates their tolerogenic capacity^{6, 7}.

Preclinical data

Rheumatoid synovium is characterized by accumulation of immature and mature DC subsets perivascularly, in close association with T cells and B cell follicles^{1, 8, 9}. Synovial fluid (SF) contains significant numbers of myeloid DC (mDC)¹⁰ and plasmacytoid DC (pDC; Box 1) compared to blood, signifying a role for these APC in disease perpetuation¹¹. In vitro studies suggest that DC migrate into the joint in response to locally produced cytokines and chemokines, or differentiate locally from myeloid progenitors in response to growth factors contained within synovial fluid (SF)^{12, 13}.

DC may contribute to ongoing inflammation through presentation of autoantigens, as suggested by animal models of autoimmune arthritis¹⁴, or production of pro-inflammatory factors (Figure 1A). Joint DC and monocyte-derived DC can present human cartilage glycoprotein 39 (HCgp39), and epitopes from SF to antigen-specific T cells, respectively^{15, 16}. Indeed, synovial DC show evidence of activation in vivo: upregulation of MHC, co-stimulatory molecules, RelB⁹, expression of receptor activator of nuclear factor- κ B (RANK) and its ligand (RANKL)¹⁷, and heightened production of pro-inflammatory cytokines when stimulated ex vivo (IL-1, IL-6, TNF α) with immune complexes or TLR agonists¹. DC migrating into SF may undergo activation in response to locally produced cytokines, or to endogenous factors released from dying cells during inflammation¹⁸. DC may also indirectly contribute to RA pathogenesis. CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) is a negative regulator of T cell activation also expressed on Treg¹⁹. CTLA-4 expression on Treg suppresses DC activation by downregulating the costimulatory molecules CD80 and CD86¹⁹. CTLA-4 polymorphisms have been associated with RA, reflecting either a lack of effector T cell blockade or reduced Treg activity²⁰.

RA synovium is characterized by formation of ectopic lymphoid organs resembling germinal centers (GC). They contain plasma cells expressing activation-induced cytidine deaminase (AID) and producing anti-cyclic citrullinated peptide (ACPA) antibodies²¹. ACPA and rheumatoid factor (RF) are presumed to bind to Fc receptors on macrophages and DC, inducing their activation and production of pro-inflammatory cytokines²². pDC also accumulate in rheumatoid synovium and through production of type I IFN may enhance autoantibody production²³. Indeed a subset of RA patients express a type I IFN signature associated with ACPA production²⁴.

RA was initially considered to be Th1 driven. Identification of Th17 cells and the cytokines IL-17 and IL-23 within affected tissues and/or fluids, has implicated both Th1 and Th17 cells in its pathogenesis^{25, 26}, particularly as the p40 subunit (common to both IL-12 and IL-23), and the IL-23 specific subunit p19 are essential for joint inflammation in the collagen-induced arthritis mouse model²⁷. DC are required for differentiation of Th1 and Th17 subsets from naïve precursors (Figure 1A). Th1 cells produce IFN γ , activating macrophages and enhancing production of pro-inflammatory cytokines (IL-1, IL-6 and TNF α). IL-17 stimulates fibroblasts, endothelial and epithelial cells to produce IL-6 and IL-8, recruits neutrophils and monocytes^{28, 29}, and in animal models, induces osteoclastic bone resorption^{30, 31}. Synovial tissue DC express IL-12p70 and IL-23p19, cytokines essential for full differentiation of Th1 and Th17 cells respectively, providing a mechanism by which their production is locally facilitated or perpetuated²³ (Figure 1A). Although IL-17 has been located in RA synovial fluid and membrane, with expression predicting disease progression radiologically^{30, 32}, other reports suggest that it and Th17 cells are not abundant in rheumatoid synovial fluid³³. Furthermore, variants of *IL12B* (encoding the IL-12p40 subunit) and *IL23R*, previously associated with inflammatory diseases, do not appear to play a major role in RA risk³⁴. Recently, Brentano et al., found abundant expression of the p19 but not the p40 subunit of IL-23 in RA synovial lining, indicating that bioactive IL-23 is not produced by synoviocytes³⁵. Furthermore, levels

of bioactive IL-23 were not statistically significant between RA and osteoarthritis synovial fluids³⁵. As transgenic expression of p19 in mice leads to systemic inflammation³⁶, it may contribute to RA through p40 independent mechanisms³⁵. In summary, further verification for a role of Th17 cells and related cytokines in RA pathogenesis is required.

With the development of animal models that selectively deplete DC *in vivo*, the specific role of DC in pathogenesis of autoimmune arthritis can be addressed^{3, 37}. Nevertheless, DC can be exploited to induce tolerance *in vivo*. In animal models, immunomodulation of immature DC with immunosuppressive cytokines, genetic engineering, proteins and drugs propagates tolerance by skewing immune responses away from pro-inflammatory Th1 responses toward Th2 responses, or through induction of IL-10 producing Treg, anergy or AICD (reviewed in³⁸). Human immature DC can be rendered tolerogenic *in vitro* by pre-exposure to autologous AC (which express self-antigens) skewing T cell priming and preventing the activation of memory T cells⁶. Ligation of individual apoptotic cell receptors on human DC (CR3, CR4; Figure 1B) inhibits IL-12 production, elicits TGF β and specifically causes T cell anergy and AICD (Figure 1B⁷). DC can also be targeted *in situ* using antibodies towards C-type lectin receptors³⁹. When complexed to antigens, antibodies to DEC-205 induce Treg in animal models⁴⁰. Similarly, C-type lectin receptors like DcIR (dendritic cell immunoreceptor) negatively regulate DC⁴¹. Comparison of these different approaches to induce TDC is warranted to identify those most efficacious in inducing tolerance.

Future clinical development

Patients who do not respond to disease modifying anti-rheumatic drugs (DMARDs; e.g. methotrexate) require addition of biological agents targeting TNF α , IL-1 (produced by joint constituents, including DC), B cells or co-stimulatory molecules on DC. Anti-TNF targeted therapies ameliorate clinical symptoms and also reduce frequencies of peripheral activated mDC and pDC *in vivo*, and *in vitro* diminish their maturation and their ability to produce pro-inflammatory cytokines and chemokines^{13, 42}. These observations reinforce the strategy of targeting mediators of inflammation and bone resorption, particularly at the level of the DC (reviewed in Table 1). Below we review emerging therapies focusing on DC and related targets to treat RA.

Tolerogenic DC

The administration of TDC, or even Treg, may be options for recalcitrant disease. In the first human study to test TDC, we showed that injection of influenza matrix protein peptide-pulsed immature DC transiently induced antigen-specific, IL-10 producing, CD8⁺ Treg that blocked IFN γ production and cytolytic function by effector CD8⁺ T cells⁴³. Thomas et al. (University of Queensland, Brisbane, Australia) are injecting autologous, monocyte-derived DC 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 *DR-B1* alleles and are ACPA⁺⁴⁴. The DC are pretreated with BAY 11-7082, an inhibitor of RelB nuclear translocation, which renders them tolerogenic and then subsequently pulsed with citrullinated peptides⁴⁵. If determined to be safe, it may be possible to advance TDC therapy with the addition of costimulatory blockade e.g. abatacept which maintains DC in their immature, tolerogenic form and has proven synergistic effects in animal models of transplantation⁴⁶.

Inhibitors of costimulation

Treg employ several mechanisms to maintain self-tolerance: production of TGF β and IL-10, inhibition of cell metabolism and blockade of DC function via CTLA-4 and sequestration from effector T cells (reviewed in^{19, 47}). Abatacept, a CTLA4-Ig fusion protein that modulates T

cell costimulation, has proven efficacious in DMARD- and anti-TNF therapy resistant RA patients. The fusion protein may also enhance the induction in mDC and pDC of indoleamine 2',3' dioxygenase (IDO) an enzyme which metabolizes tryptophan to kynurenine and which induces the differentiation of Treg from naïve T cells^{48–50}.

Inflammatory cytokines

IL-6—We and others identified IL-6, a prototypic inflammatory cytokine in RA synovial fluid⁵¹. Tocilizumab, an antibody that binds the IL-6 receptor, has been proven efficacious for the treatment of RA in combination with DMARDs and is currently in phase III trials⁵² (Table 1).

IL-23—IL-23, is required for the maturation of Th17 cells (Figure 1A). Neutralizing antibodies to p40, ustekinumab and ABT-874, have shown promise in patients with psoriasis and inflammatory bowel disease but await testing in RA⁵³. Targeting the p19 component of IL-23 may be a more selective way to block Th17 differentiation while keeping Th1 cells unaffected, as they are also required for immunity against pathogens.

IL-17—Clinical trials are underway targeting IL-17 as well as IL-22, a product of Th17 cells. Unlike Th1 cells, Th17 cells are resistant to Treg-mediated suppression in animal models of autoimmunity⁵⁴. However, they directly stimulate production of cytokines (IL6 and TNF α) at the site of inflammation, which abrogate Treg-mediated suppression. Current anti-IL-1 and IL-6 therapeutics may ameliorate inflammation by also inhibiting Th17 differentiation.

Inhibitors of osteoclastogenesis

RANKL is expressed by DC, T cells (e.g. Th17 cells) and fibroblast-like cells and interaction with RANK on osteoclast progenitors induces osteoclastogenesis. Denosumab, an antibody against RANKL has shown retardation of radiologic progression in RA⁵⁵.

Small molecule inhibitors

The intracellular kinases JAK-3 and Syk kinases are involved in signaling through the common γ chain of cytokine receptors and/or receptors, which have immuno-receptor tyrosine-based activation motifs (e.g. Fc γ receptor). As rheumatoid synovial tissue DC express JAK3, (in addition to STAT1, STAT4 and STAT6⁵⁶), these inhibitors could conceivably block DC activation⁵³. JAK3 and Syk inhibitors in RA have shown encouraging results⁵³. Mutations in *STAT4*, a transcription factor that transduces signals from IL-12, IL-23 and type 1 interferon in T cells and monocytes, and *STAT3*, a critical transcription factor for Th17 cell differentiation have been linked to RA. These transcription factors in addition to those governing Th17 differentiation (ROR γ t, aryl hydrocarbon receptors) are potential attractive targets for RA. A caveat of inhibiting transcription factors, however, is the potential of side effects since the pathways are common across multiple cells.

Outlook

DC are potential tools to treat autoimmunity because of their natural ability to induce tolerance in vivo. The identification of relevant antigens in RA and optimal approaches to generate TDC will be required to maximize their immunomodulatory activity. Studies linking genetic alterations with RA make it conceivable that therapy can be further tailored to gain maximum benefit. Approaches to target inhibitory receptors on DC (e.g. CR3, DEC-205) with antigen fused to antibodies are another option. Advantage could be taken of other DC subsets (pDC), to induce tolerance. When activated via TLRs or following engagement of CD80/CD86 with CTLA-4, pDC express IDO and induce Treg^{49, 57}. Induction of IDO in animal models of arthritis controls accumulation of pathogenic T cells at the site of inflammation^{58, 59}, and IDO-expressing DC are able to reverse arthritis⁵⁹. The combination of established therapies along

with active immunomodulation through the use of DC-based therapies may further improve our ability to ameliorate autoimmunity in RA.

Acknowledgments

Some of the studies cited in this review were supported by NIH grant RO1 AIO71078 and an Alliance for Lupus research grant to NB.

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Figure 1. DC: mediators of Immunity and tolerance

A. Role of DC in differentiation of TH1/TH17 cells: potential effectors in RA. Cytokine production by DC and the subsequent differentiation of naïve T cells is determined by locality and exposure to environmental stimuli (pathogens, necrotic debris, and immune complexes). DCs activate naïve T cells through three signals: presentation of antigenic peptide on MHC molecules, co-stimulatory molecules (CD80/86) and immunomodulatory cytokines. The production of IL-12 by DC strongly favors differentiation of naïve T cells towards the Th1 pathway. Th1 cells produce IFN γ , which is required for cell mediated immunity. Th1 cells also activate B cells to differentiate into plasma cells. DCs produce B cell activating factor (e.g. BAFF) mediating proliferation of the antibody producing B cells. DC products, activated TGF β and the pro-inflammatory cytokines (IL1, IL-6) induce the differentiation of Th17 cells from naïve T cells. DC can augment local TGF β levels by converting exogenously produced latent TGF β (TGF β associated with latency associated protein (LAP)). The binding of LAP to $\alpha_v\beta_8$ on DC results in its processing and activation of TGF β ⁶⁰. IL-23, an IL-12 family member produced by DC, and IL-21 produced endogenously by Th17 cells, are necessary for proliferation and further maturation. Th17 cells produce IL-17, a pro-inflammatory cytokine that drives inflammation and bone resorption. IL-17 induces expression of RANK on precursor osteoclasts. RANKL expression is upregulated on osteoblasts and mesenchymal cells by IL-17, while TNF α and IL-1 induce RANKL expression on synovial fibroblasts. Interaction of RANKL on these cells with RANK on osteoclast progenitors induces osteoclastogenesis, generation of multinucleated osteoclasts which initiate bone resorption. In addition, cytokines secreted by DC, Th1 and Th17 cells activate macrophages and induce nonhematopoietic cells to produce inflammatory cytokines (IL-1, IL-6 and TNF α), chemokines and matrix metalloproteinases (MMP) which altogether lead to tissue destruction and inflammation.

B. For apoptotic cell uptake, DCs express a variety of receptors: CD91, the integrins $\alpha v\beta 3$ and $\alpha v\beta 5$, scavenger receptors (e.g. CD36), the $\beta 2$ integrins, MER, $\beta 2$ GPI receptor, CD14, ABC-1, Lectins, Stabilin, BAI, and Tim-4. Upon uptake of apoptotic cells or apoptotic microparticles, DCs are rendered tolerogenic. Tolerogenic DC downregulate co-stimulatory molecules (CD80/86) and on interaction with T cells, induce anergy or activation induced cell death. DCs can also produce TGF β , a cytokine necessary for differentiation of naïve T cells to Treg⁶¹. Treg have a critical role in the maintenance of self-tolerance by several mechanisms including the production of anti-inflammatory cytokines TGF β and IL-10. These and other factors block the proliferation and induce the death of CD4⁺ T cells. Cytotoxic T lymphocyte antigen 4 (CTLA-4), a molecule constitutively expressed on Treg, has a key task in maintaining self tolerance¹⁹. CTLA-4 interacts with the co-stimulatory molecules CD80 and CD86 on DCs, delivering inhibitory signals which result in their down-regulation. This interaction also inhibits maturation of DC thereby diminishing the potency of DC-dependent activation of effector T cells.