ARTIGO DE REVISÃO

DENDRITIC CELL SUBSETS: THEIR ROLES IN RHEUMATOID ARTHRITIS

Maria C. Lebre, Paul P. Tak

Introduction

Dendritic cells (DC) play a pivotal role in orchestrating T cell immunity and tolerance due to their ability to stimulate naive T cells and direct effector cell function.\(^1\) Human DC have been divided into two different subsets: myeloid (m) or conventional (c) DC and plasmacytoid (p) DC. These subsets constitute a heterogeneous population that consists of differences in tissue distribution, phenotype and function.\(^2\)

Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with the destruction of affected joints and represents one of the most common autoimmune-related diseases, affecting as much as 1% of Western populations. Clinically, RA manifests as a symmetric polyarthritis associated with swelling and pain in multiple joints, often ini-
DENDRITIC CELL SUBSETS: THEIR ROLES IN RHEUMATOID ARTHRITIS

Dendritic cells (DCs) are crucial components of the immune system, playing a central role in the regulation of immune responses. They are known for their ability to facilitate the differentiation of naive T cells into effector cells and play a key role in the development of autoimmune diseases such as rheumatoid arthritis (RA). In RA, DCs are found in the synovial fluid and tissue, contributing to the pathogenesis of the disease.

**DC subsets**

As mentioned above, DCs constitute a heterogeneous population of antigen-presenting cells (APCs) characterized by differences in tissue distribution, phenotype, and function. It has become possible to identify the different subsets within a DC population through their differential expression of surface markers. In human peripheral blood (PB), the DC subsets include CD1c+ and CD16+ mDCs, one of plasmacytoid origin CD123+ pDC, which are involved in the production of type I IFNs.

**DC subsets in RA**

Since the discovery of changes in the DC subsets in other autoimmune diseases (e.g., systemic lupus erythematosus, SLE), various studies have been performed that addressed both local and systemic levels of DC subsets in RA.

DCs have been identified in rheumatoid synovium, both by several groups over the last 15 years, and their origin, function, and potential role in the pathogenesis are not fully understood. One of the first reports described an mDC population (CD33+CD14-) present in inflamed RA synovium, which has been described but not well functionally characterized. It has been reported that CD1c+ mDCs specifically express CCR9, TLR3, and high levels of CD40. The increased CD40 expression by the CD16+ and CD1c+ mDC subsets possibly reflects a continuum of activation. CD123+ pDCs are present in synovial fluid and SF. When pDCs are exposed to immune complexes consisting of anti-double stranded DNA or upon viral or bacterial infection, high amounts of type I IFN, IFN-α and IFN-β are produced.

Another DC subset, that is absent in the peripheral blood, is the CD1a+ mDC. This DC subset originates from blood-derived CD14+ monocytes and is called mo-DC. The ability of monocytes to differentiate into DC was originally demonstrated by Sallusto and Lanzavecchia, who reported the generation of DC from human peripheral monocytes after *in vitro* culture with GM-CSF + IL-4. Over the last 10 years, this method has prompted numerous studies on human DC that were previously hampered by the difficulties in working with *ex vivo*-isolated human DC, and it has proven to be an extremely powerful tool for the study of human DC differentiation and maturation processes.
Myeloid DC in RA

**CD1c⁺BDCA1⁺ mDC**

As stated above, CD1c⁺ mDC are able to produce IL-12p70 in response to TLR ligands and CD40L.7 Recently, CD1c⁺ mDC have been associated with induction of chemotaxis in response to TLR ligands, due to their capacity to produce elevated levels of various chemokines and in particular CXCL8/IL-8.9

In RA patients, CD1c⁺ mDC are present in different compartments: PB, and SF(MC Lebre et al. Am J Pathol. in press and Figure 1, left panel). We have shown that circulating PB CD1c⁺ mDC were significantly reduced in RA compared to healthy controls with reduced CD62L expression. In RA SF, these cells were also present, but they displayed a more mature phenotype compared to PB. In addition, the number of PB circulating mDC in RA was significantly inversely correlated to C-reactive protein.18 In RA ST these cells are in the vicinity of T cells and express the cytokines IL-12p70 and IL-23p19, important for the induction/expansion of the Th1 and Th17 T cell subsets, respectively (MC Lebre et al. Am J Pathol. in press). Importantly, most of the CD1c⁺ mDC present in RA ST are CD83⁺ and DC-LAMP⁻. This DC subset may represent recently activated DC (because they express cytokines) that not yet up-regulated the maturation markers. Another explanation is that mature DC have migrated, upon activation, from the inflamed ST into the draining lymph node.

**CD1a⁺ mDC**

PB CD14⁺ monocytes are able to differentiate into CD1a⁺ mDC (mo-DC).13 CD1a is one of the classical markers that is absent in all blood mDC and pDC subsets, and it is considered the specific marker for mo-DC.14 Recently, it was reported that mo-DC do exist and differentiate in vivo in a *Leishmania* infection model.19 This report provided for the first time evidence that in vitro differentiated mo-DC is not an in vitro artifact but reflect a population that is present in vivo.

We (Figure 2A) and others have demonstrated the presence of CD1a⁺ mDC in RA synovium.20-24 Later, mature CD1a⁺ mDC were reported to be primarily located in the perivascular areas and ectopic lymphoid-like structures within the RA ST.25 Altogether, a role for CD1a⁺ mDC in disease initiation and perpetuation has been proposed.

Interestingly, this DC subset has been associated with the presentation of human cartilage glycoprotein 39 (HCgp39, or YKL-40), an RA candidate autoantigen, both in vivo and in vitro.27,28 Presentation of the immunodominant epitope of HCgp39 by synovial DC, in the context of the shared epitope, was associated with characteristic histological features of follicular synovitis and was found to be highly specific for RA.29

CD1a⁺ mDC have also been reported to express high levels of Jak3, STAT4, and STAT6 in seropositive (rheumatoid factor positive) RAST.24 This study suggested that these markers might be alternative markers for DC in their early stages of activation, providing a tool for identifying RA at the level of the synovium. Moreover, the authors proposed that Jak3 inhibition might be a potential therapeutic target to prevent DC maturation in RA, thereby influencing T cell activation. Recently, the same group has extended their initial observations and reported that in RA ST Jak3⁺ DC were not confined to a single DC subset, with cells having phenotypes consistent with both myeloid- and plasmacytoid-type DC. Moreover, the activation status of these DC might suggest that DC were maturing or were fully matured,30 which is in line with our own observations (MC Lebre et al. Am J Pathol. in press).

DC may also be involved in the

![Figure 1. Expression of three DC subsets in RA synovial tissue. Immunohistochemistry staining of frozen sections from a representative RA ST using specific antibodies against CD1c/BDCA1 (mDC), CD141/BDCA3 (mDC) and CD304/BDCA4 (pDC). Arrows indicate red positive staining. Original magnification 250x.](image)
process of joint destruction in RA. The receptor activator of NF-κB (RANK)/RANK ligand (RANKL) pathway is critical in this process. In this respect, it is of particular interest that in RA ST immature CD1a+ mDC expressed both RANK and RANKL, while some mature DC-LAMP+ DC expressed only RANK. In this study, RANK expression appeared to be limited to the sites of inflammation (RA synovium). As RANK/RANKL interactions could be important for DC-T cell interactions during the inflammatory process, therapeutic control of these targets may have both positive and negative consequences for the immune system.

Several studies using in vitro generated RA mo-DC (CD1a+ mDC) allowed more detailed investigations of the roles of these cells in RA. The first study demonstrated that in contrast to normal PB DC precursors, mo-DC derived from RA SF were resistant to the immunosuppressive effect of IL-10 in vitro. This unresponsiveness might be regulated through modulation of cell surface IL-10R1 expression or signaling. These data imply that synovial CD1a+ mDC maintain their inflammatory potential, even in the presence of an anti-inflammatory cytokine, contributing in this way to RA synovial inflammation. Interestingly, the existence of DC progenitors and mDC growth factors in RA SF supports the concept that RA SF may be a reservoir for joint-associated DC and reveals a compelling mechanism for the amplification and perpetuation of DC-driven responses in the RA joint, including inflammatory-type Th1 responses and probably Th17 responses.

Moreover, RA SF inducible heat shock protein (ihsp)70 exhibited chaperoning potential, as indicated by the capture of ihsp70 present in RA SF in the surface of normal mo-DC. These data indicate that ihsp70 might chaperone autologous antigens into immature RA SF DC via hsp receptors (hspR), and that cross-talk between DC co-expressing hsp/hspR could reflect a disease process in RA. Several studies have investigated the role of Fcγ receptors (FcγR) in RA mo-DC. Mature mo-DC from RA patients showed a markedly increased production of IL-1, IL-6, TNF-α, and IL-10 compared with DC from healthy controls. When mo-DC (immature and mature) were triggered by FcγR the production of pro-inflammatory cytokines IL-1 and IL-12p70 decreased. Triggering of FcγR-independent mechanisms using IFN-γ increased the production of pro-inflammatory and Th1 cytokines, which was more pronounced in RA. FcγR dependent pathways influence cytokine production by DC. These data suggest that a skewed balance towards pro-inflammatory and Th1 cytokines in RA can, at least partly, be restored by triggering FcγR on DC in RA and may lead to new strategies to abrogate Th1-driven inflammatory processes in RA. In addition, immature DC from patients with active RA but not from patients with inactive RA or healthy controls markedly up-regulated FcγRII. Mature DC from patients with active RA also lacked the physiological down regulation of FcγRII that occurs upon maturation in control groups. FcγR-dependent stimulation of DC using antigen-IgG immune complexes (IC) significantly increased TNF-α production by DC from healthy controls, but significantly decreased TNF-α by RA DC. Overlapping expression patterns between FcγRII and DC-LAMP in the ST of patients with RA may imply that in vivo, mature DC express increased levels of FcγRIlb. Moreover, RA mo-DC have increased expression of CCL18, CCL19 and CCL17 and this expression is partly regulated by FcγR triggering and results in an inhibitory DC subtype in RA upon FcγR-mediated triggering. Of particular interest, RA mo-DC lacked the IL-13-
mediated increase of FcγRII expression, with clear functional consequences. RA DC co-cultured with IL-4 already displayed an inhibitory DC phenotype, but this inhibitory phenotype was not augmented by the addition of IL-13. The defective FcγRII regulation was further substantiated by the finding that IL-13-generated DC from healthy controls increased antigen uptake capacity, whereas RA IL-13-generated DC did not. These data suggest that IL-13, by regulating the expression of FcγRII in normal subjects but not in RA, potentially contributes to the chronic pro-inflammatory immune reaction in RA. 39 Finally, it was reported that the functional variant of the inhibitory FcγRIIb (CD32B) is associated with the rate of radiological joint damage and DC function in RA. 40

TLRs are involved in the regulation of DC activation and cytokine production. 41 In this respect, TLR2- and TLR4-mediated stimulation of mo-DC from RA patients resulted in markedly higher production of inflammatory mediators (TNF-α and IL-6) compared to DC from healthy controls. 42 The authors suggested that various TLR ligands in the joint may trigger multiple TLRs simultaneously, favoring the breakthrough of tolerance in RA.

Another cytokine that is increased by mo-DC in RA upon TLR triggering is macrophage migration inhibitory factor (MIF). 43 MIF is elevated in the serum of patients with RA to concentrations that are sufficient to induce leukocyte activation in vitro. 44 MIF production by DC may thus play a potential role in the amplification of the inflammatory loop.

Despite recent data indicating that mDC may be over-represented in RA, relatively little is known about the mechanisms promoting differentiation along specific DC pathways within distinct joint microenvironments. 44 Immature human mo-DC transdifferentiated into functional osteoclasts in the presence of M-CSF and RANKL. Importantly, this process was greatly enhanced by RA SF and involved pro-inflammatory cytokines such as IL-1 or TNF-α, as well as components of the extracellular matrix as hyaluronic acid. 45 DC-derived osteoclasts may thus represent key therapeutic targets in inflammation-induced bone resorption.

In addition, a unique natural killer (NK) cell subset (CD3 CD56bright) that accumulates in lymph nodes and chronically inflamed tissues triggered CD14+ monocytes to differentiate into potent Th1-promoting DC. This process required direct contact of monocytes with NK cells and it was mediated by GM-CSF and CD154 derived from NK cells. It is noteworthy that RA SF, but not osteoarthritis SF, induced monocytes to differentiate into DC. However, this process occurred only in the presence of NK cells. 46 NK cells might play a role in the maintenance of Th1-mediated inflammatory diseases such as RA by providing a local milieu for monocytes to differentiate into DC. 46

We have recently observed that RA synovial fibroblasts or their soluble factors present in conditioning medium are able to induce the generation of CD1a- mDC from peripheral blood monocytes (MC Lebre et al., unpublished observations). Taking together, these data suggest an important role for the synovial microenvironment in the commitment of monocyte-derived cells and might support the generation of the synovial CD1a- mDC pool.

**CD141 (BDCA3)- mDC**

To present no data on this peculiar DC subset in RA has been reported, except that CD141/
CD16+ mDC

Randolph et al. have demonstrated that CD16+ monocytes are able to differentiate into DC in vitro, in the absence of exogenous cytokines, by using an experimental system of reverse transendothelial migration. So far, the existence of CD16+ mDC in RA has not yet been reported, probably due to the lack of specific antibodies that characterized this DC subset. In this respect, the CD16 molecule is also expressed by human NK cells. NK cells are phenotypically defined as CD3–CD56+ lymphocytes. In healthy controls, CD56dimCD16bright cells represent at least 90% of all NK cells. As the majority of the SF NK cells lack CD16 expression is tempting to speculate that in RA ST CD16+CD14- are CD16+ mDC (Figure 2B, single positive cells seen in green).

Plasmacytoid DC in RA

CD123+/CD303(BDCA2)+/CD304(BDCA4)+ pDC

Plasmacytoid DC constitute a rare population of immature DC, which lack myeloid markers, show plasmacytid morphology, and differentiate in vitro into mature DC following stimulation with CD40L, viruses, and bacterial DNA. Importantlly, pDC have been shown to induce T-dependent and -independent B cell differentiation into antibody-producing plasma cells.

In 2004, Lande et al. reported for the first time the presence of pDC in RA. In this study, the percentage of pDC, identified as a population of Lin–CD123++ cells, was 4- to 5-fold higher in RA SF and psoriatic arthritis (PsA) SF compared with osteoarthritis SF. The morphological and immunophenotypic characterization of isolated pDC from RA SF showed that these cells were in an immature state, most likely due to inhibitory factors present in RA SF. However, pDC were still able to undergo maturation when exposed ex-vivo to viral agent or unmethylated DNA. In addition, CD123+ and BDCA2+ pDC were detected by immunohistochemistry in RAST in which expression of the IFN-α-inducible protein MxA was also found, suggesting production of type I IFN by maturing pDC.

DENDRITIC CELL SUBSETS: THEIR ROLES IN RHEUMATOID ARTHRITIS

Differentiated DC and other APC are characterized by the nuclear location of RelB, a member of the nuclear factor (NF-κB/Rel family. Nuclear RelB CD123+ pDC were located in perivascular regions of RA, with similar frequency compared to nuclear RelB–CD123- mDC, but not normal ST sublining. This study also reported that the numbers and phenotypes of SF pDC were similar compared to those of normal PB pDC. In terms of antigen presentation capacity, PB pDC were less efficient than mDC. However, RA SF pDC efficiently activated resting allogeneic PB T cells. Thus, pDCs are recruited to RA ST and comprise an APC population that might contribute significantly to the local inflammatory environment.

We have shown that circulating PB CD303+/BDCA2+ pDC (and also CD1c+ mDC) numbers were significantly reduced in RA patients compared with healthy controls and displayed an immature phenotype with low CD62L expression. In RA SF pDC were present with the mDC:pDC ratio significantly exceeding that in matched peripheral blood. pDC isolated from RA SF also displayed an immature phenotype however, displayed a more mature phenotype (increased expression of CD80, CD83 and CD86) compared with PB mDC. Since pDC maturation is incomplete in the inflamed synovial compartment, immature pDC in SF may contribute to the perpetuation of inflammation via sampling of the inflamed synovial environment, and in situ presentation of arthritogenic antigen.

To extend these observations we have extensively analyzed the distribution and phenotype of pDC within, and between RA, PsA and inflammatory OA synovia (MC Lebre et al. Am J Pathol. in press). CD304/BDCA4+ pDC numbers exceeded CD1c+ mDC only in RA, with the majority of infiltrating DC displaying an immature phenotype. Analysis of in situ cytokine expression revealed that pDC expressed IL-15, IL-18, IFN-α and IFN-β. In general, our results indicate that synovial DC (CD304/BDCA4+ pDC and CD1c+ mDC) might play an important role in synovial inflammation, in part via production of inflammatory cytokines. Importantly, type I IFNs released by pDC might also play a role in (auto-)antibody production by B cells. In line with this hypothesis is of particular interest that synovial pDC numbers were especially increased in RA patients who were positive anti-citrullinated peptide antibody (ACPA) and that synovial pDC numbers are positively correlated with the ACPA serum levels (MC Lebre et al. Am J Pathol. in press). Thus, immunomodulation by targeting synovial DC may provide a novel anti-rheumatic strategy.
Targeting DC in RA

RA is one of the most extensively studied diseases with regards to the tissue-specific attack of the joints leading to joint and bone destruction. In line with the studies on the potential roles of DC in RA, agents that inhibit DC differentiation and function might have therapeutic potential in the treatment of RA.

In this respect, the first report on the effect of therapy in RA DC showed that non-steroidal anti-oestrogens inhibited the differentiation of synovial macrophages into DC and the capacity of SF macrophage-derived DC to stimulate allogeneic T cells.58

The biologic agent anti-TNF has proven to be effective in treating patients with RA. 59 Analysis of the phenotype of circulating DC in RA patients before and after treatment with infliximab (at 24 h and 6 months) was recently undertaken and the correlation between these changes and the clinical response to treatment was assessed.60 A decrease in circulating CD11c+ mDC and, to a lesser extent, CD123+ pDC percentages was found after infliximab therapy. The expression of CD83, the most important activation marker for DC, was also shown to be decreased 24 h after infliximab therapy. After 6 months of treatment, all patients showed significant clinical improvement and expression of the activation marker on DC remained low. In conclusion, this study supports the role for TNF-α blockade in preventing the maturation of circulating DC and in reducing the expression of their activation markers. Although the clinical response to infliximab was not observed after 24 h, circulating DC activation was strongly reduced by anti-TNF-α therapy. After 6 months of treatment, all patients showed significant clinical improvement and expression of the activation marker on DC remained low. In conclusion, this study supports the role for TNF-α blockade in preventing the maturation of circulating DC and in reducing the expression of their activation markers. Although the clinical response to infliximab was not observed after 24 h, circulating DC activation was strongly reduced by anti-TNF-α therapy. After 6 months of treatment, all patients showed significant clinical improvement and expression of the activation marker on DC remained low. In conclusion, this study supports the role for TNF-α blockade in preventing the maturation of circulating DC and in reducing the expression of their activation markers.

DC in experimental arthritis

Data on the role of DC subsets in experimental arthritis are lacking. The only study that points to a role for DC in collagen-induced arthritis (CIA) in mice (the most common animal model for human RA used) was demonstrated by Leung et al.63 These authors demonstrated that presentation of collagen-derived peptides by mature bone-marrow derived-DC is sufficient for the induction of arthritis in DBA/1 mice and that CIA could be inhibited by treatment with TNF antagonists. Importantly, CIA was antigen-specific, as transfer of control, unpulsed DC, or DC pulsed with ovalbumin did not induce arthritis. In contrast to other experimental arthritis models, DC-induced arthritis localized to the site of injection and did not spontaneously generalize to uninvolved joints, despite the demonstration of circulating collagen-reactive T cells. Histological analysis also revealed that DC induced arthritis (DCIA) exhibited extensive synovial hyperplasia and the appearance also resembled those of complete Freund’s adjuvant-induced CIA and RA ST. Furthermore, collagen-pulsed DC were observed in the T cell areas of popliteal and inguinal lymph nodes and some of the CD4+ T cells associated with these DC were activated. This supports the role for DC in the inductive phase of arthritis in this model, involving activation of T cells in local lymph nodes.

In a recent study with adjuvant-induced arthritis (AA) in rats, putative DC in normal rat synovial rich tissues (SRT) were characterized.64 Flow cytometry showed that approximately 25% of the cells expressed CD45 and that approximately 60% of these CD45+ cells expressed surface and/or cytoplasmic MHC class II molecules. Three subsets of putative APC were identified: CD45+MHCIIlo, CD45+MHCIIhi and CD45+MHCII-. The MHCIIhi cells had the phenotype CD11c+, CD163+ and CD68+, similar to that of rat DC.65 However, further
studies are required to define the precise cellular origins of DC and the factors responsible for the maturation of DC in rat inflammatory arthritis. T cell–induced inflammation in synovium is accompanied by an increase in mDC, macrophages and an incompletely characterized subset of MHC IIhiCD11b- non-lymphoid cells. The presence of many MHC IIhi monocyte-like cells in inflamed SRT suggests that differentiation of monocytes is deviated towards DC, while the smaller proportion of “indeterminate” cells and the greater numbers of cells that expressed co-stimulatory molecules suggested that these cells will be different functionally from those in normal SRT.

Most of the present data on DC in experimental arthritis reported the use of DC as a therapeutic tool to ameliorate arthritis. In this respect, vaccination of mice with CIA with immature or LPS-activated DC had no significant effect on the disease course; administration of antigen-loaded, TNF- or IL-10-modulated DC propagated in GM-CSF with or without IL-4 resulted in a delayed onset of arthritis and a lower clinical score. The response was antigen-specific, since TNF-treated DC pulsed with a control antigen did not modify the disease course. A specific decrease in the collagen-specific «Th1-associated» IgG2a response was observed, whereas IgG1 titers were unaffected. These findings provide a rationale for immunotherapy using DC in RA.

In addition, it was recently reported that repetitive immature DC injections triggered the expansion of a novel regulatory population with high immunomodulatory properties, able to protect mice from CIA. These regulatory T cells were characterized by the expression of the CD49b molecule and produced the anti-inflammatory cytokine IL-10. Thus, together these data demonstrated that immature DC can expand and activate a novel regulatory population of CD49b+ T cells, with high immunosuppressive potential able to mediate protection against a systemic autoimmune disease.

To gain a better understanding of the abilities and mechanisms by which immunomodulatory DC might influence the outcome of T cell responses, several immunomodulatory DC (TNF-, IL-10-, or dexamethasone-stimulated bone marrow-derived DC) were studied side by side for their ability to modulate T cell responses and autoimmune diseases. This report showed that differentially modulated DC display a different composition of molecules involved in T cell activation. Although all DC subsets analyzed were able to inhibit the induction of CIA in mice, the modulation of the underlying immune response was different. Vaccination with TNF- or IL-10-modulated DC altered the Th1/Th2 balance as evidenced by the induction of IL-5- and IL-10-secreting T cells and the concomitant reduction of the IgG2a-IgG1 ratio against the immunizing antigen. In contrast, DC modulated with dexamethasone did not affect the ratio of IL-5-producing versus IFN-α-producing cells and tended to affect the antibody response in a non-specific manner. These data indicate that distinct mechanisms can be used by distinct DC subsets to change the outcome of autoimmunity.

In another model of experimental arthritis, namely antigen-induced arthritis in C57BL/6 mice, it was shown that injection with antigen-(methylated BSA, mBSA)-exposed BAY 11-7082-treated DC resulted in suppressed inflammation and erosion, but not in those that received control antigen-exposed BAY 11-7082-treated DC. Clinical improvement was dependent on IL-10 and was associated with antigen-specific suppression of the delayed-type hypersensitivity reaction and switching of anti-mBSA antibody isotype from IgG2b to IgG1 and IgA. Suppression could be reversed by intra-articular administration of IL-1α and could be restored by a second injection of mBSA-exposed BAY 11-7082-treated DC. In conclusion, BAY 11-7082-treated DC induced antigen-specific immune suppression in this model of inflammatory arthritis, even after full clinical expression of the disease. Such DC may have potential as antigen-specific therapy for autoimmune inflammatory arthritis, including RA.

Recently, the possibility of blocking antigen presentation of the type II collagen (CII)-derived immunodominant arthritogenic epitope CII259-273 to specific CD4 T cells by inhibition of antigen uptake in HLA-DR1-transgenic mice in vitro and in vivo was addressed. The authors showed that CII accumulated in membrane fractions of intermediate density corresponding to late endosomes. When DC and macrophages were treated with cytochalasin D or amiloride the intracellular appearance of CII was prevented and antigen presentation of CII259-273 to HLA-DR1-restricted T cell hybridomas was blocked. These data suggest that CII was taken up by DC and macrophages predominantly via macropinocytosis. Administration of amiloride in vivo prevented activation of CII-specific polyclonal T cells in the draining popliteal
lymph nodes.\textsuperscript{71} This study suggests that selective targeting of CII internalization in professional APC prevents activation of autoimmune T cells, constituting a novel therapeutic strategy for the immunotherapy of RA.

Finally, a recent study investigated the potential of LF 15-0195 (LF), an NF-kb inhibiting agent, to generate immature tolerogenic DC that could be used for antigen-specific immunotherapy \textit{in vivo} (in mice CIA).\textsuperscript{72} Treatment of DC progenitors with LF resulted in a population of tolerogenic DC that was characterized by low expression of MHC class II, CD40, and CD86 molecules, as well as by poor allostimulatory capacity in a mixed leukocyte reaction. The efficacy of LF-treated DC in preventing arthritis was substantiated by histological examination, which revealed a significant decrease in inflammatory cell infiltration in the joints.\textsuperscript{72} This study demonstrated that \textit{in vitro}-generated antigen-specific immature DC may have important potential as a tolerogenic vaccine for the treatment of autoimmune arthritis.

\textbf{Concluding remarks}

As DC are APC which are key players in initiating specific immune responses by linking innate immunity with adaptive immunity, it is of particular interest to investigate the potential role(s) of these cells and design DC-based strategies to modulate the arthritic process. Thus, DC are of great interest for clinical applications in autoimmunity; a DC subset that can induce antigen/disease-specific tolerance would be highly desirable. Therefore, it is important to dissect the functional characteristics of DC subsets in arthritis in order to reveal whether (or which) DC subset is the problem, the solution or both.

Intense research of the immunobiology of RA has stimulated the introduction of novel approaches aimed at blocking inflammatory cytokine pathways in the synovial joint (e.g. TNF-\(\alpha\)). However, an ever-increasing number of inflammatory molecules are being identified which contribute to RA pathology. As these inflammatory molecules have redundant effects on activating neighboring cells, therapies aimed at blocking multiple pathways may be required. Strategies aimed at identifying DC-derived molecules (that may represent ideal targets for gene therapy and systemic targeted therapies) involved in initiation/perpetuation of arthritis, T cell stimulation and B cell differentiation into plasma cell antibody production, hold great promise for future treatment of RA.

However, the functional roles of DC subsets in CIA and in RA remain to be established. Achieving a detailed understanding of the specific DC functions in RA holds potential for modulating DC for immunotherapy by down-regulating the autoimmune response.

\textbf{Acknowledgements}

The authors which to acknowledge Saïda Aarrass for excellent technical assistance and EULAR for providing financial support (EULAR Young Investigator Award 2005, to M.C.L.) and also the Dutch Arthritis Foundation (2008, nr. 0701014, to P.P.T. and M.C.L.)

\textbf{Correspondence to:}
Maria Cristina Lebre, PhD
Academic Medical Center/University of Amsterdam
Division of Clinical Immunology and Rheumatology, K0-134
PO. Box 22700, 1100 DE Amsterdam
The Netherlands
Tel. +31 20 5666806
Fax +31 20 6919658
E-mail: c.lebre@amc.uva.nl

\textbf{References:}


40. Radstake TR, Franke B, Wenink MH et al. The functional variant of the inhibitory Fc gamma receptor IIb (CD16b) is associated with the rate of radiologic joint damage and dendritic cell function in rheumatoid arthritis. Arthritis Rheum 2006;54:3828-3837.