Dendritic cells manipulating immune responses: Understanding the role of Flt3L and Flt3-dependent DCs in rheumatic diseases
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chapter EIGHT

General Discussion

Parts adapted from:
Fms-like tyrosine kinase 3 ligand-dependent dendritic cells in autoimmune inflammation

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Dendritic cells in the center court of immune regulation: understanding the importance of Flt3L and Flt3L-dependent dendritic cells in arthritis

General discussion
The immune system developed to protect against invading pathogens and to help tissue repair after injury. However in systemic autoimmune diseases the mechanisms that regulate the balance between recognition of pathogens and avoidance of self-attack are impaired. Studying the characteristics of the microenvironment affecting immune cell differentiation, leading to activation of adaptive immunity, is fundamental for understanding the break of tolerance in autoimmunity. The precise cellular and molecular mechanisms leading to autoimmune disease and which factors determine which organs are involved continue to be poorly understood and these are key questions in immunology. Moreover, not only the initiation of the autoimmune process remains elusive but also the factors contributing to the perpetuation of the inflammatory process are not fully understood. In autoimmune diseases the ability to control and resolve inflammation is partly lost, resulting in continuous immune activation without any obvious infection, with different degrees during flares and quiescent phases (1). It is unlikely that only one mechanism is responsible for sustaining autoimmunity and recent evidence has suggested that both innate and adaptive mechanisms participate in this process. Studying the bridge between innate and adaptive immune response might provide further insight into the genesis and pathogenesis of autoimmunity.

Dendritic cells bridging innate and adaptive immune response

The dark side of dendritic cells: the role of dendritic cells in rheumatoid arthritis and autoimmunity
Dendritic cells (DCs) are present in all tissues and are involved in the initiation of immune responses (2). They recognize pathogens and various danger signals (see Chapter 1), which leads to the upregulation of co-stimulatory molecules, cytokine production, and pathogen-specific T cells activation/differentiation. Additionally, DCs communicate with various immune cells (e.g., natural killer cells (NKs)) bridging the innate and adaptive arm of the immune response.

Clinical and experimental evidence has shown that prolonged presentation of self-antigens (3;4) and defects in tolerogenic DC functions are fundamental events for the development of destructive autoimmune diseases. In rheumatoid arthritis (RA), DCs have been shown to infiltrate synovial tissue (ST) and synovial fluid (SF) (5) and they are able to take up, process and present antigen locally, contributing to disease perpetuation (6;7). Studies of DCs from SF of RA patients show that these
cells display an activated phenotype as they express high levels of HLA-DR and co-stimulatory molecules (8).

Chronic inflammatory conditions due to improper eradication of pathogens, autoimmune processes and chronic allograft rejections are associated with the genesis of organized lymphoid tissue. Interestingly, DCs in RA inflamed tissues are associated with T cells in structures resembling germinal centers where they can stimulate self-reactive T cells (9). DCs have also been involved in promoting synovial inflammation due to their ability to secrete pro-inflammatory cytokines and chemokines (10;11). Assuring immune homeostasis requires a complex interaction of soluble and cell-associated components. Understanding this network may lead to the identification of novel therapeutic targets for the treatment of autoimmune disease.

**Flt3L-dependent dendritic cells - the ones to blame?**

The fms-like thyrosine kinase 3 receptor (FLT3) and its ligand (Flt3L) are key regulators of DC commitment and development (12;13) (see Chapter 1). FLT3 is expressed on the surface of early multipotent hematopoietic progenitors, committed DC precursors, and differentiated DCs in lymphoid tissues. FLT3 expression and its signaling is essential for steady-state DC development and homeostasis as loss of FLT3 or its ligand resulted in a substantial reduction in DC numbers in mouse lymphoid tissues (14). Conversely Flt3L administration leads to increased numbers of these cells in both mice and humans (15;16). The role of FLT3 signaling in autoimmune diseases is not fully understood, although in recent years Flt3L has been linked to primary Sjögren’s syndrome (pSS) and RA. In pSS patients, Flt3L was reported to be increased in the serum and B-cells expressing FLT3 can be found in both peripheral blood and salivary glands of these patients. Furthermore, Flt3L was found to promote survival and proliferation of anti-IgM stimulated B-cells isolated from both pSS patients and healthy individuals (HI) (17). Studies in mouse models of RA have demonstrated that intra-articular expression of Flt3L in the knee joint aggravates the severity of peptidoglycan-induced arthritis and FLT3 signaling blockade can reduce the severity of arthritis in an antigen-induced arthritis model (18). Flt3L was also recently listed in a panel of cytokines found to be elevated during the preclinical stage of RA (19). To understand the importance of the FLT3/Flt3L axis and whether this pathway is deregulated in RA contributing to disease induction/maintenance, we studied in Chapter 2 in detail the FLT3 and Flt3L expression levels, as well as the major sheddase for Flt3L (TACE) in 3 different compartments in RA patients compared to disease controls. We observed that the levels of Flt3L in RA serum, SF and ST are significantly elevated compared to gout patients and HI. We have identified monocytes and DCs as main Flt3L producers in RA and also showed that these cells express the FLT3 receptor. Co-expression of FLT3 and its ligand in the same cell suggests a possible autocrine stimulatory mechanism as
already reported for primary acute myeloid leukemia (AML) (20). Binding of Flt3L to FLT3 triggers the phosphatidylinositol 3-Kinase (PI3K) and Ras pathways, leading to increased cell proliferation and the inhibition of apoptosis (21;22). Taking into account that DCs are increased at the site of inflammation in RA and that Flt3L is particularly important in driving DC differentiation we hypothesized that the high levels of Flt3L in RA might contribute to DC differentiation. Indeed, local DC differentiation occurs in the RA synovium supporting the concept that the synovial compartment may be a reservoir for joint-associated DCs revealing an important mechanism for the amplification and perpetuation of DC-driven responses in the joint of RA patients, including inflammatory-type Th1 responses (6). Whether Flt3L participates in this differentiation process still needs to be addressed. However since DCs can respond to but also produce Flt3L, it remains unclear whether the high Flt3L levels observed in RA patients are a cause or a consequence of elevated DC numbers. A good way to address this question would be to monitor Flt3L serum levels at different stages of disease and to correlate them with DCs numbers. It has also been reported that overexpression of FL3T leads to IL-6 induction (23). Besides being involved in inflammatory responses, IL-6 has pleiotropic effects on hematopoietic cells (24). IL-6 has been shown to acts in synergy with IL-3 to stimulate proliferation of primitive hematopoietic progenitors (25). Thus, the induction of IL-6 in FLT3 overexpressed cells may also play a role in sustaining cell proliferation. Soluble Flt3L levels are highly dependent on TNF converting enzyme (TACE) activity (26). We observed the same TACE expression levels between RA and gout patients and increased soluble Flt3L expression in RA, suggesting that TACE biological activity might be increased in RA patients. Blocking this molecule could have beneficial results in RA by reducing Flt3L and TNF levels. Since TACE is involved in TNF processing (27) and TNF has an important role in RA, several studies have been conducted using TACE inhibitors. TACE inhibitor(s) have shown efficacy in the collagen-induced arthritis (CIA) mouse model both prophylactically and therapeutically (28). It would be interesting to monitor Flt3L levels in the above mentioned studies to better understand the Flt3L importance in disease development/progression. Macrophages are critically involved in the pathogenesis of RA (29). Importantly we have demonstrated (Chapter 2) that in vitro IFNγ–differentiated macrophages (mimicking M1 type macrophages mainly present in RA ST) are important sources of Flt3L. Since synovial sublining macrophages are a reliable biomarker for response to therapy in RA (30), we also evaluated the impact of effective therapy, prednisone treatment, on Flt3L serum levels. We observed a positive correlation between disease activity score (DAS28) and Flt3L serum levels that might reflect the reduction in the numbers of the main source(s) of Flt3L: circulating monocytes (31)/DCs and/or ST macrophages. Overall, we have demonstrated that FLT3/Flt3L pathway is deregulated in RA and might be of importance for RA pathogenesis.
We investigated the importance of FLT3/Flt3L axis in more depth in Chapter 3 by making use of a constitutive knockout mouse for Flt3L where we were able to study in more detail how Flt3L-dependent signaling affects CIA induction and development. Mice lacking this molecule have severe reductions in DC numbers and are protected from disease indicating that Flt3L-dependent DCs play a crucial role in the pathogenesis of CIA. In addition, CIA severity was dramatically reduced in Flt3L-/- mice indicating the importance of Flt3L-dependent DCs in the initiation of adaptive immune responses. We observed a reduction in synovial cellular infiltration and cartilage damage in Flt3L-/- mice. We observed quantitative and qualitative alterations in the adaptive immune response, namely reduction of cytokine production by T cells and collagen specific antibody production by B cells. Immune responses are initiated in the T cell areas of secondary lymphoid organs where naive T lymphocytes meet DCs, which present antigens taken up in peripheral tissues (32). As previously shown (14), Flt3L−/− mice have a severe reduction in DC numbers in lymphoid organs. We observed reductions in MHCII+CD11C+ DCs as well as specific reductions in CD11bl+CD11c+ DC, CD8α+ and CD8α− DC and CD103+ DC subsets in the LNs of Flt3L−/− mice after CIA induction. In the steady-state, these subsets require Flt3L for their generation. However not all DC subsets depend on Flt3L for their development (33) (see Chapter 1), and not all DCs subsets in these mice are affected (34). In Chapter 3 we have demonstrated that the density and quality of DCs present in the secondary lymphoid organs might account for the changes in the magnitude and class of the T and B cell responses observed in Flt3L−/− mice during CIA. Together, Flt3L-dependent DCs seem necessary for CIA induction and contribute to disease severity.

In Chapter 4 we aimed to identify which Flt3L-dependent DC subset is required for the initiation of CIA. DCs are instrumental in the initiation of immune responses by transporting antigen from the periphery to lymphoid organs presenting them to T and B cells. In this chapter we identified dermal cross-presenting CD103+ DCs as crucial for the initiation of CIA. Mice lacking this subset by constitutive (Flt3L-/- and Batf3 -/- ) or chemical deletion (CEP-701 treatment) are protected from disease showing reduced cellular infiltration and cartilage destruction. A decrease in T cell (especially CD8+ T cells) and B cell responses (antibody production) was also observed. The importance of T cells for the initiation and maintenance of CIA has been established before (35;36). Therapies targeting co-stimulatory pathways operative between antigen-presenting cells and T cells have shown clinical improvement and protection against progressive joint destruction in RA patients with disease reduction comparable to anti-TNF agents while perhaps providing a better safety profile (37;38). However many questions still remain over the exact role of T cell function in the pathogenesis of disease and particularly regarding the role of CD8+ T cells. Studies indicate that CD8+ T cells are necessary for the establishment of germinal centers observed in nearly 50% of RA patients (39). Despite the decisive role these structures have been suggested to play in the initiation and maintenance of the disease process and a correlation between
the phenotypical structure of these ectopic lymphoid structures and enhanced auto-
antibody production as well as disease severity suggested by several studies (40;41),
this remains controversial (42;43). B cells require cognate interaction with T helper
cells in the germinal center of lymphoid follicles to generate protective antibodies.
The observed decrease of antibody production in mice lacking CD103+ DCs after CIA
induction could be due to a reduction in T cell help. Nevertheless, recent evidence has
shown that B cells receive additional help from invariant natural killer T cells, DCs, and
various granulocytes, including neutrophils, eosinophils, and basophils (44-46). These
cells can deliver T cell-independent B-cell helper signals at the mucosal interface and
in the marginal zone of the spleen to initiate rapid innate-like antibody responses
suggesting a more direct role for DCs in antibody production. It has also been shown
that the recognition of antigens on the surfaces of antigen-presenting cells by B cells
results in efficient formation of an immune synapse (47) which has been proven to be
much more active and dynamic than that predicted from the response of B cells to
Ags in solution (48). In addition, DCs are also associated with plasmablast survival (49).

It has been suggested that functional differences between DCs in guiding immune
responses might depend on not only their subset but also of the microenvironment
of cytokines and/or inflammatory mediators (50). In homeostatic conditions CD103+
DCs have been shown to exert a tolerogenic function (51) but we (Chapter 4) and
others have shown that this subset is also able to initiate an inflammatory immune
response (52). Overall, our data indicate that activation of Flt3L-dependent DCs,
especially CD103+ DCs is necessary for T and B cell activation and CIA development.
Targeting this DC subset might be of interest and valuable in individuals at risk of
developing autoimmune disorders.

**Dendritic cells deciphering the signals**

The chronic inflammatory response is heterogeneous and generates different types
of cellular infiltrates depending on factors such as antigenic stimulus and affected
tissue. Changing tissue microenvironments shapes the phenotypes of the resident
and infiltrating cells determining cell heterogeneity in a temporal and spatial manner.
RA is characterized by a systemic inflammatory state, in which immune cells and
soluble mediators play a crucial role. During arthritis a number of changes in joint
tissues can be observed. RA is characterized by key histological features including
intimal lining layer hyperplasia, synovial sublining infiltration with mononuclear cells,
increased vascularity and fibrin deposition (53). DCs constitute a big portion of
the synovial mononuclear infiltrate and several subsets have been identified in RA
patients (10). In Chapter 5 we identified for the first time CD141+CLEC9A+DCs at
the site of inflammation in RA and demonstrated that through interferon (IFN)λ1/
IL-29 these cells can induce an increase in chemokine mRNA expression in T cells,
contributing to the perpetuation of the inflammatory process.
Increased expression of IFN inducible genes in RA ST was an early finding of molecular studies of RA pathogenesis (54;55). In addition to the known type I and II groups of IFNs, a novel group of IFNs was recently discovered. This family comprises three cytokines, designated interleukin-28A (IL-28A), IL-28B, and IL-29, also called IFNλ2, IFNλ3, and IFNλ1 that form the type III IFNs family (56;57). Type III IFNs are expressed in several tissues and are produced by human peripheral blood mononuclear cells (PBMC) mainly by DCs upon infection with viruses and/or after stimulation with poly (I:C) or lipopolysaccharide (LPS) (58). Recently it has been reported that RA patients have an IFNλ1/IL-29 serum signature (59). Importantly, mouse CD8α+ DCs and human CD141+ DCs are the major producers of IFNλ in response to poly IC (60). In Chapter 5 we have identified CD141+ DCs as the major contributors to IFNλ1/IL-29 production in RA ST both at the gene and protein levels. IFNλ cross-linking activates the same intracellular signaling pathways as type-I IFNs (61). In addition, IFNλ has been shown to induce ELR(-) CXC chemokine mRNA in PBMCs, in an IFN-gamma-independent manner (62). It is generally accepted that chemokines and their receptors are essential for the recruitment and positioning of cells in the inflamed synovium (63;64). In vitro studies have suggested that chemokines including CC chemokine receptor (CCR)1, CCR2, CCR5, CC chemokine ligand (CCL)2/monocyte chemoattractant protein (MCP)-1, CCL5/RANTES and CXCL8/IL-8, are intimately involved in cell migration toward the synovial compartment in RA (65). More recently other chemokines such as CXCL10 have also been identified in RA synovium (66;67). CXCL10 is categorized functionally as a Th1-chemokine binding to the receptor CXCR3 and regulating immune responses through the activation and recruitment of leukocytes, such as, T cells, eosinophils, and monocytes (68). Several studies have shown that serum and/or tissue expression of CXCL10 are increased in autoimmune diseases such as arthritis (69-71). Furthermore, reports have demonstrated that CXCL10 and CXCR3 have important roles in leukocyte homing to inflamed tissues (39), synovial fibroblast invasion (72) and bone destruction (40;41) in RA patients leading to the perpetuation of inflammation, and therefore, tissue damage. The regulation of T cell infiltration in the synovium is an important aspect of RA progression. The immune system plays an essential role not only in protecting the host against infections but also in monitoring the health of cells, and responds to ones that have been injured and die, even under sterile conditions. This process is initiated when dying cells expose intracellular molecules that can be recognized by cells of the innate immune system. We propose that RNA released from necrotic cells in the arthritic joint or lesional skin activates TLR3 on CD141+ DCs, as previously shown for other cell types in the joint and during acute inflammatory events (73;74), leading to IFNλ1/IL-29 production that induces CXCL10 mRNA expression on T cells. This mechanism might be a novel pathway to sustain inflammation in RA. An important area of future research will be the molecular identification of endogenous adjuvants and their receptors as well as elucidation of their mechanism of action and contribution to immune responses.
Dendritic cells listening to the dead cell talk

The interaction between dying cells and phagocytes reflects the involvement of inflammation in normal tissue homeostasis (75). Alterations of this equilibrium due to the inappropriate death of non-inflammatory cells (76) or deficient clearance of dying or dead cells by phagocytes (77;78) can lead to autoimmune disease, as well as sustained inflammation. Once inflammation is set or an immune response is mounted, resolution of inflammation requires the clearance of effector cells (1;79). Therefore, understanding the pathways for cell death and clearance is key for the exploration and therapeutic approach aimed at resolving inflammation. Germline-encoded pattern recognition receptors (PRRs) are responsible for sensing the presence of pathogens. PRRs are also responsible for recognizing endogenous molecules released from damaged cells, termed damage-associated molecular patterns (DAMPs)(see Chapter 1). In Chapter 5 we have shown an example of how DCs sense the surrounding microenvironment (TLR3 activation) and modulate immune responses (CXCL10 induction) but other receptors besides TLRs can also be involved in this process. CLEC9A, group V C-type lectin-like receptor is important for transmitting information from necrotic cells to CD8+ T cells and defines a pathway by which adaptive immune response can be elicited in the absence of infection (80). It has been demonstrated that there is a collaborative recognition of distinct microbial components by different classes of innate immune receptors. Importantly, the addition of poly I:C to apoptotic cells enhanced the cytotoxicity of antigen-specific CD8+ T cells (81). CD8+ T cells have been shown to play an important role in psoriatic arthritis (PsA) pathology (82). PsA is a common chronic inflammatory joint disease in which both inflammation and tissue damage contribute to disease outcome (83). Innate and adaptive immune mechanisms appear to fulfill complementary roles in the pathogenesis of PsA. T cells, in particular the CD8+T cell population (namely IL-17 producing), are likely to be involved in the progression of disease (84). In Chapter 6 we aimed to assess whether CLEC9A expression might be modulated after response to adalimumab therapy compared to placebo, where T cell numbers were reduced, studying three different compartments (skin, ST and peripheral blood). We observed that CLEC9A protein expression in ST is significantly reduced after 4 weeks of adalimumab therapy compared to placebo and there is a positive correlation between CLEC9A and T cell numbers. Moreover PsA ST CLEC9A+ cells were in close contact with CD8+ T cells (in non-biological treated patients) suggesting a possible interaction. The reduction of synovial inflammation associated with clinical improvement of the joint after adalimumab treatment was accompanied by a decrease in T cell numbers, as well as reduced expression of MMPs (85). Studies with TNF blockers have suggested that the observed cellular changes after treatment could be explained by alterations in cell migration due to reduced neoangiogenesis and decreased expression of adhesion molecules and chemokines (86-88). Here we
suggest an additional mechanism for adalimumab induced T cell reduction by which adalimumab might target CLEC9A expressing cells (CD141+ DCs and CD14+CD16- monocytes) and thus reducing antigen specific T cell responses in PsA ST. CLEC9A possesses a cytoplasmic immunoreceptor tyrosine-based activation-like motif that can recruit Syk kinase, and using receptor chimeras, it has been shown that this receptor can induce proinflammatory cytokine production and potentially contributes to the maintenance of the inflammatory process (89). Therefore reducing CLEC9A expression might contribute not only to a decrease in T cell numbers but also to a reduction in proinflammatory cytokine production in PsA ST. Investigating the impact of effective therapies on modulation of different DC subsets with different PRR expression, different phenotypes and maturation states, and mechanisms involved in migration and recirculation could provide valuable insight into how T cell responses may be manipulated. Targeting CLEC9A or CLEC9A-expressing cells might be promising in reducing inflammation in PsA patients by modulating (cross) presentation, T cell responses and pro-inflammatory cytokine production.

**Flt3L - more than just a DC growth factor**

In the previous Chapters 3 and 4 we have demonstrated the importance of Flt3L in tissue inflammation by reducing DC numbers. DCs infiltrate bone adjacent tissues during inflammation, where their interactions with T cells constitute a key component of the inflammatory infiltrate at active disease sites in human and experimental RA (90;91). It has previously been shown that DCs share a common FLT3+ progenitor with osteoclasts (OCs) (90) and can also de-differentiate into OCs in the RA synovial microenvironment (92). Exacerbated bone resorption associated with chronic inflammation has been considered, until now, to be the result of enhanced constitutive osteoclastogenesis from bone marrow monocyte/macrophage precursors under the control of M-CSF. Despite evidence supporting the role of DCs in the development as well as the perpetuation of inflammatory processes in RA, it is still unclear whether they play a direct role in inflammation-induced osteoclastogenesis and bone loss. In Chapter 7 we investigated if FLT3/Flt3L axis might be directly involved in bone damage in both human and animal models of RA, and whether specific targeting of this system would be beneficial for the prevention of bone damage in arthritis. It has been proposed that the contribution of DCs to osteoclastogenesis is only indirect and linked to their ability to activate naive T cells, which then produce RANKL and stimulate OC differentiation (93). However, it has been shown in mice that Flt3L can directly induce OC differentiation by substitution for M-CSF (94). In Chapter 7 we have demonstrated that Flt3L can induce osteoclastogenesis from human PBMCs and that blocking FLT3 leads to a reduction of the resorption capacity of M-CSF generated OCs. Of note, Flt3L-generated OCs could not resorb bone. The lack of resorption capacity could not be explained by differences in cathepsin K (protease
involved in bone resorption) and Atp6v0d2 (essential component of the OC-specific proton pump that mediates extracellular acidification in bone resorption) mRNA expression and numbers of nuclei between the different culture conditions, supporting the notion that in humans M-CSF is required for resorption capacity (95;96). Despite promising in vitro data using the FLT3 inhibitor CEP-701 affecting bone resorption capacity of M-CSF-generated OCs and previous observations that Flt3L−/− mice are protected from CIA showing reduced cellular infiltration and cartilage damage (Chapter 3), in vivo administration of CEP-701 did not protect mice from bone damage. This might perhaps be explained by CEP-701 administration before the onset of disease, to low disease severity, short duration of the experiment or route of CEP-701 administration. Further studies are necessary to evaluate the impact of FLT3/Flt3L axis in bone damage in in vivo mouse models of RA. Flt3L seems to have a more secondary role in osteoclastogenesis compared to M-CSF. We have reported in Chapter 2 that Flt3L is increased in RA serum compared to healthy individuals and in RA SF compared to paired serum. Importantly, in combination with exclusive pathways of differentiation, cellular plasticity seems to play an important role within the myeloid lineage. Since both in mice and in humans Flt3L leads to an increase in DC numbers and these cells express FLT3 (and can transdifferentiate into OCs), mouse models where Flt3L could be induced (as observed in human RA) would provide better models to study CEP-701 effect. We propose that Flt3L alone is insufficient to support normal osteoclastogenesis in vivo, but it might have an additive effect in combination with M-CSF. Taken together, we have shown that Flt3L can induce OC formation in RA patients and that CEP-701 might have a potential therapeutic effect in preventing bone loss. Manipulating DC numbers or function may have tremendous implications, because in addition to DCs important roles in regulating innate and adaptive immunity, a direct contribution by these cells to inflammation-induced bone loss may provide promising therapeutic targets not only for controlling inflammation but also for preventing bone destruction. Further studies are necessary to better understand the mechanism by which Flt3L regulates osteoclastogenesis in RA.

Connecting the dots - looking at dendritic cell subsets, the missing link?

Autoimmune diseases such as RA have a complex pathogenesis and multifactorial etiology. An increasing body of evidence has highlighted the importance of environmental factors in the development of RA in genetically predisposed individuals. Some of these environmental factors have been identified, while others are hypothesized and not yet proven, and it is likely that most have still to be identified. Cigarette smoke (97;98), obesity (98) and periodontitis (99) are amongst the most studied environmental risk factors. Because of their location at the border zones of the organism and close proximity to the environment, DCs are regarded
as important sentinels of the immune system. DCs residing at peripheral sites are known to integrate antigen specificity with environmentally responsive immune control. DC subsets differ in their specialized functions, including the location of activity, cytokine profiles, types of antigens detected, migratory or tissue resident status and presence during immunological homeostasis or during inflammation. We tried to integrate all the knowledge gained within this thesis and elaborate a schematic representation where Flt3L and Flt3L-dependent DCs might contribute to arthritis development and maintenance (Figure 1).

Aiming at dendritic cells-FLT3 inhibition as a treatment for autoimmunity

DCs presenting autoantigens have been shown to cause organ specific T cell-mediated autoimmune diseases, such as type 1 diabetes and autoimmune myocarditis (100). Different compounds have been examined in vitro and in vivo and reported as potential FLT3 inhibitors (101-103). Recent studies using FLT3 inhibitors have shown efficacy in mouse models of autoimmune diseases by targeting DCs (104;105). In Chapter 4 we have shown that pretreating mice with the FLT3 inhibitor CEP-701 before the onset of disease protects mice from CIA. Treatment with FLT3 inhibitors leads to a reduction of total DC numbers by acting on progenitor cells and inducing apoptosis in a significant fraction of the more mature DCs (104). This latter finding is particularly relevant since for FLT3 inhibition to have an effect on ongoing disease, mature DCs would need to be dependent on this pathway. Moreover, it indicates that constant T cell activation by DCs is required to maintain/sustain the inflammatory process. In Chapter 7 we propose that FLT3 inhibitors might also have a role in bone damage in arthritis, acting on the resorption capacity of OCs. It is also important to point out that the DC depletion was reversible in vivo and that DC numbers returned to normal after discontinuation of therapy. No serious toxicity effects were reported and both in vitro colony-forming capacity and repopulation of bone marrow stem/progenitor cells were maintained. Importantly, treated mice were able to mount a proper immune response to Listeria infection in a manner similar to control counterparts, demonstrating that no severe immunosuppression was present (104). FLT3 inhibitors are currently being used in the clinic. FLT3 is frequently mutated in patients with AML and FLT3 inhibitors have shown therapeutic activity in AML patients (106). Since oral bioavailability and Phase II data in humans are available, if proven efficacious and safe in autoimmunity, this may facilitate the development of FLT3 inhibitors for the treatment of immune-mediated inflammatory diseases. Obviously, much more work needs to be done before we get to this stage. Nevertheless, we could envisage 2 different routes of administration; systemic and intra-articular (see Figure 2). FLT3 inhibitors could perhaps constitute a new and safe therapeutic approach for the treatment of autoimmune diseases such as RA.
Figure 1. Schematic representation of the arthritic process via Flt3L and Flt3L-dependent DCs. In Chapter 2 and 3 we have highlighted the importance of Flt3L DCs in induction and maintenance of the arthritic process. In Chapter 4 we proposed that peripheral migratory Flt3L-dependent CD103+ DCs contribute to arthritis induction in a mouse model of RA and in Chapter 5 we have identified CD141+ DCs, human homologue of mouse CD103+ DCs, at the site of inflammation in arthritis (ST in RA and lesional skin in PsA) and shown that this subset contributes to the inflammatory process via IFNα1/IL-29 induction and CXCL10 mRNA expression on T cells. CD141+ DCs are regarded as the human cross-presenting DCs. They have an enhanced ability to take up dead or necrotic cell antigens via CLEC9A, sense nucleic acids with TLR3 and 8, and cross present to CD8+ T cells in vitro. These cells might be involved not only in the initiation of the (auto)immune process but also in sustaining inflammation. In Chapter 6 we have shown that CLEC9A expression in ST of PsA patients is decreased after adalimumab treatment compared to placebo correlating with reduced T cell numbers. Lastly, in Chapter 7 we have shown that Flt3L might contribute to the arthritic process not only via DCs but also by interfering with osteoclastogenesis and osteoclast function.
Concluding remarks

DC subsets are emerging as cells endowed with particular functions, either in terms of specific cytokine secretion profile or of signals provided to other neighboring cells through distinctive surface molecules during cell-to-cell contacts. An increased awareness of the intricate relationship between DCs (and their different subsets) and other cell types of the adaptive immune system is vital for understanding how inflammatory processes are initiated, maintained and suppressed. Manipulating specific DC subsets or molecules specifically expressed by particular DC subsets might constitute promising therapeutic targets for the prevention and treatment of autoimmune diseases.
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