Research article

Rheumatoid arthritis synovium contains plasmacytoid dendritic cells

Lois L Cavanagh¹, Amanda Boyce¹, Louise Smith¹, Jagadish Padmanabha¹, Luis Filgueira², Peter Pietschmann³ and Ranjeny Thomas¹

¹Centre for Immunology and Cancer Research, University of Queensland, Princess Alexandra Hospital, Brisbane, Australia
²Institute of Anatomy, University Irchel-Zurich, Zurich, Switzerland
³Department of General and Experimental Pathology, University of Vienna, Vienna, Austria

Corresponding author: Ranjeny Thomas, rthomas@cicr.uq.edu.au

Received: 11 Jun 2004 Revisions requested: 29 Jul 2004 Revisions received: 13 Oct 2004 Accepted: 26 Oct 2004 Published: 11 Jan 2005

Arthritis Res Ther 2005, 7:R230-R240 (DOI 10.1186/ar1467)

© 2004 Cavanagh et al.; licensee BioMed Central Ltd.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is cited.

Abstract

We have previously described enrichment of antigen-presenting HLA-DR+ nuclear RelB+ dendritic cells (DCs) in rheumatoid arthritis (RA) synovium. CD123+HLA-DR+ plasmacytoid DCs (pDCs) and their precursors have been identified in human peripheral blood (PB), lymphoid tissue, and some inflamed tissues. We hypothesized recruitment of pDCs into the inflamed RA synovial environment and their contribution as antigen-presenting cells (APCs) and inflammatory cells in RA. CD11c+ myeloid DCs and CD123+ pDCs were compared in normal and RA PB, synovial fluid (SF), and synovial tissue by flow cytometry, immunohistochemistry, and electron microscopy and were sorted for functional studies. Nuclear RelB-CD123+ DCs were located in perivascular regions of RA, in a similar frequency to nuclear RelB+CD123+ DCs, but not normal synovial tissue sublining. Apart from higher expression of HLA-DR, the numbers and phenotypes of SF pDCs were similar to those of normal PB pDCs. While the APC function of PB pDCs was less efficient than that of PB myeloid DCs, RA SF pDCs efficiently activated resting allogeneic PB T cells, and high levels of IFN-γ, IL-10, and tumor necrosis factor α were produced in response to incubation of allogeneic T cells with either type of SF DCs. Thus, pDCs are recruited to RA synovial tissue and comprise an APC population distinct from the previously described nuclear RelB+ synovial DCs. pDCs may contribute significantly to the local inflammatory environment.

Keywords: dendritic cells, plasmacytoid, rheumatoid arthritis, TNF

Introduction

Plasmacytoid dendritic cells (pDCs) are a distinct population of antigen-presenting cells (APCs) with the capacity for potent antigen-presenting function and production of large amounts of cytokines, including tumor necrosis factor (TNF)-α and IFN-α. Human pDCs can be identified by cell-surface expression of MHC molecules, the α-chain of the IL-3 receptor (CD123), and the presence of blood dendritic-cell (DC) antigens known as BDCA2 and BDCA4 in a proportion of cells [1]. In comparison with CD11c+ myeloid DCs, pDCs display a distinct set of chemokine and Toll-like receptors [2-4]. In response to viruses and CpG DNA, pDCs become activated to produce IFN-α and their APC function is enhanced [5-8]. While pDCs were first demonstrated in the T-cell areas of lymph nodes [5,9], precursors of this DC population have been isolated from several sources, including normal peripheral blood (PB), thymus, fetal liver, and bone marrow [10]. Although they do not reside in normal peripheral tissues, pDCs have been shown to infiltrate certain inflamed tissues and tumor sites, including the skin in psoriasis and lupus, the cerebrospinal fluid in multiple sclerosis, and melanoma and ovarian carcinoma [11-15]. While pDCs play an important effector role in viral disease, being the major producers of IFN-α and having a primary role in innate immunity, there is also evidence that they may play an immunoregulatory role, through the induction of Th2 (T helper 2)-type cytokines [9,16-18].
The synovial autoimmune reaction of rheumatoid arthritis (RA) is characterized by lymphocyte, macrophage, and DC infiltration that can progress to the development of lymphoid tissue in established disease [19-21]. DCs are likely to contribute to the formation and maintenance of such organized lymphoid tissue and antigen presentation in RA and other autoimmune lesions [22-24]. We have previously shown that the effector site in RA synovial tissue is enriched in differentiated myeloid DCs, which express nuclear RelB [21,25]. Translocation of RelB to the nucleus of myeloid DCs is associated with APC function, particularly through increased expression of MHC molecules CD86 and CD40 [26].

The proinflammatory cytokines TNF-α and IL-1β are key contributors to the inflammatory cytokine cascade in RA [27,28]. This relates to a number of actions, but activation of the endothelium by TNF-α is particularly important in cellular recruitment to the synovium [29-31]. Since RA is characterized by endothelial activation, leukocyte recruitment, and the development of high endothelial venules, we hypothesized that pDCs would be enriched in inflamed but not normal synovium. Since the functional role of pDCs in disease pathogenesis is only partly understood, we also wished to address whether these cells represent a population distinct from the described nuclear RelB+ synovial DCs, and whether they may contribute as APCs or inflammatory cells in RA [21].

Materials and methods

Patients and controls

Thirty patients who fulfilled the American College of Rheumatology criteria for RA were included [32]. Of these, 10 provided synovial fluid (SF) samples and 27 provided PB samples. Of the 30 patients, 80% were seropositive, 62% were female, and 73% were taking at least one disease-modifying antirheumatic drug or low-dose prednisone or both. Synovial tissue was obtained at arthroscopy from seven patients with RA, of whom three were untreated and four were taking at least one disease-modifying antirheumatic drug or low-dose prednisone. Synovial tissue was obtained at arthroscopy from seven patients with RA, of whom three were untreated and four were taking at least one disease-modifying antirheumatic drug or low-dose prednisone. The duration of disease ranged from 0.5 to 18 years. In addition, we studied synovial tissue from four healthy individuals with non-specific knee pain undergoing arthroscopy, one patient who had had psoriatic arthritis for 8 years, and one patient who had had ankylosing spondylitis for 30 years. Each patient with spondyloarthopathy was taking sulphasalazine. No patient in the study was taking biologics. Synovial tissue was provided by Dr Malcolm Smith (Repatriation Hospital, Adelaide, Australia). PB Buffy coats prepared from 30 healthy donors were obtained from the Red Cross Blood Transfusion Service (Brisbane, QLD, Australia). The study was approved by the Research Ethics Committee of the Princess Alexandra Hospital.

Culture medium and cell isolation

All cells were cultured in RPMI 1640 (Gibco, Life Technologies, Mulgrave, VIC, Australia) supplemented with 10% FCS (CSL Ltd, Parkville, VIC, Australia), 0.3 mg/ml L-glutamine (Trace Biosciences, Castle Hill, NSW, Australia), 0.12 mg/ml benzylpenicillin (CSL), and 10 µg/ml gentamicin (Delta West, Pharmacia and Upjohn, Spring Hill, QLD, Australia). The monoclonal antibodies used in this study include FITC, phycoerythrin (PE), and purified anti-CD11c, CD14-PerCP, PE, biotinylated and purified anti-CD123, CD86-FITC (all from BD Pharmingen, San Diego, CA, USA), BDCA2-FITC (Miltenyi Biotech, San Francisco, CA, USA), HLA-DR-biotin (Coulter Immunotech, Fullerton, CA, USA), CD40-FITC (Biolegend, San Diego, CA, USA), CD80-FITC (Cymbus Biotech, Chandlers Ford, Hants, UK), CD68 (Kp-1, DAKO, Carpinteria, CA, USA), RelB (C-19, Santa Cruz Biotech, Santa Cruz, CA, USA), and biotinylated Ulex europaeus agglutinin I (Vector Laboratories, Burlingame, CA, USA).

Mononuclear cells were prepared from normal or RA PB or RA SF by density gradient centrifugation over Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) as described elsewhere [33]. T cells were purified from PB mononuclear cells by passing the cells over a nylon wool column, followed by immunomagnetic depletion of remaining monocytes, DCs, B cells, and NK (natural killer) cells using monoclonal antibodies against CD14, CD16, CD19, CD56, and HLA-DR (all from BD Pharmingen), followed by goat antimouse immunoglobulin magnetic beads, then passage through a strong magnetic field (MACS, Miltenyi Biotech), and collection of the unbound fraction. On analysis by flow cytometry, the unbound fraction routinely contained 95–98% CD3+ T cells. DC-enriched non-T cells were produced by immunomagnetic depletion of T, B, and NK cells from non-T cells, by incubation with monoclonal antibodies against CD19, CD16, CD56, and CD3.

Flow cytometric analysis and selection of cells by cell sorting

To enumerate CD123+ and CD11c+ subsets of DCs, mononuclear cells from normal PB or RA SF were stained for four-colour flow cytometry as described elsewhere [33], using monoclonal antibodies against CD14-PECy5, CD11c-FITC, CD123-PE, and HLA-DR-APC. Live CD14- HLA-DR+ mononuclear cells were gaged for analysis. Subset percentages are expressed as percentage of total mononuclear cells. Listmode data were analyzed using Winlist 2.0 software (Verity Software House, Topsham, ME, USA). For sorting, PB or SF DC-enriched non-T cells stained with the same four markers were sorted using the MoFlo flow cytometer (DAKO), gating on CD14-HLA-DR+ and either CD123+CD11c+ or CD11c+CD123+ cells, respectively. For phenotypic analysis, mononuclear cells from PB or SF were stained with CD14-PECy5, CD123-PE.
or CD11c-PE, HLA-DR-APC, and either a fourth monoclonal antibody or isotype control monoclonal antibody conjugated with FITC. DCs were gated as described above.

Electron microscopy
Electron microscopy of freshly sorted cells was carried out as described elsewhere [5]. After fixation in 2.5% glutaraldehyde in phosphate-buffered saline, the cells were post-fixed with an aqueous solution of 1% OsO4 containing 1.5% K4Fe(CN)6. Subsequently, the specimens were dehydrated in an alcohol series and embedded into epon. Ultrathin sections (50 nm) were contrasted with lead citrate and uranyl acetate and studied with a CM100 electron microscope (Philips, Eindhoven, The Netherlands).

Mixed lymphocyte reactions and cytokine analysis
Various numbers of sorted PB or RA SF DCs were incubated with 10⁵ allogeneic PB T cells in triplicate wells for 5 days, as described elsewhere [33]. Supernatants were removed from some cultures and [³H]thymidine (1 μCi/well, ICN Biochemicals) was added to the remainder for the final 18 h. Cells were harvested onto glass-fiber filter mats and the incorporation of [³H]thymidine was determined by liquid scintillation spectroscopy (Packard Topcount, Packard Instrument Co, Meriden, CT, USA). IFN-γ, IL-10, and TNF-α were measured in supernatants by ELISA using OptEIA ELISA kits (BD Pharmaningen).

Immunohistochemistry
Frozen or paraffin-embedded sections of synovial tissue from patients with untreated active RA were obtained by arthroscopic biopsy and supplied by Malcolm Smith (Repatrination Hospital, Adelaide, Australia). Normal synovial tissue was obtained from arthroscopy from patients undergoing arthroscopy for nonspecific knee pain and in whom no abnormality was found. After fixation with acetone, sections were incubated with anti-CD11c or anti-CD123 using an immunoperoxidase technique, and revealed with diaminobenzidine (brown). Frozen sections were double-stained with U. europaeus agglutinin I (Ulex), a lectin that specifically binds endothelial cells (fast red), and anti-CD123 (brown), using a double, immunoperoxidase–immunoalkaline phosphatase technique as described elsewhere [34]. Formalin-fixed paraffin-embedded sections were antigen-retrieved in 10 mM citrate buffer at pH6 in an autoclave, then stained with anti-CD123 (diaminobenzidine, brown) alone, or in combination with anti-ReIB (BCIP, DAKO, purple). Sections were counterstained with hematoxylin except when they had been double-stained for CD123 and ReIB and were photographed using a transmitted-light microscope (Leitz Diaplan, Leica, Germany). To quantitate infiltration by CD123⁺ DCs, the number of CD123⁺Ulex cells was counted in sections double-stained with CD123 and Ulex. Cells were counted in each of the entire sections from three patients and three normal controls at high power, and for each biopsy this number was corrected for the area of the section to obtain the number per mm². To quantitate infiltration by CD11c⁺ cells, the number of these cells was counted in three high-power fields of the synovial sublining in sections from three patients and three normal controls.

Statistical analysis
Differences were analyzed using unpaired Student's t-tests.

Results
CD123⁺ nuclear RelB⁺ DCs are located in perivascular regions of RA synovial tissue
We have previously shown that synovial tissue in RA and spondyloarthropathy is enriched in differentiated myeloid DCs that express CD33, CD11c, MHC class II, costimulatory molecules, and nuclear RelB [21,25]. Translocation of RelB to the nucleus is associated with maturation and APC function of myeloid DCs [26]. These nuclear RelB⁺ DCs are absent in normal synovial tissue and are rare in RA SF [21,23]. To determine whether RA synovial tissue was infiltrated by CD123⁺ pDCs in addition to CD11c⁺ myeloid cells, frozen synovial tissue sections, either normal or from patients with RA or spondyloarthropathy, were stained with CD11c or CD123. CD11c⁺ cells were found both in the lining layer and adjacent to vessels in the sublining of normal synovial tissue. In contrast, CD123 only stained endothelial cells in the normal tissue (Fig. 1a,1b). In RA synovial tissue, CD11c again stained cells adjacent to vessels, now within lymphoid aggregates in the sublining. A population of CD123⁺ cells with dendritic appearance was also stained adjacent to CD123⁺ blood vessels in RA (Fig. 1c,1d). Cells expressing TNF-α in RA synovial tissue were found in a similar location in serial sections (data not shown), as demonstrated previously [35]. To confirm the perivascular CD123⁺ cells in synovial tissue, normal and RA synovial tissue were double-stained with the endothelial cell marker Ulex agglutinin (red) and with CD123 (brown). Whereas all CD123⁺ structures in normal synovial tissue colocalized with Ulex agglutinin (orange), single-stained CD123⁺ cells (brown) were located in perivascular lymphoid aggregates and within the lumen of occasional blood vessels in RA synovial tissue (Fig. 1e,1f). These CD123⁺ cells are similar in appearance to those previously demonstrated as CD123⁺ pDCs in human tonsil, in that they are smaller than CD11c⁺ myeloid DCs, with shorter dendritic processes, and cell clusters gave the appearance of locally proliferating cells (Fig. 1g) [5,36]. While some macrophages can express CD123, there was no colocalization in synovial tissue of CD123 and CD68 (data not shown). However, aside from the dendritic morphology, we cannot exclude that some of the CD123⁺ cells stained are mast cells [37]. To determine whether CD123⁺ cells in synovial tissue were also nuclear RelB⁺, formalin-fixed tissue was double-stained for RelB.
and CD123 without hematoxylin counterstaining. No CD123+ cells had translocated RelB to the nucleus, although some expressed cytoplasmic RelB (Fig. 1h,1i). In contrast, nuclear staining of RelB was evident in adjacent CD123+ cells (Fig. 1h, arrows). All patients with RA showed similar infiltration by pDCs and no differences in the cell numbers or location were noted between patients with RA or spondyloarthropathy (data not shown).

We quantitated pDCs in normal or RA synovial tissue by counting CD123+Ulex cells in synovial tissue sections from patients with RA or normal controls stained with CD123 and Ulex as shown in Fig. 1. Whereas no pDCs infiltrated the normal tissue, approximately 22 pDCs per mm² were identified within the RA tissue (Fig. 2). This number is similar to the number of nuclear RelB+ differentiated DCs identified previously in RA synovial tissue [38]. In contrast, CD11c+ cells infiltrated both normal and RA synovial tissue, with significantly larger numbers in RA ($P < 0.05$) (Fig. 2). We conclude that the CD123+ cell population is most likely a pDC population that infiltrates RA and spondyloarthropathy but not normal synovial tissue and that it is distinct from the described nuclear RelB+ DCs [21,36,39]. CD11c+ cells comprise immature and differentiated myeloid DCs as well as monocytes [1,34]. Differentiated nuclear RelB+ DCs are found within the CD11c+ DC population in RA and other inflammatory arthritides but not in normal synovial tissue [21].

**CD11c+ and CD123+ DCs in RA SF**

Workers in our laboratory have previously shown that RA SF is enriched in CD11c+CD33brightCD14- myeloid DCs with efficient APC function [25,40]. However, when freshly isolated, only a small proportion of SF CD33brightCD14- DCs have translocated RelB to the nucleus. RA and normal PB mononuclear cells contain similar proportions of CD33brightCD14- DCs [25]. To examine plasmacytoid and myeloid DCs in parallel, we compared RA SF with RA and healthy, control PB for the proportion of CD123+ and CD11c+ HLA-DR+CD14- DCs. After purification of mononuclear cells from either normal or RA PB or RA SF by gradient centrifugation, cells were stained with CD123-PE, CD11c-FITC, CD14-PECy5, and HLA-DR-APC. Polymorphonuclear cells were excluded on the basis of forward and side light-scatter. Since basophils and monocytes can also express CD123, potential CD123+ non-DCs were excluded by gating CD14-hHLA-DR+ DCs [10]. By four-color analysis, CD14-HLA-DR+CD123+ and CD11c+ DC populations could be distinguished (Fig. 3). The percentages of CD123+CD11c+ pDCs in RA PB and normal PB were low and did not differ from each other. This observation contrasts with the reduction in pDCs observed in blood from patients with systemic lupus erythematosus [41]. CD11c+CD123 myeloid DCs were more common than CD123+ DCs in patient and control blood ($P < 0.005$), in keeping with previous studies of normal PB [1]. RA SF contained a significantly greater percentage of...
CD11c+ DCs than normal or RA PB (P < 0.005) – in accord with previous studies using the markers CD33 and CD14 [40]. The proportion of CD11c+ DCs in RA SF was higher than that of RA SF CD123+ DCs (P < 0.05). Although the difference was small, the percentage of CD123+ DCs in RA SF was higher than in RA or control PB (P < 0.05). The data show that CD123+ DCs are present in RA SF, and that the ratio of CD11c+ to CD123+ DCs is similar in RA SF to that in normal or RA PB (approximately 10:1). In RA synovial tissue, mature myeloid nuclear RelB+ and CD123+ DCs have infiltrated perivascular lymphoid aggregates in similar numbers. Previously, similar numbers of immature and mature myeloid DCs were identified in RA synovial tissue [42]. Thus pDCs make up about 30% of DCs within RA synovial tissue. The present and previously published data, taken together, show that both pDCs and myeloid DCs are recruited to RA synovium, with an enrichment of pDCs in synovial tissue relative to blood or SF.

**CD123+ PB DCs are immature whereas SF pDCs show signs of activation**

In normal PB, pDCs circulate as precursors with the potential for recruitment into tissues in response to chemokines [2,43]. These precursors exhibit a characteristic plasmacytoid morphology on electron microscopy, a cell-surface phenotype characterized by expression of the BDCA2 antigen, by low levels of costimulatory molecule expression, and by the potential for IFN-α production in response to viral or immunostimulatory CpG DNA motifs [1]. We therefore analyzed the characteristics of sorted RA SF CD123+ DCs and compared them with control PB CD123+ DCs. On electron microscopic examination, freshly sorted PB and SF CD123+ DCs appeared similar, with a smooth surface and abundant rough endoplasmic reticulum in the cytoplasm. The nucleus was nonlobulated and abundant in euchromatin and contained a distinct nucleolus (Fig. 4).

CD123+ PB DCs were morphologically distinct from the CD123+ pDCs, with a lobulated nucleus and some phagocytic vesicles. CD123+ DCs from SF showed more membrane ruffling and phagocytic activity than those from PB (Fig. 4). Thus SF CD123+ DCs morphologically resemble CD123+ DCs in PB, whereas CD11c+ SF DCs display a greater level of ruffling and phagocytic activity, consistent with their enhanced level of activation, than CD11c+ circulating precursors [21].

On four-color flow cytometric analysis, gated RA SF CD14- HLA-DR+CD123+ DCs expressed low levels of CD40, CD80, and CD86. All or the majority of SF CD123+ DCs expressed the BDCA2 marker of immature pDC precursors [1]. This cell-surface phenotype closely resembles that of control PB CD123+ DC precursors, although BDCA2 was consistently expressed at high levels only by a subset of CD123+ HLA-DR+ cells in PB (Fig. 4b). No PB or SF cells expressed the DC differentiation marker CD83 (data not shown). However, SF CD123+ and CD11c+ DCs expressed higher levels of cell-surface HLA-DR than the corresponding cells in PB, suggesting some cellular activation within the SF environment [5,10,44]. Thus CD123+ pDCs comprise a small proportion of RA SF mononuclear cells, which are predominantly immature but show some evidence of activation in situ. These observations regarding phenotype and PB and SF numbers are consistent with findings in two recent studies [39,45].

**CD123+ and CD11c+ SF DCs are efficient APCs**

We have previously shown that freshly isolated CD33brightCD14 CD11c+ SF DCs efficiently stimulate resting T cells in allogeneic mixed lymphocyte reactions [21]. In contrast, whereas freshly isolated CD11c+ PB DCs are efficient APCs in mixed lymphocyte reactions, CD123+ PB DCs usually require prior activation in the presence of IL-3.
and CD154 for acquisition of APC function in this assay. To analyse the functional capability of RA SF DCs, CD11c+ and CD123+ DCs were sorted from either normal PB or RA SF and incubated with freshly isolated normal allogeneic PB T cells. Freshly isolated PB CD11c+ but not CD123+ DCs efficiently stimulated allogeneic T-cell proliferation and IFN-γ and IL-10 production in mixed lymphocyte reactions. Addition of IL-3 made no difference to the T-cell proliferation in response to CD123+ DCs (data not shown), suggesting that death of the APCs was not responsible. In contrast, both freshly isolated CD11c+ and CD123+ SF DCs efficiently stimulated proliferation and IFN-γ and IL-10 production by resting normal allogeneic T cells (Fig. 5). A recent study demonstrated the capacity of RA SF to inhibit pDC differentiation in vitro [39]. The current studies are consistent, in that SF pDCs showed only some evidence of activation in situ, but once incubated in mixed lymphocyte reactions in the absence of SF they displayed enhanced APC function relative to that of PB pDCs. Whereas stimulation of mixed lymphocyte reactions either by CD11c+ or by CD123+ PB DCs resulted in little TNF-α production, stimulation by either of these DCs from RA SF resulted in high levels of TNF-α secretion (Fig. 5). The data indicate that pDCs have the capacity for enhanced APC function relative to PB pDCs once removed from the RA SF environment. Furthermore, at the time of antigen presentation by SF DCs to T cells, production of a number of cytokines by either T cells or DCs may be stimulated, including TNF-α, and this appears to be a characteristic of RA synovial DCs rather than the subtype of stimulating DCs.

**Discussion**

Ongoing inflammation in RA involves positive feedback loops between activated T cells, B cells, DCs, macrophages, and their products, with destructive consequences for parenchymal cells. Clinical and animal data indicate that effector-site DCs play an important proinflammatory role in the perpetuation of autoimmune disease and contribute to the lymph-node-like organization of that tissue [22,46]. This role may be effected by local antigen presentation to CD4+ and CD8+ effector cells, but DC cytokine and chemokine secretion are also important [47,48]. TNF-α and IL-1β are important downstream proinflammatory and destructive cytokines in RA for somatic cells, whose release is promoted by activation of macrophages. IL-10 is highly expressed in RA, and IFN-γ is an important T-cell effector cytokine [49,50].

In the current studies, we show that, in addition to the previously described population of nuclear RelB+ DCs, a further population of nuclear RelB CD123+ pDCs is located in perivascular regions of RA but not normal synovial tissue sublining. Moreover, pDCs were located within blood vessels, and both DC populations were observed in perivascular areas in which cells producing TNF-α were colocated [35]. Adherence of CD123+ and CD11c+ DCs to TNF-α-activated endothelium was higher than to resting endothelium in vitro (data not shown). TNF-α plays an important role in the recruitment of other leukocytes to RA synovial...
tissue [29], and this most likely pertains to the recruitment of pDCs to RA but not normal synovial tissue through expression of adhesion molecules such as intercellular adhesion molecule (ICAM)-1, CD62-E, and CD62-P and interaction with their ligands on pDCs [9,11,51-54]. Furthermore, TNF-α up-regulates synthesis of chemokines by endothelial cells [55]. During experimentally elicited allergic rhinitis, CD123+HLA-DR+ pDCs have been shown to be recruited to human nasal mucosa [11].

The gene for MxA is specifically induced by IFN-α and therefore identifies a population of activated pDCs. In contrast, BDCA2 is a marker of immature pDCs. MxA+ pDCs have previously been demonstrated in involved lupus skin
and inflamed tonsil [13]. In RA synovial tissue, BDCA2 was shown to stain fewer cells than CD123 or MxA, suggesting differentiation in situ of a large proportion of pDCs into cells with a capacity for production of IFN-α and other cytokines. Together, the current and previous studies demonstrate recruitment of pDCs to normal lymphoid organs as well as inflammatory sites, with local differentiation, but no recruitment to normal peripheral tissues. In contrast, CD11c+ myeloid precursors populate normal resting tissues, as shown here, but additional CD11c+ myeloid cellular recruitment takes place at inflammatory sites, where RelB nuclear translocation takes place [21,56]. We have previously shown that, like synovial pDCs, CD123+ DCs in the T-cell area of human tonsil are also nuclear RelB [23]. The data suggest either that activation of pDCs is not associated with nuclear translocation and transcriptional activity of RelB or that conditions in tonsil and synovium do not induce sufficient RelB translocation for detection by immunohistochemistry [26,57]. As preliminary studies in vitro demonstrate induction of RelB in PB pDCs after stimulation with lipopolysaccharide and CpG, and reduced production of IFN-α by pDCs in RelB-deficient mice, it is likely that RelB activation does accompany pDC activation. However, RelB translocation might be quantitatively reduced or RelB might be more rapidly degraded in the nucleus of pDCs than of myeloid DCs in inflamed tissues [58].

Of relevance to the RA inflammatory lesion, stimulation of blood pDC precursors with signals including CD154, influenza virus, or CpG oligonucleotides induces production of large amounts of cytokines, including IFN-α, IFN-β, and TNF-α; induction of DC differentiation; and stimulation of APC function [3,39,44,59]. Although inhibitory effects of SF on DC function, and thus on T-cell proliferation and cytokine production, are confirmed here [21,39,60], factors in the RA SF environment, such as IL-3 and CD154,
may be sufficient to precondition the SF pDCs for efficient APC function ex vivo [36,44]. IFN-γ, IL-10, and TNF-α were produced in mixed lymphocyte reactions stimulated by myeloid or pDCs derived from SF but not PB, potentially by DCs or by T cells or both. In the tissue, as a result of antigen presentation by myeloid DCs or pDCs, key effector cytokines may be produced in perivascular areas in RA, located strategically close to endothelial cells, as well as incoming leukocytes. It is not known whether pDCs are capable, like myeloid DCs, of migration from synovial tissue to draining lymph nodes. However, it seems probable that pDCs conditioned by local IL-3 and CD154, or even viral or bacterial products transported to the synovium, predominantly play local proinflammatory and antigen-presenting roles, through secretion of cytokines such as IFN-α and possibly TNF-α [61,62].

Conclusion

pDCs are recruited to RA synovial tissue and comprise an APC population distinct from the previously described nuclear RelB+ synovial DCs. The APC function of pDCs is greater in SF than in PB. Activated pDCs and interacting T cells may contribute significantly to the inflammatory environment in RA.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

LC, RT, LF, and PP conceived the experiments and LC, AB, LS, JP, and LF carried them out. LC, RT, and LF wrote the manuscript.

Acknowledgements

We thank Malcolm Smith (Repatiation Hospital, Adelaide, South Australia) for providing synovial tissue. This research was supported by grant 210237 from the National Health and Medical Research Council of Australia and by a grant-in-aid from the Princess Alexandra Hospital Foundation. Dr Thomas and Ms Smith were supported by the Arthritis Foundation of Queensland.

References

1. Dziok G, Fuchs A, Schmidt P, Remer S, Zysk M, Miitlenyi S, Buck DW, Schmitz J: BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J Immunol 2000, 165:6037-6046.
2. Vandenbroucke B, Bendriess-Vermare N, Massacrier C, Homey B, de Bouteiller O, Briere F, Jallat C: The inducible CXCR3 ligands control plasmacytoid dendritic cell responsiveness to the constitutive chemokine stromal cell-derived factor 1 (SDF-1)/CXCL12. J Exp Med 2003, 198:823-830.
3. Bauer M, Redecke V, Ellwart JW, Scherer B, Kremer JP, Wagner H, Lipford GB: Bacterial CpG-DNA triggers activation and maturation of human CD11c+, CD123+ dendritic cells. J Immunol 2001, 166:5000-5007.
4. Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Bazan F, Liu YJ: Subsets of human dendritic cell precursors express different Toll-like receptors and respond to different microbial antigens. J Exp Med 2001, 194:863-869.
5. Grouard G, Rissoan MC, Filgueira L, Durand I, Banchereau J, Liu YJ: The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. J Exp Med 1997, 185:1101-1111.
6. Kadowaki N, Antonenko S, Liu YJ: Distinct CpG DNA and polyinosinic-polycytidylic acid double-stranded RNA, respectively, stimulate CD11c-negative and CD11c-positive dendritic cell precursors and CD11c+ dendritic cells to produce type I IFN. J Immunol 2001, 166:2291-2295.
7. Kadowaki N, Antonenko S, Lau JY, Liu YJ: Natural interferon alpha/beta-producing cells link innate and adaptive immunity. J Exp Med 2000, 192:219-226.
8. Blom B, Ho S, Antonenko S, Liu YJ: Generation of interferon alpha-producing dendritic cell (Pre-DC2) from human CD34+ hematopoietic stem cells. J Exp Med 2000, 192:1785-1796.
9. Verma M, Jarrossay D, Facchetti F, Aleardi O, Nakajima H, Lanzavecchia A, Colonna M: Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon [see comments]. Nat Med 1999, 5:919-923.
10. Olweus J, BitMansour A, Wanke R, Thompson PA, Carballido J, Picker LJ, Lund-Johansen F: Dendritic cells induce autoimmune diabetes and myeloid dendritic cell lineage of myeloid origin. Proc Natl Acad Sci USA 1997, 11:12551-12556.
11. Jahnsen FL, Lund-Johansen F, Dunne JF, Farkas L, Haye R, Brandtzaeg P: Experimenter-induced recruitment of plasmacytoid (CD123(high)) dendritic cells in human nasal allergy. J Immunol 2000, 165:4062-4066.
12. Pashenkov M, Huang YM, Kostulas V, Haglund M, Soderstrom M, Link H: Two subsets of dendritic cells are present in human cerebrospinal fluid. Brain 2001, 124:480-492.
13. Farkas L, Baeiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL: Plasmacytoid dendritic cells (natural interferon-alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. Am J Pathol 2001, 159:237-243.
14. Zou W, Machelon V, Coulomb-L’Hermin A, Bovak J, Nome F, Isaeva T, Wei S, Kryszek B, Durand-Gasselin I, Gordon A, et al.: Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. Nat Med 2001, 7:1339-1346.
15. Verma W, Bonecchi R, Facchetti F, Bianchi D, Sozzani S, Festa S, Berenzi A, Celli M, Colonna M: Recruitment of immature plasmacytoid dendritic cells (plasmacytoid monocytes) and myeloid dendritic cells in primary cutaneous melanosomas. J Pathol 2003, 200:255-268.
16. Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S, Antonenko S, Liu YJ: The nature of the principal type 1 interferon-producing cells in human blood. Science 1999, 284:1835-1837.
17. Kuwana M, Kaburaki J, Wright TM, Kawakami Y, Ikeda Y: Induction of antigen-specific human CD4+ T cell anergy by peripheral blood DC2 precursors. Eur J Immunol 2001, 31:2547-2557.
18. Gillet M, Liu YJ: Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. J Exp Med 2002, 195:699-704.
19. Wagner UG, Kurtz PJ, Wahner A, Bracketz M, Berry DJ, Goronzky JJ, Weyand CM: The role of CD8+ CD40L+ T cells in the formation of germinal centers in rheumatoid synovitis. J Immunol 1999, 161:8390-8397.
20. Kim HJ, Krenn V, Steinhauser G, Beren M, Thomas R: Plasma cell development in synovial germinal centers in patients with rheumatoid and reactive arthritis. J Immunol 1999, 162:3053-3062.
21. Pettit AR, MacDonald KPA, O'Sullivan B, Thomas R: Differentiated dendritic cells expressing nuclear RelB are predominantly located in rheumatoid synovial tissue perivascular mononuclear cell aggregates. Arthritis Rheum 2000, 43:791-800.
22. Ludewig B, Odermatt B, Landmann S, Hengartner Z, Zinkernagel RM: Dendritic cells induce autoimmune diabetes and maintain disease via de novo formation of local lymphoid tissue. J Exp Med 1998, 188:1493-1501.
23. Thompson AG, Pettit AR, Padmanabha J, Mansfield H, Frazier H, Strutton GM, Thomas R: Nuclear RelB+ cells are found in normal lymphoid organs and in peripheral tissue in the context of inflammation, but not under normal resting conditions. Immuno Cell Biol 2002, 80:164-169.

Available online http://arthritis-research.com/content/7/2/R230
24. Ma-Krupa W, Jeon MS, Spoerl S, Tedder TF, Goronzky JJ, Weyand CM: Activation of arterial wall dendritic cells and breakdown of self-tolerance in giant cell arteritis. J Exp Med 2004, 199:173-183.

25. Thomas R, Quinn C: Functional differentiation of dendritic cells in rheumatoid arthritis: Role of CD86 in the synovium. J Immunol 1996, 156:3074-3086.

26. O’Sullivan BJ, Thomas R: CD40 ligation conditions dendritic cell antigen-presenting function through sustained activation of NF-kappaB. J Immunol 2002, 168:5491-5498.

27. Feldmann M, Charles P, Taylor P, Maini RN: Biological insights from clinical trials with anti-TNF therapy. Springer Semin Immunopathol 1998, 20:211-226.

28. Gabay C, Arend WP: Treatment of rheumatoid arthritis with IL-1 inhibitors. Springer Semin Immunopathol 1998, 20:229-246.

29. Paleolog EM, Hunt M, Elliott MJ, Feldmann M, Maini RN, Woody J: Description of vascular endothelium by mononuclear anti-tumor necrosis factor alpha antibody in rheumatoid arthritis. Arthritis Rheum 1996, 39:1082-1091.

30. Tak PP, Taylor PC, Breedveld FC, Smeets TJ, Daha MR, Kluin PM, Meinders AE, Maini RN: Decrease in cellularity and expression of adhesion molecules in synovial lymphoid tissues of rheumatoid arthritis patients with rheumatoid arthritis. Arthritis Rheum 1996, 39:1077-1081.

31. Weninger W, Carlsen HS, Goodarzi M, Moazed F, Crowley MA, Baekkevold ES, Cavanagh LL, von Andrian UH: Recruitment to nonlymphoid tissues: a role for endothelium-dependent tissue factor expression of vascular endothelium by mononuclear anti-tumor necrosis factor alpha antibody in rheumatoid arthritis. Arthritis Rheum 1996, 39:1077-1081.

32. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988, 31:315-324.

33. Cavanagh LL, Saal RJ, Grimmert KL, Thomas R: Proliferation in monocyte-derived dendritic cell cultures is caused by progenitor cells immune to myeloid differentiation. Blood 1998, 92:1598-1607.

34. Pettit AR, Quinn C, MacDonald KP, Cavanagh LL, Thomas G, Townsend W, Handel M, Thomas R: Nuclear localization of RELB (associated with effective antigen-presenting cell function). J Immunol 1997, 159:3861-3869.

35. Pettit AR, Weedon H, Ahern S, Zehntner S, Frazer IH, Slavotenc J, Au V, Smith MD, Thomas R: Association of clinical, radiological and synovial immunopathological response to anti-rheumatoid treatment in rheumatoid arthritis. Rheumatology 2001, 40:1243-1255.

36. Cella M, Facchetti F, Lanzavecchia A, Colonna M: Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. Nat Immunol 2000, 1:305-310.

37. Wollin DE, De Keyser F, De Wever N, Baeten D, Van Damme N, Verbruggen M, Guelvicer C, Veys EMA: A comparative phenotypic analysis of rheumatoid nodule macrophages and rheumatoid synovial macrophages with special reference to adhesion molecules and activation markers. Ann Rheum Dis 1998, 57:480-486.

38. Voussef PP, Triantafillou S, Parker A, Coleman M, Roberts-Thomson PJ, Ahern MJ, Smith MD: Effects of pulse methylprednisolone on cell adhesion molecules in the synovial membrane in rheumatoid arthritis. Reduced E-selectin and intercellular adhesion molecule 1 expression. Arthritis Rheum 1996, 39:1970-1979.

39. Laszik Z, Zensen PJ, Cummings RD, Tedder TF, McEver RP, Moore KL: P-selectin glycoprotein ligand-1 is broadly expressed in cells of myeloid, lymphoid, and dendritic lineage and in some nonhematopoietic cell lines. Blood 1996, 88:3010-3021.

40. Goebeler M, Yoshimura T, Toksoy A, Ritter U, Brocker EB, Gilleron R: The chemokine repertoire of human dermal microvascular endothelial cells and its regulation by inflammatory cytokines. J Invest Dermatol 1997, 108:445-451.

41. McWilliam AS, Nelson D, Thomas JA, Holt PG: Rapid dendritic cell recruitment is a hallmark of the acute inflammatory response at mucosal surfaces. J Exp Med 1994, 179:1331-1336.

42. O’Sullivan BJ, MacDonald KP, Pettit AR, Thomas R: RelB nuclear transcription regulates B cell MHC molecule, CD40 expression, and antigen-presenting cell function. Proc Natl Acad Sci USA 2000, 97:11421-11426.

43. Mannen F, Berberich-Siebelt F, Berberich I, Denk A, Serfling E, Marienfeld R, Berberich S, McFarlin JE, Schulze-Koops H, Kurz H: Decrease in cellularity and expression of the markers BDCA-2 and BDCA-4 and production of interferon-alpha by plasmacytoid dendritic cells in systemic lupus erythematosus. Arthritis Rheum 2003, 48:2524-2532.

44. Page G, Lebecque S, Missiacc P: Anatomic localization of immature and mature dendritic cells in an ectopic lymphoid organ: correlation with selective chemokine expression in rheumatoid synovium. J Immunol 2002, 168:5333-5341.

45. Krug A, Uppaluri R, Facchetti F, Domer BG, Sheehan KC, Schreiber RD, Cella M, Colonna M: IFN-producing cells respond to CXC chemokines in the presence of CXCL12 and secrete inflammatory mediators upon activation. J Immunol 2002, 168:8079-8083.

46. Kohrgruber N, Halanek N, Groger M, Winter D, Rappersberger K, Schmitt-Egenolf M, Stirling G, Maurer D: Survival, maturation, and function of CD11c+ and CD11c+ peripheral blood dendritic cells are differentially regulated by cytokines. J Immunol 1999, 163:2350-2355.

47. Van Kirms CH, Matsyszak MK, Gaston JS: Characterization of plasmacytoid dendritic cells in inflammatory arthritis synovial fluid. Rheumatology (Oxford) 2004, 43:453-460.

48. Xia W, Pinto CE, Kradin RL: The antigen-presenting activities of la+ dendritic cells shift dynamically from lung to lymph node after an airway challenge with soluble antigen. J Exp Med 2003, 198:2591-2598.

49. Dahlen E, Dawe K, Ohlsson L, Hedlund G: Dendritic cell and macrophages are the first and major producers of TNF-alpha in pancreatic islets in the nonobese diabetic mouse. J Immunol 1998, 160:3585-3593.

50. Cernak JJ, Spawlski JB, Thomas R, McFarlin JE, Schulze-Koops H, Davis LS, Fujita K, Lipsky PE: Elevated interleukin-10 levels in patients with rheumatoid arthritis. Arthritis Rheum 1995, 38:96-104.

51. Dahlen E, Dawe K, Lorange CJ, Kradin RL: Induction of CD4 and CD8 T cells by plasmacytoid dendritic cells activated by in fluenza virus and CD40L drive a potent TH1 polarization. Nat Immunol 2001, 2:8142-8147.

52. Blomberg S, Eloranta ML, Magnusson M, Alm GV, Ronnblom L: Expression of the markers BDCA-2 and BDCA-4 and production of interferon-alpha by plasmacytoid dendritic cells in systemic lupus erythematosus. Arthritis Rheum 2003, 48:2524-2532.

53. Goebeler M, Yoshimura T, Toksoy A, Ritter U, Brocker EB, Gilleron R: The chemokine repertoire of human dermal microvascular endothelial cells and its regulation by inflammatory cytokines. J Invest Dermatol 1997, 108:445-451.
61. MacDonald KPA, Nishioka N, Lipsky PE, Thomas R: Functional CD40-ligand is expressed by T cells in rheumatoid arthritis. *J Clin Invest* 1997, 100:2404-2414.
62. Schrijver IA, Melief MJ, Tak PP, Hazenberg MP, Laman JD: Antigen-presenting cells containing bacterial peptidoglycan in synovial tissues of rheumatoid arthritis patients coexpress costimulatory molecules and cytokines. *Arthritis Rheum* 2000, 43:2160-2168.