Characterization of plasmacytoid dendritic cells in inflammatory arthritis synovial fluid


Objective. To examine the phenotype of dendritic cell subsets in synovial fluid and peripheral blood from patients with rheumatoid arthritis (RA) or spondyloarthropathy (SpA).

Methods. Multiparameter flow cytometry was used to identify and characterize dendritic cells in mononuclear cell populations isolated from synovial fluid and peripheral blood.

Results. Synovial fluid contained two subsets of dendritic cells (DC), myeloid and plasmacytoid. These subsets could also be identified in peripheral blood, but there were lower numbers of DC in peripheral blood compared with synovial fluid. Plasmacytoid DC were distinguished from the myeloid subset by high expression of CD123 and lack of expression of CD11c. In comparison with myeloid dendritic cells, the plasmacytoid subset were less mature, similar to those in peripheral blood. They failed to express CD83 and DC-LAMP, and had relatively low levels of CD40 and CD86. Comparison of dendritic cells in synovial fluid from RA and SpA patients showed increased numbers of the plasmacytoid subset in SpA.

Conclusions. This is the first demonstration of the plasmacytoid subset of dendritic cells in synovial fluid. Since these cells are major producers of type I interferons, their increased numbers in SpA might be relevant to pathogenesis, but the immature phenotype in SpA synovial fluid may also indicate that conditions for maturation of this subset do not pertain in SpA synovium.

Key words: Dendritic cells, Plasmacytoid, Spondyloarthropathy, Rheumatoid arthritis, Chemokine receptors, Trafficking.

Dendritic cells (DC) have been recognized as the pre-eminent antigen-processing and antigen-presenting cells; they are necessary for the activation of naïve CD4+ and CD8+ T cells and are able to determine the nature of the immune response they elicit through the production of cytokines which affect T-cell differentiation (reviewed by Banchereau and Steinman [1]). Increased numbers of DC have been reported in the synovium and synovial fluid in inflammatory arthropathies where they are well placed to direct local immune responses and inflammation [2–4]. Recently it has been possible to identify subsets within dendritic cell populations through their differential expression of surface markers. One particular subset, the plasmacytoid DC (PDC), was originally recognized on morphological grounds in tissue sections [5], but it is now possible to identify these cells and their precursors in peripheral blood and to examine their growth requirements and their effects on immune responses. Unlike myeloid DC (MDC), so called because they can be derived in vitro from CD14+ monocytes by culture with interleukin-4 (IL-4) and granulocyte-macrophage colony-stimulating factor (GM-CSF), PDC respond poorly to these cytokines but respond instead to IL-3 and express high levels of CD123, the α chain of the IL-3 receptor [6]. Their principal functional characteristic is the ability to make large quantities of type I interferons (α and β) [7], and they are likely therefore to play an important role in the immune response to viruses, recognizing viruses and their nucleic acids through Toll-like receptor (TLR) 9. In contrast, MDC respond best to bacterial infection, recognizing LPS via TLR4 and producing IL-12 and tumour necrosis factor-α (TNFα) [8], whereas PDC do not express TLR4.

PDC are present in increased numbers in psoriatic skin, and in contact dermatitis [9], and they are believed to be an important source of type I interferons in skin lesions of patients with systemic lupus erythematosus (SLE) [10]. However, the presence of PDC in inflamed joints has not to our knowledge been previously reported. We used multicolour flow cytometry to identify DC in peripheral blood and synovial fluid mononuclear cells (PBMC/SFMC) and noted two distinct populations, one of which was found to have a phenotype characteristic of PDC. This allowed us to investigate their phenotype in more detail and to determine their proportions in SFMC from patients with different forms of inflammatory arthritis.

Materials and methods

Patients

We analysed synovial fluid from 7 rheumatoid arthritis (RA) patients (4 seropositive for rheumatoid factor, 5 erosive) and 14 patients with spondyloarthropathy (SpA) (5 with psoriatic arthritis and 9 with reactive arthritis or undifferentiated SpA). By definition all had active synovial inflammation requiring therapeutic aspiration. We also analysed peripheral blood from 10 RA patients and 7 patients with SpA. All patients gave informed consent and the work was approved by Addenbrooke’s Hospital Local Research Ethics Committee.

Peripheral blood (PB) and synovial fluid (SF) samples were placed in universal tubes containing 10 U/ml preservative-free heparin. Synovial fluid was incubated for 30 min with 10 U/ml hyaluronidase (Sigma, Poole, UK) prior to isolation of mononuclear cells. Mononuclear cells were isolated from both SF and PB by density gradient centrifugation over Ficoll-Hypaque (Amersham Biosciences, Amersham, UK), washed and resuspended in heat-inactivated foetal calf serum (First Link, UK)
Table 1. Monoclonal antibodies used in this study

<table>
<thead>
<tr>
<th>Name</th>
<th>Clone</th>
<th>Conjugate</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2</td>
<td>MT910</td>
<td>FITC</td>
<td>1/200</td>
<td>Dako, Ely, UK</td>
</tr>
<tr>
<td>CD3</td>
<td>UCHT1</td>
<td>FITC</td>
<td>1/100</td>
<td>Dako</td>
</tr>
<tr>
<td>CD4</td>
<td>RPA-T4</td>
<td>CyChrome</td>
<td>1/20</td>
<td>BD Pharmingen, Oxford, UK</td>
</tr>
<tr>
<td>CD8</td>
<td>DK25</td>
<td>FITC</td>
<td>1/250</td>
<td>Dako</td>
</tr>
<tr>
<td>CD11c</td>
<td>BU15</td>
<td>PE</td>
<td>1/100</td>
<td>Caltag, Buckingham, UK</td>
</tr>
<tr>
<td>CD14</td>
<td>CRIS-6</td>
<td>FITC</td>
<td>1/150</td>
<td>Biosource, Nivelles, Belgium</td>
</tr>
<tr>
<td>CD20</td>
<td>2H7</td>
<td>FITC</td>
<td>1/10</td>
<td>BD Pharmingen</td>
</tr>
<tr>
<td>CD40</td>
<td>5C3</td>
<td>PE</td>
<td>1/5</td>
<td>BD Pharmingen</td>
</tr>
<tr>
<td>CD80</td>
<td>L307.4</td>
<td>PE</td>
<td>1/5</td>
<td>BD Pharmingen</td>
</tr>
<tr>
<td>CD83</td>
<td>HB15c</td>
<td>PE</td>
<td>1/10</td>
<td>BD Pharmingen</td>
</tr>
<tr>
<td>CD86</td>
<td>2331 (FUN-1)</td>
<td>PE</td>
<td>1/20</td>
<td>BD Pharmingen</td>
</tr>
<tr>
<td>CD94</td>
<td>HP-3D9</td>
<td>FITC</td>
<td>1/100</td>
<td>BD Pharmingen</td>
</tr>
<tr>
<td>CD123</td>
<td>9F5</td>
<td>PE</td>
<td>1/50</td>
<td>BD Pharmingen</td>
</tr>
<tr>
<td>CCR5</td>
<td>2D7/CCR5</td>
<td>PE</td>
<td>1/5</td>
<td>BD Pharmingen</td>
</tr>
<tr>
<td>CCR7</td>
<td>150153</td>
<td>PE</td>
<td>1/5</td>
<td>R&amp;D Systems, Abingdon, UK</td>
</tr>
<tr>
<td>DC-LAMP</td>
<td>104.G4</td>
<td>PE</td>
<td>1/40</td>
<td>Beckman, High Wycombe, UK</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>TU36</td>
<td>APC</td>
<td>1/5</td>
<td>BD Pharmingen</td>
</tr>
<tr>
<td>BDCA-2</td>
<td>AC144</td>
<td>FITC</td>
<td>1/11</td>
<td>Miltenyi Biotec, Bisley, UK</td>
</tr>
</tbody>
</table>

Characterization of DC phenotype in peripheral blood and synovial fluid

Four-colour flow cytometry was used to detect DC in mononuclear cells from SF samples. The antibodies used are listed in Table 1. All incubations and washes were carried out in FACS buffer [phosphate-buffered saline (PBS)/0.1% bovine serum albumin (BSA)/0.01% sodium azide]. Cells were incubated with the appropriate concentration of antibody to visualize T cells, monocytes, B cells, natural killer (NK) cells and γδ T cells. These antibodies were as follows: anti-CD2, anti-CD3, anti-CD8, anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94. Next cells were stained with allophycocyanin (APC)-conjugated anti-CD14, anti-CD20 and anti-CD94.

Results

Identification and characterization of DC subsets in PBMC and SFMC

Dendritic cells (DC) in PB and SF mononuclear cells were identified by three-colour flow cytometry as cells that were dim or negative for antigens expressed on T (CD2, CD3 and CD8), B (CD20) or NK cells (CD94), and on monocytes (CD14), but positive for CD4 and major histocompatibility complex (MHC) Class II. Using this technique, in several instances two distinct populations of DC were clearly visible in SF; the first expressed high levels of MHC Class II, and was dim for antigens expressed on T, B and NK cells and monocytes, whereas the second expressed somewhat lower levels of MHC Class II, but was completely negative for antigens expressed on the other cell types, as shown in Fig. 1A which presents data from SFMC of a patient with undifferentiated SpA. The second population also expressed higher levels of CD4. That these populations were indeed phenotypically different was shown by staining for expression of CD11c and CD123 (IL-3 receptor a chain). The first population was uniformly positive for CD11c, and expressed relatively low levels of CD123, whereas the second population was predominantly CD11c negative and stained brightly for CD123. This phenotype is similar to that reported for so-called plasmacytoid dendritic cells (PDC) [6], as compared with the ‘classical’ myeloid DC (MDC) which are CD11c+ and CD123dim.

Fig. 1B presents data from PBMC from the same patient. As noted in previous studies [2, 3], the numbers of DC in PB are much lower than in SF, and for this reason two populations cannot readily be discerned. However, as with the SFMC, when expression of CD11c and CD123 was examined, two populations were again clearly present, both CD11c+ and CD11c−, and CD123hi and CD123dim.

The phenotype of the PDC in SFMC is shown in more detail in Figs 2A and 2B; these data are also from a patient with
undifferentiated SpA. In Fig. 2A, PDC were identified by gating on cells negative for antigens expressed on other cell lineages, positive for MHC Class II and expressing high levels of CD4, and analysed for expression of markers of dendritic cell maturation and chemokine receptors. In Fig. 2B, PDC in the same sample of SFMC were positively identified by staining with an antibody to BDCA-2, a Type II C-type lectin which has recently been shown to be expressed specifically by PDC. The analysis showed that both gated and BDCA-2+ cells had the same CD11c–,CD123hi phenotype characteristic of PDC, and did not express CD83 or DC-LAMP, two markers of DC maturation. There were low levels of expression of CD40 and CD86, but both CCR5 and CCR7 were clearly expressed. For comparison, PDC in PBMC from the same patient (Fig. 2C) could also be identified by staining with BDCA-2, and again had a phenotype identical to the PDC in SF. Thus PDC from both PBMC and SFMC have phenotypic properties consistent with immaturity.

This pattern of expression of surface markers by SF PDC was consistently seen in other patients, and contrasts with that seen when MDC within the same SFMC samples were analysed. This comparison is shown in Fig. 3; a median of 87% of MDC expressed CD40 compared with 8% of PDC (P = 0.0079), and whilst PDC did not express any CD83 or DC-LAMP, around 25% of myeloid DC expressed these proteins. Expression of CCR5 and CCR7 also varied in the two subsets (Fig. 4); CCR5 expression was higher on PDC than on MDC (91% vs. 65%), whilst CCR7 expression was also higher on PDC than on MDC (80% vs. 42%). Although these differences were large, statistical significance was not achieved due to the relatively small number of cases examined. Note that previous studies have shown that PDC in PB are CCR5+,CCR7+ whereas MDC are CCR5+,CCR7– [11], so that the changes seen in SF represent up-regulation of CCR7 and the beginning of down-regulation of CCR5 by MDC, as compared with an unchanged level of expression of both CCR5 and CCR7 by PDC, consistent with a lack of maturation of these cells.

Differences in the proportions of different DC subsets in PBMC and SFMC from patients with different forms of inflammatory arthritis

Fig. 5 presents analysis of DC from healthy controls and RA or SpA patients, together with paired SF from RA and SpA patients. Fig. 5a and b compare the proportions of DC that express CD11c and high levels of CD123 respectively. Whereas the majority of DC in PB or SF from RA patients were CD11c+, SF DC in SpA patients showed a much wider range of proportions of CD11c+ cells, with only ~50% of DC being positive in some cases. Since CD11c is characteristic of MDC, this suggests that a higher proportion of these cells are present in RA SF as compared with
FIG. 2. Analysis of the surface phenotype of plasmacytoid DC in synovial fluid and peripheral blood. PDC, identified by high CD4 expression, moderate MHC Class II expression, and lack of expression of other lineage markers (A), or by staining with an antibody to BDCA-2 (B) were analysed by flow cytometry for their expression of additional cell surface antigens. PDC in paired PBMC were also analysed following identification by staining with an antibody to BDCA-2 (C). Quadrants were set using isotype-matched control antibodies.
FIG. 3. Expression of maturation markers by myeloid DC (MDC) and plasmacytoid DC (PDC) in synovial fluid. DC subsets were gated as described in Fig. 2A, and examined for their surface expression of CD40, CD83 and DC-LAMP. Median values are indicated by horizontal lines. Statistically significant differences between groups are indicated by brackets and asterisks: *P < 0.05, **P < 0.01.

FIG. 4. Expression of chemokine receptors by myeloid DC (MDC) and plasmacytoid DC (PDC) in synovial fluid DC subsets was defined as described in Fig. 2A, and examined for their surface expression of CCR5 and CCR7. Median values are indicated by horizontal lines.

FIG. 5. Proportions of DC from peripheral blood of healthy controls, and peripheral blood and synovial fluid of patients with RA and SpA, which express CD11c or high levels of CD123. The total DC population, identified by expression of CD4 and MHC Class II together with dim or negative expression of other lineage markers, was then analysed for expression of (a) CD11c and (b) CD123. Median values are indicated by horizontal lines. Statistically significant differences between groups are indicated by brackets and asterisks: *P < 0.05.
Discussion

In this paper we have identified two populations of DC in SF. One population was shown to be the myeloid subset of DC, which expresses CD11c, whereas the other was the more recently described plasmacytoid subset, which does not express CD11c but expresses high levels of CD123. Whilst several previous investigators have commented on the increased numbers of DC in SF and synovial tissue in inflammatory arthritis [2-4] those observations were confined to the myeloid subset (or failed to distinguish between subsets), so that this is to our knowledge the first report on the presence and phenotype of the plasmacytoid subset of dendritic cells in SF. Several questions arise in relation to our findings: why are PDC more numerous in SpA SF as compared with RA SF; why do SF PDC fail to mature in SF (unlike MDC); and what are the implications of these findings for our understanding of the pathogenesis of joint inflammation in SpA and RA?

Increased numbers of PDC in SpA SF might reflect increased recruitment to the joint. Recruitment of DC to the joint is influenced primarily by available chemokines and expression of appropriate chemokine receptors by DC. Although immature PDC express both CCR5 and CCR7 (unlike MDC which express only CCR5), neither of these receptors is functional, and it is only upon maturation that CCR7 is further up-regulated and becomes functional, whilst CCR5 is down-regulated [11]. Indeed, the only chemokine to which blood PDC respond by chemotaxis is CXCL12, which acts through CXCR4 [11], although in patients with neurological inflammation, blood PDC have also been shown to respond to MCP-1 (CCL2) [12]. Since CXCL12 (SDF-1) is known to be produced by the synovium, this is the most likely chemokine to be involved in recruiting PDC into the joint. Indeed, in preliminary studies we have shown that SF PDC populations do express CXCR4, consistent with their immature phenotype. However, CXCL12 is expressed in the synovium of both RA [13, 14] and SpA patients [15], so production of this chemokine cannot account for the higher numbers of PDC observed in SF from SpA patients. Very recently it has been shown that the response to SDF-1 is greatly enhanced by chemokines which bind to CXCR3 including CXCL9 (Mig) and CXCL11 (I-tac) [16], so differential expression of these chemokines could also play a role in recruitment to the SpA joint. Once recruited, PDC require IL-3 to survive, and undergo rapid apoptosis in culture if no IL-3 is present [6]; IL-3 has been shown to be present in SF of both reactive arthritis (ReA) and RA patients, but levels have been found to be higher in RA so that the availability of this growth factor also does not account for the increased numbers of PDC in SpA SF.

Differences in treatment, or disease duration, between patient groups might affect the relative proportions of different DC subsets in SF. Although SpA patients had a shorter duration of arthritis than those with RA, we mainly studied patients with chronic undifferentiated SpA or chronic ReA, rather than those with acute disease, so our findings do not reflect differences between acute and chronic arthritis. Shodell and Siegal [17] showed that prednisolone reduced the number of PDC present in the blood of healthy individuals and patients (and hence the amount of interferon-α (IFNα) produced upon stimulation of PBMC with virus, PDC being the main source of IFNα, although high doses (30 mg/day prednisolone) were used in that study. In our study none of the patients received more than 10 mg/day prednisolone. Disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate or leflunomide might also have an effect on particular DC subsets; in our study there were examples of SpA patients treated with these drugs who had high numbers of PDC in SF, and we could also identify PDC in the peripheral blood of both RA and SpA patients, so it is unlikely that differential exposure to DMARDs accounts for the difference between RA and SpA patients.
As noted, one of the principal characteristics of PDC is their ability to produce large amounts of IFNα and IFNβ in response to viral and bacterial infection [7]. Kadokawa et al. [18] demonstrated that addition of neutralizing antibody to IFNα or IFNβ decreased the viability of the PDC, suggesting that the Type I interferons they produce may act as an autocrine survival signal. In this case the presence of bacterial products in the joint, as is known to be the case in ReA, could lead to activation of PDC, release of IFNα and increased survival, possibly accounting for the higher numbers of PDC in SpA, and particularly in ReA where the highest numbers of PDC were observed. In addition to Type I interferons, PDC also make TNFα in response to infection, and this acts to increase expression of co-stimulatory molecules. However, we found that PDC in the joint expressed low levels of CD80 and CD86 (data not shown), arguing against the notion that they had responded to bacterial products in the joint by producing TNFα since this would have resulted in up-regulation of CD80 and CD86. It is possible that low-level exposure to bacteria or bacterial products might induce survival of PDC through production of Type I interferon, but fail to produce TNFα and consequent up-regulation of co-stimulatory molecules.

Finally, PDC might actually be recruited to RA joints in the same way as to SpA joints, but be retained in synovium and induced to mature there, rather than trafficking through the tissue into SF. Expression of functional CCR7, which occurs on PDC maturation, would allow PDC to remain in the synovial tissue or migrate to draining lymph nodes, so that they would not be present in the SF. Indeed, in contrast to the CD83−, DC-LAMP− PDC which we noted in SF, Page et al. [19] observed a subset of CD83+ DC-LAMP+ cells in rheumatoid synovial tissue with ‘a plasmacytoid-like appearance, and a remote nucleus in a large cytoplasm’, although these authors did not further characterize these cells. Also, in a review article Cavanagh and Von Andrian [20] mentioned unpublished observations of PDC in rheumatoid synovial tissue. These observations suggest that mature PDC may be present in the rheumatoid synovium, and that conditions in the tissue are conducive to their maturation. In contrast PDC would be recruited to the SpA synovium, fail to mature, and pass through in an immature state into SF. A major signal for PDC maturation is CD40 ligand (CD40L), expressed by T cells, and the organized lymphoid aggregates of the chronic RA synovium could provide more opportunities for PDC contact with CD40L+ T cells and subsequent maturation. IL-3 induces up-regulation of CD40 on PDC, and cells which respond in this way to IL-3 in synovium would then be able to receive signals from T cells expressing CD40L, and mature. Cells failing to up-regulate CD40 would not respond to CD40L and move into SF, consistent with our finding of relatively low CD40 expression by the SF population of PDC. To answer these questions, immunohistochemical studies of synovial tissue should be carried out, in order to detect the presence of PDC, identified by expression of high levels of CD123 and BDCA-2, and determine their expression of CD40, and whether they are associated with T cells.

PDC have been reported at other peripheral non-lymphoid sites of inflammation, where they may contribute to disease pathogenesis. Both MDC and PDC were shown to be enriched in the cerebrospinal fluid (CSF) of patients with inflammatory neurological diseases such as multiple sclerosis and optic neuritis. PDC numbers were especially high in the CSF of patients with neuroborreliosis, caused by the spirochaete *Borrelia burgdorferi*, and patients with bacterial meningitis [12, 21]. These PDC were of a similar phenotype to those found in the blood and those we have studied in the synovial fluid, i.e. expressing low levels of CD40 and CD86, and moderate levels of MHC Class II and CCR5 [22, 23]. These findings may suggest that PDC tend to accumulate in tissues affected by inflammatory disorders secondary to microbial infection.

Functional characterization of PDC from the inflamed joint has not yet been carried out. Isolation of DC from synovial fluid has, in the past, relied on sorting, by flow cytometry, cells that were negative for antigens expressed on other cell lineages. Therefore, it is likely that both MDC and PDC were present in the sorted population. It would be important to determine if synovial PDC produce Type I interferons, and to compare their ability to stimulate T cells with that of SF MDC.

The authors have declared no conflicts of interest.

### Acknowledgement

This work was supported by the UK MRC.

### References


