Distribution and clinical significance of blood dendritic cells in children with juvenile idiopathic arthritis

E Smolewska,[1,2] J Stańczyk,[1] H Brózik,[2] M Biernacka-Zielińska,[1,2] B Cebula,[3] T Robak,[3] P Smolewski[3]

[1] Departament of Paediatric Cardiology, Medical University of Lodz, Lodz, Poland;
[2] Rheumatological Clinic for Children, Medical University of Lodz, Lodz, Poland;
[3] Department of Haematology, Medical University of Lodz, Lodz, Poland

Correspondence to:
Dr E Smolewska, Department of Paediatric Cardiology, II Chair of Pediatrics and Cardiology, Medical University of Lodz, Sporna 36/50, 91-738 Lodz, Poland; e.smolewska@wp.pl

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ABSTRACT

Background: A role for dendritic cells (DC) in the development of adult rheumatoid arthritis has been suggested. To date, this problem has been poorly explored in juvenile idiopathic arthritis (JIA).

Objective: To analyse distribution and maturation status of blood DC (BDC) in JIA.

Methods: Absolute BDC counts were assessed by the "single platform" method in peripheral blood (PB) of 47 untreated children with JIA and 32 healthy controls. Moreover, BDC were investigated in JIA synovial fluid (SF). When the panel of monoclonal antibodies against BDC antigens (BDCA) was used, three BDC subpopulations were determined: myeloid type 1 (mDC1; BDCA-1+/HLA-DR+/CD19−), myeloid type 2 (mDC2; BDCA-3+/HLA-DR+/CD14−) and plasmacytoid (pDC; BDCA-2+/HLA-DR+/CD123+).

Results: A considerable deficiency of all subtypes of BDC was found in the PB of children with JIA. BDC counts in JIA SF were significantly higher than in PB both from children with JIA ($p<0.001$) and healthy children ($p<0.001$). SF BDC, especially mDC1 and mDC2 subtypes, had significantly higher expression of maturation markers (CD40, CD80, CD86 or CD83 antigens) than those from PB. A smaller number of PB BDC at diagnosis correlated significantly with poor response to treatment.

Conclusions: A deficiency of BDC in PB is accompanied by enrichment of SF with those cells. Probably, circulating BDC migrate to joints where they undergo maturation and help to mediate and maintain the local immune response. Interestingly, the level of PD BDC deficiency seems to influence the outcome in children with JIA.

Dendritic cells (DC) are specialised antigen-presenting cells, which originate from the bone marrow CD34+ stem cells. They circulate via the blood to different tissues,[1] where, after maturation, they initiate and regulate T- and B-cell responses.[2 3] Mature DC are characterised by several markers, including CD40, CD80, CD83 or CD86 antigen expression.[4]

According to recent findings, freshly isolated peripheral blood DC (BDC) can be divided into three subsets: plasmacytoid DC (pDC) and two myeloid subsets—mDC1 and mDC2.[2 5–7] pDC express specific BDC antigens, BDCA-2 and BDCA-4, and are CD123+/CD11c−/CD4+/CD2−/CD45RO+. These cells typically produce interferon α (IFNα).[8] Moreover, activated pDC produce IFNγ, tumour necrosis factor α (TNFα)[8 9] and express toll-like receptors 7 and 9 (TLR7 and TLR9).[6]

The mDC1 subpopulation has been shown to express the CD1c+(BDCA-1+)/HLA-DR+/CD11c^{bright}/CD123^{dim}/CD4+/CD2+/CD45RO+ immunophenotype as well as myeloid lineage markers (CD13, CD33) and Fc receptors (CD32 and CD64). The more recently described BDC subpopulation, mDC2, is characterised by BDCA-3+/CD11c^{low}/CD1c−/CD123^{dim}/CD2− immunophenotype and lack of Fc receptors.[1 10 11] Both mDC1 and mDC2 subtypes have been shown to release proinflammatory cytokines (eg, TNFα, interleukin (IL) 1β, IL6 or IL8) in response to TLR2 and TLR4 stimulation; however, mDC1 are much stronger inducers of T-cell proliferation than mDC2.[6]

Several reports suggest that synovial DC in rheumatoid arthritis (RA) may be involved in the initiation, maintenance and/or regulation of the inflammatory response. First reports showed the presence of DC in both peripheral blood (PB) and synovial fluid (SF) in RA.[12–14] In 1995 Randen *et al* reported an expansion of follicular DC forming germinal centres in the synovium of patients with RA and juvenile idiopathic arthritis (JI)A.[15] More recently, precursor DC correlating with the presence of soluble receptor for TNF, p55, were found in RA SF.[16]

Only a few reports, however, show the diversity of DC origin in RA. Despite myeloid DC (CD11c+), RA SF was reported to contain also lymphoid-origin CD123+/CD11c− cells.[17] The same DC subtypes were detected in RA PB, but in much lower prevalence. Moreover, CD123+/RelB− DC were found in RA, but not in normal synovial tissue.[8]

In 2004, an anti-BDCA-2 antibody was used for the first time to detect pDC in RA SF.[18] Most recently, the ability of BDCA-2+ pDC from RA synovium to produce IFNα and TNFα in response to TLR stimulation was described.[19]

Much less is known about BDC in JIA. The cause of JIA is still unclear and apparently differs from adult RA in several clinical and, most likely, some biological extents. So far, only BDCA-2+/CD123+ and CD11c+ DC have been investigated in children with JIA.[8] Therefore, we performed comprehensive evaluation of all three BDC subtypes, mDC1, mDC2, and pDC in this disease. Importantly, the objective, direct flow cytometric assessment of absolute BDC counts, the so called "single-platform" method, was used here for the first time in patients with RA/JIA. Moreover, differentiation status of BDC and their correlation with outcome was also studied for the first time in JIA.

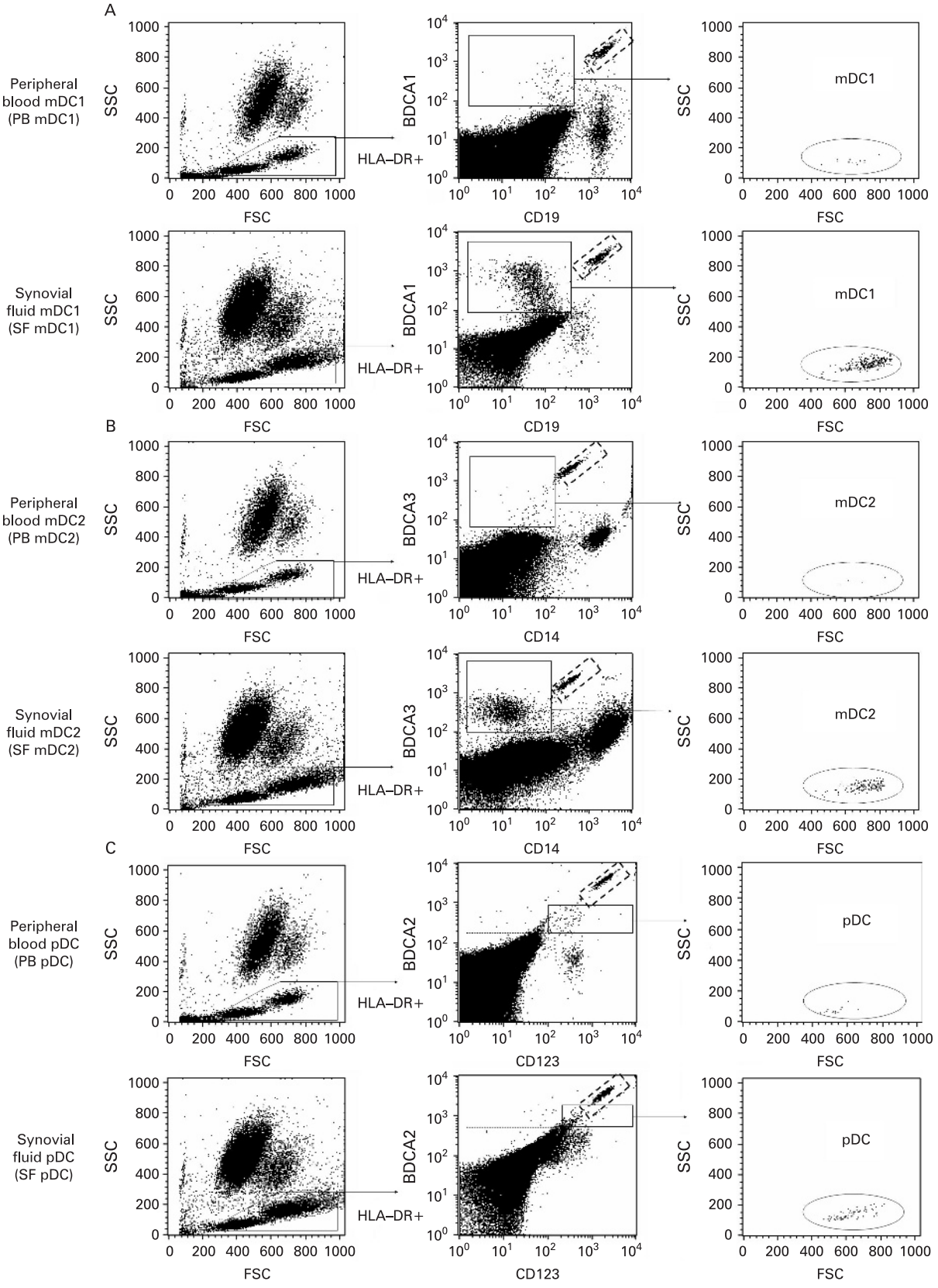

Figure 1 Detection of blood dendritic cell (BDC) subtypes in whole blood and synovial fluid (SF) samples of children with juvenile idiopathic arthritis, stained according to the "lyse-no wash" protocol, performed by flow cytometry. BDC have been gated from the population of lymphocytes and monocytes based on side scatter (SSC) versus forward scatter (FSC) distribution. Next, from HLA-DR+ cells from peripheral blood (PB) mononuclear cells gate (not shown on scattergrams), three subpopulations of BDC have been discriminated. There were two myeloid subtypes with the following immunophenotypes: (A) BDCA-1+/CD19−/HLA-DR+ (mDC1) and (B) BDCA-3+/CD14−/HLA-DR+ (mDC2). (C) The third, plasmacytoid population (pDC) was characterised by BDCA-2+/CD123+/HLA-DR+ phenotype. The BDC rate was calculated as a percentage of BDC among the whole population of mononuclear cells detected using SSC vs FSC discrimination. The absolute BDC counts were calculated by the single-platform assay using fluorescent polystyrene beads (indicated by dashed-line boxes on the scattergram) kit and presented as a number of PB BDC or SF BDC per μl.

PATIENTS AND METHODS

Patients

The study was performed in 47 newly diagnosed, previously untreated children with JIA, including 29 girls and 18 boys, median age 14 years (range 3–16). Thirty-two age- and sex-matched healthy children served as controls (20 girls and 12 boys, median age 14.5 years (4–17). The diagnosis was established according to the Durban criteria.[20] Three types of JIA onset were distinguished: polyarticular, oligoarticular and systemic disease. Moreover, three stages of JIA activity were defined: low activity (limitation of joints motion, without pain or swelling, no extra-articular symptoms, erythrocyte sedimentation rate (ESR) <20 mm/1st h, C-reactive protein (CRP) <10 mg/l), moderate activity (moderate intensity of arthritis, and/or slight temperature, ESR 20–60 mm/1st h, CRP 10–30 mg/l) and high activity (two or more clinical symptoms: morning stiffness, pain and/or swelling of joints, hepatosplenomegaly, fever, rash, and ESR >60 mm/1st h, CRP >30 mg/l).[21]

Response to subsequently introduced treatment was evaluated according to the improvement criteria proposed by Giannini *et al*[22] (at least three of six core set criteria with ⩾30% improvement and no more than one of the remaining variables worsened by 30%). Lack of improvement was defined as a resistant disease.

The study was conducted in accordance with the Helsinki Declaration and was approved by the local ethics committee. All specimens were collected with the written consent of the parents.

Immunophenotyping

PB samples for BDC (PB BDC) evaluation were collected by venepuncture, during routine laboratory investigations. About 6–8 ml of blood was drawn from the patients. BDC in SF (called here SF BDC, as these cells were characterised by the same BDC antigens as the PB population) were evaluated in samples obtained during therapeutics biopsy when joints were affected. In those children PB samples were collected at the day of joint biopsy.

Table 1 Clinical and laboratory characteristics of children with juvenile idiopathic arthritis

Characteristics	No of patients (%)/ median value (range)
Total number	47
Gender (girls/boys)	29 (61.7)/18 (38.3)
Age (years)	14 (3–16)
Type of onset	
Systemic disease	7 (14.9)
Polyarthritis	18 (38.3)
Oligoarthritis	22 (46.8)
Activity of the disease	
Low	18 (38.3)
Moderate	10 (21.3)
High	19 (40.4)
Leucocytes (g/l)	8.7 (3.8–22.5)
Platelets (g/l)	360.0 (210.0–657.0)
Erythrocyte sedimentation ratio/1st h	20.5 (2–121)
Serum C-reactive protein (mg/ml)	1.2 (0.01–12.4)
Patients with rheumatoid factor	6 (12.8)
Patients with antinuclear antibodies	14 (29.8)

Cell immunophenotype was determined using a panel of monoclonal antibodies (mAbs) directed against BDC-specific antigens: fluorescein isothiocyanate-conjugated BDCA-1, BDCA-2, and BDCA-3 (Miltenyi Biotec, Bergish Gladback, Germany), phycoerythrin-conjugated CD14, CD19 (Caltag Laboratories, Burlingame, CA, USA), and CD123 (Miltenyi Biotec), as well as tri-colour-conjugated HLA-DR (Caltag Laboratories). Moreover, mAbs, against DC maturation markers, such as CD40 antigen, CD80, CD83 and CD86 costimulatory molecules, were also used. Isotype controls were performed with the respective mouse IgG1 mAbs (all Caltag Laboratories).

The immunophenotype was assessed by four-colour flow cytometry (FACScan, Becton-Dickinson, San Jose, CA, USA) and analysed with standard emission filters (FL1–FL4).

Three main subpopulations of BDC were distinguished by their antigen expression profile: two myeloid types–mDC1 (BDCA-1+/CD19−/HLA-DR+) (fig 1A) and mDC2 (BDCA-3+/CD14−/HLA-DR+) (fig 1B). As was previously found, these subpopulations strictly corresponded with mDC characterised by BDCA-1+/CD11c+/HLA-DR+ immunophenotype for mDC1 and BDCA-3+/CD32−/CD64−/HLA-DR+ for mDC2 subtype.[23] The third subpopulation, pDC, was discriminated based on BDCA-2+/CD123+/HLA-DR+ immunophenotype (fig 1C).

BDC rates were assessed as a percentage of particular BDC subsets among the whole PB or SF population of mononuclear cells, based on the side scatter versus forward scatter discrimination method. The absolute BDC counts were calculated by the single-platform assay, using the fluorescent polystyrene beads kit (TruCOUNT Tubes; Becton-Dickinson), in the PB/SF samples stained according to the "lyse-no wash" protocol. Results were presented as a number of PB BDC or SF BDC per μl (fig 1). In brief, 50 μl of whole blood was added directly to the TruCOUNT tubes, containing a known number of fluorescent beads. The FACS Lysing Solution (Becton-Dickinson) was subsequently added in order to lyse and, in this way, to eliminate interfering red blood cells. Then, appropriate mAbs were added to the tubes for detecting particular DC antigens. To avoid a loss of cells no washing was performed during the whole procedure (the "lyse-no wash" method). In each sample 100 000 cells were measured. The absolute DC number (cells/μl) was determined by comparing the number of cellular events to the number of bead events. Thus, the number of measured DC was divided by the number of fluorescence beads counted and multiplied by the TruCOUNT bead concentration.

$$\text{Absolute DC count} = \frac{\text{Number of counted DC}}{\text{Number of fluorescent beads counted during measurement}} \times \frac{\text{Total number of fluorescent beads in the TruCOUNT tube (known from the manufacturer's description)}}{\text{Sample volume (50 μl)}}$$

Other laboratory variables

In all cases morphological measures of PB cells, the level of antinuclear antibodies (ANA), the presence of rheumatoid factor (RF), CRP level and ESR were also assessed by standard methods.

Table 2 Comparison of the absolute number of blood dendritic cells (BDC) and their subpopulations in the peripheral blood of children with juvenile idiopathic arthritis (JIA) and of healthy controls

BDC	JIA (n = 47)	Control (n = 32)	p Value
TDC (%)*	0.9 (0.3–2.4)	1.8 (0.8–6.2)	
TDC (cells/μl)†	80.7 (9.4–445.8)	378.3 (91.6–1306.0)	<0.001
mDC1 (%)	0.4 (0.1–1.1)	0.7 (0.4–1.5)	0.005
mDC1 (cells/μl)	30.2 (5.7–185.7)	191.7 (47.9–432.0)	<0.001
mDC2 (%)	0.04 (0.01–0.1)	0.06 (0.02–1.0)	0.015
mDC2 (cells/μl)	3.7 (0.5–72.4)	11.2 (2.9–54.4)	0.006
pDC (%)	0.04 (0.05–1.3)	0.8 (0.4–3.7)	<0.001
pDC (cells/μl)	23.2 (0.7–339.0)	161.2 (56.7–827.0)	<0.001

*BDC rates were assessed as a percentage of particular BDC subsets among the whole peripheral blood or synovial fluid population of mononuclear cells based on the side scatter versus forward scatter discrimination method.
†The absolute BDC counts were calculated by the single-platform assay, using a fluorescent polystyrene bead kit (see text).
mDC, myeloid BDC; pDC, plasmacytoid BDC; TDC, total rate/absolute number of BDC.

Statistics

For the statistical analysis of the data, quantitative variables were presented as medians and ranges. The non-parametric Wilcoxon pair test was used for comparison of the results (eg, assessment of differences between DC numbers in PB and SF in each patient). The correlation between features was evaluated using the Spearman R test. Comparisons and correlations were considered significant when $p<0.05$.

RESULTS

Table 1 shows the characteristics of the examined group of children with JIA.

Twenty-two children had oligoarticular disease, 18 polyarticular and seven systemic disease type of onset. The activity of the disease was considered low in 18, moderate in 10 and high in 19 children with JIA. Abnormal ANA levels were detected in 11 children with JIA with oligoarticular and three with systemic disease. RF positivity was found only in six children; all with polyarticular type of onset.

Deficiency of BDC in peripheral blood of children with JIA

We found a profound deficiency of PB BDC in children with JIA (median total PB BDC 100.7 cells/μl) compared with healthy controls (median 378.3 cells/μl; $p<0.001$). This was found also for the PB BDC rates and all subpopulations examined (table 2).

Correlation of pretreatment numbers of PB BDC with clinical response and laboratory variables of children with JIA

PB BDC numbers were slightly, but not significantly lower in children with higher disease activity ($p>0.05$; data not shown). This concerned total counts of PB BDC and all their subtypes. According to the type of onset there was a slight prevalence of total PB BDC count in the polyarticular, compared with the oligoarticular type. The median total numbers of PB BDC were 82.4 cells/μl versus 65.2 cells/μl, respectively ($p>0.05$).

Interestingly, when the effect of treatment was assessed after 12 months from diagnosis, the pretreatment absolute total numbers of PB BDC showed predictive significance. Thus, children with JIA resistant to subsequently introduced treatment had significantly lower PB BDC number at diagnosis (median 1215.9 cells/μl; range 574.9–1437.0 cells/μl) than those with good clinical and laboratory response (2739.2 cell/μl; range 460.3–3565.6 cell/μl; $p = 0.037$).

According to laboratory variables, total PB BDC counts correlated negatively with the numbers of platelets ($R = -0.67$; $p<0.001$), ANA titres ($R = -0.45$; $p = 0.021$) and the presence of RF ($p = 0.035$). This was mainly owing to mDC1 (negative correlation with platelets, ANA and RF: $p = 0.004$, 0.011 and 0.012, respectively) and pDC (negative correlation with ANA titres: $p = 0.023$). Additionally, the absolute numbers of both PB mDC1 and pDC correlated negatively with CRP levels ($p = 0.013$ and 0.035, respectively) and ESR values ($p = 0.007$ and 0.009, respectively).

Increased number of BDC in JIA SF

BDC counts in JIA SF (SF BDC) were significantly higher than in PB BDC in both children with JIA ($p<0.001$) and healthy children ($p<0.001$). The SF BDC number was significantly higher than the total PB BDC count ($p<0.001$). Similarly, statistically significant differences were found for all SF BDC subtypes (table 3). According to BDC rates, the differences were significant for all except PB pDC (table 3).

Table 3 Comparison of the absolute number of blood dendritic cells (BDC) and their subpopulations in matched samples of peripheral blood and synovial fluid of children with juvenile idiopathic arthritis

BDC	Peripheral blood (n = 22)	Synovial fluid (n = 22)	p Value
TDC (%)	0.9 (0.3–1.4)	5.5 (1.2–55.4)	<0.001
TDC (cells/μl)	62.1 (19.6–205.6)	1437.0 (132.6–3565.6)	<0.001
mDC1 (%)	0.3 (0.1–1.0)	3.5 (0.5–13.0)	<0.001
mDC1 (cells/μl)	20.4 (6.2–128.0)	561.5 (42.1–1761.0)	<0.001
mDC2 (%)	0.03 (0.0–0.1)	1.9 (0.1–6.5)	<0.001
mDC2 (cells/μl)	3.4 (0.5–46.6)	268.1 (33.8–1305.5)	<0.001
pDC (%)	0.3 (0.1–0.9)	0.5 (0.1–41.2)	NS
pDC (cells/μl)	23.2 (6.5–92.3)	187.1 (11.4–2925.5)	0.018

mDC, myeloid BDC; NS, no significant differences; pDC, plasmacytoid BDC; TDC, total rate/absolute number of BDC.

Table 4 Absolute number of blood dendritic cells (BDC) and their subpopulations in synovial fluid (SF) according to particular types of juvenile idiopathic arthritis onset and (JIA) activity

	JIA activity				JIA onset		
BDC (cell/μl)	Low (a) (n = 5)	Moderate (b) (n = 7)	High (c) (n = 10)	p Value*	Oligoarticular (n = 14)	Polyarticular (n = 8)	p Value
SF TDC	1037.7 (132.6–2230.2)	1437.0 (574.9–2739.2)	2155.6 (744.2–3565.6)	a vs c; 0.005 b vs c; 0.023	744.2 132.6–2230.2	1615.1 574.9–3565.6	0.001
SF mDC1	399.7 (42.1–813.8)	636.9 (254.5–1109.1)	1221.5 (342.3–1761.0)	a vs c ; <0.001	254.5 42.1–1761.0	873.0 636.9–1109.1	0.016
SF mDC2	128.0 (33.8–614.1)	268.1 (202.3–922.7)	393.3 (125.9–1305.5)	a vs c; 0.035	177.3 33.8–922.7	202.3 78.7–1305.5	NS
SF pDC	121.9 (45.2–406.8)	90.4 (11.4–291.1)	363.8 (324.6–2925.5)	a vs c; 0.003 b vs c; <0.001	56.7 11.4–363.8	187.1 30.4–2925.5	0.027

*Only significant differences shown.
mDC, myeloid BDC; NS, no significant differences; pDC, plasmacytoid BDC; TDC, total number of BDC.

Correlation of SF BDC counts with JIA activity and the type of JIA onset

Absolute numbers of SF BDC depended on JIA activity (table 4). Thus, the highest median total number of SF BDC was found in children with high disease activity (low vs moderate vs high activity; $p<0.03$). Consequently, absolute counts of particular SF BDC subtypes in children with high JIA activity were significantly higher than in those with low activity of the disease (table 4).

Moreover, SF BDC counts differed according to the type of JIA onset (table 4). They were significantly higher in polyarticular than in oligoarticular disease (median total SF BDC 1615.1 cell/μl vs 744,2 cell/μl; $p = 0.001$). Only two patients with systemic disease had joint exudation and were available for the assessment of SF BDC amount. The total numbers of SF BDC were 1437.0 cells/μl and 2739.2 cells/μl, respectively (median 2088.1 cells/μl), and these patients were not included in the statistical analysis. For particular SF BDC subsets, children with polyarticular disease had significantly higher number of both SF mDC1 and SF pDC than those with oligoarticular type of onset ($p = 0.016$ and $p = 0.027$, respectively) (table 4).

Synovial fluid in JIA is highly enriched with mature BDC of mDC2 and pDC subtypes

SF mDC1 cells showed significantly higher expression than PB mDC1 cells of all maturation markers examined (fig 2). Thus, 68.1% of CD40+ mDC1 were found in SF, compared with 10.2% CD40+ mDC1 in PB ($p = 0.038$) (fig 2). The median percentages of both CD80+ and CD86+ mDC1 were significantly higher in SF than in PB (82.3% vs 4.4% and 92.1% vs 13.1%, respectively; $p = 0.003$ and $p = 0.005$, respectively). The CD83+ mDC1 cell rate in SF (72.0%) was significantly higher than in PB (11.5%; $p = 0.012$) (fig 2).

Significant differences in CD40+ cell rates between SF and PB were noticed also for mDC2 subtype (medians 77.9% vs 0.9%, respectively; $p = 0.002$). The rates of CD80+ mDC2 and CD86+ mDC2 in SF versus PB were 27.9% versus 2.5% and 92.1% versus 13.1%, respectively ($p = 0.025$ and 0.003, respectively). The percentage of CD83+ mDC2 in SF and PB did not differ significantly between those two compartments; however, the CD83+ mDC2 rate was about four times higher in JIA SF than in PB (6.3% vs 1.5%; $p>0.05$) (fig 2).

The percentage of pDC expressing CD40 antigen was significantly higher in JIA SF than PB (medians 38.5% vs

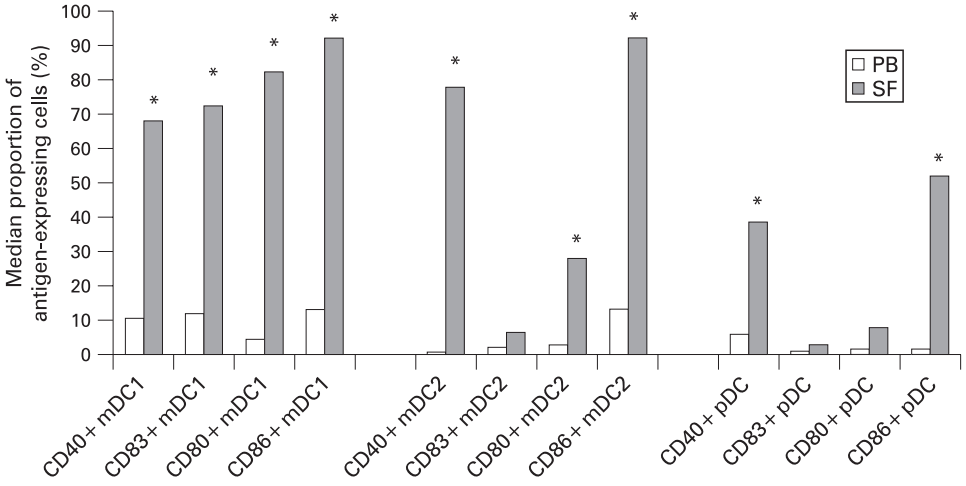

Figure 2 Proportion of cells expressing maturation markers. For each subtype the rates of CD40+, CD83+, CD80+ and CD86+ were calculated as a proportion of the whole absolute cell number of particular subtype and a number of particular antigen-expressing cells. Blood dendritic cells of peripheral blood (PB) and synovial fluid (SF) samples were assessed. mDC, myeloid DC; pDC, plasmacytoid DC.

* Statistically significant differences

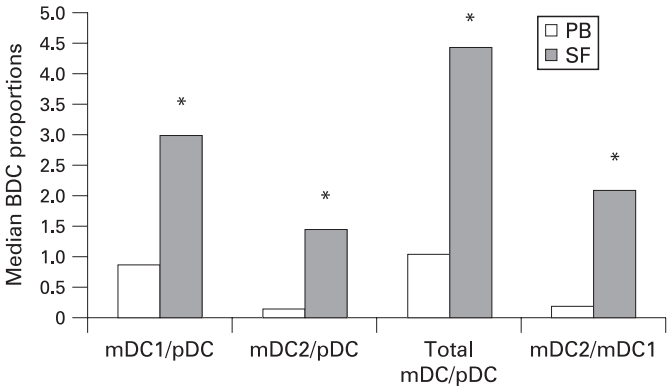

Figure 3 Differences in proportion between particular blood dendritic cell (BDC) subtypes in peripheral blood (PB) and synovial fluid (SF). mDC, myeloid DC; pDC, plasmacytoid DC.

5.3%, respectively; p = 0.037). The rates of CD80+ pDC did not differ significantly between PB and SF (medians 7.4% vs 1.3%; p>0.05); pDC in PB from the majority of children with JIA lacked CD80 expression. Moreover, a significant proportion of pDC did not express CD80 also in SF. Significantly higher percentages of CD86+ pDC in SF than in PB were found (medians 51.6% vs 1.3%; p = 0.002). In the majority of children with JIA there was no CD83 expression found on pDC cells in either PB or SF compartments. In contrast, CD83 was expressed on part of the mDC2 cells. For the pDC subtype, CD83+ cell rates were higher in SF than in PB of children with JIA, but this difference was not significant (medians 5.5% vs 0.5%; p>0.05) (fig 2).

DISCUSSION

As far as we know, this is the first study assessing absolute counts and maturation status of three subtypes of BDC, defined by the expression of BDCA-1, -2 and -3 antigens in children with JIA. So far only BDCA-2+/CD123+ DC have been investigated in adult patients with RA.[18 19] More recently, these cells were assessed in JIA.[8] In regard to myeloid DC, only the whole population with CD11c+ antigen expression was studied both in RA[9 17] and JIA.[8] Drugs used for the treatment of either RA or JIA, especially steroids,[24] may significantly influence the proportion or number of DC. Therefore we limited the study to previously untreated children with JIA.

A significantly lower frequency of both myeloid and plasmacytoid (BDCA-2+) DC in the PB of patients with RA in comparison with healthy people was recently reported by Jongbloed *et al*.[19] This finding is in agreement with our observations on children with JIA. We found a profound deficiency of all BDC subtypes in children with JIA in comparison with healthy controls.

Moreover we observed a significant enrichment of SF with cells of immunophenotype identical with BDC detected in PB. According to recent findings in RA, pDC can migrate from PB into inflamed joint tissues, acting as antigen-presenting cells.[18] They are chemoattracted and recruited from PB probably by chemokines CXCL-10, -11 and -12. Here we found, that all three subtypes of BDC are present in SF, which may also be due to migration from the circulation into this compartment. Certainly, further intensive studies are needed to elucidate the mechanisms of this phenomenon requires.

Gattorno *et al* assessed the presence and characteristics of type I IFN- and TNFα- producing cells in SF from inflamed JIA joints as well as in the PB of children with active JIA. In JIA SF they detected BDCA-2+/CD123+ cells producing IFN (pDC), and myeloid CD11c+/CD14− cells which produced TNFα. pDC were more frequently represented in JIA SF than in PB, as we also found in our study. Those pDC were localised at inflammatory lymph-follicular-like structures of the synovium, whereas myeloid DC were mostly found at the lining and sublining layers. Thus, both those types of DC are likely to participate in the rheumatological process.

In patients with RA the mDC/pDC ratio was significantly higher than in matched PB samples.[19] Consequently, in our series this ratio was more than four times higher in SF than in PB when the BDC numbers were analysed, and even 10 times higher when measured by BDC percentages. Moreover, the mDC2/pDC ratio was 14 times higher in SF than in PB, whereas the mDC1/pDC ratio in SF was over three times higher than in PB. Additionally, the SF mDC2/mDC1 ratio was 10 times higher than in PB samples (fig 3). This indicates that JIA SF is distinctly enriched with mDC2 subtype. The mDC2 is a rare subtype of BDC, and there are only a few observations describing its behaviour in various pathological states.[5 6 25] Thus, further studies would give us better understanding of the significance and function of mDC2, including their role in development and/or maintenance of the rheumatoid process in JIA.

The differentiation/maturation status of SF DC has not been established yet in JIA, whereas in RA this problem has been the subject of several studies. Expression of CD80, CD86 and other markers of DC differentiation in RA was examined by Thomas and Quinn.[14] They defined large population $CD33^{dim}/CD14^{dim}$ precursors of DC, as well as smaller subset of $CD33^{bright}/CD14^{dim}$ mature, myeloid-origin DC. CD80 expression was minimal on DC from RA SF. CD86 expression was found on a subset of synovial DC. These cells are probably located in the synovium after migration through the endothelium to present RA-induced antigens to T cells. In RA, myeloid SF DC had more mature phenotype than those from PB.[18] Conversely, pDC in RA SF were found to be less mature than myeloid DC, probably owing to the presence of soluble inhibitory factors.[17–19] On the other hand, several mediators of inflammation directing DC maturation were detected in RA SF, including IFNγ, TNFα or IL10.[6 19] SF DC remained responsive to TLR agonists, showing ex vivo maturation and releasing cytokines. Thus, the exact mechanisms leading to local inhibition of DC maturation in inflamed joints need to be explored.[6 17–19]

In our children with JIA both SF mDC1 and mDC2 expressed more mature phenotype than those BDC circulating in PB. This fact strongly suggests the participation of these BDC subtypes in maintaining an inflammatory response in the JIA joints. SF pDC showed strong expression of both CD40 and CD86 costimulatory antigens, whereas CD80 expression was similarly low in SF and PB, and CD83 antigen was scarcely expressed on these cells. This observation is to some extent contradictory to the findings in patients with RA and may demonstrate some differences in the pathology of RA and JIA, indicating that the role of pDC in JIA pathogenesis is also very likely.

There is further evidence that DC activation correlates with development and maintenance of the rheumatoid process. Thus, interactions of different signalling pathways may contribute to the survival of inflammatory cells within RA or JIA joints.[26] Most recently, activated mDC and pDC with expression of transcription factors representing the Jak/STAT pathway were

found in RA synovial tissue.[27] The presence of these cells correlated with RF positivity and with generally worse outcome of patients. Moreover, increased expression of receptor activator of NF-κB (RANK) was found on cells with features of mature mDC (CD11c+/CD86+).[26]

DC status/counts was found to be associated with some measures of the rheumatoid process. Jongbloed *et al* showed an inverse correlation between the number of mDC circulating in RA PB and the CRP serum level.[19] We confirmed a similar correlation in JIA. Additionally, we found that absolute numbers of SF BDC, but not PB BDC, correlated with high disease activity and polyarticular type of JIA onset.

Importantly, our data indicate that a lower total PB BDC number at the diagnosis may be related to poor response to treatment in children with JIA. This is an original and interesting observation, and certainly needs to be confirmed in further studies comprising larger group of patients.

Finally, how to establish the most precise method of BDC number assessment is still important and an unresolved problem. So far, the prevalence of DC has been expressed as a percentage of DC among all mononuclear cells.[7 8 17 26] Recently, data for the absolute numbers of DC in RA were presented.[19] However, the authors used an indirect method of DC enumeration, based on the percentage of DC in the white blood cell count assessed during routine morphology in a separate sample. In contrast, in our study a direct, objective assessment of absolute BDC counts was made for the first time in a rheumatoid disorder. This so called "single-platform" method of DC calculation was recently introduced by Ma *et al*.[28] The prevalence of BDC assessed in our study by the traditional method did not correspond well with the absolute number counted using the "single-platform" assay, and we recommend the latter method for quantitative expression of the number of BDC as being more accurate and reliable.

In conclusion, our data show that the number of PB BDC is significantly decreased in JIA, probably owing to their accumulation in tissues affected by the inflammatory process. It is likely that circulating BDC migrate to joints, where they undergo maturation. SF in JIA is highly enriched with mature BDC, especially mDC1 and mDC2 subtypes. Most probably those SF BDC participate in mediation of a local immune response, and, in turn, in maintenance of prolonged inflammation in JIA. Moreover, the level of deficiency of circulating PB BDC may influence clinical response to treatment in a patient with JIA.

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E Smolewska, J Stanczyk, H Brózik, M Biernacka-Zielinska, B Cebula, T Robak and P Smolewski

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