ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LVII, 23 SECTIO D 2002

[1] Department of Clinical Immunology, University School of Medicine, Lublin
Zakład Immunologii Klinicznej Akademii Medycznej w Lublinie
[2] Department of Rheumatology, University School of Medicine, Lublin
Katedra i Klinika Reumatologii Akademii Medycznej w Lublinie

JACEK TABARKIEWICZ[1], KAMILA WOJAS[1], MAŁGORZATA SIEKLUCKA[1], JOLANTA PARADA-TURSKA[2], MARCIN MAZUREK[2], AGNIESZKA BOJARSKA-JUNAK[1], JACEK ROLIŃSKI[1], LESZEK SZCZEPAŃSKI[2]

Rheumatoid synovial fluid is enriched in BDCA-1 positive and BDCA-2 positive dendritic cells compared with peripheral blood

Płyn stawowy chorych na reumatoidalne zapalenie stawów zawiera większy odsetek komórek dendrytycznych BDCA-1 i BDCA-2 pozytywnych w porównaniu do krwi obwodowej

INTRODUCTION

Dendritic cells (DC) are potent antigen presenting cells (APC) that take up, sequester and present antigens (Ags) in the primary and secondary immune responses [8]. They are believed to play a crucial role in the initiation and maintenance of T-cell immunity [1, 5, 8]. DC are present in lymphoid and non lymphoid tissues [5]. Circulating DCs precursors home to lymphoid and non-lymphoid tissues where they reside as immature cells [1, 5]. At the immature stage DCs are well equipped to acquire antigens (Ags) but express low levels of the requisite MHC and costimulatory molecules needed for T lymphocytes stimulation [1, 5]. Following Ags engulfment and processing, dendritic cells migrate to secondary lymphoid organs where they mature and become APCs able to select and activate naive Ag-specific T cells and induce Ag-specific immune response.

Studies of dendritic cells have been hampered by their scarcity in peripheral blood and lack of specific cell surface markers [1, 3, 5]. From studies of DC it became evident, that they do not represent a homogeneous cell population. DC are a mixture of at least two populations myeloid and lymphoid dendritic cells. Nowadays, markers for circulating dendritic cells are available: BDCA-1 for myeloid and BDCA-2 for lymphoid. BDCA-1 (CD1c) is expressed on a subpopulation of human dendritic cells, which are of monocytoid morphology [3]. These cells are CD4+, Lin–, CD11c^{bright},

CD123^{dim}, CD45RO+, CD2+ and express myeloid markers (CD13, CD33) as well as Fc receptors (CD32, CD64, FceRI). BDCA-2 is specifically expressed on human lymphoid (plasmacytoid) dendritic cells. Phenotyping of BDCA-2 positive cells shows these cells are CD4+, Lin–, CD11c–, CD123^{bright}, CD45RA+, CD2– and expressing neither myeloid lineage markers like CD13 and CD33, nor Fc receptors [3]. These cells are circulating in blood and home to lymphoid and non-lymphoid tissues.

It is no surprise that DC are also the most efficient APC for endogenous self antigens and initiate autoimmune diseases in mice after incubation with autoantigens [5]. Dendritc cells may prolong or exacerbate local immune-based inflammatory such as occur in arthritis and increased numbers of DC in rheumatoid synovial fluid (SF) and synovial tissue (ST) have been described [2, 4, 8, 10, 12]. The aim of our study was to examine numbers and proportion of myeloid (BDCA-1 positive) and lymphoid (BDCA-2 positive) dendritic cells in peripheral blood and synovial fluid of patients with rheumatoid arthritis (RA).

MATERIAL AND METHODS

Subjects

Peripheral blood was obtained from 19 patients having rheumatoid arthritis. All of them met the American Rheumatism Association criteria for classical RA.

All patients was receiving treatment at the time of study; the majority were on methotrexate and the remainder on disease modifying agents or cyclophosphamide. Informed consent was obtained from each individual.

Isolation of the peripheral blood and synovial fluid mononuclear cells

Peripheral blood and synovial fluid were obtained into sterile heparinized syringes. Mononuclear cells were separated by gradient centrifugation (Gradisol-L, Aqua Medica, Poland) and washed twice in phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}, containing 0.5% bovine serum albumin (BSA) and 2mM EDTA.

Phenotyping of the cells

The cell surface antigens in each case were determined on fresh cells at the time of sample submission. The following directly conjugated monoclonal antibodies (mAbs) were used: mouse anti-human BDCA-1-FITC (Miltenyi-Biotec, Germany), BDCA-2-FITC (Miltenyi-Biotec, Germany), CD123-PE (Becton Dickinson, USA), CD19-CyChrome (Pharmingen, USA). Immunofluorescent staining was prepared according to manufacturers' protocol. Cells were collected using a FACSCalibur flow cytometer equipped with 488-nm argon laser (Becton Dickinson) and analysed with CellQuest Software. We collected 300,000 of total events.

Statistical analysis

Wilcoxon-non-parametric test and Statistica 5.0 PL software were applied to statistical analysis.

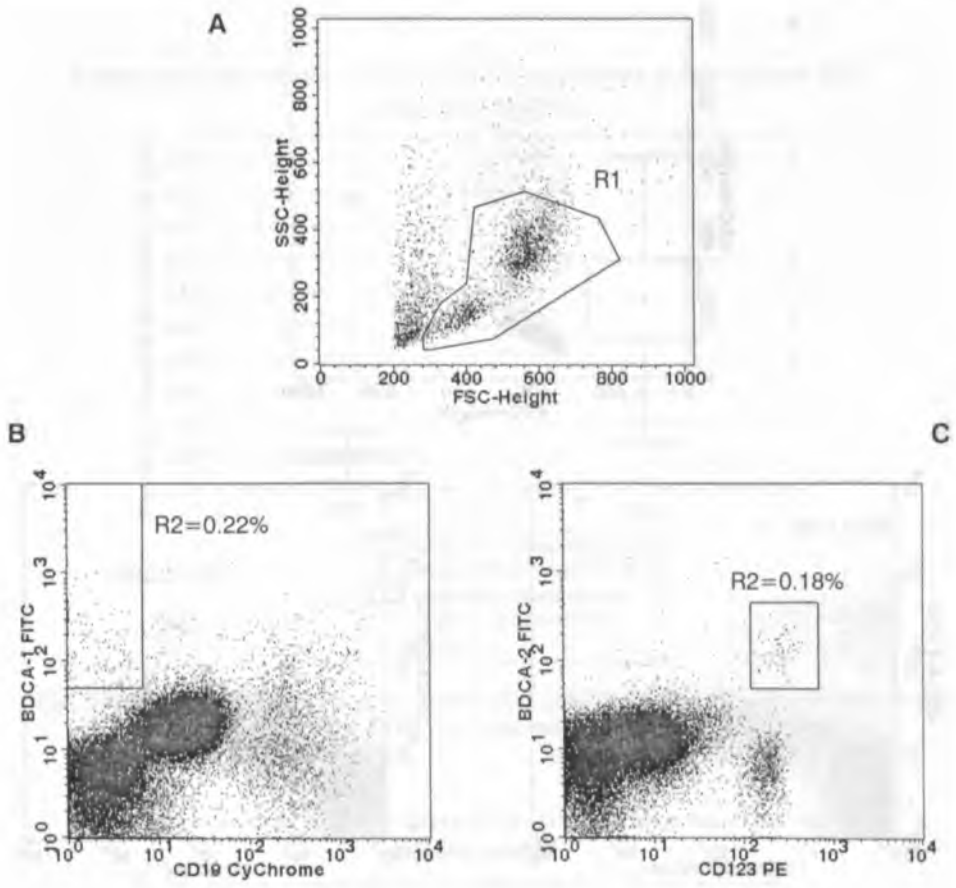

Fig.1. Identification of circulating DC by flow cytometry in peripheral blood of one patient with rheumatoid arthritis. A: The mononuclear cells analysis region (R1) applied to light scatters. B: The R1 gated events were then analysed for BDCA-1 and CD19 staining and BDCA-1+/CD19– cells were counted as circulating myeloid DC. C: The R1 gated events were then analysed for BDCA-2 and CD123 staining and BDCA-2+/CD123+ cells were counted as circulating lymphoid DC.

RESULTS

BDCA-1 and BDCA-2 are novel human circulating dendritic cells antigens, for myeloid and lymphoid respectively [3]. In our study we identified myeloid DC as BDCA-1 positive and CD-19 negative cells and lymphoid DC as BDCA-2 and CD123 double positive cells. The count of DC were expressed as a proportion of DC to mononuclear cells. The mononuclear cells (MC) analysis region for peripheral blood mononuclear cells (PBMC) (Fig. 1) and synovial fluid mononuclear cells (SFMC) (Fig. 2) was applied to light scatters and the gate was set to exclude dead cells and debris. Our results

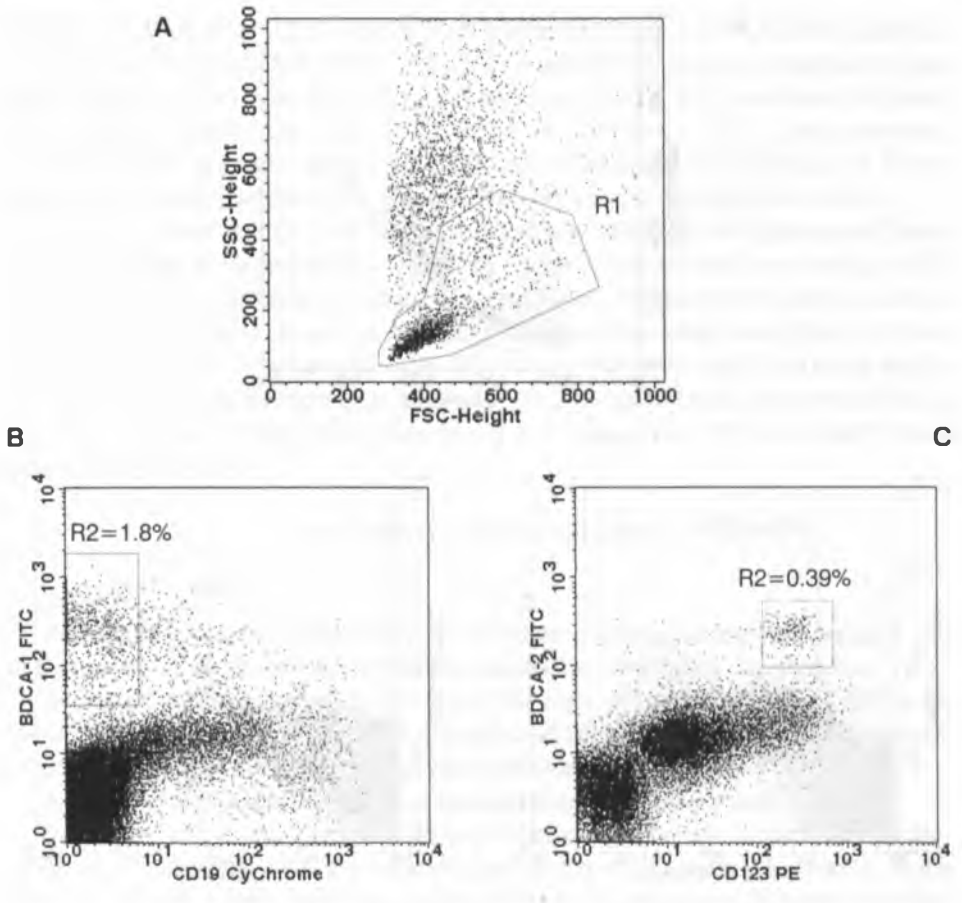

Fig. 2. Identification of circulating DC by flow cytometry in rheumatoid synovial fluid of one patient with rheumatoid arthritis. A: The mononuclear cells analysis region (R1) applied to light scatters. B: The R1 gated events were then analysed for BDCA-1 and CD19 staining and BDCA-1+/CD19– cells were counted as circulating myeloid DC. C: The R1 gated events were then analysed for BDCA-2 and CD123 staining and BDCA-2+/CD123+ cells were counted as circulating lymphoid DC.

show that total number of DC was significantly higher (p=0.002) in rheumatoid SF (2.2%±1.79) than in PB (0.34%±0.32). Comparison of dendritic cells' subsets between rheumatoid SF and PB demonstrates that the number of myeloid (BDCA1+/CD19–) demdritic cells was significantly higher (p=0,002) in rheumatoid synovial fluid than in peripheral blood, 0.18%±0.14 and 1.73%±1.29 in that order (Fig. 3). Figure 4 illustrates that percentage of lymphoid DC (BDCA2+/CD123+) was also significantly higher (p=0.04) in SF (0,47%±0,88) in comparison to PB (0.17%±0.23). We also noticed that in rheumatoid synovial fluid number of myeloid dendritic cells was significantly higher (p=0.002) than lymphoid dendritic cells and in peripheral

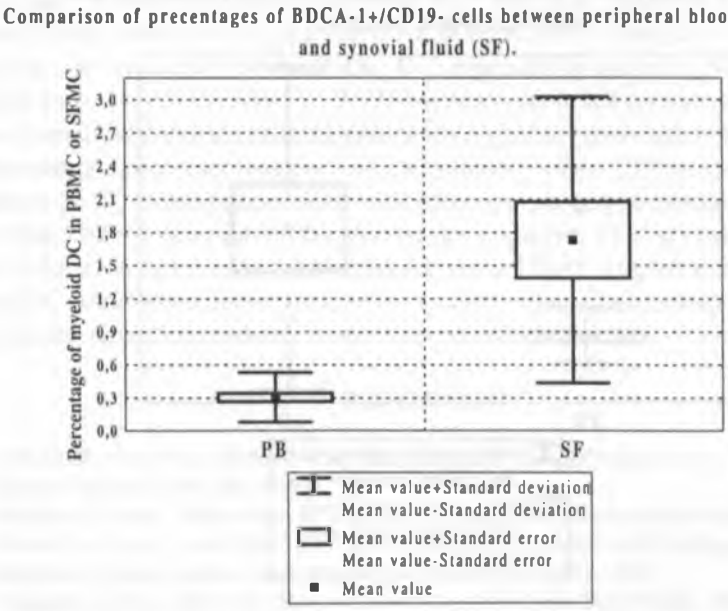

Fig. 3. Comparison of percentages of myeloid DC (BDCA-1+/CD19–) between peripheral blood (PB) and synovial fluid (SF)

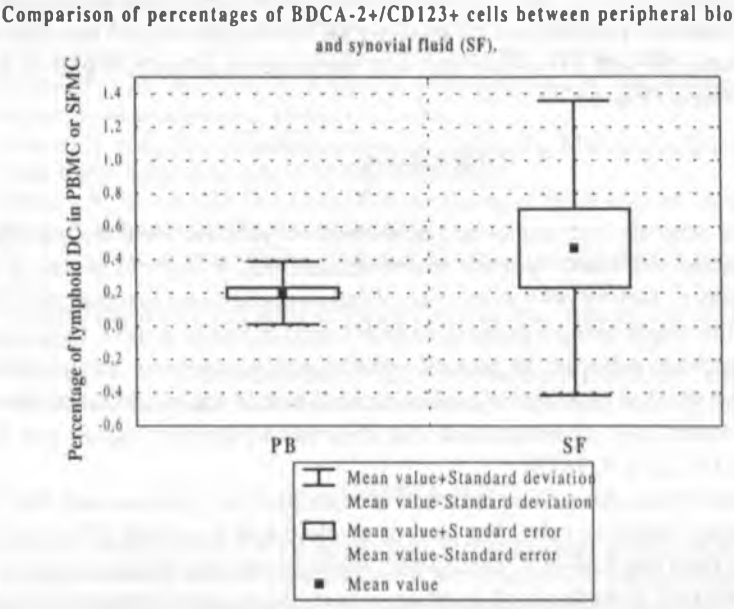

Fig.4. Comparison of percentages of lymphoid DC (BDCA-2+/CD123+) between peripheral blood (PB) and synovial fluid (SF)

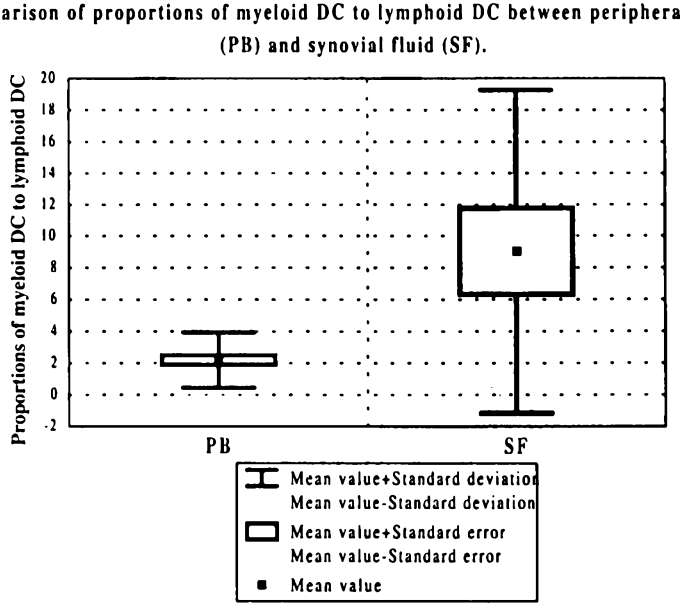

Fig.5. Comparison of proportions of myeloid DC to lymphoid DC between peripheral blood (PB) and synovial fluid (SF)

blood was not such difference. We find that rheumatoid SF is enriched in myeloid and lymphoid DC and the proportions of myeloid DC to lymphoid DC are different ($p=0.012$) between SF and PB of patients' with rheumatoid arthritis, 9.05 ± 10.2 and 2.2 ± 1.7 respectively (Fig. 5).

DISCUSSION

Rheumatoid arthritis is characterised by persistent synovitis, local destruction of bones and cartilage and many systemic manifestation. RA is likely to be result of a complex interplay of factors both at the site of inflammation and systemically. Dendritic cells are the major antigen presenting cells found in rheumatoid synovium and synovial fluid and are expected to present arthritogenic antigens to autoreactive T lymphocytes and for that reason could take a crucial role in the initiation of disease. Previous researchers have demonstrated that rheumatoid synovial tissues and fluid are enriched in DC [2, 4, 8, 9, 12].

We found that rheumatoid SF is enriched in both BDCA-1 positive and BDCA-2 positive circulating dendritic cells. The sum of myeloid and lymphoid DC in inflammatory synovial fluid was 0.49–6.77% of SFMC, while in peripheral blood account for 0.09–1.22% of PBMC. Zvailfler et al. and Bergroth et al. revealed comparable results using different surface antigens to characterise DC [2, 12]. Our results show that DC

circulating in PB could migrated into the synovium through postcapillary venules. The reason for accumulation of dendritic cells in synovial fluid is not yet clearly explained. Some researches suggest that it could be caused by changed synovial environment causing chemotaxis of circulating DC, especially by the local production of cytokines such GM-CSF, TNF-α [7, 9, 11]. We also concluded that proportion of myeloid to lymphoid dendritic cells is significantly higher in rheumatoid SF than in PB. It is interesting due to fact that myeloid DC stimulate mainly Th1 depended immune response [1, 5], which is associated with pathogenesis and perpetuating of arthritis [6], while Th2 response inhibit the disease development. The fact that myeloid dendritic cells are accumulating in rheumatoid synovial fluid in higher number than lymphoid DC could be an important clue for understanding of immunopathogeneseis of RA and for future methods of immunotherapy of this disease.

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STRESZCZENIE

Komórki dendrytyczne (DC) są komórkami prezentującymi antygen (APC), które inicjują pierwotną odpowiedź immunologiczną zależną od limfocytów T. Poprzednie badania donoszą, że DC biorą udział w patogenezie wielu chorób człowieka. Krew obwodowa i płyn stawowy pobrane zostały od 19 pacjentów chorych na RZS. Komórki mononuklearne krwi obwodowej i

płynu stawowego inkubowano z przeciwciałami monoklonalnymi anty-CD19, anty-CD123, anty-BDCA-1, anty-BDCA-2. Określono odsetek mieloidalnych i limfoidalnych DC we krwi obwodowej i płynie stawowym. Odsetek mieloidalnych i limfoidalnych DC był statystycznie istotnie wyższy w płynie stawowym niż we krwi obwodowej. Stwierdziliśmy także różnicę w proporcji mieloidalnych do limfoidalnych komórek dendrytycznych pomiędzy krwią obwodową a płynem stawowym. W płynie stawowym odsetek mieloidalnych DC był istotnie wyższy niż komórek linii limfoidalnej. Określenie odsetka komórek dendrytycznych mieloidalnych i limfoidalnych we krwi obwodowej i płynie stawowym oraz różnic w ich wzajemnej proporcji sugeruje udział komórek dendrytycznych w patogenezie RZS i podtrzymywaniu niekorzystnej dla przebiegu choroby odpowiedzi immunologicznej typu Th1.