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Co-stimulatory and adhesion molecules of dendritic cells in rheumatoid arthritis

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Abstract

Dendritic cells (DCs) in the rheumatoid arthritis (RA) joint mediate the immunopathological process and act as a potent antigen presenting cell. We compared the expression of co-stimulatory and adhesion molecules on DCs in RA patients versus controls with traumatic joint lesions and evalulated the correlation between the immunophenotypical presentation of DCs and the clinical status of the disease. Samples of peripheral venous blood, synovial fluid (SF) and synovial tissue (ST) were obtained from 10 patients with RA at the time of hip or knee replacement and from 9 control patients with knee arthroscopy for traumatic lesions. Clinical status was appreciated using the DAS28 score. Blood, SF and dissociated ST cell populations were separated by centrifugation and analyzed by flow cytometry. Cells phenotypes were identified using three-color flow cytometry analysis for the following receptors HLA-DR, CD80, CD83, CD86, CD11c, CD18, CD54, CD58, CD3, CD4, CD8, CD19, CD20, CD14, CD16, CD56. HLA-DR molecules, co-stimulatory receptors CD80, CD86, CD83 and adhesion molecules CD18, CD11c, CD54, CD58, were analyzed by two-color immunofluorescence microscopy on ST serial sections. In patients with active RA (DAS28>5.1) we found a highly differentiated subpopulation of DCs in the ST and SF that expressed an activated phenotype (HLA-DR, CD86+, CD80+, CD83+, CD11c+, CD54+, CD58+). No differences were found between circulating DCs from RA patients and control patients. Our data suggest an interrelationship between clinical outcome and the immunophenotypical presentation of DCs. Clinical active RA (DAS28>5.1) is associated with high incidence of activated DCs population in the ST and SF as demonstrated by expression of adhesion and co-stimulatory molecules.

Keywords: dendritic cells - co-stimulatory molecules - adhesion molecules - synovial fluid - synovial tissue

Introduction

Rheumatoid arthritis (RA) is a severe autoimmune inflammatory disease of multifactorial origin. The immune response predominantly occurs in the synovial tissue (ST) and fluid (SF). Its pathogenic mechanism is complex and involves the collaboration of many cells of the immune system. The disease process involves abnormal presentation of antigen by antigen-presenting cell (APC) and activation of autoreactive T cells. It is generally agreed that the first step in the immune response –

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the antigen-presenting process- plays an important role. This idea is supported by several arguments: histological studies demonstrating APC clustered with T cells close to blood vessels, evidence of CD4+ T cells in the SF and ST, the association of the disease with certain HLA-DR alleles [1].

Effective APCs have the capacity to provide all the necessary signals required for the activation and proliferation of CD4+ T cells [2]. For many years the macrophage has been regarded as the main accessory cell for the immune response. Later it has became evident that dendritic cells (DCs) can also act as a very potent APC [3,4]. They express large amounts of surface class II HLA antigens [5] particularly HLA-DR and lack macrophage differentiation antigens like CD14 or CD68 [6,7]. The importance of co-stimulatory signals has been highlighted by several studies [8]. Expression of CD80 or CD86 varies amongst different APC types and may be modulated by activation or environmental factors such as cytokines [9,10]. Activated DCs express both CD80 and CD86, similar to activated B and T cells and macrophages; during inflammatory reactions, the inappropriate expression of co-stimulatory molecules may arise [11-14].

In RA, synovial fibroblasts and macrophages produce granulocyte-macrophage colony-stimulating factor (GM-CSF) which stimulates the full differentiation and activation of DCs. A large number of DCs is found in synovial effusions of patients with RA [15-17]. They comprise up to 5% SF mononuclear cells, compared to less than 1% of peripheral blood cells [18]. DCs are found in the SF of patients with other forms of inflammatory arthritis, but their number is significantly less than in RA and some data suggested that RA articular DCs present antigen more efficiently that those isolated from other sites [19]. DCs have also been detected in the ST, especially near dense lymphoid aggregates and high endothelial venules [20]. Synovial DCs migrate from the peripheral blood, presumably under the influence of a chemotactic gradient [21]. There is evidence that mature DCs in the RA joint mediate the immunopathological process. Once in the joint, they can act as a potent APC for an "arthrogenic" antigen. However, their derivation, function and potential role in the pathogenesis of the disease is incomplete understood [22].

Previous reports indicate that immunopathological features of RA ST (including DCs phenotype) reflect clinical disease activity [23-26]. We compared the expression of costimmulatory and adhesion molecules on DCs in RA patients versus controls with traumatic joint lesions and looked for a possible correlation between the immunophenotypical presentation of DCs and the clinical status of the disease.

Materials and methods

Subjects

Ten RA patients were recruited from the Deptartment of Rheumatology and Internal Medicine, "St. Maria" Hospital, Bucharest, Romania between June and December 2001, diagnosed according to the 1987 revised criteria of the American College of Rheumatology. All patients were receiving disease-modifying agents (such as gold, methotrexat or leflunomide), 2 were receiving prednisone 10 mg daily. None was receiving TNF-α antagonist therapy. Clinical status was appreciated using the DAS28 score. Patients variables are presented in Table 1.

All patients were operated for hip or knee replacement in Department of Orthopedics, "St. Pantelimon" Hospital, Bucharest, Romania, and samples of peripheral venous blood, synovial fluid and synovial membrane were obtained. As controls we analysed samples of peripheral venous blood, synovial fluid and synovial membrane obtained during knee arthroscopy from 9 patients with traumatic lesions.

Table 1 Patient variables.

Sex M/F	3/7
Age (mean +/- SD)	51 +/- 5.7 years
Disease duration (mean +/-SD)	52 +/- 15.3 months
Disease activity measures	
Tender joint count (mean +/- SD)	18.6 +/- 6.2
Swollen joint count (mean +/- SD)	10.7 +/- 5.3
ESR (mm/h) (mean +/- SD)	40.5 +/- 10.3
DAS28 (mean)	5.38

Flow cytometry

Monoclonal antibodies anti-CD11a PE, -CD11c PE, -CD14 PE, -CD18 FITC, CD54-FITC, -CD58 PE, -CD80 PE, -CD83 FITC, -CD86 FITC, -CD123 PE, HLA-DR PerCP, as well as lin1 FITC (a cocktail of mAbs against CD3, CD14, CD16, CD19, CD20 and CD56) were obtained from BD Biosciences, together with the appropriate control antibodies.

Mononuclear cells from anticoagulated peripheral blood, synovial fluid and dissociated synovial tissue were isolated by density gradient centrifugation (Sepcel, Victor Babes Institute, Bucharest).

For flow cytometry analysis, aliquots of cells (approx.10^5 cells) were washed then resuspended in PBS and stained for 30 min with specific monoclonal antibodies (10µl). Cells were then washed and fixed with paraformaldehyde 1%, and analyzed using a FACSVantage SE cytometer (BD) and CellQuest software.

Cells were analysed for their relative size and granularity (forward – FSC, and side scatter – SSC) and surface marker expression. Peripheral blood DCs were identified based upon their lack of reactivity with lin1, intense HLA-DR expression, as well as CD11c or CD123 reactivity. HLA-DR positive SF or ST mononuclear cells were also gated on a FSC vs SSC dot-plot for further membrane marker expression analysis.

Immunofluorescence

Monoclonal antibodies against HLA-DR, CD11c, CD54, CD58, CD80, CD83 and CD86 labelled with FITC or PE were obtained from BD Biosciences, together with the appropriate control antibodies. Cryostat serial sections of synovial tissues (5µm) were prepared from the bone-cartilage interface. Immunostaining consisted of incubation with the labelled antibody 2 hours at 37°C. Slides were counterstained with Hoechst 33342 (Molecular Probes) then observed under a Nikon Eclipse TE 300 microscope.

Statistical analysis

The proportion of cells expressing a particular marker was scored through flow cytometry, expressed as mean ± one standard deviation for triplicate samples. 200 cells were counted by microscopy for each sample, and positive cells expressed as percentage of total cells. Results are presented as mean ± one standard deviation for triplicate samples.

Results

Flow cytometry

Proportions of DC in peripheral blood cells were counted using the former's lack of lin1 reactivity as well as co-expression of HLA-DR with either

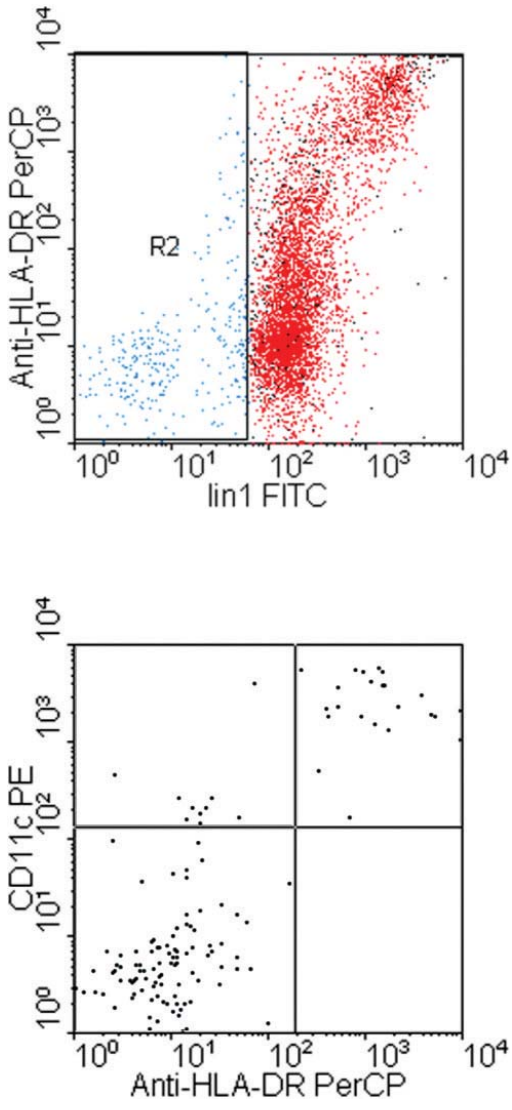

Fig. 1 Cells in region R2 in dot-plot a (lin1 low/negative) were analyzed for CD11c expression: 40% of lin1$^-$ cells were positive for CD11c and expressed high levels of HLA-DR, indicative of DC lineage (b).

Table 2 Flow cytometry analysis of DC in synovial fluid and tissue in RA patients (percentages of gated mononuclear population, expressed as mean ± SD of triplicate samples).

Patient	DAS 28	Synovial tissue					
		CD11c	CD54	CD58	CD80	CD83	CD86
1	4,2	22,20 ± 2,89	12,30 ± 1,85	13,40 ± 1,21	40,10 ± 4,41	16,20 ± 1,94	34,30 ± 3,09
2	4,8	22,40 ± 2,91	14,30 ± 2,15	14,40 ± 1,30	42,30 ± 4,65	22,50 ± 2,70	29,90 ± 2,69
3	5,7	35,50 ± 4,62	19,30 ± 2,90	16,70 ± 1,50	44,80 ± 4,93	36,20 ± 4,34	63,50 ± 5,72
4	6,2	47,80 ± 6,21	24,20 ± 3,63	34,20 ± 3,08	57,90 ± 6,37	43,60 ± 5,23	74,40 ± 6,70
5	4,1	24,20 ± 3,15	11,90 ± 1,79	13,10 ± 1,18	39,30 ± 4,32	11,90 ± 1,43	29,50 ± 2,66
6	6,7	55,20 ± 7,18	29,90 ± 4,49	27,80 ± 2,50	75,20 ± 8,27	39,60 ± 4,75	68,50 ± 6,17
7	7,2	65,30 ± 8,49	37,20 ± 5,58	42,10 ± 3,79	82,10 ± 9,03	47,20 ± 5,66	74,10 ± 6,67
8	4,6	18,90 ± 2,46	10,20 ± 1,53	15,60 ± 1,40	48,20 ± 5,30	22,60 ± 2,71	42,20 ± 3,80
9	5,2	50,40 ± 6,55	26,10 ± 3,92	16,70 ± 1,50	56,90 ± 6,26	32,10 ± 3,85	58,20 ± 5,24
10	5,1	46,70 ± 6,07	22,50 ± 3,38	18,30 ± 1,65	59,20 ± 6,51	33,90 ± 4,07	52,40 ± 4,72
Patient	**DAS 28**	**Synovial fluid**					
		CD11c	**CD54**	**CD58**	**CD80**	**CD83**	**CD86**
1	4,2	-	-	-	-	-	-
2	4,8	-	-	-	-	-	-
3	5,7	48,30 ± 3,38	55,30 ± 4,42	48,30 ± 2,42	29,20 ± 1,75	11,20 ± 0,73	14,30 ± 0,57
4	6,2	54,20 ± 3,79	58,90 ± 4,71	53,40 ± 2,67	41,20 ± 2,47	15,40 ± 1,00	16,20 ± 0,65
5	4,1	-	-	-	-	-	-
6	6,7	66,70 ± 4,67	48,20 ± 3,86	66,20 ± 3,31	53,20 ± 3,19	14,30 ± 0,93	18,60 ± 0,74
7	7,2	64,20 ± 4,49	59,30 ± 4,74	68,40 ± 3,42	51,20 ± 3,07	23,10 ± 1,50	20,10 ± 0,80
8	4,6	31,20 ± 2,18	36,70 ± 2,94	32,60 ± 1,63	23,20 ± 1,39	9,70 ± 0,63	6,50 ± 0,26
9	5,2	46,70 ± 3,27	45,80 ± 3,66	38,70 ± 1,94	28,50 ± 1,71	12,40 ± 0,81	8,30 ± 0,33
10	5,1	-	-	-	-	-	-

CD11c or CD123 (Fig.1). No significant difference was found when comparing control subjects to RA patients.

Synovial fluid and tissue isolated mononuclear cells were analysed for adhesion and co-stimulatory molecules expression. Results (Table 2) show that subjects with the highest DAS28 score – patients 6 and 7 – had also the highest percentage of both ST and SF cells positive for CD11c, CD54 and CD58 adhesion molecules (Fig. 2). Although there is a good correlation in the way percentages of positive cells in SF and ST compare among patients, differences were noted in the expression levels of a particular marker when the two kind of samples arising from the same patient are considered. This fact might be due to the higher proportion of cellular debris generated by the mechanical dissociation method employed for tissue samples preparation, or to differences in the relative amounts of synoviocytes impurification. Another possible cause could be the preferential localization of distinct mononucleated cell subsets in the joint tissue or synovial fluid, hypothesis that could not be tested in our experimental set-up.

Patient 8 showed significantly lower proportions of adhesion molecules-expressing cells, within both compartments, as compared to cases 6 and 7, consistent with his inferior clinical score.

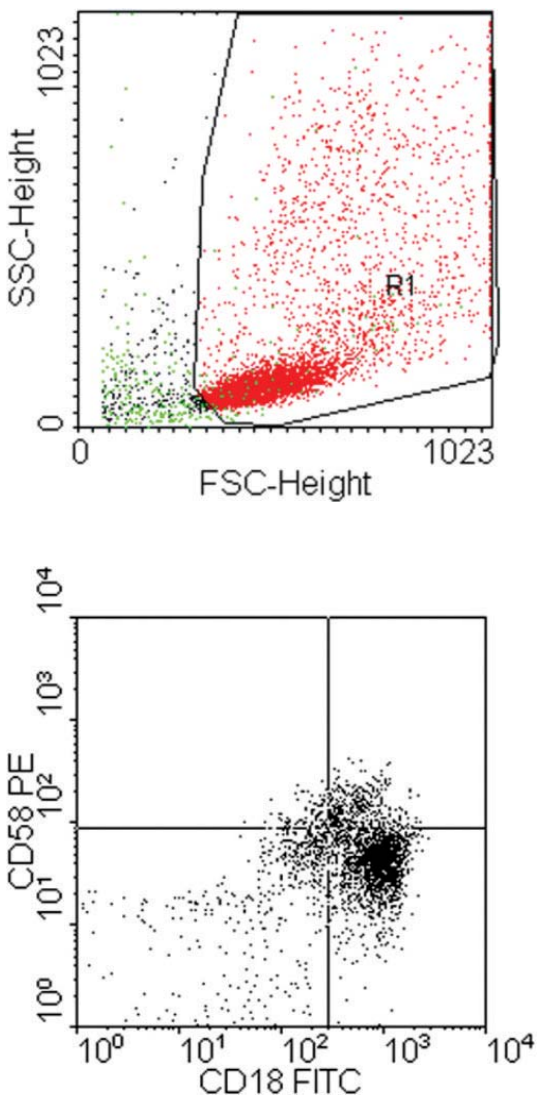

Fig. 2 Mononuclear cells were gated on a FSC vs SSC dot-plot (a) and analyzed for CD18 and CD58 expression. For patient 6, 80% of the cells are CD18 positive and 27.8% are CD58 positive (b).

Proportions of co-stimulatory molecules-positive (CD80+ and CD86+) activated (CD83+) DC showed a similar correlation to the clinical score in ST samples, being significantly higher in all patients with active RA (DAS28 > 5.1) than in those with mild clinical presentation (DAS < 5.1). SF samples displayed the same trend of surface markers expression.

All patients had significantly higher proportions of the studied cell subpopulations than controls (Fig. 3).

Microscopy

HE stained sections of ST showed various degree of organized focal aggregate of lymphocytes, synoviocyte proliferation, mononuclear cell infiltration and neoangiogenesis, related to the patient's condition (Fig. 4).

By immunofluorescence analysis, in patients with active RA (DAS28>5.1) we found a highly differentiated subpopulation of DC (HLA-DR, CD86, CD80, CD11c, CD54, CD58 positive) in the synovial tissue to express an activated phenotype (CD83 positive), while patients with inferior DAS28 scores had lower proportions of ST DCs (Table 3).

Suggestive slides from patients 7 and 8 are presented in Fig. 5 and 6, where differences between DCs proportions and distribution are easily noticed.

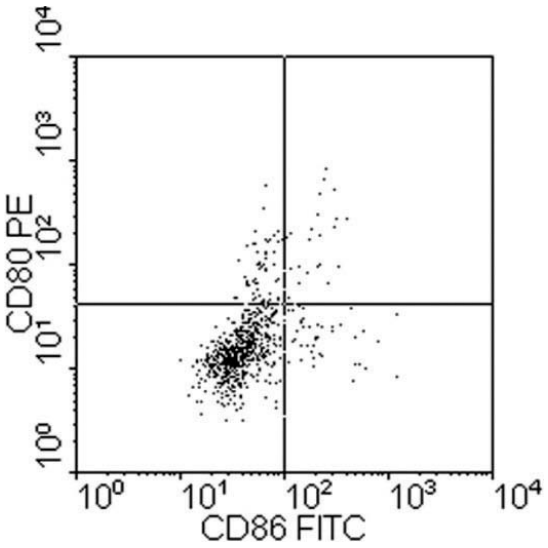

Fig. 3 Using a strategy similar to the one depicted in fig. 2, gated mononuclear cells from a control subject were found to express low amounts of CD80 and CD86.

Table 3 Percentages of synovial cells expressing adhesion and co-stimulatory membrane molecules – immunohistochemistry (* mean of 9 normal synovial tissues). Mean ± SD.

Patient	HLA-DR	CD11c	CD54	CD58	CD80	CD83	CD86
1	40,2 ± 3,62	28,2 ± 2,26	13,1 ± 1,31	9,6 ± 0,86	14,2 ± 1,56	5,3 ± 0,42	7,8 ± 0,70
2	56,3 ± 5,07	31,2 ± 2,50	18,9 ± 1,89	8,8 ± 0,79	13,8 ± 1,52	11,4 ± 0,91	2,3 ± 0,21
3	78,4 ± 7,06	43,2 ± 3,46	25,2 ± 2,52	14,6 ± 1,31	22,5 ± 2,48	21,2 ± 1,70	3,4 ± 0,31
4	79,5 ± 7,16	56,3 ± 4,50	29,2 ± 2,92	18,6 ± 1,67	34,2 ± 3,76	22,3 ± 1,78	11,2 ± 1,01
5	54,3 ± 4,89	38,6 ± 3,09	19,2 ± 1,92	16,3 ± 1,47	12,8 ± 1,41	9,5 ± 0,76	9,4 ± 0,85
6	68,4 ± 6,16	59,6 ± 4,77	26,4 ± 2,64	19,6 ± 1,76	34,6 ± 3,81	22,4 ± 1,79	8,4 ± 0,76
7	86,5 ± 7,79	63,3 ± 5,06	45,4 ± 4,54	28,3 ± 2,55	37,5 ± 4,13	26,1 ± 2,09	15,2 ± 1,37
8	24,2 ± 2,18	45,4 ± 3,63	14,3 ± 1,43	12,6 ± 1,13	12,1 ± 1,33	14,2 ± 1,14	14,3 ± 1,29
9	72,1 ± 6,49	50,1 ± 4,01	33,3 ± 3,33	12,9 ± 1,16	34,5 ± 3,80	12,3 ± 0,98	12,6 ± 1,13
10	61,2 ± 5,51	46,8 ± 3,74	27,2 ± 2,72	14,7 ± 1,32	32,2 ± 3,54	21,1 ± 1,69	14,9 ± 1,34
Ctrl	8,9 ± 0,80	2,34 ± 0,19	1,66 ± 0,17	1,08 ± 0,10	0,44 ± 0,05	1,2 ± 0,10	0,22 ± 0,02

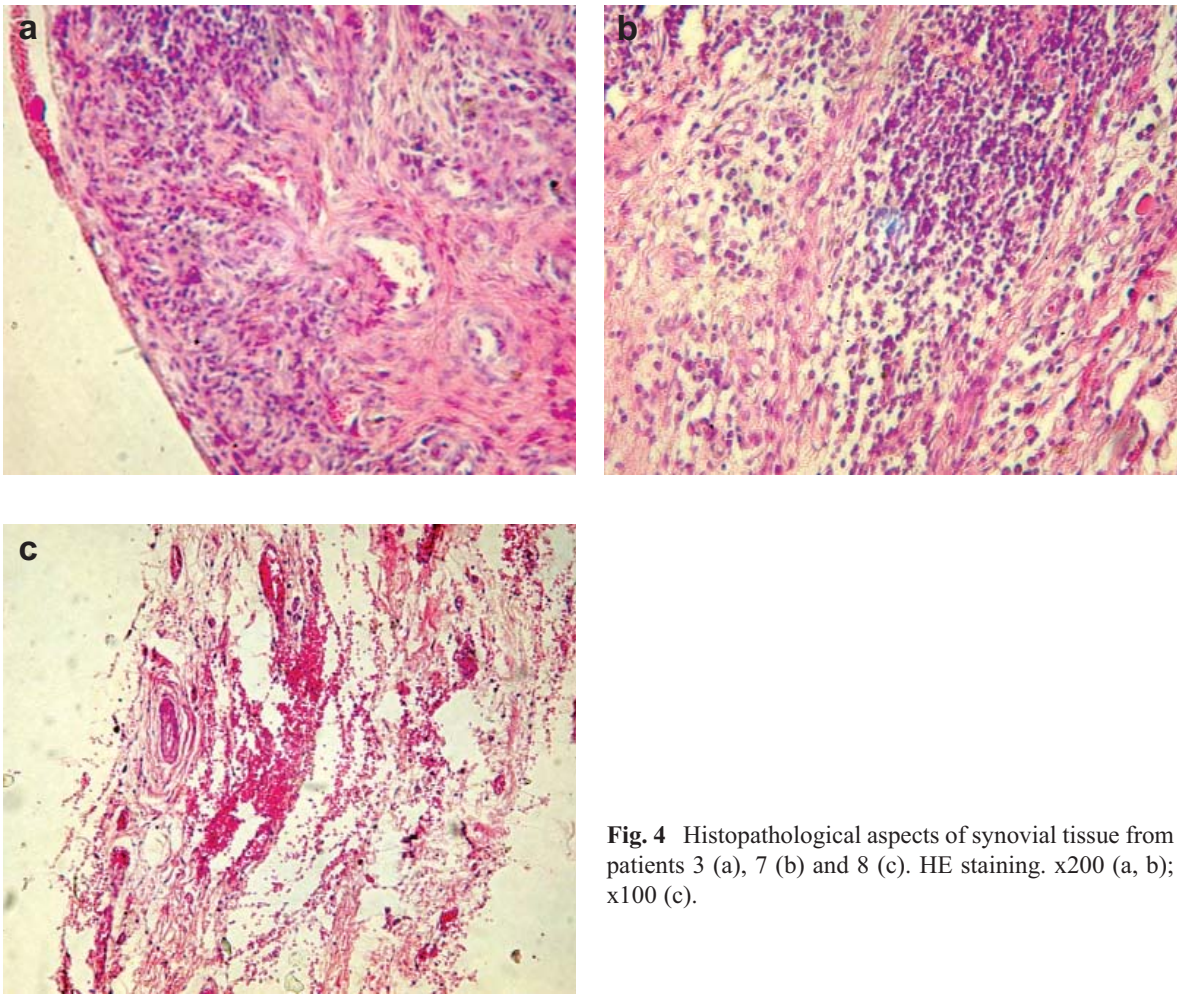

Fig. 4 Histopathological aspects of synovial tissue from patients 3 (a), 7 (b) and 8 (c). HE staining. x200 (a, b); x100 (c).

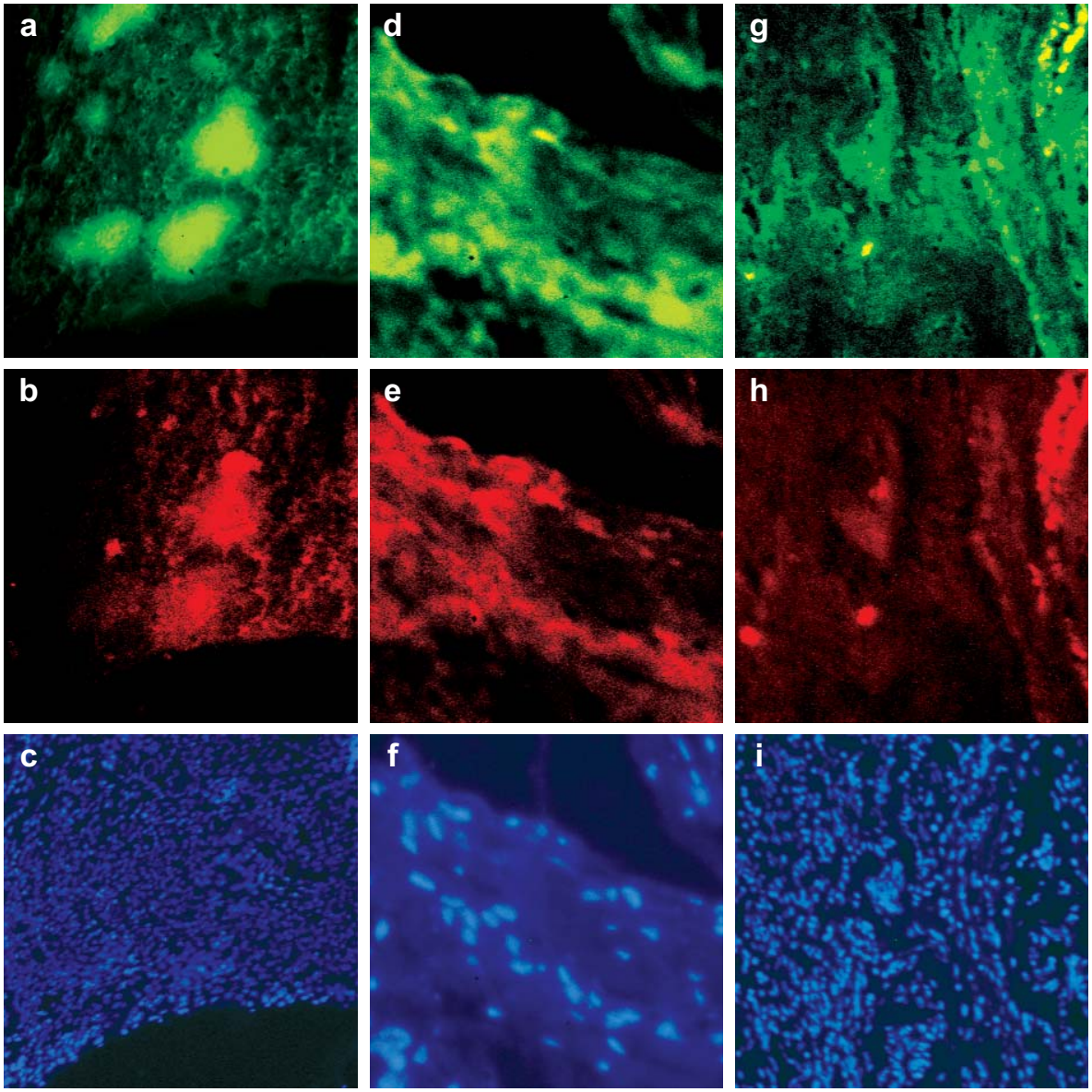

Fig. 5 Immunofluorescence images of three synovial tissue slides (a-c, d-f and g-i) stained for lin1 (a), CD11c (b), HLA-DR (d), CD80 (e), CD86 (g), CD54 (h) and Hoechst 33342 (c, f and i) from a patient with a mild clinical form (patient 8, DAS28 = 4,6). x100 (a–c, g–i); x200 (d–f).

Discussion

The synovial histopathological aspects in RA are characterized by organized focal aggregates of lymphocytes, macrophages and DCs that can progress to germinal centre-like structures, in addition to the synovial inflammatory process (synoviocyte proliferation, pannus formation, angiogenesis, cytokine production, mononuclear cell infiltration) [27-29]. DCs play an important role in the formation and maintenance of inflammation [30-32]. Mature DCs may be central to the development and maintenance of perivascular aggregates in synovial inflammation,

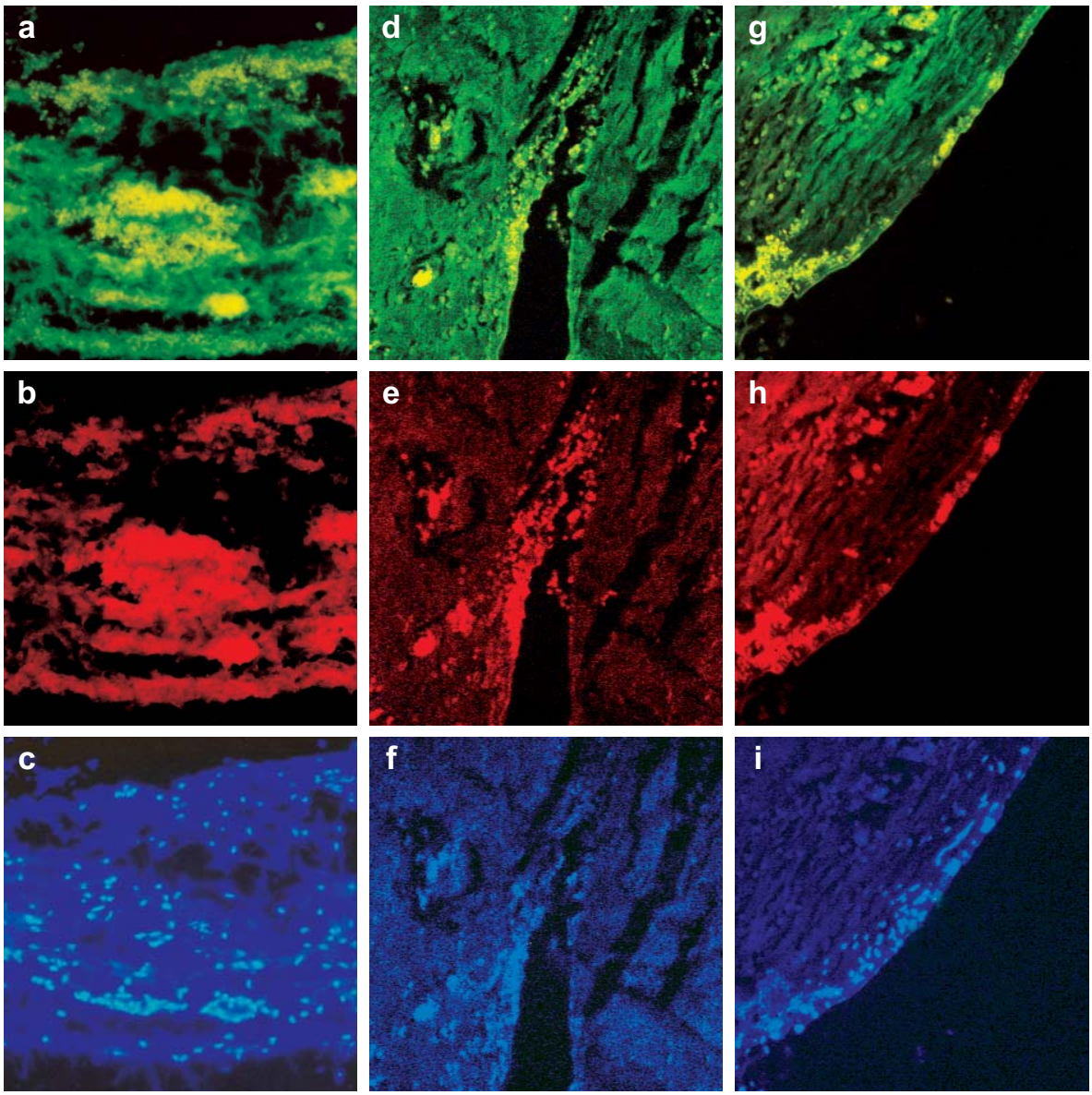

Fig. 6 Immunofluorescence images of three synovial tissue slides (a-c, d-f and g-i) stained for lin1 (a), CD11c (b), HLA-DR (d), CD58 (e), CD86 (g), CD80 (h) and Hoechst 33342 (c, f and i) from a patient with active RA (patient 7, DAS28 = 7,2). x100.

the formation of organized lymphoid structures, the perpetuation of RA inflammatory and erosive activity [33,34].

Previous serial and cross-sectional studies of synovial immunopathology have suggested that at least some immunopathological features of RA ST inflammation, including macrophages, lymphocytic infiltration, cytokine, adhesion molecules, correlate with clinical activity [35-38]. Correspondingly, infiltration of ST by macrophages and the expression of macrophage-derived cytokines in ST of patients with RA have been noted [39-41]. A recent study demonstrates a close correlation between differentiated DCs infiltration, lymphocytic infiltration and vascularity in ST from patients not only with RA, but also with osteoarthritis and

spondyloarthropathy, suggesting common factors mediating the migration of DCs and lymphocytes into damaged joints [42].

The relationship of differentiated DCs infiltration of ST to clinical activity has been poorly studied. This study demonstrates that DCs markers of differentiation can also correlate with the clinical status of the disease.

In the current study of 10 patients with RA, we evaluated the expression of costimmulatory and adhesion molecules on DCs in peripheral blood, SF and ST, as markers of their differentiation, with the immunophenotypical presentation of DCs from control patients and we compared this data with the patient's clinical scoring.

The results have suggested the enhanced functional ability of SF and ST DCs from RA patients to provide adhesion and co-stimulatory signals. DCs from control patients did not express such differentiation markers. We found a proportion of ST DCs in RA patients higher than previously reported [18,27], possibly due to our experimental design. The enhanced adhesion and co-stimulatory ability of SF and ST DCs may play an important role in the pathogenesis of the synovial immune response and its localization to the joint [43-45].

Despite the relatively small number of patients, the indices of disease activity correlated across the set of ST and SF with the differentiation of DCs. The expression of DCs adhesion and co-stimulatory molecules correlated with the clinical status of the disease as evaluated by the DAS28 score. This is a complex disease activity score which includes clinical data (tender joint count, swollen joint count) and biological data (ESR).

However, the current study is limited in its capacity to evaluate the complete implication of DCs in the pathogenesis of RA: the small number of patients, variability in patient's factors at study entry (disease duration, previous DMARD treatment [46,47]). It has to be continued in order to allow statistical correlations and to prove the importance of adhesion and co-stimulatory molecules expression on DCs as targets for future immunotherapies.

Circulating DCs from rheumatoid arthritis patients are phenotypically similar with peripheral blood DCs from controls patients. The data demonstrate an interrelationship between clinical outcome and the immunophenotypical presentation of DCs. Clinical active RA (DAS28>5.1) is associated with high incidence of activated DCs population in the ST and SF as demonstrated by high expression of adhesion and co-stimulatory molecules. Upregulated co-stimulatory activity in the synovium may be important in determining the localization of inflammation to the joints in RA and could provide new ways to evaluate and monitor disease activity, as well as therapeutic targets for immunotherapy.

Acknowledgements

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