Synovial osteoprogenitor phenotype in patients with rheumatoid arthritis

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, autoimmune joint inflammation, which results in disability due to irreversible joint destruction. Current treatments can slow the progression of the disease, but are still ineffective in a number of individuals and mainly target inflammation. Since there is increasing evidence on the ability of mesenchymal cells to promote regeneration and suppress inflammation, we focused on the phenotype of non-hematopoietic progenitor populations in synovial tissue of patients with arthritis.

AIMS

The aim of the present study was to identify cell populations within the synovial infiltrate which are altered in patients with severe forms of RA which underwent surgical treatment.

Specific aims

- to assess the proportions of cell subpopulations in the synovial compartment
  - immune cells (lymphocytes, myeloid cells, macrophages)
  - stromal populations containing bone and cartilage progenitors
- to assess the associations of proportions of target populations with disease activity

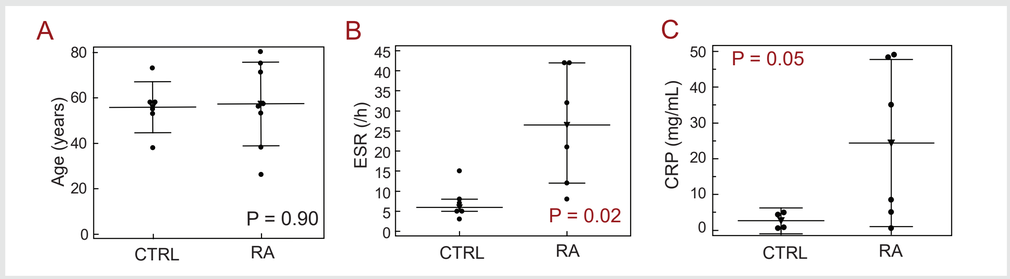
METHODS

We analyzed cellular composition of synovial tissue obtained from 9 RA patients undergoing surgery, and 7 control patients undergoing arthroscopic treatment, after obtaining approval from the Ethics Committee and informed consent from participants. Cells were released by collagenase digestion and analyzed by flow cytometry after labeling with two panels:

1. CD3-FITC, CD14-PE, 7-AAD, CD11b-PECy7, CD235a-APC, CD19-APCeF780
2. CD140a-PE, 7-AAD, CD105-PECy7, CD45/CD31/CD235a-APC, CD200-APCeF780.

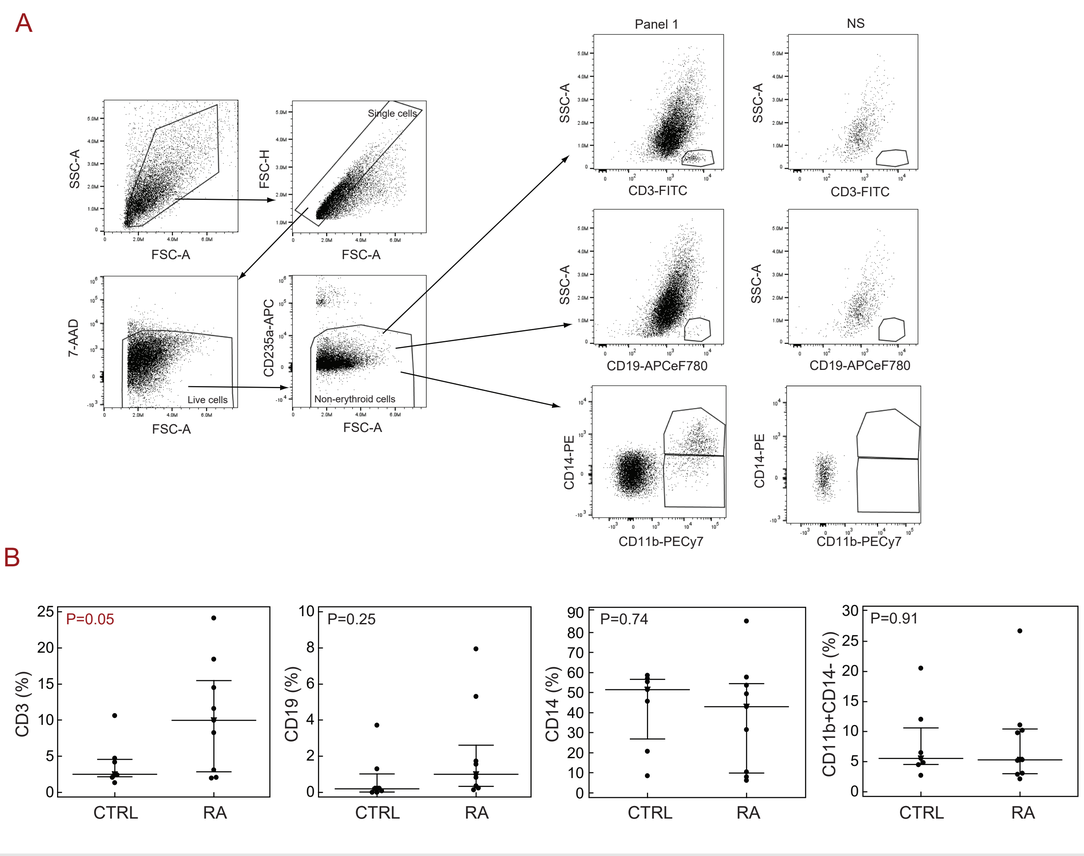
RESULTS

Patient characteristics



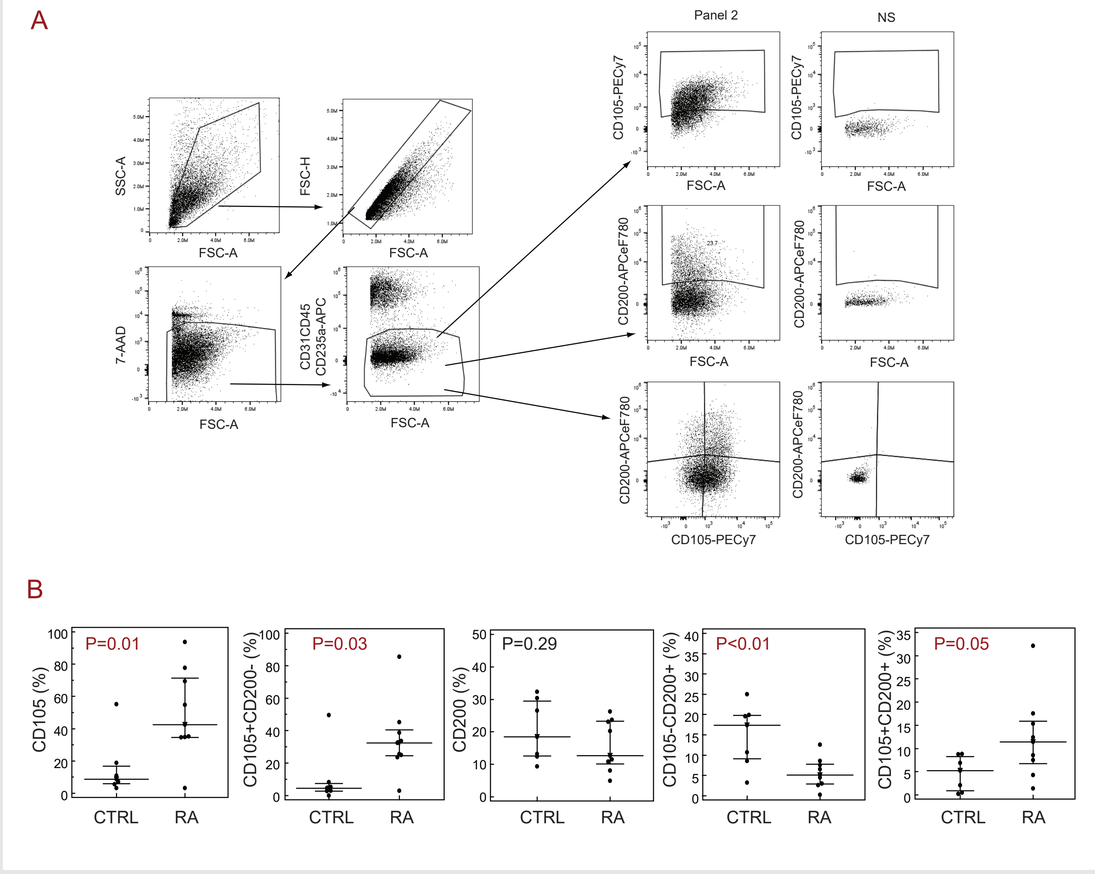
(A) Age distribution of control (CTRL) and RA patients (RA), (B) Erythrocyte sedimentation rate (ESR) in CTRL and RA patients, and (C) C-reactive protein concentration in CTRL and RA patients. Markers represent individual values, horizontal lines are median±IQR, P values, Mann-Whitney test. There were 2 female and 5 male CTRL patients, whereas all 9 RA patients were females. In CTRL group synovial tissue was harvested from knee joints during arthroscopy, whereas in RA group synovial tissue was harvested from 4 wrist joints and 1 elbow joint during open surgical synovectomy, and 3 knees and 1 hip joint during prostesis placement surgery. All RA patients had disease duration of more than 5 years, and in 3 of them disease appeared in childhood (as juvenile idiopathic arthritis).

Proportions of hematopoietic cells in the synovia of RA patients



(A) Flow cytometry analysis of hematopoietic cell populations in the synovial tissue from control (CTRL) and RA patients. Cells were flushed from the synovial tissue after collagenase digestion. Cell populations were delineted amongst single, live cells (7-AAD$^-$). Myeloid lineage was delineated as CD11b$^+$CD14$^-$, and granulocytes as CD11b$^+$CD14$^+$, whereas T- (CD3$^+$) and B-lymphcytes (CD19$^+$) were delineated according to staining for the surface markers and scatter properties. Positive populations were delineated according to the signals of non-stained cells. (B) Proportions of hematopoietic lineage cells amongst control (CTRL) and RA patients. Markers, individual values; horizontal lines, median±IQR; P values, Mann-Whitney test.

Proportions of osteoprogenitor cells in the synovia of RA patients



(A) Flow cytometry analysis of non-hematopoietic osteoprogenitor populations in the synovial tissue from control (CTRL) and RA patients. Cells were flushed from the synovial tissue after collagenase digestion. Cell populations were delineted amongst single, live cells (7-AAD$^-$). Non-haemopoietic cells (CD45$^-$CD31$^-$CD235a$^-$) were further subdivided according to the expression of mesenchymal markers CD200 and CD105. Positive populations were delineated according to the signals of non-stained cells. (B) Proportions of osteoprogenitor lineage subpopulations amongst control (CTRL) and RA patients. Markers, individual values; horizontal lines, median±IQR; P values, Mann-Whitney test.

Association of proportions of cell populations and disease activity

		CD3	CD19	CD14	CD11b	CD200	CD105	CD200$^+$105$^-$	CD200$^-$105$^+$	CD200$^+$105$^+$	ESR	CRP
CD3	r		0.397	0.002	0.494	0.176	-0.199	-0.071	-0.059	0.082	0.221	**0.823**
	P		0.115	0.993	0.044	0.513	0.445	0.795	0.829	0.762	0.489	**0.001**
CD19	r	0.397		-0.093	-0.044	0.032	0.349	-0.109	0.335	0.418	0.186	0.355
	P	0.115		0.722	0.866	0.905	0.169	0.688	0.204	0.108	0.562	0.234
CD14	r	0.002	-0.093		-0.098	**-0.521**	-0.294	-0.179	-0.415	**-0.547**	0.411	-0.036
	P	0.993	0.722		0.708	**0.039**	0.252	0.506	0.110	**0.028**	0.184	0.908
CD11b	r	0.494	-0.044	-0.098		0.180	-0.450	0.353	**-0.527**	-0.110	0.032	0.322
	P	0.044	0.866	0.708		0.506	0.070	0.180	**0.036**	0.684	0.922	0.283
CD200	r	0.176	0.032	**-0.521**	0.180		0.166	**0.581**	0.042	**0.532**	-0.445	0.117
	P	0.513	0.905	**0.039**	0.506		0.526	**0.015**	0.874	**0.028**	0.128	0.691
CD105	r	-0.199	0.349	-0.294	-0.450	0.166		-0.340	**0.894**	**0.720**	-0.395	-0.135
	P	0.445	0.169	0.252	0.070	0.526		0.182	**<0.0001**	**0.001**	0.182	0.647
CD200$^+$105$^-$	r	-0.071	-0.109	-0.179	0.353	**0.581**	-0.340		**-0.561**	0.145	-0.262	-0.251
	P	0.795	0.688	0.506	0.180	**0.015**	0.182		**0.019**	0.580	0.386	0.387
CD200$^-$105$^+$	r	-0.059	0.335	-0.415	**-0.527**	0.042	**0.894**	**-0.561**		**0.500**	-0.257	0.110
	P	0.829	0.204	0.110	**0.036**	0.874	**<0.0001**	**0.019**		**0.041**	0.397	0.708
CD200$^+$105$^+$	r	0.082	0.418	**-0.547**	-0.110	**0.532**	**0.720**	0.145	**0.500**		-0.348	-0.037
	P	0.762	0.108	**0.028**	0.684	**0.028**	**0.001**	0.580	**0.041**		0.244	0.899
ESR	r	0.221	0.186	0.411	0.032	-0.445	-0.395	-0.262	-0.257	-0.348		**0.738**
	P	0.489	0.562	0.184	0.922	0.128	0.182	0.386	0.397	0.244		**0.015**
CRP	r	**0.823**	0.355	-0.036	0.322	0.117	-0.135	-0.251	0.110	-0.037	**0.738**	
	P	**0.001**	0.234	0.908	0.283	0.691	0.647	0.387	0.708	0.899	**0.015**	

Correlation table showing associations of proportions of analyzed populations, erythrocyte sedimentation rate (ESR) and serum concentration of C-reactive protein (CRP). Association was assessed by Sperman's rank correlation. ρ, correlation coefficient; P, statistical significance.

CONCLUSIONS

- T lymphocytes represent small proportion of cells in synovial tissue, which is increased in rheumatoid synovia and associated with systemic inflammatory activity
- Myeloid lineage cells abundant in synovial tissue of both healthy subjects and patients with arthritis
- Composition of non-hematopoietic cells, containing bone and cartilage progenitors, is altered in the synovial tissue of patients with RA.
- Proportion of CD200$^+$ population amongst non-hematopoietic cells is significantly decreased in synovial tissue from patents with RA. This population according to experimental studies, represent earliest bone and cartilage progenitors (skeletal stem cells, *Chan CKF et al Cell 160, 285–298*). Particular decrease is prominent in CD200$^+$CD105$^-$ cells.
- Proportion of CD105$^+$ non-hematopoietic cells is significantly increased in synovial tissue from patents with RA. Up regulation of CD105 is described in RA and inflammatory conditions, so this population could correspond to hypertrophic synoviocytes.
- CD200 is involved in control of myeloid cell development and activity (*Amouzegar A et al. Stem Cells 35, 1532–41,* so decrease in proportion of CD200$^+$ cells could favor pro-inflammatory and pro-osteoclastic milleu in RA.

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