INCREASED OSTEOCLASTOGENEIS IN PATIENTS WITH RHEUMATOID ARTHRITIS

P. Dufort[1], M. Durand[1], H. Allard-Chamard[1], M.A. Gallant[1], S. Komarova[2], M. Manolson[3], R. Harrisson[3], J. Dixon[4], S. Sims[4], L. Kurgan[5], G. Boire[1] and A. J. de Brum-Fernandes[1]

[1]Division de Rhumatologie , Département de Médecine, Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke [2]Faculty of Dentistry, McGill University;

[3]Dental Research Institute, Faculty of Dentistry, University of Toronto; [4]Department of Physiology and Pharmacology, University of Western Ontario;

[5]Department of Electrical and Computer Engineering, University of Alberta

Introduction

Osteoclasts (OCs) are important cells for bone and joint destruction in Rheumatoid Arthritis (RA). OC-driven bone resorption depends both on osteoclastogenesis and on indidual OC activity.

Objective

Our objective is :

1) Determine if the capacity to generate osteoclasts from peripheral blood mononuclear cells and to correlate it with the presence and activity of RA.

Methods

Subjects:

Patients and Controls: 139 patients with RA were recruited from the outpatient clinic at the Division of Rheumatology, Centre Hospitalier Universitaire de Sherbrooke. RA was defined according to the ACR criteria and active disease was defined as a DAS28 higher than 2.6. Fourty-one self-reported healthy controls were recruited from the local population.

Osteoclast studies:

Peripheral blood mononuclear cells were separated from whole blood by Ficoll gradient and the number of $CD14^+$ cells was determined by flow cytometry. The number of OCs was evaluated after 21 days of culture in the presence of RANKL and M-CSF. OC apoptosis was evaluated by colorimetric assay (TACS™ TdT Blue Label kit). Bone resorption was quantified on cortical bone slices stained with toluidine blue.

Analysis:

The following tests were used: Student's T-Test and ANOVA for continuous parametric variable(s), Mann-Whitney test and Kruskal-Wallis test for continuous non parametric variable(s) and the Pearson's chi-square test for categorical variables.

Results

Descriptive analysis:

Table 1. Main descriptive analysis of the controls and patients

Table 1. Baseline characteristics of the controls and patients.†				
Characteristics	Control (N=41)	RA-total (N = 139)	RA-active (N = 77)	RA-inactive (N = 62)
Age -- yr	57.1±1.2	60.8±1.0*	62.1±1.3*	59.2±1.5
Female sex -- no. (%)	23 (56.1)	94 (67.6)	59 (76.6)*	35 (56.5)
Menopause -- no. (%)				
Menopause	NA	64 (68.1)	43 (72.9)	21 (61.8)
Pre-menopause	NA	4 (4.3)	2 (3.4)	2 (5.9)
Body-mass index‡	27.6±0.9	26.6±0.4	26.6±0.6	26.5±0.6
Ethnic group -- no. (%)¶				
Caucassian	38 (92.7)	133 (95.7)	72 (93.5)	61 (98.4)
Other	3	6	5	1
Smoking status -- no. (%)				
Ever smoke	15 (36.6)	84 (60.4)**	42 (54.5)**	42 (67.7)**
Alcohol status -- no. (%)	32 (78.0)	67 (48.6)***	35 (45.5)**	32 (51.6)**
Medication -- no. (%)				
NSAIDs	NA	57 (41.0)	32 (41.6)	25 (40.3)
Biphosphonates	NA	42 (30.2)	29 (37.7)	13 (21)$^+$
Anti-TNF	NA	39 (28.1)	24 (31.2)	15 (24.2)
Sulfasalazin	NA	23 (16.5)	16 (20.8)	7 (11.3)
Methotrexate	NA	118 (84.9)	66 (85.7)	52 (83.9)
Antimalarial drugs	NA	101 (72.7)	50 (64.9)	51 (82.3)$^+$
Prednisone	NA	24 (17.3)	17 (22.1)	7 (11.3)
Calcium (supplement)	NA	15 (10.8)	9 (11.7)	6 (9.7)
Vitamin D (supplement)	NA	11 (7.9)	8 (10.4)	3 (4.8)

† Plus-minus values are means ±SEM.
‡ Body-mass index is the weight in kilograms divided by the square of the height in meters.
¶ Ethnic groups are self-reported.
* P≤0.05 for the comparison between this group and the control group.
** P≤0.01 for the comparison between this group and the control group.
*** P≤0.001 for the comparison between this group and the control group.
$^+$P≤0.05 for the comparison between this group and the RA active group.

Quantitative analysis:

Statistical analysis of the number of osteoclast precursors cells ($CD14^+$) and mature osteoclasts for RA patients and controls (see Figure 1). No significant difference was found between osteoclast precusor cell numbers in RA patients and controls. However, osteoclastogenesis was significantly higher in patients with inactive RA compared to both controls and patients with active RA.

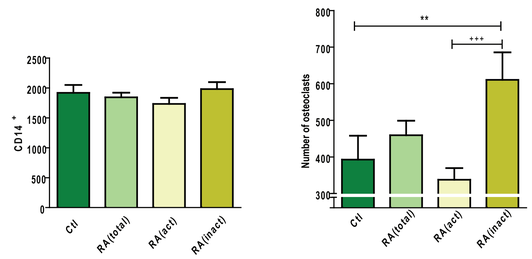

Figure 1. Statistical analysis of $CD14^+$ cells and osteoclastogenesis in control and RA patients. (** P≤0.01 for a comparison with the control group. $^{+++}$ P≤0.001 for the comparison between active and inactive RA groups.)

Osteoclast physiology was analyzed using apoptosis and resorption assays. RA patients showed less apoptosis than controls. Bone resorption was significantly higher in patients with inactive RA compared with patients with active RA and controls (see Figure 2).

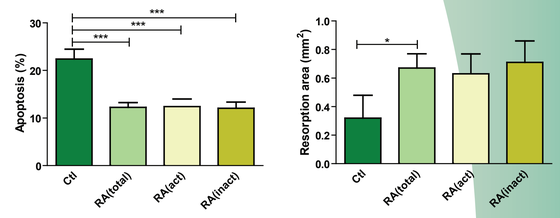

Figure 2. Statistical analysis of osteoclasts apoptosis and resorption. (* P≤0.05 for a comparison with the control group. *** P≤0.001 for a comparison with the control group.)

The ratio number of osteoclasts/$CD14^+$ cells was calculated to determine if osteoclast's precursors had a greater capacity to trigger osteoclastogenesis. This ratio was significantly higher in patients with inactive RA compared with controls (see Figure 3). The ratio resorption/number of osteoclasts indicates the osteoclasts activity. The ratio was significantly higher in RA patients compared to controls (see Figure 3).

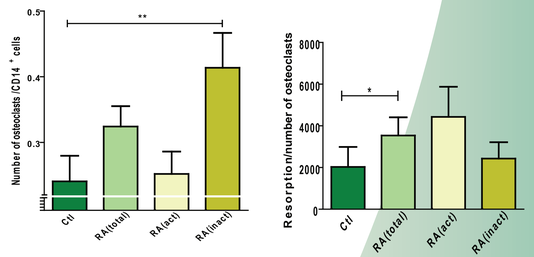

Figure 3. Statistical analysis of both ratios: Number of osteoclasts/$CD14^+$ cells and resorption/number of osteoclasts. (* P≤0.05 for a comparison with the control group. ** P≤0.01 for a comparison with the control group.)

Conclusion

Patients with inactive RA present higher *in vitro* osteoclastogenesis when compared to patients with active RA or to a control population. These results cannot be explained by a higher number of osteoclast precursors, by higher apoptosis rates or by the medication taken. We are presently prospectively assessing osteoclastogenesis in a longitudinal cohort of RA patients to better understand the relationship between disease activity an osteoclastogenesis.

Acknowledgements

Pfizer

CANADIAN ARTHRITIS NETWORK | LE RÉSEAU CANADIEN DE L'ARTHRITE

IRSC CIHR Instituts de recherche en santé du Canada | Canadian Institutes of Health Research

Centre hospitalier universitaire de Sherbrooke
Centre de recherche clinique Étienne-Le Bel

UNIVERSITÉ DE SHERBROOKE
Faculté de médecine