RANK expression is reduced in circulating monocytes from ankylosing spondylitis patients

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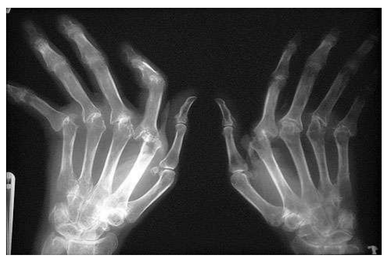
Introduction

Rheumatoid arthritis (RA) and ankylosing spondylitis (AS) are chronic systemic inflammatory diseases and share pathogenic pathways like the TNFα pathway.

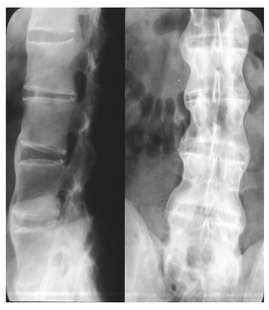
Rheumatoid Arthritis (RA)

- Joint inflammation originates in the synovial membrane of peripheral joints
- Bone and joint destruction



Ankylosing Spondylitis (AS)

- Primary site of inflammation located at the enthesis or at the subchondral bone marrow
- Bony spurs formation (syndesmophytes)



The aim of this study is to understand how systemic inflammation determines the pathological consequences that cause bone resorption and erosion in RA and bone formation in AS. Our hypothesis is that there are differences in osteoclast differentiation pathways between AS and RA.

Patients and Methods

Untreated RA or AS patients with active disease and age and gender-matched healthy donors were recruited for this study.

Whole blood was collected, RANKL cell surface expression was evaluated by flow cytometry and anti-human CD66b (granulocytes), CD3 (T cells) and CD19 (B cells) antibodies were used. Absolute number of leucocytes, RANKL positive cells and median fluorescence intensity (MFI) of RANKL were determined.

Peripheral blood mononuclear cells were isolated, CD14 and CD16 anti-human antibodies were used to gate different monocyte subpopulations and RANK surface expression was assessed by flow cytometry.

Serum was collected for cytokine and bone turnover marker quantification.

Results (I)

Thirty-five patients were recruited along with 14 age and gender matched controls. Fourteen AS patients, 6 women and 8 men, with a mean age of 46±16 years and ASDAS-ESR of 3.4±0.9 were enrolled in this study as well as 21 RA patients, 15 women and 6 men, with a mean age of 45±14 years and DAS28-ESR of 5.4±1.5. The characteristics of each group are summarized in Table 1.

Table 1 – Summary of RA and AS patients characteristics

	AS	RA	p-value
Sample size	14	21	-
Gender (% women)	42.9	71.4	0.1716
Age (years)	46 ± 14	45 ± 14	0.9691
Symptoms duration (years)	15 ± 10	2 ± 3	<0.0001*
ESR (mm/h)	28.4 ± 29.3	47.6 ± 38.3	0.0639
CRP (mg/dL)	1.2 ± 1.5	2.5 ± 4.6	0.6577
NSAIDs (% Yes)	61.5	45.5	1.0000
DAS28	-	5.4 ± 1.5	nc
Tender joint count	-	6.6 ± 7.1	nc
Swollen joint count	-	9.2 ± 8.8	nc
HAQ	-	1.8 ± 0.7	nc
RF (% Pos)	-	52.4	nc
ACPA (% Pos)	-	35	nc
Erosive (% Yes)	-	33.3	nc
BASDAI	5.6 ± 2.2	-	nc
ASDAS	3.4 ± 0.9	-	nc
BASFI	4.7 ± 2.2	-	nc
Sacroileitis (% Yes)	91.7	-	nc

Data are represented as mean±standard deviation unless stated otherwise; *p-value<0.05 is considered significant; RA – rheumatoid arthritis; AS – ankylosing spondylitis; NSAIDs - non-steroidal anti-inflammatory drugs; ESR – erythrocyte sedimentation rate; CRP – C-reactive protein; DAS – disease activity score; HAQ – health assessment questionnaire; RF – rheumatoid factor; ACPA - anti-citrullinated protein antibody; BASDAI – Bath ankylosing spondylitis disease activity index; ASDAS – ankylosing spondylitis disease activity score; BASFI – Bath ankylosing spondylitis functional index ; nc - not comparable

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Results (II)

We found that RANKL T and B lymphocyte expression was significantly higher in AS and RA patients when compared to healthy donors (p=0.0445 and 0.0016, respectively). We also found a positive correlation between RANKL expression in T cells and HAQ index in RA patients (p<0.01, Spearman r=0.9341 , data not shown).

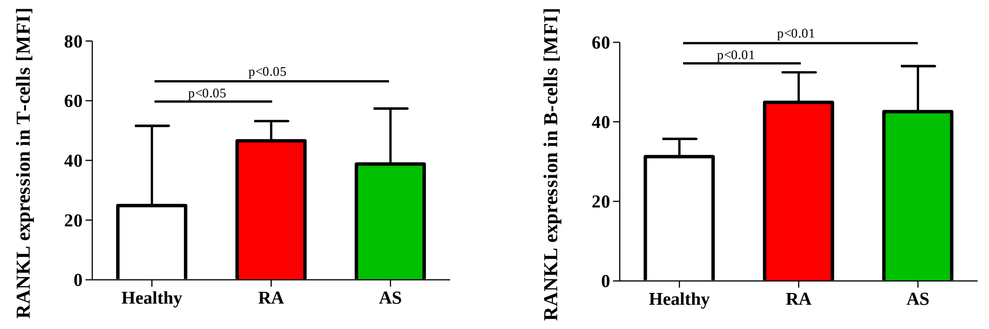

Fig.1 – Flow cytometry data on RANKL expression. RA and AS patients have increased RANKL surface expression in T and B lymphocytes when compared to healthy donors. RANKL surface expression is given by median fluorescence intensity (MFI); Bar represents median and line interquartile range. RANKL – receptor activator of NF-kB; RA – rheumatoid arthritis; AS – ankylosing spondylitis.

Frequency of both classical ($CD14^{bright}CD16^{negative}$) and non-classical ($CD14^{dim}CD16^{positive}$) was increased in AS and RA patients when compared to healthy donors (p<0.0001 and p=0.0227, respectively; data not shown).

We also found that in the classical subpopulation RANK expression was lower in AS patients as compared to healthy controls (p=0.0073; Fig.2A). Moreover, in the intermediate subpopulation this decrease was also found when comparing AS and RA patients (p=0.0203, Fig.2B).

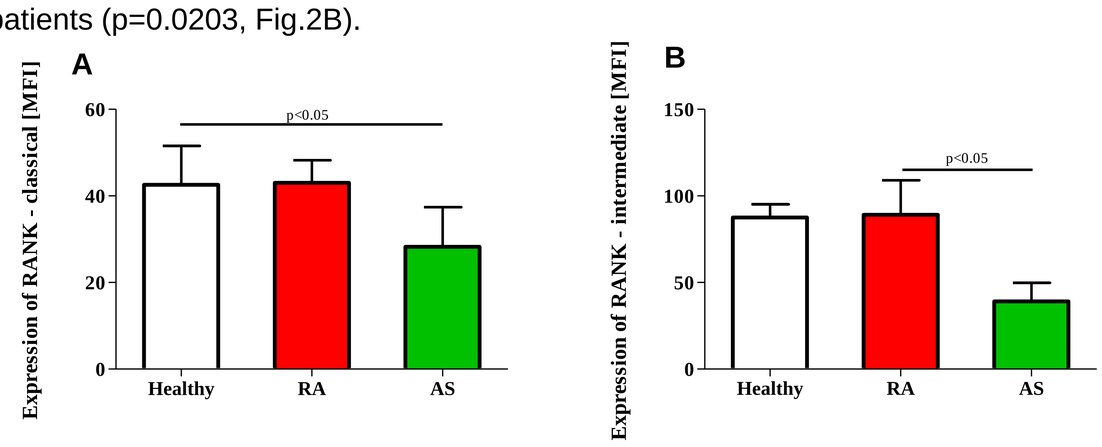

Fig. 2 – (A) RANK expression is lower in the circulating classical $CD14^{bright}CD16^{negative}$ monocyte subpopulation in AS patients than in healthy donors. (B) RANK expression in intermediate subpopulation $CD14^{bright}$ $CD16^{positive}$ is lower in AS patients when compared to RA patients. Bar represents median and line interquartile range. MFI – median fluorescence intensity; RANK – receptor activator of nf-κβ; RA – rheumatoid arthritis; AS – ankylosing spondylitis

When analyzing the circulating levels of cytokines in AS we found that there were no differences when compared to healthy controls. When comparing to RA we found that cytokines related to B and T cell activation (like IL-1β, IL-4 and IL-6) and cytokines from the IL-17 axis, namely IL-12 (p70) and IL-17, were significantly lower in AS when compared to RA (data not shown). No differences were found regarding sRANKL, OPG, sRANKL/OPG ratio or DKK-1 levels between groups (data not shown).

Regarding circulating bone turnover markers we detected that CTX-I and P1NP serum levels were also lower in AS patients than in healthy controls (p=0.0100 and p=0.0195, respectively; Fig 3).

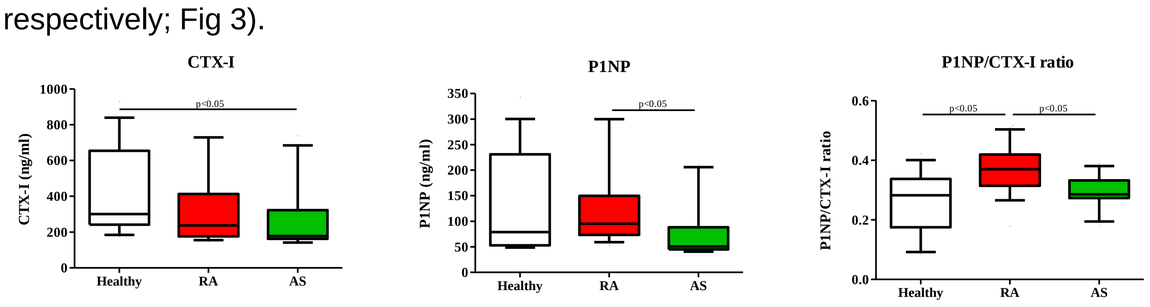

Fig. 3 – AS patients have lower circulating levels of CTX-I than healthy donors and lower levels of P1NP than RA patients. The P1NP/CTX-I ratio is decreased in both healthy donors and AS patients when compared to RA patients. Bone turnover markers CTX-I and P1NP were analyzed in the serum of healthy donors, RA and AS patients. Box line represents median and whiskers percentile 10 and 90. RA – rheumatoid arthritis; AS – ankylosing spondylitis; CTX-I - carboxy-terminal type I collagen crosslinks; P1NP - type I procollagen amino-terminal-propeptide.

Discussion

AS patients:
- do not differ from controls with regards to RANKL surface expression ;
- have less RANK expression at the surface of osteoclast precursors;
- have reduced markers of both bone formation and bone resorption;

In conclusion, despite comparable osteoclastogenic stimuli between AS and RA patients, RANK expression is reduced in AS circulating monocytes which may contribute to the bone forming phenotype observed in AS patients.