Senescent T cells promote bone loss in Rheumatoid Arthritis

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Background/Purpose

To study the influence of aged $CD28^-$ T cells on systemic osteoporosis in rheumatoid arthritis (RA) patients.

Methods

Prospective, cross-sectional study on 100 patients with RA [mean age 61.9 (±SD 11.2), 75% female, median time since diagnosis 162.4 (range 0-552) months, SDAI 12.7 (±SD9.3), 81% and 50% received synthetic and/or biologic DMARDs, respectively; 24% used corticosteroids, 16% were treated with biphosphonates]. Bone mineral density (BMD) was determined by lumbar spine (LS) and total hip DEXA and laboratory markers of bone metabolism included bone specific alkaline phosphatase, osteocalcin, osteoprotegerin, β-crosslaps and soluble RANKL. PBMCs were retrieved at the same day of BMD measurement and were stained with anti-RANKL, CD3, CD4, CD8, CD45RA, CD45RO and/or CD28 mAbs to measure surface expression of RANKL on T cells and to determine the prevalence of T cell subsets by flow cytometry. *In vitro* RANKL regulation assays were performed using human TNF-α (100ng/ml), IL-6 (100ng/ml), IL-15 (100ng/ml) or solid-phase anti-CD3 (10ng/ml).

Results

A reduced BMD as determined by DEXA was found in 63% of RA patients (13% with osteoporosis, 50% with osteopenia). The prevalences of aged $CD4^+CD28^-$ and $CD8^+CD28^-$ T cells inversely correlated with T-scores of LS ($corr_{coeff}$=-0.235, p=0.028 and $corr_{coeff}$=-0.266, p=0.012, respectively) and hip ($corr_{coeff}$=-0.235, p=0.025, $corr_{coeff=}$-0.253, p=0.016 respectively). Patients with a T-score below -1.0 tended to have higher prevalences of circulating $CD4^+CD28^-$ (2.2% [0.1–41.2] vs. 0.5% (0–17.6), p=0.065) and $CD8^+CD28^-$ T cells [44.8 ± 20.7 vs. 37.4 ± 20.1, p=0.134] than patients with normal bone mass. No association was found between frequencies of aged T cells and blood parameters of bone metabolism.

RANKL expression was higher in $CD4^+CD28^-$ T cells (3.8 [0.2-57.9]) compared to naïve $CD4^+CD28^+CD45RA^+$ (2.2 [0.2-30.5], p<0.001) and memory $CD4^+CD28^+CD45RO^+$ (2.8 [0.2-38], p=0.009) T cells. In the $CD8^+$ T cell population surface expression of RANKL was higher on memory (4.4 [0.5-44.5]) compared to naïve 3.3 [0.5-41.3], p<0.001) and aged T cells (2.2 [0-20.1], p<0.001).

In cell culture experiments IL-15 and anti-CD3 stimulation increased RANKL expression on all T cell subsets. IL-15 stimulation showed largest effects on memory $CD4^+$ and $CD8^+$ T cells [4.5-fold and 6-fold higher expression, respectively compared to unstimulated cells, p<0.05] compared to aged [3.9-fold and 5-fold, respectively, p<0.05] and naïve T cells [1.5-fold and 3.8-fold, respectively, p<0.05]. Also, activation by anti-CD3 had the largest effect on RANKL expression on memory $CD4^+$ and $CD8^+$ T cells [7.8-fold and 7.5-fold, respectively, p<0.05] compared to naïve [5.2-fold and 4.7-fold, respectively, p<0.05] and aged cell subsets [2.9-fold and 3.2-fold, respectively, p<0.05]. IL-6 and TNF-α had no effect on RANKL.

Conclusion

Aged $CD28^-$ T cells are linked with the occurrence of systemic bone loss in RA. Increased expression of RANKL on $CD4^+CD28^-$ T cells compared to other T cell subsets is compatible with direct stimulation of osteoclastogenesis by aged T cells in RA.