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RANKL expression in human T-lymphocytes requires cooperative signaling through the T-cell receptor and adhesion molecule CD2

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Introduction

T-lymphocytes contribute to osteolysis in rheumatic diseases through their production of the osteoclastogenic cytokine RANKL. Mitogenic stimulation of lymphocytes has been shown to induce expression of RANKL; however, the extracellular events leading to its production have not been identified.

Aim

We sought to determine whether cell-to-cell interactions through adhesion molecules such as lymphocyte function-associated antigen 2 (CD2) were necessary to promote RANKL secretion from T-cells.

Patients and methods

Human CD4+ and CD8+ T cells were purified by negative selection. PBMC from rheumatoid arthritis (RA) patients were obtained from Conversant Healthcare Systems. Cells were cultured in medium supplemented with human serum, IL-2, IL-7, M-CSF and 1alpha,25-dihydroxyvitamin D3 in the presence of various combinations of bead bound anti-CD3, anti-CD2 and anti-CD28 antibodies for 4 days. Total RANKL was determined by osteoprotegrin capture sandwich ELISA and secreted cytokines by Meso Scale Discovery assay. T-cell activation was determined by flow cytometry using CD69 and CD25.

Results

RANKL secretion by healthy donor PBMC was first detected after 72 hr incubation with anti-CD3/CD2/CD28 antibodies. Anti-CD3/CD28 antibodies failed to induce detectable levels of RANKL. Both CD4+ and CD8+ T-cells produced RANKL only in response to the combined cross-linking of CD3 and CD2 whereas cross-linking of CD3 and CD28 was insufficient to promote RANKL expression even though the T-cells were activated. Those conditions (anti-CD3/CD2) that led to increased RANKL secretion induced significantly lower levels of TNF-α and IL-4 as compared to the combinations of anti-CD3/CD28 or anti-CD3/CD2/CD28 antibodies. Anti-CD2/CD28 antibodies did not induce RANKL secretion. PBMC from RA patients also secreted RANKL in a CD3/CD2-dependent manner and at levels similar to PBMC from healthy donors.

Conclusions

Our results demonstrate that T-lymphocytes can generate RANKL following the co-ligation of the TCR/CD3 and the adhesion molecule CD2 in the absence of the co-stimulatory receptor CD28, suggesting that interactions between T-cells and non-traditional APCs (e.g., synovial fibroblasts) could lead to the production of osteoclastogenic cytokines without the need for other co-stimulatory signals.

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