

Circulating RANKL/OPG in polymyalgia rheumatica

L. Pulsatelli¹, P. Dolzani^{1,3},
T. Silvestri^{1,4}, L. Boiardi²,
C. Salvarani², P. Macchioni²,
A. Facchini^{1,3}, R. Meliconi^{4,5}

¹Laboratorio di Immunologia e Genetica and ⁵Modulo di Reumatologia, Istituti Ortopedici Rizzoli, Bologna, Italy; ²Unità Operativa di Reumatologia, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy; ³Dipartimento di Medicina Interna e Gastroenterologia and ⁴Dipartimento di Medicina Interna, Cardioangiologia, Epatologia, University of Bologna, Italy. Lia Pulsatelli, PhD; Paolo Dolzani, PhD; Tania Silvestri, MD; Luigi Boiardi, MD; Carlo Salvarani, MD; Pierluigi Macchioni, MD; Andrea Facchini, MD; Riccardo Meliconi MD.

This work is supported by grants from Ricerca Corrente I.O.R and from the University of Bologna.

Institution to which the work should be attributed: Istituti Ortopedici Rizzoli, Laboratorio di Immunologia e Genetica, Bologna, Italy.

Please address correspondence and reprint requests to: Riccardo Meliconi, MD, Laboratorio di Immunologia e Genetica, Istituto di Ricerca Codivilla-Putti, I.O.R., Via di Barbiano 1/10, 40136 Bologna, Italy. E-mail: labimge@alma.unibo.it

Received on October 31, 2006; accepted in revised form on February 16, 2007.

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Key words: Polymyalgia rheumatica, osteoprotegerin, RANKL.

Competing interests: Dr. Riccardo Meliconi has received speaker fees, travel grants and research support from Pfizer, Wyeth, Schering-Plough and Abbott.

ABSTRACT

Objective. To evaluate whether RANKL/OPG balance is modified in PMR patients, either in the active phase of the disease or during corticosteroid treatment.

Methods. Circulating levels of RANKL and OPG were assayed by enzyme-linked immunosorbent assay in PMR patients with active untreated disease and in patients treated by corticosteroids over a 12-month follow-up period.

Results. We found no statistically significant differences in circulating levels of OPG between PMR patients either in the active phase of the disease or during all follow-up period compared to normal controls. On the other hand, systemic production of sRANKL is increased and is not modulated by corticosteroid treatment.

Conclusions. In PMR increased levels of sRANKL may be related to bone osteoporosis. Further investigations are necessary to evaluate the relationship between the RANK/RANKL/OPG system and bone turnover in PMR patients.

Introduction

Novel members of the TNF/TNF receptor superfamily, the receptor activator of nuclear factor- κ B ligand (RANKL), its receptor RANK, and the decoy receptor osteoprotegerin (OPG) have been identified as paracrine mediators of both the immune system and bone metabolism (1). The balance of RANK/RANKL and OPG is critical for osteoclastogenesis modulation and physiological bone remodeling.

Polymyalgia rheumatica (PMR) is a common disorder in the aged population. The pathogenesis of PMR is poorly understood, but evidence indicates that immunological mechanisms are involved (2). In untreated PMR, systemic inflammation and high levels of circulating cytokines (IL-6) induce an increase in bone turnover.

The aim of this study was to evaluate whether RANKL/OPG balance was modified in PMR patients, either in the active phase of the disease or during corticosteroid treatment.

Patients and methods

We studied thirty-six patients with ac-

tive, non-treated PMR (mean age: 77 years, range: 70-83), diagnosed according to the Healey criteria (2).

For the follow-up study, we obtained samples at baseline and at 1, 3, 6, 12 months over one year of steroid treatment from 17 patients.

Eighteen age-matched healthy subjects (mean age: 77 years, range: 70-86) selected according to the SENIEUR protocol (3), which describes the admission criteria to immunogerontological studies based on clinical information, laboratory data and pharmacological interference, were analyzed as normal controls (NC).

Informed consent from patients and approval by the ethical committee of the hospital were obtained.

OPG and sRANKL assay

OPG and soluble RANKL (sRANKL) concentration were assayed using highly sensitive, commercial sandwich enzyme immunoassay (ELISA) kits (Immundiagnostik, Bensheim, Germany) following the manufacturer's instructions. The detection limit was 0.140 pmol/l, for the OPG assay and 0.08 pmol/l for the sRANKL assay.

Statistical analysis

Non-parametric analysis of variance (ANOVA) tests for multiple comparison of paired data were used. The Wilcoxon test was applied when ANOVA was significant. The non-parametric Mann-Whitney U test was used to compare variables between groups. The Statistica for Windows package was used to perform statistical analysis (Statsoft Inc., Tulsa, OK).

Results

We found no statistically significant differences in circulating OPG levels between PMR patients in the active phase of the disease (median: 5.4 pmol/l, range: 1.8-23.3) and NC (median: 5.0 pmol/l, range: 2.4-9.3). Conversely, sRANKL concentrations were significantly higher in PMR patients (median: 0.23 pmol/l, range: 0-1.38) compared to NC (median: 0 pmol/l, range: 0-0.5) ($p < 0.01$). In follow-up samples, sRANKL levels were significantly higher than in NC at all times,

while OPG levels did not show any increase compared to NC and to pretreatment values (Fig. 1).

Discussion

In this study, we demonstrated that in PMR patients the systemic production of sRANKL is increased and is not modulated by corticosteroid treatment, while OPG production does not show any modification compared to age-related controls.

The role of the RANKL/RANK/OPG system has extensively been demonstrated in rheumatic diseases, in which local and systemic bone remodeling is one of the main hallmarks (4).

An elevated production of RANKL has been shown in synovial tissue in RA (5), whereas conflicting results have been reported regarding circulating levels of sRANKL and OPG (6, 7). In ankylosing spondylitis, respectively lower and higher circulating levels of OPG and sRANKL have been found (8, 9).

PMR is not characterized by focal joint bone loss like RA, but alteration in bone turn-over was observed during the active phase of the disease (10, 11) probably related to the intense systemic inflammation and immunological activation. In particular, PMR is known to be associated with elevated levels of IL-6 (12), which has a definite role in promoting bone resorption (13). In line with this previously reported data, we found high levels of circulating sRANKL without modification in OPG concentration in untreated PMR patients.

The elevated sRANKL concentrations found in untreated PMR patients appeared not to be modulated throughout 12 months of corticosteroid therapy. The role of corticosteroid treatment in modulating RANKL/OPG production or levels is debated. Recently, Makrygiannakis and coworkers (14) reported that, in patients with inflammatory arthritis, intraarticular corticosteroids down-regulated RANKL synovial expression consequently to their ability to decrease inflammation. Interestingly, studies performed on untreated and corticosteroid-treated PMR patients have shown that the effect of inflammation on bone might be more

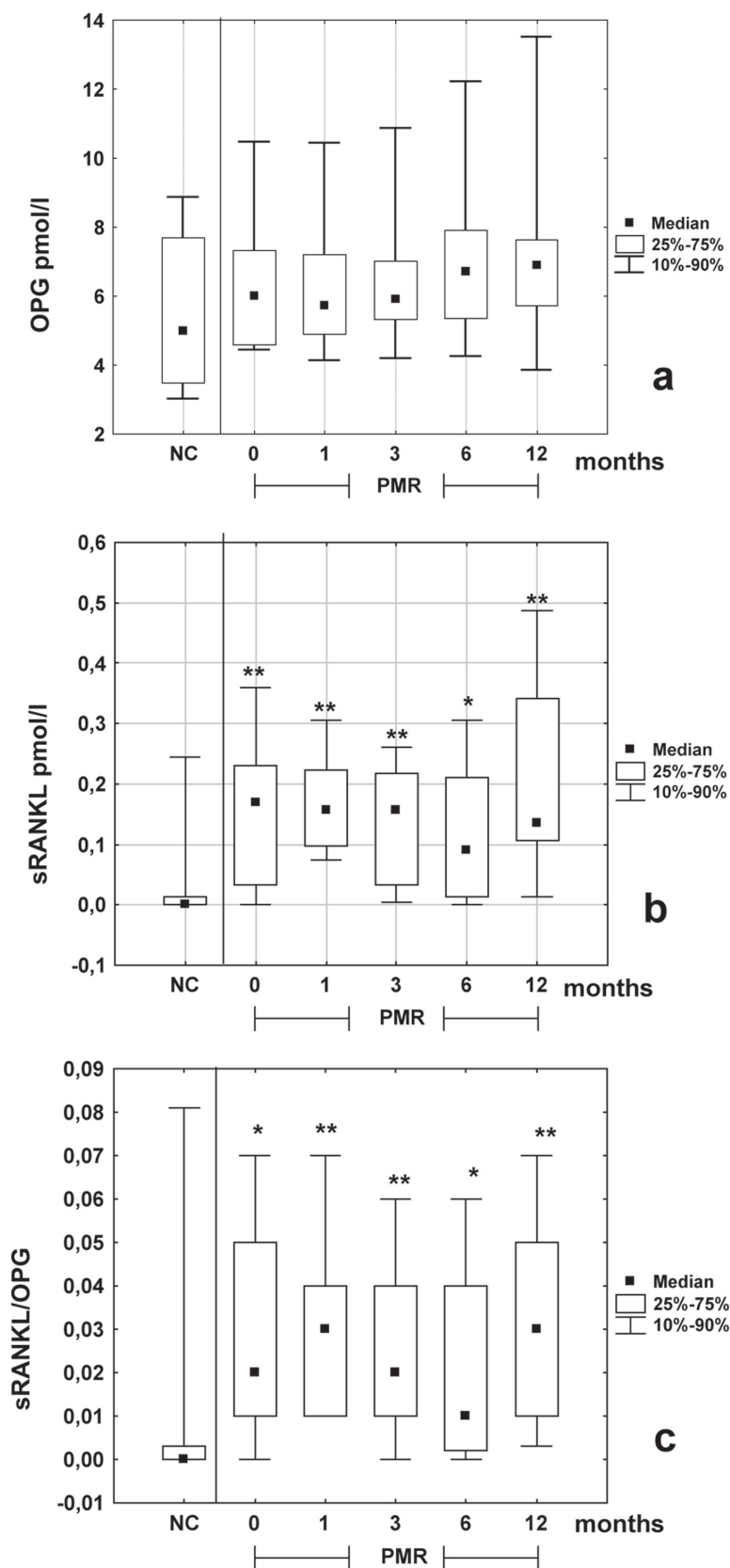


Fig. 1. Circulating concentration of OPG (a), sRANKL (b), and the ratio of sRANKL/OPG (c) in the normal subject group (NC) and in 17 patients with polymyalgia rheumatica (PMR) at diagnosis and during corticosteroid treatment period. Boxes show 25th and 75th percentiles. Squares within boxes show medians. Vertical lines below and above boxes show 10th and 90th percentiles. Significant differences are indicated: * $p < 0.05$, ** $p < 0.01$ vs NC.

detrimental than the effect of steroid treatment (10).

Therefore, taking also in account the role of RANKL in the immune response, we could hypothesize that the lack of sRANKL modulation by corticosteroid treatment described in the present report, might be due to its involvement in immunological mechanisms that corticosteroids are not able to modulate. It is noteworthy that in PMR other features of chronic immune activation are not fully controlled by corticosteroid treatment (12, 15).

An interesting consideration is prompted by evidence that VEGF, another soluble factor whose elevated spontaneous secretion by PBMC from PMR patients is not modulated by corticosteroids, has recently been identified as an inducer of RANK in endothelial cells (16). Thus, the elevation of RANK by VEGF and the concomitant presence of high sRANKL concentration might effectively contribute to enhancing immune response in the vessels of PMR patients. The vascular involvement is also strengthened by the evidence that another specific endothelial factor, von Willebrand factor, has been showed to be elevated in the serum of PMR patients and not modulated by corticosteroid therapy (17).

In conclusion, further investigation into the cellular source of systemic RANKL might clarify its role in PMR. The present results and previous evi-

dence suggest two main topics for the future studies: the relationship between the RANK/RANKL/OPG system and bone turnover in PMR patients, and on the other hand, the involvement of endothelial cells in the pathogenic mechanism of this vasculitic syndrome.

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