SIR, We read with interest the article by Haynes et al. [1] reporting the expression of osteoprotegerin (OPG) and receptor activator of nuclear factor κB ligand (RANKL) in synovial tissues from patients with arthritis, including rheumatoid arthritis (RA). They showed that OPG was expressed predominantly in macrophages in the synovial lining layer and in endothelial cells, and expression was decreased in patients with active RA compared with those with osteoarthritis (OA), inactive RA and spondyloarthropathies. In contrast, RANKL expression was seen in active RA synovial tissues, particularly in T-cell-rich regions. They concluded that this deficiency of OPG expression in the inflamed joint of RA patients may be important in the development of joint erosions.

We have recently studied OPG and RANKL expression in cultured fibroblast-like synoviocytes (FLS) from patients with active RA or OA. We observed that the expression of these two molecules, which are critical for bone metabolism, is regulated differently in RA and OA. Synovial tissues were obtained from the operated knee joints in eight active RA patients and five OA patients with informed consent. After two passages in culture, all cells showed fibroblast-like morphology and were used for subsequent experiments. Total RNA was isolated and was used for reverse transcription–polymerase chain reaction (RT-PCR) analysis and northern blot analysis, as described previously [2].

The PCR amplification of OPG cDNA was performed with 5'-TGGAGTAGTCAAGCGAGAG-3' as the sense primer and 5'-TGAAGCAGCTTTTTTTTTCAT-3' as the antisense primer (product size 1220 base pairs). The primers for RANKL cDNA amplification were 5'-AAGGAGCCTGTGAAAGGAA-3' (sense) and 5'-TAAAGGAGTTGAGACCT-3' (antisense) (524 base pairs). As shown in Fig. 1A, OPG expression in the FLS of RA patients was significantly lower than in FLS of OA patients. This result is consistent with the immunohistochemical findings of Haynes et al., who showed that OPG expression was barely detectable in the synovial tissue from active RA, while OPG expression was detected in OA patients [1]. When FLS from our RA patients were treated with tumour necrosis factor α (TNF-α), OPG message was up-regulated (Fig. 1B), which was compatible with the previous report that FLS from RA patients express OPG upon activation of proinflammatory cytokines such as TNF-α and interleukin β [3]. However, the regulation of OPG expression in FLS from our OA patients was different from that in RA patients. Treatment of FLS from our OA patients with TNF-α had no effect or only a slight inhibitory effect on OPG expression (Fig. 1B).

In contrast to the decreased OPG expression in RA, RANKL expression was observed in cultured FLS obtained from our RA patients (Fig. 2A). Although less pronounced, RANKL was
expressed in cultured FLS from OA patients as well. Increased RANKL expression in cultured FLS derived from RA patients has been reported previously [4, 5]. In the presence of TNF-α, RANKL expression from FLS was significantly decreased, as demonstrated by RT-PCR analysis and northern blot analysis (Fig. 2B and C). On the other hand, treatment of FLS from OA patients had no effect on the induction of RANKL.

RANKL is the main osteoclast-stimulating factor and OPG functions as a decoy receptor for RANKL, thereby acting as an inhibitor of RANKL-mediated bone resorption. It is therefore suggested that the balance between RANKL and OPG is important in the regulation of the bone microenvironment [6]. Without stimulation, FLS from our OA patients expressed a significantly increased level of OPG and a decreased level of RANKL compared with active RA patients. OPG expression in FLS from OA patients would be important in the protection of bone destruction. The expression of OPG and RANKL in FLS from OA patients was not significantly altered even in the presence of the potent inflammatory cytokine TNF-α. In contrast, RANKL, but not OPG, was abundantly expressed in FLS from our RA patients without stimulation. In addition, RANKL and OPG expression in FLS from RA patients was significantly modulated in the presence of TNF-α. RANKL-dominant expression, along with the lack of resistance against TNF-α of FLS from RA patients, may contribute to the pathogenesis of bone loss in RA.

The authors have declared no conflicts of interest.

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Reply

Harashima et al. produce some interesting results concerning OPG and RANKL mRNA production by RA and OA synovial fibroblasts. Unfortunately, there are several reasons why it is difficult to relate the results of Harashima et al. to the results presented in our recent paper [1].

No clinical details are provided on the RA or OA patients other than a statement that the RA patients are ‘active’. It would be helpful to know about disease duration, previous and current treatment, joint scores and levels of inflammatory markers.

All the results from Harashima et al. relate to mRNA production, and no evidence is presented to show that this mRNA results in OPG and RANKL protein production.

In addition, all the results relate to type II synovial fibroblasts grown in tissue culture; there are no results relating to other relevant inflammatory cell synovial infiltrates. Our immunohistochemical labelling results show that the major sources of OPG protein are type I synovial macrophages and endothelial cells [1], while the major source of RANKL protein was the lymphocyte [2]. We were unable to demonstrate significant OPG or RANKL protein production by type I synoviocytes by immunohistochemistry on synovial tissue.

Finally, the effects of tumour necrosis factor-α (TNF-α) on OPG and RANKL mRNA production shown by Harashima et al. are not very impressive and do not appear to correlate with the clinical situation. These authors suggest that TNF-α treatment of RA fibroblasts increased OPG mRNA and decreased RANKL mRNA, yet the active RA synovial fibroblasts (in a clinical situation in which increased TNF-α is expected in the synovial membrane) showed lower OPG and higher RANKL mRNA levels than OA fibroblasts. In addition, this would suggest that anti-TNF treatments would decrease OPG mRNA and increase RANKL mRNA levels in synovial fibroblasts, leading to a situation in which bone erosion should be increased. This is not what clinical trials on anti-TNF treatment have suggested [3].

Perhaps the conclusion that can be drawn is that results from studies undertaken in in vitro cell culture systems on isolated cell populations from synovial tissue do not necessarily correlate with what is seen in vivo in whole-tissue systems and should be interpreted with caution.

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Statins and lupus erythematosus

Sir, I read with interest the article of Wajed et al. [1] regarding the prevention of cardiovascular disease in systemic lupus