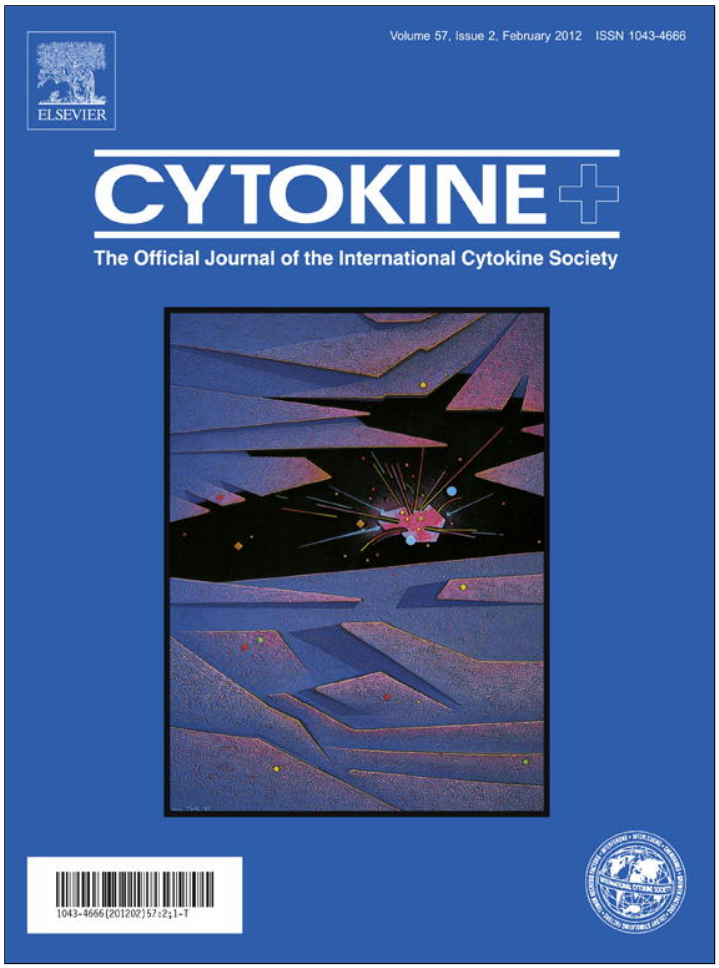
Depth and volume of resorption induced by osteoclasts generated in the presence of RANKL, TNF-alpha/IL-1 or LIGHT

Guillaume Mabilleau, Florence Pascaretti-Grizon, Michel-Félix Baslé, Daniel Chappard



Cytokine 57 (2012) 294–299

Contents lists available at SciVerse ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/issn/10434666

Depth and volume of resorption induced by osteoclasts generated in the presence of RANKL, TNF-alpha/IL-1 or LIGHT

Guillaume Mabilleau [a,b,*], Florence Pascaretti-Grizon [a], Michel Félix Baslé [a], Daniel Chappard [a]

[a] INSERM, U922 – LHEA "Remodelage osseux et Biomatériaux", IRIS-IBS, Institut de Biologie en Santé, CHU d'Angers, 49933 Angers, France
[b] Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Nuffield Orthopaedic Centre, Oxford OX3 7LD, United Kingdom

ARTICLE INFO

Article history:
Received 13 September 2011
Received in revised form 18 November 2011
Accepted 20 November 2011
Available online 14 December 2011

Keywords:
Vertical scanning profilometry
Osteoclasts
Bone resorption
RANKL
TNF-alpha

ABSTRACT

Rheumatoid arthritis (RA) is associated with pathological bone destruction mediated by osteoclasts. Although RANKL has been reported as a crucial factor for osteoclastogenesis, several other factors increased in RA support osteoclast formation and resorption in the absence of RANKL such as TNF-alpha and LIGHT. To date, *in vitro* bone resorption experiments are reported as the mean area of bone resorption per cortical or dentine slices and do not provide any information about depth and volume of resorption. The aims of this study were to assess these parameters by light microscopy and vertical scanning profilometry (VSP). Peripheral blood mononuclear cells were used as a source of osteoclast precursors and were cultured for up to 21 days in the presence of RANKL, TNF-alpha/IL-1 or LIGHT. Mean area, depth and volume of resorption were assessed by light microscopy and vertical scanning profilometry. As expected, RANKL induced large resorption pits (10,876 ± 2190 μm^2) whereas TNF-alpha/IL-1 and LIGHT generated smaller pits (respectively 1328 ± 210 and 1267 ± 173 μm^2) with no noticeable differences between these two cytokines. Depth and volume of resorption measured by VSP showed that RANKL promoted deep resorption pits resulting in large volume of resorption. Interestingly, although mean area of resorption was similar between TNF-alpha/IL-1 and LIGHT, the depth and volume of resorption of these lacunae were significantly increased by 2-fold with TNF-alpha/IL-1. These results provide evidence that although LIGHT appeared elevated in the synovial fluid of RA patients, its role in bone resorption is less than TNF-alpha/IL-1 or RANKL.

1. Introduction

Osteoclasts are multinucleated cells, tartrate resistant acid phosphatase positive originating from the hematopoietic lineage (CFU-GM) and capable of resorbing bone by digging eroded lacunae [1,2]. Several factors have been implicated in osteoclastogenesis. Among them, the triade composed of receptor activator of nuclear factor κB ligand – RANKL, receptor activator of nuclear factor κB – RANK and osteoprotegerin – OPG appeared crucial for osteoclast development [3–6]. RANKL is a member of the tumour necrosis factor (TNF) superfamily that is expressed on osteoblasts, stromal and T-cells and interacts with its receptor, RANK, expressed at the surface of osteoclast precursors [5,7–9]. The interaction between RANK and RANKL results in the fusion of osteoclast precursors and the activation and survival of mature osteoclasts. OPG acts as a decoy receptor for RANKL and blocks RANKL-mediated osteoclast differentiation and stimulation of osteoclast resorbing activity [10,11]. Involvement of the RANKL/RANK pathway has been recently reported in bone disorders characterised by increased bone resorption such as post-menopausal osteoporosis [12,13], rheumatoid arthritis (RA) [14,15] and bone metastases [16,17].

In the last decade, evidences have emerged that other members of the TNF superfamily were also capable of supporting osteoclast formation and resorption. TNF-alpha and LIGHT (homologous to lymphotoxins exhibiting inducible expression and competing with herpes simplex virus glycoprotein D for herpes-virus entry mediator [HVEM], a receptor expressed by T lymphocytes) have been reported to promote osteoclast formation independently of RANKL. However, although it is thought that LIGHT-generated osteoclasts are capable of bone resorption [18], TNF-alpha-generated osteoclasts require additional factors such as IL-1 beta to resorb bone [18,19]. Nevertheless, activation of these newly-formed osteoclasts does not require RANKL. As these two mediators are increased in the synovial fluid of RA patients [20,21], it is believed that they participate to the bone destruction associated with RA.

To date, *in vitro* bone resorption experiments involve the culture of fully differentiated osteoclasts or osteoclast precursors onto bovine cortical, dentine slices or calcified materials. Bone

* Corresponding author at: INSERM, U922 – LHEA, Institut de Biologie en Santé, CHU d'Angers, 4 rue Larrey, 49933 Angers Cedex 09, France. Tel.: +33 244 688 349; fax: +33 244 688 350.
E-mail address: guillaume.mabilleau@univ-angers.fr (G. Mabilleau).

1043-4666/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cyto.2011.11.014

resorption is as such often reported as the mean area of bone resorption per slice, i.e. the projection of a tri-dimensional network of resorption lacunae into a two-dimensional plane. Although this parameter is important to understand the effect of different mediators on the extent of osteoclast activity, it neither provides any information neither on the depth nor the volume of bone resorption. The recent development of surface microscopies such as vertical scanning profilometry (VSP) may help to overcome this issue. We previously reported that this technique is of greater importance to ascertain accurately the depth and volume of resorption pits [22].

The aims of this study were to assess by light microscopy and VSP, the mean area, depth and volume of resorption per resorption lacuna created by osteoclast generated by RANKL, TNF-alpha/IL-1 and LIGHT. Our results indicated that RANKL was the most potent agents to induce large and deep lacunae. On the other hand, although TNF-alpha/IL-1 beta and LIGHT exhibited the same area of resorption per lacuna, the depth and volume of resorption were dramatically different.

2. Material and methods

2.1. Reagents

Recombinant human macrophage-colony stimulating factor (M-CSF), recombinant human TNF-alpha, recombinant human IL-1 beta and recombinant human LIGHT were purchased from R&D Systems Europe (Abingdon, UK). Recombinant human soluble RANKL was purchased from PeproTech Ltd. (London, UK). Cytokines were aliquoted the day of purchased and store at −80 °C until use. All other chemicals used in this study were purchased from Sigma–Aldrich (Poole, UK).

2.2. Osteoclast generation

Peripheral blood mononuclear cells (PBMCs) were isolated from the buffy coat of five healthy volunteers (Etablissement Français du Sang, Angers, France), as described previously [23]. Briefly, blood was diluted 1:1 in α-minimal essential medium (MEM) (Invitrogen, Paisley, UK), layered over Histopaque (Sigma–Aldrich Chemicals, Poole, UK), and centrifuged (693*g*) for 20 min. The interface layer was removed and resuspended in MEM supplemented with 10% heat-inactivated foetal calf serum (FCS, Invitrogen, Paisley, UK). A fraction of the cell suspension was counted in a hemocytometer following lysis of red blood cells by a 5% (v/v) acetic acid solution. In order to induce osteoclast formation and activation, human PBMCs were cultured on plastic and dentine slices as described previously [24]. All cultures were maintained for up to 21 days in the presence of 25 ng/ml recombinant human M-CSF. Recombinant human soluble RANKL (100 ng/ml, added day 7), recombinant human TNF-alpha (20 ng/ml, added day 7) and IL-1 beta (10 ng/ml, added day 18) or recombinant human LIGHT (50 ng/ml added day 7) were added in the cultures. Osteoclast formation was evidenced on plastic by tartrate resistant acid phosphatase staining at day 14 and the size and number of nuclei per osteoclast were determined as previously described [23].

2.3. Assessment of the mean area of bone resorption by optical microscopy

After 21 days in culture, the dentine slices were placed in NH_4OH (1 N) for 30 min and sonicated for 5 min to remove any adherent cells. After rinsing in distiled water, the dentine slices were stained with 0.5% (v/v) toluidine blue prior to examination by light microscopy. The surface of each dentine slice was examined for evidence of lacunar resorption by mature osteoclasts. Digital photographs of the dentine surface were taken at a magnification 4× with an Olympus DP70 digital camera linked to an Olympus BX40 microscope (Olympus, Rungis, France). A lacuna was defined as a zone of dark pixels surrounded by white pixels. The area of resorption per lacuna was measured by image analysis using ImageJ freeware. At least 30 resorption lacunae randomly chosen per dentine disks were measured. Experiments were done in triplicate and repeated three times.

2.4. Vertical scanning profilometry

After assessment of the mean area of bone resorption per lacuna, dentine slices were observed by VSP on a Wyko NT9100 interference profilometre (Veeco Instruments, Bruker, Palaiseau, France) and the whole surface of each dentine disk was scanned. This microscope is based on light interferometry and operates as a non-contact optical profilometre in vertical scanning interferometry mode (VSI) to produce 3D surfaces topography maps of the sample surface. A white light beam is split into two beams which pass through a Mirau's interferometric objective. It consists of a lens, a reference mirror and a beam splitter. The incident beam is reflected from the mirror and combines with light reflected from the sample to produce interference fringes where the best-contrast fringe occurs at best focus. The light and dark fringes are used in combination with the wavelength of the light to determine height difference between each fringe. A piezo-electric stage moves the sample vertically with a nanometre precision, which produces phase shifts in the interferograms. They are digitized by a CCD camera and data are analysed to produce a topographic surface map. The software Vision™ (release 4.10, Wyko) was use to acquire and analyse the data. In this study, the entire dentine slices surface was measured at a magnification of 200 using an *x y* motorised stage. As the slices were not perfectly plane during acquisition, the tilt was automatically compensated by a facility of the Vision™ software. It minimises the angle of the sample relative to the reference optics so that slanted samples appear flat. For each lacuna, the depth of resorption was measured on the profile of 3D models by determining the edge and the bottom of each lacuna (Fig. 1). The volume of resorption per lacuna was also determined. At least 30 lacunae randomly chosen per condition were measured.

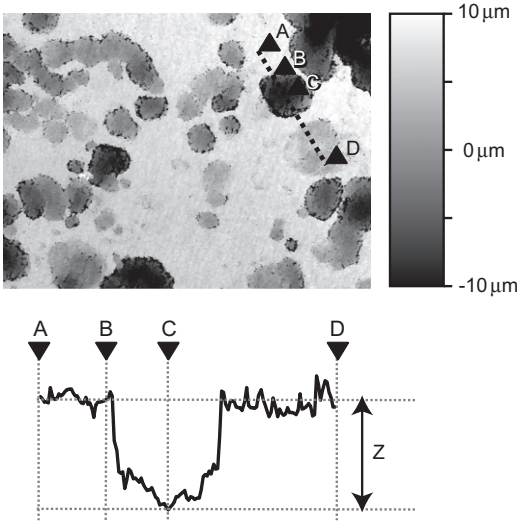


Fig. 1. Measurement of the depth of resorption lacuna. From the 2D image, it is possible to superimpose a lane (dashed black lane AD) and determine the height profile. The edge of the resorption lacuna (B) and the deeper point (C) can be defined and the difference (Z) in height between these two points calculated. Z represents the depth of the resorption lacuna.

2.5. Statistical analysis

Statistical analysis was performed with the Systat® statistical software release 11.0 (Systat Inc., San José, CA). Results were expressed as mean ± standard deviation. The non-parametric Kruskall–Wallis test was used to compare the differences between the groups. When significant differences were observed, data were subjected to Mann–Whitney U test. Differences at $p < 0.05$ were considered significant.

3. Results

3.1. Size and number of nuclei per osteoclast

As presented Fig. 2A, osteoclasts were bigger when generated in the presence of RANKL compared with TNF-alpha or LIGHT. On the other hand, osteoclasts generated in TNF-alpha and LIGHT-treated cultures appeared similar. As osteoclasts are not perfect geometrical circles, it is not possible to measure precisely the diameter of these cells. As such, the size was determined by assessing the osteoclast area (Oc.Ar) (Fig. 2B). Oc.Ar was significantly increased in RANKL-treated cultures by 3.6-fold and 3.9-fold compared with TNF-alpha- and LIGHT-treated cultures, respectively. However, no significant differences in Oc.Ar were evidenced between TNF-alpha- and LIGHT-generated osteoclasts.

The number of nuclei per osteoclast, which is an indicator of how many osteoclast precursors fused together, was significantly increased after treatment with RANKL compared with TNF-alpha and LIGHT (Fig. 2C). On the other hand, no differences in this parameter were observed between TNF-alpha and LIGHT.

3.2. Assessment of area, depth and volume of resorption lacunae

Fig. 3A represents the surface of dentine disks after RANKL-, TNF-alpha/IL-1- or LIGHT-stimulated osteoclast cultures. The extent of lacunar resorption was increased in RANKL-treated cultures compared with TNF-alpha/IL-1- or LIGHT-treated cultures and the lacuna size seemed also increased in the presence of RANKL compared with TNF-alpha/IL-1 and LIGHT. Indeed, the lacuna area was significantly increased by 8.2-fold (10,876 ± 2190 μm^2 vs. 1328 ± 210 μm^2) and 8.4-fold (10,876 ± 2190 μm^2 vs. 1267 ± 173 μm^2) in RANKL-treated cultures compared to TNF-alpha/IL-1- and LIGHT-treated cultures, respectively (Fig. 3B). On the other hand, the lacuna areas induced by TNF-alpha/IL-1 and LIGHT were similar (Fig. 3B).

Interestingly, the depth and volume of resorption per lacuna seemed increased in RANKL-treated cultures compared to TNF-alpha/IL-1 and LIGHT-treated cultures (Fig. 4). Furthermore, it also appeared that the depth and volume of bone resorption per lacuna were increased in TNF-alpha/IL-1-treated cultures compared to LIGHT-treated cultures (Fig. 4). Indeed, the depth of resorption per lacuna was significantly increased by 1.5-fold (29.7 ± 4.3 μm vs. 19.5 ± 3.9 μm) and 3.4-fold (29.7 ± 4.3 μm vs. 8.8 ± 1.3 μm) in RANKL-treated cultures compared with TNF-alpha/IL-1 and LIGHT, respectively (Fig. 5A). Furthermore, the depth of resorption per lacuna was also significantly increased in the TNF-alpha/IL-1 group by 2.2-fold compared to LIGHT. As a result of this increase in the depth of resorption, the volume of resorption per lacuna was also significantly increased by 8.9-fold (57,313 ± 15,600 μm^3 vs. 6474 ± 1150 μm^3) and 19.1-fold (57,313 ± 15,600 μm^3 vs. 2996 ± 467 μm^3) in RANKL-treated cultures compared to TNF-alpha/IL-1 and LIGHT, respectively (Fig. 5B). Moreover, the volume of resorption per lacuna was also significantly increased in TNF-alpha/IL-1-treated cultures compared to LIGHT-treated cultures by 2.2-fold (Fig. 5B).

4. Discussion

Bone resorption is a crucial process associated with the development of bone disorders such as post-menopausal osteoporosis.

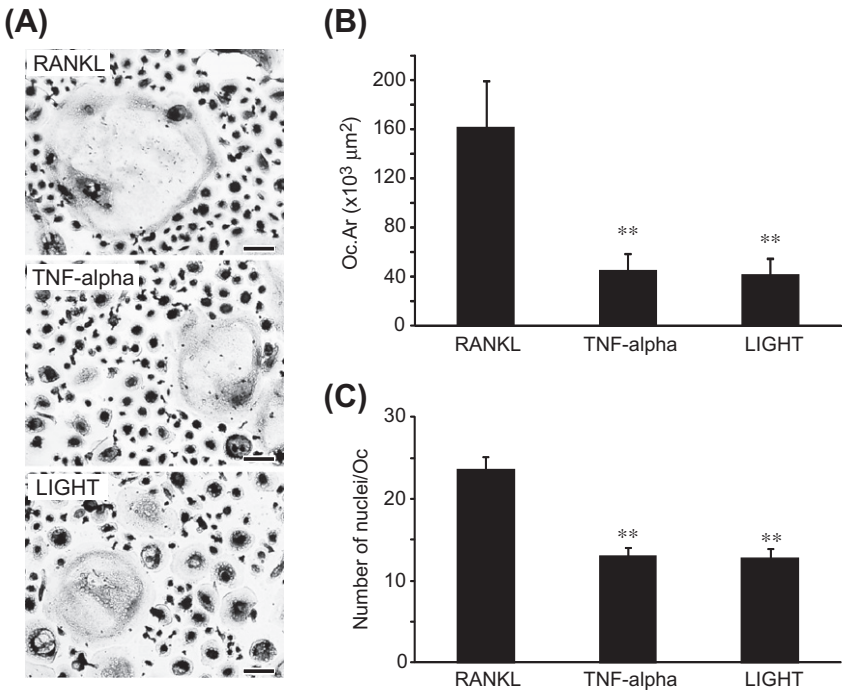


Fig. 2. Example of osteoclasts generated in the presence of RANKL, TNF-alpha/IL-1 or LIGHT (A). Osteoclast area (Oc.Ar) (B) and number of nuclei per osteoclast (C) in RANKL-, TNF-alpha/IL-1- and LIGHT-treated cultures. Bars represent 50 μm. $^{**}p < 0.05$ vs. RANKL-treated cultures.

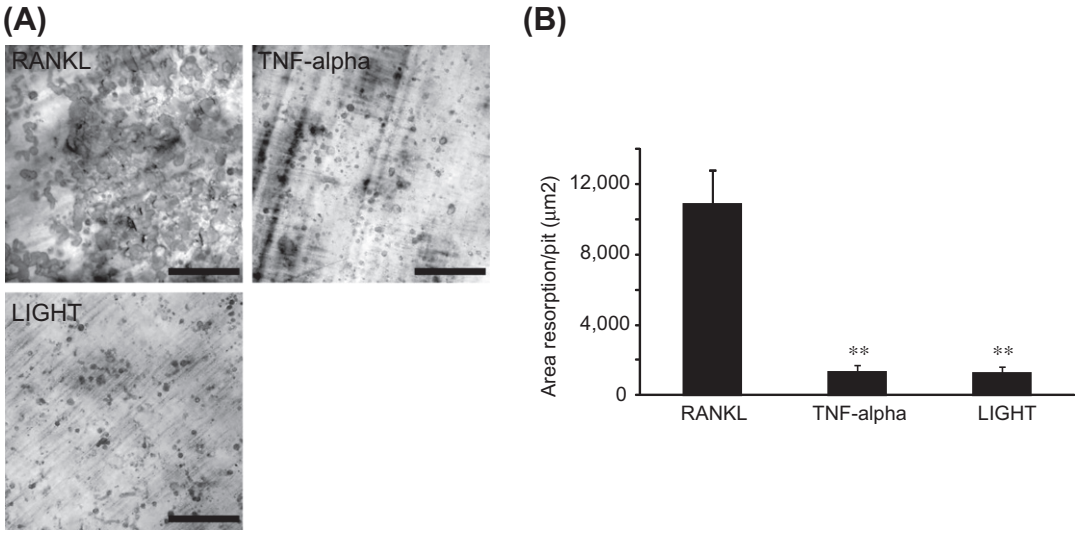


Fig. 3. Photographs of the surface of dentine disks after cultures with RANKL, TNF-alpha/IL-1 or LIGHT (A) and area resorption per lacuna (B). Bars represent 500 µm. $^{**}p < 0.05$ vs. RANKL-treated cultures.

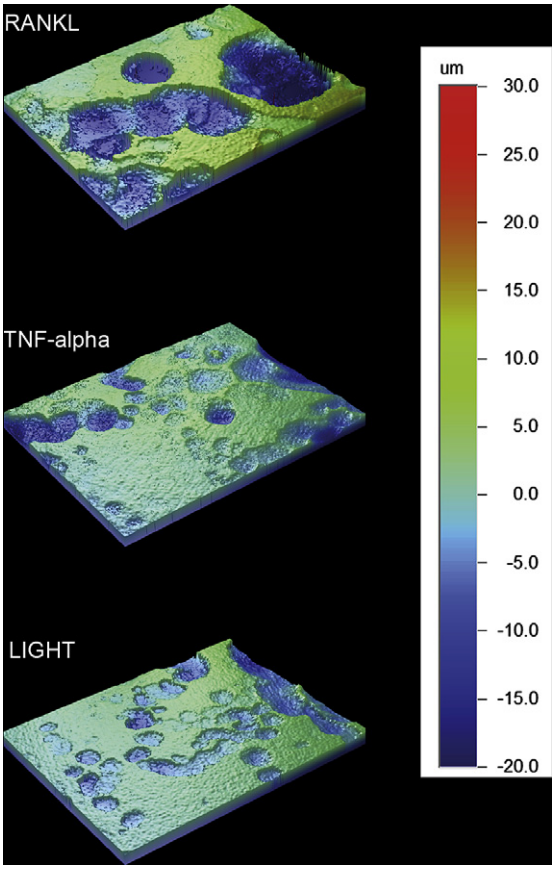


Fig. 4. 3D models of the surface of dentine disks after cultures with RANKL, TNF-alpha/IL-1 or LIGHT.

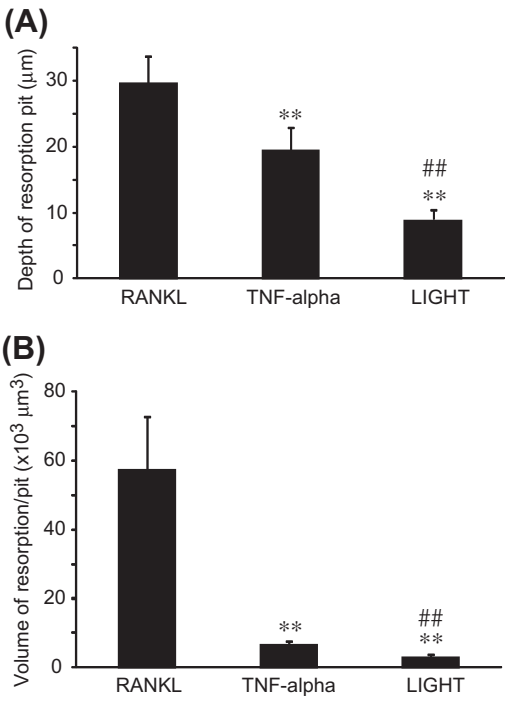


Fig. 5. Depth of resorption lacuna (A) and volume of bone resorption per lacuna (B). $^{**}p < 0.05$ vs. RANKL-treated cultures and $^{\#\#}p < 0.05$ vs. TNF-alpha/IL-1-treated cultures.

Osteoporosis is characterised by a progressive destruction of the trabecular microarchitecture resulting in increased trabecular perforations and loss of connectivity leading to reduced bone strength. Bone resorption also occurs in inflammation and similar destructions of the trabecular network have been reported in inflammatory bone disease. Increased osteoclastogenesis is also observed in malignant haematological diseases such as myeloma and B-cell lymphomas [25,26]. Interestingly, we have reported that mononucleated osteoclasts are observed in B-cell malignancies but not in myeloma [27]. In the present study, we evaluated the area, the depth and the volume of resorption per lacuna created by osteoclasts induced by different cytokines. It appeared clear that RANKL, a key factor for osteoclastogenesis, is the most potent factor to induce large and deep bone resorption lacunae. This could be in part attributed to the fact that osteoclasts generated with this cytokine are bigger. Furthermore, osteoclasts generated with RANKL often induce lacunae that superimpose slightly and create

a "resorption trench", contributing to increase the area of resorption.

The involvement of TNF-alpha and IL-6 in rheumatoid arthritis has been suspected for years and led to the development of anti-TNF and anti-IL-6 biotherapies. However, as not all RA patients respond to anti-TNF therapies [28–30], other pathways have been proposed [31,32]. Among them, LIGHT had been suggested as a possible candidate as its levels are significantly increased in RA [18,33–36]. From the present study, it appears that the area of resorption per lacuna under TNF-alpha/IL-1 treatment is reduced dramatically compared to RANKL. This could be explained by the fact that the size of osteoclasts generated in the presence of TNF-alpha is significantly reduced compared with RANKL. On the other hand, no difference in term of area resorption per lacuna was evidenced between TNF-alpha/IL-1- and LIGHT- generated osteoclasts. However, the volume of resorption per lacuna was dramatically reduced in LIGHT-treated cultures compared to TNF-alpha/IL-1. This was explained by the reduction in the depth of resorption. TNF-alpha/IL-1 was capable of inducing deep resorption whilst LIGHT was only capable of inducing a reduced eroded surface of only few micrometres. However, further investigations of the molecular mechanisms responsible for the differential response of osteoclasts between TNF-alpha/IL-1 and LIGHT are needed to elucidate why the eroded volume is smaller in LIGHT-treated cultures and hence what is the physiological role of LIGHT in pathological bone destruction. This reduction in the volume of eroded surfaces resembles the abnormalities observed in resorption in haematological disorders: in myeloma, normal sized osteoclasts are known to be generated by the RANK–RANKL system [37] and they provoke deep eroded surfaces in vivo with cortical perforations. On the other hand, other B-cell malignancies such as Waldenström disease or chronic lymphoid leukaemia induce smaller osteoclasts associated with minute erosion lacunae [38,39]. However, the cytokine network in these diseases is poorly known.

In the present study, we have also evidenced that classical 2D technique for assessing bone resorption and VSP are complementary. VSP is a useful tool to assess the depth and volume of bone resorption, parameters which cannot be investigated with classical 2D measurements. The assessment of only the mean eroded area by classical 2D measurement could lead to a false view of the pattern of bone resorption, the LIGHT/TNF-alpha/IL-1 story reported in this study being an example.

Our data suggest that the area, depth and volume of resorption per lacuna were significantly increased in RANKL-treated cultures compared with TNF-alpha/IL-1 and LIGHT-treated cultures. Although the resorption lacunae, generated in TNF-alpha/IL-1 and LIGHT-treated cultures, presented a similar surface area, the depth and volume of resorption were considerably greater in TNF-alpha/IL-1-treated cultures.

Acknowledgments

We thank A. Mieczkowska for her help with statistical analysis of this study. This work was made possible by grants from Contrat Region Pays de la Loire: Bioregos2 program.

References

[1] Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003;423:337–42.
[2] Udagawa N, Takahashi N, Akatsu T, Tanaka H, Sasaki T, Nishihara T, et al. Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. Proc Natl Acad Sci USA 1990;87:7260–4.
[3] Fujikawa Y, Quinn JM, Sabokbar A, McGee JO, Athanasou NA. The human osteoclast precursor circulates in the monocyte fraction. Endocrinology 1996;137:4058–60.
[4] Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. J Bone Miner Res 2000;15:2–12.
[5] Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998;93:165–76.
[6] Quinn JM, Elliott J, Gillespie MT, Martin TJ. A combination of osteoclast differentiation factor and macrophage-colony stimulating factor is sufficient for both human and mouse osteoclast formation in vitro. Endocrinology 1998;139:4424–7.
[7] Hsu H, Lacey DL, Dunstan CR, Solovyev I, Colombero A, Timms E, et al. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Proc Natl Acad Sci USA 1999;96:3540–5.
[8] Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Yano K, et al. RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. Biochem Biophys Res Commun 1998;253:395–400.
[9] Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 1998;95:3597–602.
[10] Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89:309–19.
[11] Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, et al. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. Endocrinology 1998;139:1329–37.
[12] Dobnig H, Hofbauer LC, Viereck V, Obermayer-Pietsch B, Fahrleitner-Pammer A. Changes in the RANK ligand/osteoprotegerin system are correlated to changes in bone mineral density in bisphosphonate-treated osteoporotic patients. Osteoporos Int 2006;17:693–703.
[13] Eghbali-Fatourechi G, Khosla S, Sanyal A, Boyle WJ, Lacey DL, Riggs BL. Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. J Clin Invest 2003;111:1221–30.
[14] Tamada K, Shimozaki K, Chapoval AI, Zhai Y, Su J, Chen SF, et al. LIGHT, a TNF-like molecule, costimulates T cell proliferation and is required for dendritic cell-mediated allogeneic T cell response. J Immunol 2000;164:4105–10.
[15] Tamada K, Shimozaki K, Chapoval AI, Zhu G, Sica G, Flies D, et al. Modulation of T-cell-mediated immunity in tumor and graft-versus-host disease models through the LIGHT co-stimulatory pathway. Nat Med 2000;6:283–9.
[16] Body JJ, Lipton A, Gralow J, Steger GG, Gao G, Yeh H, et al. Effects of denosumab in patients with bone metastases, with and without previous bisphosphonate exposure. J Bone Miner Res 2010;25:440–6.
[17] Clezardin P, Teti A. Bone metastasis: pathogenesis and therapeutic implications. Clin Exp Metastasis 2007;24:599–608.
[18] Edwards JR, Sun SG, Locklin R, Shipman CM, Adamopoulos IE, Athanasou NA, et al. LIGHT (TNFSF14), a novel mediator of bone resorption, is elevated in rheumatoid arthritis. Arthritis Rheum 2006;54:1451–62.
[19] Kim N, Kadono Y, Takami M, Lee J, Lee SH, Okada F, et al. Osteoclast differentiation independent of the TRANCE-RANK-TRAF6 axis. J Exp Med 2005;202:589–95.
[20] Manicourt DH, Poilvache P, Van Egeren A, Devogelaer JP, Lenz ME, Thonar EJ. Synovial fluid levels of tumor necrosis factor alpha and oncostatin M correlate with levels of markers of the degradation of crosslinked collagen and cartilage aggrecan in rheumatoid arthritis but not in osteoarthritis. Arthritis Rheum 2000;43:281–8.
[21] Pierer M, Brentano F, Rethage J, Wagner U, Hantzschel H, Gay RE, et al. The TNF superfamily member LIGHT contributes to survival and activation of synovial fibroblasts in rheumatoid arthritis. Rheumatology (Oxford, England) 2007;46:1063–70.
[22] Pascaretti-Grizon F, Mabilleau G, Basle MF, Chappard D. Measurement by vertical scanning profilometry of resorption volume and lacunae depth by osteoclasts on dentine slices. J Microsc 2011;241:147–52.
[23] Mabilleau G, Sabokbar A. Interleukin-32 promotes osteoclast differentiation but not osteoclast activation. PLoS ONE 2009;4:e4173.
[24] Mabilleau G, Petrova NL, Edmonds ME, Sabokbar A. Increased osteoclastic activity in acute Charcot's osteoarthopathy: the role of receptor activator of nuclear factor-kappaB ligand. Diabetologia 2008;51:1035–40.
[25] Bataille R, Chappard D, Baslé MF. Quantifiable excess of bone resorption in monoclonal gammopathy is an early symptom of malignancy: a prospective study of 87 bone biopsies. Blood 1996;87:4762–9.
[26] Bataille R, Chappard D, Baslé M. Excessive bone resorption in human plasmacytomas: direct induction by tumour cells in vivo. Br J Haematol 1995;90:721–4.
[27] Rossi JF, Chappard D, Marcelli C, Laplante J, Commes T, Baldet P, et al. Micro-osteoclast resorption as a characteristic feature of B-cell malignancies other than multiple myeloma. Br J Haematol 1990;76:469–75.
[28] Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study group. N Engl J Med 2000;343:1594–602.
[29] Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, et al. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum 2003;48:35–45.

[30] Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, et al. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med 1999;340:253–9.
[31] Takayanagi H. Osteoimmunology and the effects of the immune system on bone. Nat Rev 2009;5:667–76.
[32] Mauri DN, Ebner R, Montgomery RI, Kochel KD, Cheung TC, Yu GL, et al. LIGHT, a new member of the TNF superfamily, and lymphotoxin alpha are ligands for herpesvirus entry mediator. Immunity 1998;8:21–30.
[33] Ishida S, Yamane S, Nakano S, Yanagimoto T, Hanamoto Y, Maeda-Tanimura M, et al. The interaction of monocytes with rheumatoid synovial cells is a key step in LIGHT-mediated inflammatory bone destruction. Immunology 2009;128:e315–24.
[34] Ishida S, Yamane S, Ochi T, Nakano S, Mori T, Juji T, et al. LIGHT induces cell proliferation and inflammatory responses of rheumatoid arthritis synovial fibroblasts via lymphotoxin beta receptor. J Rheumatol 2008;35:960–8.
[35] Kang YM, Kim SY, Kang JH, Han SW, Nam EJ, Kyung HS, et al. LIGHT up-regulated on B lymphocytes and monocytes in rheumatoid arthritis mediates cellular adhesion and metalloproteinase production by synoviocytes. Arthritis Rheum 2007;56:1106–17.
[36] Kim WJ, Kang YJ, Koh EM, Ahn KS, Cha HS, Lee WH. LIGHT is involved in the pathogenesis of rheumatoid arthritis by inducing the expression of pro-inflammatory cytokines and MMP-9 in macrophages. Immunology 2005;114:272–9.
[37] Giuliani N, Colla S, Rizzoli V, Barille-Nion S, Bataille R. Do human myeloma cells directly produce the receptor activator of nuclear factor kappaB ligand (RANKL) or induce RANKL in the bone marrow microenvironment? Cancer Res 2004;64:772–3; author reply 4–5.
[38] Rossi JF, Bataille R, Chappard D, Alexandre C, Janbon C. B cell malignancies presenting with unusual bone involvement and mimicking multiple myeloma. Study of nine cases. Am J Med 1987;83:10–6.
[39] Josselin N, Libouban H, Dib M, Ifrah N, Legrand E, Basle MF, et al. Quantification of dendritic cells and osteoclasts in the bone marrow of patients with monoclonal gammopathy. Pathol Oncol Res 2009;15:65–72.