EXTENDED REPORT

Metabolic activation stimulates acid secretion and expression of matrix degrading proteases in human osteoblasts

M George, B Stein, O Müller, M Weis-Klemm, T Pap, W J Parak, W K Aicher

Web extra table W1 and figs W1–W4 can be seen on the web site at <http://www.annrheumdis.com/supplemental>

See end of article for authors' affiliations

Correspondence to: Dr W K Aicher, Research Laboratories, Centre for Orthopaedic Surgery, The University of Tübingen Medical School, Pulvermühlstrasse 5, D 72070 Tübingen, Germany; aicher@uni-tuebingen.de

Accepted 12 June 2003

Ann Rheum Dis 2004;**63**:67–70. doi: 10.1136/ard.2002.005256

Background: Both cellular and matrix components of healthy bone are permanently renewed in a balanced homeostasis. Osteoclastic bone resorption involves the expression of vacuolar-type ATPase proton pumps (vATPase) on the outer cell membrane and the secretion of matrix degrading proteases. Osteoblasts modulate the deposition of bone mineral components and secrete extracellular matrix proteins.

Objectives: To investigate the ability of osteoblasts and osteosarcoma to secrete acid and express matrix degrading proteases upon metabolic activation. To examine also the potential contribution of vATPases to proton secretion expressed on osteoblasts.

Methods: Osteoblasts were isolated from trabecular bone and characterised by reverse transcriptase-polymerase chain reaction and immunohistochemistry. Proton secretion was analysed by a cytosensor microphysiometer.

Results: Osteoblasts not only express matrix degrading proteases upon stimulation with tumour necrosis factor or with phorbol ester but they also secrete protons upon activation. Proton secretion by osteoblasts is associated partially with proton pump ATPases.

Conclusion: These data suggest that, in addition to monocyte derived osteoclasts, cytokine activated mesenchymal osteoblasts and osteosarcoma cells may contribute to the acidic milieu required for bone degradation.

Bone tissue is constantly in a process of remodelling. For bone degradation, osteoclasts express matrix degrading proteases, including different cathepsins¹ and matrix metalloproteinases (MMPs).^{2–4} Further, osteoclasts secrete protons by a vacuolar (H⁺) ATPase (vATPase).^{5–7} Mutations of a vATPase subunit cause osteopetrosis⁸ and deletion of a vATPase subunit results in osteosclerosis.⁹

Previously the vATPase activity was associated with osteoclasts only. But recently, vATPases have been described in a variety of other cell lineages as well.^{10–13} Such vATPases act in intracellular pH regulation as well as in proton secretion.^{13–14} This prompted us to study proton secretion by osteoblasts.

Parathyroid hormone has been shown to induce proton secretion by osteoblasts or osteosarcoma cells.¹⁵ We have shown that synovial fibroblasts secrete protons upon metabolic activation,¹⁶ and fibroblasts isolated from the synovial-like interface membrane can resorb bone without the help of osteoclasts.^{10–17} These results suggested that activated mesenchymal cells secrete considerable amounts of protons and thereby contribute to bone degradation under certain conditions. As osteoblasts are closely related to fibroblasts,¹⁸ we investigated osteoblasts as a second proton source in bone besides osteoclasts.

Protons have an important role in collagen degradation of demineralised bone areas or in cartilage as an acidic milieu is a prerequisite for optimal enzymatic activity of collagenolytic enzymes such as cathepsins, and also for solubilisation of collagen fibres before enzymatic degradation by collagenases.¹⁹ Consequently, enhanced proton secretion contributes

to degeneration of articular cartilage in rheumatoid arthritis¹⁶ and in other pathological conditions of the musculoskeletal apparatus as well.^{8–9} Therefore, we investigated mechanisms of proton secretion in human osteoblasts.

MATERIALS AND METHODS

Preparation of osteoblasts

Osteoblasts were isolated from trabecular bone and expanded in Dulbecco's modified Eagle's medium enriched with 20% fetal calf serum and antibiotics. Osteosarcoma lines SAOS-2 and MG-63 (ATCC) served as osteoblast controls. The cells were characterised by reverse transcriptase-polymerase chain reaction (RT-PCR; table W1 (available at <http://www.annrheumdis.com/supplemental>)) and immunohistochemistry. Secretion of MMPs was evaluated by enzyme linked immunosorbent assay (ELISA).

Cytosensor microphysiometer analysis

Proton secretion was analysed by a cytosensor microphysiometer, and pericellular acidification was measured as described previously.¹⁶ In brief, proton release of the cells after stimulation increases the acidification rate (fig W1 (<http://www.annrheumdis.com/supplemental>)). For induction experiments cells were stimulated with tumour necrosis

Abbreviations: BMP, bone morphogenic protein; MMP, matrix metalloproteinase; PMA, phorbol myristate acetate; RT-PCR, reverse transcriptase-polymerase chain reaction; TNF α , tumour necrosis factor α

factor α (TNF; 1–100 ng/ml) or phorbol myristate acetate (PMA; 0.1 ng/ml to 10 μ g/ml). To block Na^+/H^+ transmembrane proton pumps or vacuolar-type H^+ ATPases (vATPases), Amiloride (0.5 and 1 mmol/l) or Bafilomycin A1 (1 and 2 μ mol/l; Calbiochem, Bad Soden, Germany) were added to the osteoblasts, and proton secretion was recorded.

RESULTS

Functional characterisation of the osteoblasts

To characterise the cells under study, the expression of osteocalcin was detected by immunohistochemistry (fig W2A (<http://www.annrheumdis.com/supplemental>)), and osteoblast associated genes, including alkaline phosphatase, bone morphogenic protein (BMP)-2, BMP-4, osteocalcin, and osteopontin, were detected by RT-PCR analysis, confirming that the cells under investigation were osteoblast-like cells (fig W2B (<http://www.annrheumdis.com/supplemental>)).

Proton secretion by osteoblasts

Phorbol ester (PMA) activates protein kinase C and parathyroid hormone induced pericellular acidification in SAOS-2 osteosarcoma by a protein kinase C dependent pathway.¹⁵ Consequently, we tested osteoblasts and osteosarcoma cells for proton secretion upon activation with PMA. In early passage osteoblasts (passage 4–8), PMA induced a dose dependent acidification, and 35% proton secretion above equilibrium was reached (fig W3A (<http://www.annrheumdis.com/supplemental>)). After long term culture (10–12 passages) osteoblasts responded with lower sensitivity to PMA induced proton secretion (fig W3A (<http://www.annrheumdis.com/supplemental>)). Further, time course experiments showed that proton secretion was activated immediately after addition of PMA, reaching the plateau as early as 10 minutes after induction (fig 1A). PMA-induced proton secretion in SAOS-2 osteosarcoma cells confirmed that mesenchymal cells secrete protons upon metabolic activation, and a proton secretion above 40% was induced with PMA (fig W3A (<http://www.annrheumdis.com/supplemental>)).

TNF α stimulates proton secretion in osteoblasts

We next tested TNF α for its induction of proton secretion by osteoblasts. Early passage osteoblasts responded to TNF α at higher concentrations (10–100 ng/ml) with a proton secretion of 20–35% above the equilibrium level. Low concentrations of TNF (1–2 ng/ml) induced an acidification rate of about 20% above the equilibrium level. The TNF-induced proton secretion was reduced in late passage osteoblasts (fig W3B (<http://www.annrheumdis.com/supplemental>)). Further, time course experiments showed that the TNF response was delayed to some extent (fig 1B) in comparison with the PMA induced acidification (fig 1A), and the maximal proton secretion was reached 20 minutes after induction (fig 1B).

As different mechanisms may contribute to pericellular acidification, we used Bafilomycin A1, a specific vATPase blocker (1 μ mol/l, 2 μ mol/l), and Amiloride, a blocker of Na^+/H^+ exchange ATPases at a higher concentration (500 μ mol/l, 1 mmol/l), to reduce the proton secretion. Both compounds reduced proton secretion by 5 and 12% or by 10 and 12%, respectively (fig 2). This indicates that the involvement of proton pump ATPases of either the vacuolar type (vATPase) or Na^+/H^+ exchange ATPases works across the extracellular membrane of osteoblasts, thus contributing to the acidification reported here.

Expression of proteolytic enzymes in activated osteoblasts

To test whether activated osteoblasts may contribute to degradation of extracellular matrix proteins, the expression of proteolytic enzymes was investigated. In osteoblasts mRNA encoding MMP-1 was enhanced by addition of TNF ($p \leq 0.17$) and PMA ($p \leq 0.17$) about 25-fold (fig W4A (<http://www.annrheumdis.com/supplemental>)). MMP-3 encoding mRNA was stimulated by TNF ($p \leq 0.136$) and PMA ($p \leq 0.05$) on average fivefold as determined by real time quantitative RT-PCR (fig W4A). Further, TNF augmented MMP-1 ($p \leq 0.13$) and MMP-3 ($p \leq 0.02$) concentrations in osteoblast supernatants. Comparably, PMA stimulated

Figure 1 Activation of proton secretion ($r_{\max}/r_{\text{eq}}$ in per cent) in osteoblasts upon stimulation by PMA or TNF. (A) Osteoblasts were preincubated for about 20 minutes in running medium to reach metabolic quiescence characterised by a low spontaneous acidification ($r(t) = r_{\text{eq}}$, white zone). After 15 minutes of preincubation the fluctuations of the acidification value drop below 5%. Then cells are activated with PMA (grey zone), and the metabolic responses are recorded as a function of time. Each data point represents the mean value of n individual measurements of x_i ($n = 5-10$) and the error bars represent the normalised standard deviations. Addition of 1 μ g/ml PMA induced an immediate increase in acidification, reaching a plateau at about 115% after 20 minutes. Addition of 5 μ g/ml PMA induced a higher response reaching more than 120% acidification. Addition of 10 μ g/ml PMA induced an immediate acidification response and a plateau was not observed after 40 minutes of induction. (B) Osteoblasts were activated with TNF (grey zone) as described above (see (A)). Addition of 20 ng/ml TNF induced a slow increase in acidification reaching about 115% after 20 minutes. Addition of 50 ng/ml TNF induced a response reaching more than 120% acidification. Addition of 100 ng/ml TNF α at first reduced the acidification rate below the equilibrium level, then an acidification response was recorded reaching about 125% after 20 minutes

Figure 2 Blocking proton secretion by osteoblasts upon addition of Bafilomycin A1 or Amiloride. Osteoblast-like cells were incubated in medium containing 1 mM and 2 mM Bafilomycin A1 or 500 mM and 10000 mM Amiloride. Pericellular acidification was recorded by cytosensor microphysiometer. Each data point represents the mean of two individual measurements and the error bars represent the normalised standard deviations. Both the vATPase proton pump blocker, Bafilomycin A1, and the blocker of Na^+/H^+ transmembrane exchange pathways, Amiloride, reduced proton secretion by 5–12%.

MMP-1 ($p \leq 0.096$) as well as MMP-3 secretion ($p \leq 0.27$) (fig W4B (<http://www.annrheumdis.com/supplemental>)). These results indicate that cells of the osteoblast lineage may contribute to degradation of the extracellular matrix upon activation by both proton and protease production.

DISCUSSION

Previously, we showed that fibroblasts may secrete protons upon metabolic activation. To study osteoblast associated proton secretion we activated the cells by addition of PMA, as this compound has previously been associated with proton secretion in bone²⁰ and with pericellular acidification through osteoblasts, T cells, or peritoneal macrophages.^{11–15, 21} Interestingly, an overall trend towards a lower acidification response was noted in late passage osteoblasts in comparison with early passage cells or SAOS-2 in osteosarcoma cells. However, detection of osteoblast marker genes supports our suggestion that besides osteoclasts the osteoblasts may secrete protons upon activation. Addition of TNF was used as a physiological activator of the osteoblasts. TNF is a prominent product of monocytic cells and, in addition, TNF activates osteoclast resorptive activity.²² In our experiments, TNF activated proton secretion in human normal osteoblasts.

Activated osteoblasts express different matrix MMPs.^{23–24} The activation of proton secretion in the presence of enhanced MMP activity may result in a catabolic situation. Our data support these findings as expression of MMP-1 and MMP-3 were up regulated in osteoblasts. However, owing to variations in gene induction in the individual samples the statistical significance was not high. Nevertheless, induction of MMP-1 and MMP-3 were recorded in all individual experiments.

The resorptive capacity of cells in bone is principally associated with osteoclasts or tumour associated macrophages. Proton secretion by individual osteoblast cells is probably rather small as they lack ruffled membrane areas equipped with proton pumps. Of note, osteoblasts outnumber osteoclasts in bone. The lifespan of osteoclasts and their differentiation capabilities are limited at least in vitro in comparison with osteoblasts. In addition, osteoclasts are rather sensitive to apoptosis inducing signals in comparison with osteoblasts.²⁵ Although osteolysis by tumours was shown to require osteoclasts in vivo,^{26–27} osteoblasts or osteosarcomas may represent a considerable proton source in bone under specific conditions.²⁸ Consequently, for physiological bone turnover processes such as growth of

bone, wound healing, or load adaptation, osteoclasts represent the primary cells for mineral and protein matrix degradation. In chronic inflammatory processes, osteoporosis of the elderly, cancer, or under other pathological conditions the slow resorbing but apoptosis resistant osteoblast may contribute to osteolysis.

The specific contribution of different cellular proton sources for pericellular acidification by osteoblasts remains to be investigated. Enhanced glycolysis may account for pericellular acidification. This may explain in part the observation that early passage osteoblasts show higher proton secretion than the same cells at later passages or SAOS-2 cells. However, as cells are serum starved before cytosensor microphysiometry, glycolysis is probably considerably reduced under these conditions. This indicates that additional proton sources are active on osteoblasts. Such proton sources include Na^+/H^+ ion exchange ATPases or vATPases.²⁸ As we found mRNA encoding the 116 kDa subunit of the vATPase in osteoblasts (not shown) and a reduced pericellular acidification upon addition of the specific proton pump blocker Bafilomycin A1 and Amiloride to the cells, our data suggest that specific cell membrane associated ATPases participate in proton extrusion and in pericellular acidification by osteoblasts or osteosarcoma cells. Therefore, mesenchymal cells may contribute directly to tissue degradation using different proton sources, including vATPase and H^+ ion exchange ATPase activities and matrix degrading proteases. To the best of our knowledge, this is the first report of mechanisms of pericellular acidification by proton pumps on osteoblasts or osteosarcoma cells.

ACKNOWLEDGEMENTS

We thank Professor HE Gaub for experimental advice and providing the equipment, Angelika Kardinal and Andrea Mast for excellent technical support, and Professors Giehl, Martini, and Sell for suitable tissue samples. This project was supported by DFG grants Ai 16/10–1, Ai 16/14–1, and Pa 698/3–1, by the Schuler Foundation (University of Tübingen) and, in part, by institutional funding.

Authors' affiliations

M George, B Stein, W J Parak, Institute for Applied Physics and Centre for Nanoscience, Ludwig-Maximilians, University Munich, Munich, Germany
O Müller, M Weis-Klemm, W K Aicher, Centre for Orthopaedic Surgery, University of Tübingen Medical School, Tübingen, Germany
T Pap, Department of Internal Medicine, University of Magdeburg, Magdeburg, Germany

REFERENCES

- Blair HC, Sidonio RF, Friedberg RC, Khan NN, Dong SS. Proteinase expression during differentiation of human osteoclasts in vitro. *J Cell Biochem* 2000;**78**:627–37.
- Beedles KE, Sharpe PT, Wagner EF, Grigoriadis AE. A putative role for c-Fos in the pathophysiology of Paget's disease. *J Bone Miner Res* 1999;**14**:221–8.
- Sato T, del Carmen Ovejero M, Hou P, Heegaard AM, Kumegawa M, Foged NT, et al. Identification of the membrane-type matrix metalloproteinase MT1-MMP in osteoclasts. *J Cell Sci* 1997;**110**(Pt 5):589–96.
- Sato T, Foged NT, Delaisse JM. The migration of purified osteoclasts through collagen is inhibited by matrix metalloproteinase inhibitors. *J Bone Miner Res* 1998;**13**:59–66.
- Lee BS, Holliday LS, Krits I, Gluck SL. Vacuolar H^+ -ATPase activity and expression in mouse bone marrow cultures. *J Bone Miner Res* 1999;**14**:2127–36.
- Teitelbaum SL, Lam J, Takeshita S, Barker JE, Kanagawa O, Ross FP. Bone resorption by osteoclasts. Osteoclasts, integrins, and osteoporosis TNF- α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *Science* 2000;**289**:1504–8.
- Rousselle AV, Heymann D. Osteoclastic acidification pathways during bone resorption. *Bone* 2002;**30**:533–540.
- Kornak U, Schulz A, Friedrich W, Uhlhaas S, Kremens B, Voit T, et al. Mutations in the $\alpha 3$ subunit of the vacuolar H^+ -ATPase cause infantile malignant osteopetrosis. *Hum Mol Genet* 2000;**9**:2059–63.
- Scimeca JC, Franchi A, Trojani C, Parrinello H, Grosgeorge J, Robert C, et al. The gene encoding the mouse homologue of the human osteoclast-specific 116-kDa V-ATPase subunit bears a deletion in osteosclerotic (oc/oc) mutants. *Bone* 2000;**26**:207–13.

- 10 **Otsu S**, Pap T, Shigeyama Y, Aicher WK, Pap G, Gay RE, *et al*. Identification of a novel splice variant of an osteoclast-like v-ATPase beta-1 subunit in activated fibroblasts [abstract]. *Arthritis Rheum* 2000;**43**(suppl):S511.
- 11 **Heinemann T**, Bulwin GC, Randall J, Schnieders B, Sandhoff K, Volk HD, *et al*. Genomic organization of the gene coding for TIRC7, a novel membrane protein essential for T cell activation. *Genomics* 1999;**57**:398–406.
- 12 **Karet F**, Finberg K, Nelson R, Nayir A, Mocan H, Sanjad SA, *et al*. Mutations in the gene encoding B1 subunit of H⁺-ATPase cause renal tubular acidosis with sensorineural deafness. *Nat Genet* 1999;**21**:84–90.
- 13 **Smith A**, Skaug J, Choate K, Nayir A, Bakkaloglu A, Ozen S, *et al*. Mutations in ATP6N1B, encoding a new kidney vacuolar proton pump 116-kD subunit, cause recessive distal renal tubular acidosis with preserved hearing. *Nat Genet* 2000;**26**:71–5.
- 14 **Dorup J**, Scholz H, Maunsbach AB, Kaissling B. Effects of inhibition of the vacuolar-type proton pump on nephron ultrastructure and acidification in the isolated perfused rat kidney. *Exp Nephrol* 1995;**3**:180–92.
- 15 **Barrett MG**, Belinsky GS, Tashjian AH. A new action of parathyroid hormone. Receptor-mediated stimulation of extracellular acidification in human osteoblast-like SaOS-2 cells. *J Biol Chem* 1997;**272**:26346–53.
- 16 **Parak WJ**, Dannohl S, George M, Schuler MK, Schaumburger J, Gaub HE, *et al*. Metabolic activation stimulates acid production in synovial fibroblasts. *J Rheumatol* 2000;**27**:2312–22.
- 17 **Pap T**, Claus A, Ohtsu S, Hummel KM, Schwartz P, Drynda S, *et al*. Osteoclast-independent bone resorption by fibroblast-like cells. *Arthritis Res Ther* 2003;**5**:R163–73.
- 18 **Ducy P**, Schinke T, Karsenty G. The osteoblast: a sophisticated fibroblast under central surveillance. *Science* 2000;**289**:1501–4.
- 19 **Miller EJ**. Chemistry of the collagens and their distribution. In: Piez KA, Reddi AH, eds. *Extracellular matrix biochemistry*. New York: Elsevier Science, 1984:41–82.
- 20 **Belinsky GS**, Tashjian AH. Direct measurement of hormone-induced acidification in intact bone. *J Bone Miner Res* 2000;**15**:550–6.
- 21 **Nordstrom T**, Grinstein S, Brisseau GF, Manolson MF, Rotstein OD. Protein kinase C activation accelerates proton extrusion by vacuolar-type H⁺-ATPases in murine peritoneal macrophages. *FEBS Lett* 1994;**350**:82–6.
- 22 **Kobayashi K**, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S, *et al*. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. *J Exp Med* 2000;**191**:275–86.
- 23 **Varghese S**, Rydziel S, Canalis E. Basic fibroblast growth factor stimulates collagenase-3 promoter activity in osteoblasts through an activator protein-1-binding site. *Endocrinology* 2000;**141**:2185–91.
- 24 **Rifas L**, Fausto A, Scott MJ, Avioli LV, Welgus HG. Expression of metalloproteinases and tissue inhibitors of metalloproteinases in human osteoblast-like cells: differentiation is associated with repression of metalloproteinase biosynthesis. *Endocrinology* 1994;**134**:213–21.
- 25 **Hughes DE**, Dai A, Tiffée JC, Li HH, Mundy GR, Boyce BF. Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-beta. *Nat Med* 1996;**2**:1132–6.
- 26 **Clohisey DR**, Ogilvie CM, Carpenter RJ, Ramnaraine ML. Localized, tumor-associated osteolysis involves the recruitment and activation of osteoclasts. *J Orthop Res* 1996;**14**:2–6.
- 27 **Clohisey DR**, Ramnaraine ML. Osteoclasts are required for bone tumors to grow and destroy bone. *J Orthop Res* 1998;**16**:660–6.
- 28 **Mobasheri A**, Golding S, Pagakis SN, Corkey K, Pocock AE, Fermor B, *et al*. Expression of cation exchanger NHE and anion exchanger AE isoforms in primary human bone-derived osteoblasts. *Cell Biol Int* 1998;**22**:551–62.

Want to know more?

Data supplements

Limited space in printed journals means that interesting data and other material are often edited out of articles; however, limitless cyberspace means that we can include this information online. Look out for additional tables, references, illustrations.

www.annrheumdis.com

PostScript

BOOK REVIEW

Osteoarthritis. 2nd ed

K D Brandt, M Doherty, L S Lohmander. (Pp. 511. £125.) Oxford: Oxford University Press. 2003. ISBN 0-19-850967-7.

The second edition of *Osteoarthritis* is a very well documented and illustrated book. The different chapters cover perfectly the most important questions relating to osteoarthritis—epidemiology, socioeconomics, genetics, pathogenesis, clinical features, and management both in daily practice and clinical trials. In particular, there are well documented sections on specific investigations such as biological markers, magnetic resonance imaging, and ultrasonography. Moreover, two sections evaluate more fully the potential modifiers of cartilage breakdown. This book will be useful for all who are involved in osteoarthritis research and/or in charge of the management of osteoarthritic patients.

M Dougados

FORTHCOMING EVENTS

International Congress on SLE and Related Conditions

9–13 May 2004; New York, New York, USA
Contact: The Oakley Group, 2014 Broadway, Suite 250, Nashville, Tennessee 37203, USA

Tel: +1 615 322 2785
Fax: +1 615 322 2784
Email: Lupus2004@theoakleygroup.com
Website: <http://www.lupus2004.org>

IOF World Congress on Osteoporosis

14–18 May 2004; Rio de Janeiro, Brazil
IOF awards are available for scientists:
IOF Claus Christiansen Research Fellowship: 45 000
IOF Servier Young Investigator Fellowship: 40 000
Contact: Congress Secretariat at info@osteofound.org
Website: www.osteofound.org

International Society for the Study of the Lumbar Spine

31 May–5 June 2004; Porto, Portugal
Contact: International Society for the Study of the Lumbar Spine, 2075 Bayview Avenue, Room MG 323, Toronto, Ontario, Canada M4N 3M5
Tel: 00 1 416 480 4833
Fax: 00 1 416 480 6055
Email: shirley.fitzgerald@sw.ca

XIth International Conference on Behçet's Disease

27–31 October 2004; Antalya, Turkey
Contact: Congress Secretariat, Figur Congress and Organization Services Ltd. STL, Ayazmaderesi Cad. Karadut Sok. No: 7 80888 Dikilitas, Istanbul, Turkey
Tel: +90 (0212) 258 6020
Fax: +90 (0212) 258 6078
Email: behcet2004@figur.net
Website: www.behcet2004.org

4th International Congress on Autoimmunity

3–7 November, 2004; Budapest, Hungary
Deadline for receipt of abstracts: 20 June 2004
Contact: 4th International Congress on Autoimmunity, Kenes International—Global Congress Organisers and Association

Management Services, 17 rue du Cendrier, PO Box 1726, CH-1211 Geneva 1, Switzerland
Tel: +41 22 908 0488
Fax: +41 22 732 2850
Email: autoim04@kenes.com
Website: www.kenes.com/autoim2004

Vlth European Lupus Meeting

3–5 March 2005; Royal College of Physicians, London, UK
Contact: Julia Kermode, Conference organiser of the British Society of Rheumatology
Email: Julia@Rheumatology.org.uk

Future EULAR congresses

9–12 June 2004; EULAR 2004; Berlin, Germany
8–11 June 2005; EULAR 2005; Vienna, Austria
21–24 June 2006; EULAR 2006; Amsterdam, The Netherlands

Future ACR meeting

16–21 October 2004; 68th Annual Scientific Meeting; San Antonio, Texas

CORRECTION

Corrections printed in the journal also appear on the *Annals* website www.annrheumdis.com and are linked to the original publication

doi: 10.1136/ard.2002.005256corr1

Metabolic activation stimulates acid secretion and expression of matrix degrading proteases in human osteoblasts (George M, Stein B, Müller O, Weisklemm M, Pap T, Parak W J, Aicher W K. *Ann Rheum Dis* 2004;63:67–70.)

We regret that in fig 1B of this article the lines joining the data points did not appear in the shaded area of the figure. The correct figure is shown below.

Figure 1 Activation of proton secretion in osteoblasts upon stimulation by PMA or TNF.