Autoimmunity and Bone

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**ABSTRACT: Focal erosions of cartilage and bone, which occur in the joints
of patients with autoimmune inflammatory arthritis (i.e., rheumatoid
arthritis (RA) and psoriatic arthritis [PsA]), represent the most debili-
tating and irreversible components of the disease. Over the last decade,
seminal breakthroughs in our understanding of the cells and signal trans-
duction pathways central to this process have been elucidated. From this
information an established paradigm has been developed to explain focal
erosions in which osteoclasts responsible for erosions are derived from
bone marrow-derived myeloid precursors. Using the tumor necrosis fac-
tor (TNF) transgenic mouse model of erosive arthritis and anti-TNF
clinical trials with PsA patients, we have demonstrated that systemic
TNF induces the migration of CD11b$^+$ osteoclast precursors (OCP) from
the bone marrow into peripheral blood. These OCP can then enter the
joints in blood vessels, translocate across the receptor activator of NF-
κB ligand (RANKL) rich inflamed synovium, and differentiate into ac-
tive osteoclasts. In direct contrast to this, systemic lupus erythematosus
(SLE) patients appear to have an innate resistance to bone resorption.
Our hypothesis to explain this phenomenon is that systemic interferon-α
(IFN-α) diverts the bone marrow-derived myeloid precursors away from
the osteoclast lineage and stimulates their differentiation into dendritic
cells (DC). In support of this model, several labs have used microarray
analyses to define the IFN-induced transcriptome in peripheral blood
mononuclear cells (PBMC) from SLE patients. Here we propose the hy-
pothesis that systemic TNF induces osteoclastic differentiation of PBMC
in PsA patients that correlates with their erosive disease, and that the
innate immune TNF/IFN axis in patients with autoimmune disease dic-
tates their erosive phenotype. To demonstrate this, we injected wild-type
C57B/6 and TNF-Tg mice with poly I:C, which is known to induce sys-
temic IFN responses, and show its dominant effects on increasing the
number of circulating CD11b$^+$/CD11c$^+$ precursor dendritic cells (pDC),
concomitant with a dramatic reduction in CD11b$^+$/CD11c$^-$ OCP. Thus,
systemic factors produced by autoimmunity have a dramatic impact on
active myelopoiesis and bone homeostasis.**

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TNF IN AUTOIMMUNE DISEASE

It is now firmly established that the proinflammatory cytokine, tumor necrosis factor (TNF), is a central mediator of inflammation and tissue catabolism. Although this article focuses on autoimmunity and bone, no discussion on the subject would be complete without highlighting the remarkable success of anti-TNF therapy in the treatment of rheumatoid arthritis (RA), psoriatic arthritis (PsA), ankylosing spondylitis (AS), psoriasis (PS), and Crohn's disease (CD). For their seminal role in defining TNF at the apex of the proinflammatory cytokine cascade and their pioneering work in anti-TNF clinical trials, Drs. M. Feldmann and R.N. Maini received the 2003 Lasker Clinical Medical Research Award.[1] Beyond these specific achievements, the success of anti-TNF therapy via injection of a recombinant soluble receptor (etanercept, Enbrel,[TM] Wyeth Pharmaceuticals Inc., Philadelphia, PA) or antibody (infliximab, Remicade,[TM] Centocor, Inc., Horsham, PA and adalimumab, Humira,[TM] Abbott Laboratories, Abbott Park, IL) exemplifies the ultimate goal of biotechnology and dramatically underscores how complex autoimmune diseases can be effectively suppressed using a targeted therapeutic approach.

IMMUNE-MEDIATED INFLAMMATORY DISORDERS (IMIDS)

Perhaps the most remarkable feature of anti-TNF therapy is its efficacy in various autoimmune diseases, which have completely different pathologies. In an effort to simplify public awareness, the pharmaceutical industry devised a concept to explain a group of unrelated disease that share the common etiology of dysregulated TNF, and termed these conditions IMIDs.[2] We have attempted to elucidate the common mechanism through which TNF drives IMIDs, and how anti-TNF therapy ameliorates them and have pursued the central hypothesis that all autoimmune diseases lead to systemic TNF expression as a result of end organ pathology. This systemic TNF stimulates myelopoiesis and release of monocytes from the bone marrow into the blood. These peripheral blood mononuclear cells (PBMC) then home to sites of inflammation where they translocate into the parenchymal tissue and differentiate into activated macrophages, dendritic cells (DC), or osteoclasts (OC) depending on the local cytokine milieu to mediate the end organ pathology. Monocyte precursors stimulated with granulocyte macrophage colony-stimulating factor (GM-CSF) plus interleukin (IL)-4 differentiate into DC,[3] but can also form OCs if exposed to receptor activator of NF-κB ligand (RANKL) and monocyte colony-stimulating factor (M-CSF).[4] Treatment of monocytes with M-CSF alone produces tissue macrophages, and interferon-γ (IFN-γ) or IL-6 treatment shifts monocyte precursor differentiation to macrophages rather than DC and OC.[5]

THE EROSIVE VERSUS THE NONEROSIVE
PHENOTYPE IN PsA AND SLE

Inflammatory arthritis is one of the most common clinical features in autoimmune disorders manifesting as pain, stiffness redness, and swelling. Radiologic evidence of joint damage (erosions and joint space narrowing) is observed in almost all patients with RA followed for more than 5 years.[6,7] Joint damage is also very common in PsA, an inflammatory joint disease that occurs in 10–15% of psoriasis patients.[8] Gladman and colleagues noted that two-thirds of PsA patients had bone erosions on initial presentation to a rheumatologist.[9] Radiographs often show extensive bone loss manifesting as eccentric erosions, frank tuft resorption, and pencil-in cup deformities.[10] Many PsA patients have aggressive synovitis confirmed histologically as marked synovial hyperplasia, extensive vascular proliferation with a tortuous morphology, and pannus tissue penetrating deep into cartilage and bone.[11] In addition, OCs are prominently situated at the bone–pannus junction and in bone marrow-derived deep resorption cavities traversing the bone matrix,[12] which is consistent with the bi-directional attack originally described by Wooley in severely affected RA joints.[13]

Despite the presence of joint deformities on physical examination, not all forms of inflammatory arthritis degrade cartilage and resorb bone. For example, systemic lupus erythematosus (SLE) is a systemic autoimmune disorder with diverse clinical manifestations, but musculoskeletal symptoms are usually the chief complaint, with 50% of patients reporting articular pain on presentation.[14,15] Lupus patients often develop rheumatoid-like deformities (ulnar deviation, tendinopathies, and subluxation) but only 4–6% of patients display erosive changes on plain radiographs.[16,17] Histopathologic analysis of SLE synovium shows mild-to-moderate synovial hyperplasia, microvascular changes, and perivascular inflammation with mononuclear cells.[18–20] Notably, aggressive synovial tissue-invading cartilage or bone is not described in these studies. Indeed, Bywaters noted the similarity of the SLE joint pattern to that reported by Jaccoud in rheumatic fever. In Jaccoud's arthropathy, joint deformities are manually reducible and radiographs do not depict bone or joint damage. In one large study of 939 SLE patients, Jaccoud's arthropathy was identified in 43% of the patients and was associated with a benign prognosis.[21]

TNF AND IFN ARE THE MASTER CYTOKINES
OF INNATE IMMUNITY

Innate immunity is a rapidly mobilized, non-antigen-specific response to pathogens or injury that markedly changes the local tissue to defend against infection and damage, and is effectively terminated when the initiating stimulus is removed. The temporal and spatial demands of this system require that all cells contain a common germline-encoded signaling pathway that will enable

them to uniformly respond immediately. Research over the last 30 years has identified two critical cytokine pathwaysthat are indispensable for mammalian innate immunity. The first of these is the TNF pathway, derived from the phylogenetically ancient Toll receptor family, whose evolutionary conservation extends from drosophila to man.[22] Essentially, all mammalian cells express TNF receptors and are capable of responding within seconds to appropriate ligands (TNF and lymphotoxin), primarily through NF-κB, JNK, and p38 signaling.[23] The other potent molecules regulating the innate immune response are the type-I INF, which were originally discovered as the intracellular immune defense against viral infection.[24] Similarly, the IFN pathway relies on a ubiquitously expressed receptor that signals through an immediate biochemical cascade known as the JAK-STAT pathway.[25] More recently it has been recognized that when these innate immune responses are chronically activated, the host becomes highly susceptible to a variety of IMIDs. As described above, overexpression of TNF leads to IMIDs. It has been discovered that dysregulation of IFN-α is a major component of SLE.[3] The most compelling data to support this come from microarray studies of PBMC from SLE patients, which demonstrated a remarkably consistent IFN-induced transcriptome.[26,27] Preclinical studies also support an important role for IFN signaling in the development of lupus nephritis as the susceptibility genes in lupus-prone mice have been mapped to IFN-inducible loci.[28–33] Banchereau and colleagues have proposed a unifying hypothesis to explain the presence and etiologic role of IFN in SLE.[34] In this model, an environmental insult (i.e., viral infection) stimulates plasmacytoid DC differentiation, which processes high levels of IFN. This IFN drives myeloid DC differentiation, which thus provides the antigen-presenting cells for autoantibody production. These autoantibodies then induce B-cell stimulation and plasmacytoid DC differentiation to complete the autoimmune vicious cycle.

Considering the role of IFN in DC differentiation and its critical role as a negative regulator of osteoclastogenesis to maintain bone homeostasis,[35] here we propose that the IFN axis controls the erosive versus nonerosive phenotypes of arthritis in SLE patients. In most of these patients, systemic IFN dominantly biases myelopoiesis toward DC and away from OC such that there are very few osteoclast precursors (OCP) in an inflamed joint that have the potential to form bone-resorbing OC. Here we provide the first experimental evidence for this model by stimulating systemic IFN in wild-type C57Bl/6 and TNF-transgenic (TNF-Tg) mice.

METHODS

Animal Studies and Collection of PBMC

Under an approved protocol by the University of Rochester Committee for Animal Resources, we induce systemic IFN in five 12-week-old female

C57Bl/6 (Jackson Labs, Bar Harbor, ME) or TNF-Tg mice,[36] via administration of acute poly (I:C) (Sigma, St. Louis, MO), as described previously.[37] Briefly, PBS placebo or 100 mg of polyI:C was injected into the mice via intraperitoneal (i.p.) injection once daily for 3 days. Afterward, the mice were euthanized, and pooled PBMC were collected via a ficoll gradient, as we described previously.[38]

$CD11b^+/CD11c^+$ pDC and $CD11b^+/CD11c^-$ OCP Frequency and TRAP Osteoclastogenesis Assays

OCP and precursor dendritic cells (pDC) frequency were determined simultaneously, immediately after monocyte purification of PBMC, by two-color FACS, as we described previously.[38] The murine-specific antibodies used were CD11b-PE (clone M1/70), CD11c-APC (clone HL3), (BD Biosciences, San Jose, CA). The percentage of $CD11b^+/CD11c^+$ pDC and $CD11b^+/CD11c^-$ OCP was determined by gating on the populations that contained a fluorescence signal greater than 99% of the isotype controls.

TRAP Staining and Quantitation of Osteoclasts

Tartrate-resistant acid phosphatase (TRAP) osteoclastogenesis was performed, as we described previously.[38] Briefly, PBMC from TNF-Tg mice and their wild-type littermates were cultured in α-modified essential medium (GIBCO BRL, Grand Island, NY) with 10% fetal calf serum (FCS, Hyclone Laboratories, Logan, UT), RANKL (100 ng/mL), and M-CSF (10 ng/mL) for 4 and 6 days, respectively. Cells were fixed and stained for TRAP using the Diagnostics Acid Phosphatase Kit (Sigma) to identify OCs. The OCs were quantified by manual tracing of the photographed OC area, as we described previously.[39]

RESULTS AND DISCUSSION

Systemic IFN Increases the Circulating pDC Frequency and Reduces OCP Frequency

In order to test our hypothesis that systemic IFN mediates a decrease in erosions by biasing myelopoiesis away from OC and toward DC differentiation, we used the well-established polyI:C injection model, which induces an IFN antiviral innate immune response. Wild-type C57B/6 mice and their TNF-Tg littermates were given X injections of poly I:C for 3 days and then PBMC were harvested for analysis by fluorescence-activated cell sorting (FACS) to
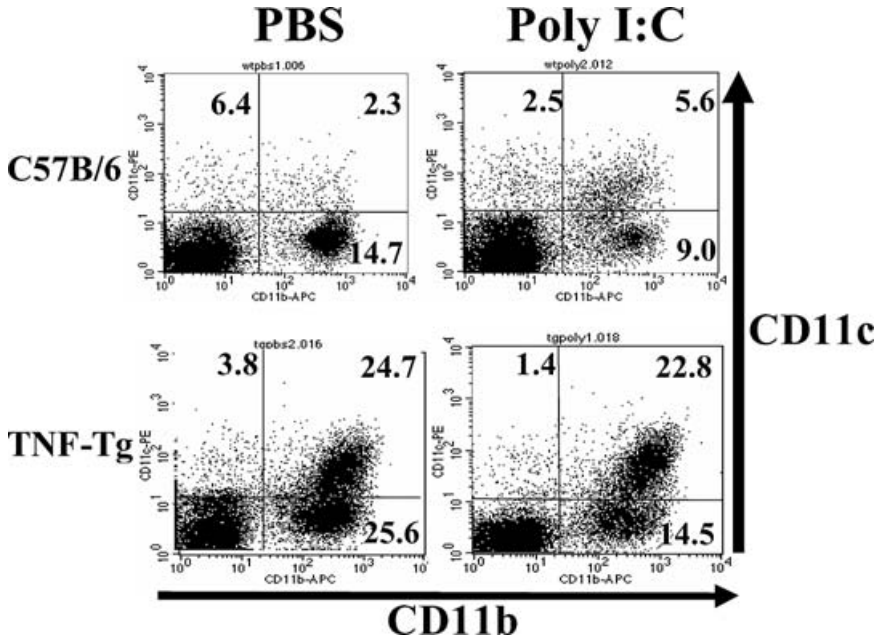
FIGURE 1. PolyI:C treatment alters pDC and OCP frequency in PBMC. Wild-type C57Bl/6 mice and their TNF-Tg littermates ($n = 5$) were treated with PBS placebo or polyI:C (100 mg i.p./day for 3 days) and PBMC from each strain were isolated and pooled together. The PMBC were labeled by antibodies against CD11b and CD11c, and analyzed by two-color FACS, as described in "Methods" section. The percentage of single and double positive cells in each quadrant is indicated.

determine the CD11b$^+$/CD11c$^+$ pDC and CD11b$^+$/CD11c$^-$ OCP frequency. Consistent with our previous reports,[38,40] FIGURE 1 shows that TNF-Tg mice with erosive arthritis have a significant increase in circulating CD11b$^+$ PBMC compared to their nontransgenic littermates. Here, we see that this is due to an increase in both CD11b$^+$/CD11c$^+$ pDC and CD11b$^+$/CD11c$^-$ OCP. Also evident are the effects of the polyI:C treatment, which resulted in a 2.4-fold increase in CD11b$^+$/CD11c$^+$ pDC in wild-type mice. This increase was associated with a 38.8% decrease in CD11b$^+$/CD11c$^-$ OCP in wild-type mice and a 43.4% decrease in TNF-Tg mice.

To confirm these findings, the PBMC were cultured in M-CSF and RANKL to induce osteoclastogenesis, which was quantified by TRAP staining. Consistent with our previous findings,[38,40] osteoclastogenesis in the TNF-Tg PBMC cultures peaked on day 4, while osteoclastogenesis in the wild-type PBMC cultures peaked on day 6. Quantification of the TRAP$^+$ OC in these cultures demonstrated that the polyI:C treatment significantly reduced the number of OCP in both strains (FIG. 2). This finding suggests that systemic IFN can bias
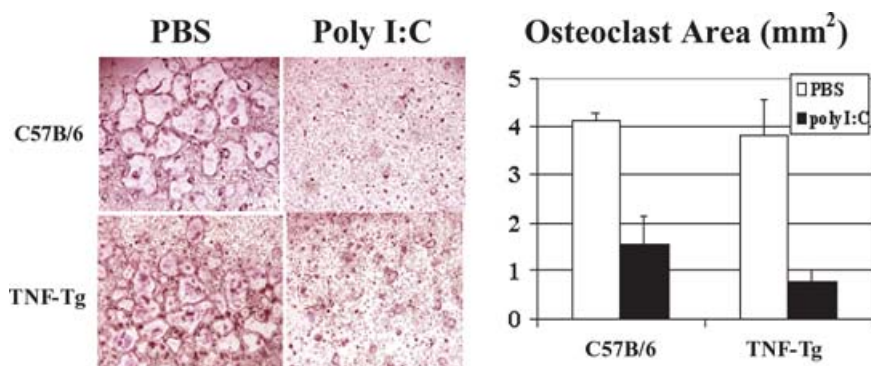
FIGURE 2. PolyI:C treatment dramatically reduces the osteoclastogenic potential of PBMC. The PBMC from C57B/6 and TNF-Tg mice treated with PBS placebo or polyI:C described in FIGURE 1 were cultured in M-CSF and RANKL until the peak in osteoclastogenesis (day 6 and day 4, respectively), and then were fixed and stained for TRAP activity. Representative microphotographs of the cultures ($n = 4$) are shown together with the quantified mean osteoclast area ± SEM. The osteoclast area in the cultures of PBMC from polyI:C-treated mice was significantly less than that of the PBS controls ($P < 0.05$).

myelopoiesis toward DC differentiation at the expense of OCP, even under conditions that favor osteoclastogenesis. Although future studies are needed to determine the precise stage of myelopoiesis that is affected by IFN and whether the IFN has a direct effect on the inhibition of erosive arthritis, these findings support our hypothesis that high IFN levels in SLE patients pushes myeloid differentiation away from osteoclastogenesis.

ACKNOWLEDGMENTS

REFERENCES

1. FELDMANN, M. & R.N. MAINI. 2003. Lasker Clinical Medical Research Award. TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. Nat. Med. **9:** 1245–1250.
2. WILLIAMS, J.P. & J.A. MEYERS. 2002. Immune-mediated inflammatory disorders (I.M.I.D.s): the economic and clinical costs. Am. J. Manag. Care. **8:** S664.
3. PASCUAL, V., J. BANCHEREAU & A.K. PALUCKA. 2003. The central role of dendritic cells and interferon-alpha in SLE. Curr. Opin. Rheumatol. **15:** 548–556.

4. Boyle, W.J., W.S. Simonet & D.L. Lacey. 2003. Osteoclast differentiation and activation. Nature **423:** 337–342.

5. Delneste, Y., P. Charbonnier, N. Herbault, *et al.* 2003. Interferon-gamma switches monocyte differentiation from dendritic cells to macrophages. Blood **101:** 143–150.

6. Kaarela, K., R. Luukkainen & S. Koskimies. 1993. How often is seropositive rheumatoid arthritis an erosive disease? A 17-year followup study. J. Rheumatol. **20:** 1670–1673.

7. Kaarela, K. & S. Sarna. 1993. Correlations between clinical facets of outcome in rheumatoid arthritis. Clin. Exp. Rheumatol. **11:** 643–644.

8. Mease, P.J. 2002. Tumour necrosis factor (TNF) in psoriatic arthritis: pathophysiology and treatment with TNF inhibitors. Ann. Rheum. Dis. **61:** 298–304.

9. Gladman, D.D., V.T. Farewell & C. Nadeau. 1995. Clinical indicators of progression in psoriatic arthritis: multivariate relative risk model. J. Rheumatol. **22:** 675–679.

10. Resnick, D. & G. Niwayama. 1989. Psoriatic arthritis. *In* Bone and Joint Imaging. Resnick, Ed.: 320. WB Saunders. Philadelphia.

11. Fearon, U., K. Griosios, A. Fraser, *et al.* 2003. Angiopoietins, growth factors, and vascular morphology in early arthritis. J. Rheumatol. **30:** 260–268.

12. Ritchlin, C.T., S.A. Haas-Smith, P. Li, *et al.* 2003. Mechanisms of TNF-alpha- and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. J. Clin. Invest. **111:** 821–831.

13. Bromley, M., H. Bertfield, J.M. Evanson, *et al.* 1985. Bidirectional erosion of cartilage in the rheumatoid knee joint. Ann. Rheum. Dis. **44:** 676–681.

14. Kaposi, M. 1872. New reports on knowledge of systemic lupus erythematosus. Arch. Dermat. u. Syph. **4:** 36–78.

15. Stevens, M. 1983. The Clinical Management of Systemic Lupus Erythematosus. *In* P.H. Shur, Ed.: 63–84. Grune & Stratton. New York.

16. Esdaile, J.M., D. Danoff, L. Rosenthall & A. Gutkowski. 1981. Deforming arthritis in systemic lupus erythematosus. Ann. Rheum. Dis. **40:** 124–126.

17. Grigor, R., J. Edmonds, R. Lewkonia, *et al.* 1978. Systemic lupus erythematosus. A prospective analysis. Ann. Rheum. Dis. **37:** 121–128.

18. Goldenberg, D.L. & A.S. Cohen. 1978. Synovial membrane histopathology in the differential diagnosis of rheumatoid arthritis, gout, pseudogout, systemic lupus erythematosus, infectious arthritis and degenerative joint disease. Medicine (Baltimore) **57:** 239–252.

19. Labowitz, R. & H.R. Schumacher, Jr. 1971. Articular manifestations of systemic lupus erythematosus. Ann. Intern. Med. **74:** 911–921.

20. Natour, J., L.C. Montezzo, L.A. Moura & E. Atra. 1991. A study of synovial membrane of patients with systemic lupus erythematosus (SLE). Clin. Exp. Rheumatol. **9:** 221–225.

21. Molina, J.F., G. Citera, D. Rosler, *et al.* 1995. Coexistence of human immunodeficiency virus infection and systemic lupus erythematosus. J. Rheumatol. **22:** 347–350.

22. Locksley, R.M., N. Killeen & M.J. Lenardo. 2001. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell **104:** 487–501.

23. Chen, G. & D.V. Goeddel. 2002. TNF-R1 signaling: a beautiful pathway. Science **296:** 1634–1635.

24. Arend, W.P. 2001. The innate immune system in rheumatoid arthritis. Arthritis. Rheum. **44:** 2224–2234.

25. AARONSON, D.S. & C.M. HORVATH. 2002. A road map for those who don't know JAK-STAT. Science **296:** 1653–1655.
26. BENNETT, L., A.K. PALUCKA, E. ARCE, *et al*. 2003. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J. Exp. Med. **197:** 711–723.
27. BAECHLER, E.C., F.M. BATLIWALLA, G. KARYPIS, *et al*. 2003. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc. Natl. Acad. Sci. USA **100:** 2610–2615.
28. DRAKE, C.G., S.J. ROZZO, H.F. HIRSCHFELD, *et al*. 1995. Analysis of the New Zealand black contribution to lupus-like renal disease. Multiple genes that operate in a threshold manner. J. Immunol. **154:** 2441–2447.
29. KOTZIN, B.L. 1996. Systemic lupus erythematosus. Cell **85:** 303–306.
30. ROZZO, S.J., T.J. VYSE, C.G. DRAKE & B.L. KOTZIN. 1996. Effect of genetic background on the contribution of New Zealand black loci to autoimmune lupus nephritis. Proc. Natl. Acad. Sci. USA **93:** 15164–15168.
31. ROZZO, S.J., T.J. VYSE, K. MENZE, *et al*. 2000. Enhanced susceptibility to lupus contributed from the nonautoimmune C57BL/10, but not C57BL/6, genome. J. Immunol. **164:** 5515–5521.
32. VYSE, T.J., S.J. ROZZO, C.G. DRAKE, *et al*. 1997. Control of multiple autoantibodies linked with a lupus nephritis susceptibility locus in New Zealand black mice. J. Immunol. **158:** 5566–5574.
33. VYSE, T.J., S.J. ROZZO, C.G. DRAKE, *et al*. 1998. Contributions of Ea(z) and Eb(z) MHC genes to lupus susceptibility in New Zealand mice. J. Immunol. **160:** 2757–2766.
34. BANCHEREAU, J., V. PASCUAL & A.K. PALUCKA. 2004. Autoimmunity through cytokine-induced dendritic cell activation. Immunity **20:** 539–550.
35. TAKAYANAGI, H., S. KIM, K. MATSUO, *et al*. 2002. RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-beta. Nature **416:** 744–749.
36. KEFFER, J., L. PROBERT, H. CAZLARIS, *et al*. 1991. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. Embo J. **10:** 4025–4031.
37. SANTIAGO-RABER, M.L., R. BACCALA, K.M. HARALDSSON, *et al*. 2003. Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. J. Exp. Med. **197:** 777–788.
38. LI, P., E.M. SCHWARZ, R.J. O'KEEFE, *et al*. 2004. Systemic tumor necrosis factor alpha mediates an increase in peripheral CD11bhigh osteoclast precursors in tumor necrosis factor alpha-transgenic mice. Arthritis. Rheum. **50:** 265–276.
39. HUANG, W., R.J. O'KEEFE & E.M. SCHWARZ. 2003. Exposure to receptor-activator of NFkappaB ligand renders pre-osteoclasts resistant to IFN-gamma by inducing terminal differentiation. Arthritis. Res. Ther. **5:** R49–R59.
40. LI, P., E.M. SCHWARZ, R.J. O'KEEFE, *et al*. 2004. RANK signaling is not required for TNFalpha-mediated increase in CD11(hi) osteoclast precursors but is essential for mature osteoclast formation in TNFalpha-mediated inflammatory arthritis. J. Bone. Miner. Res. **19:** 207–213.