4. Yoder, M. C., Mead, L. E., Prater, D., Krier, T. R., Mroueh, K. N., Li, F., Krasich, R., Temm, C. J., Prchal, J. T., Ingram, D. A. (2007) Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* **109,** 1801–1809.
5. Colombo, E., Calcaterra, F., Cappelletti, M., Mavilio, D., Della Bella, S. (2013) Comparison of fibronectin and collagen in supporting the isolation and expansion of endothelial progenitor cells from human adult peripheral blood. *PLoS One* **8,** e66734.
6. Calcaterra, F., Taddeo, A., Colombo, E., Cappelletti, M., Martinelli, A., Calabrese, S., Mavilio, D., Cetin, I., Della Bella, S. (2014) Reduction of maternal circulating endothelial progenitor cells in human pregnancies with intrauterine growth restriction. *Placenta* **35,** 431–436.
7. Rivera, L. B., Bergers, G. (2015) Intertwined regulation of angiogenesis and immunity by myeloid cells. *Trends Immunol.* **36,** 240–249.
8. Facciabene, A., Motz, G. T., Coukos, G. (2012) T-Regulatory cells: key players in tumor immune escape and angiogenesis. *Cancer Res.* **72,** 2162–2171.
9. Hur, J., Yang, H. M., Yoon, C. H., Lee, C. S., Park, K. W., Kim, J. H., Kim, T. Y., Kim, J. Y., Kang, H. J., Chae, I. H., Oh, B. H., Park, Y. B., Kim, H. S. (2007) Identification of a novel role of T cells in postnatal vasculogenesis: characterization of endothelial progenitor cell colonies. *Circulation* **116,** 1671–1682.
10. Rouhl, R. P. W., Mertens, A. E. C. S., van Oostenbrugge, R. J., Damoiseaux, J. G. M. C., Debrus-Palmans, L. L., Henskens, L. H. G., Kroon, A. A., de Leeuw, P. W., Lodder, J., Tervaert, J. W. C. (2012) Angiogenic T-cells and putative endothelial progenitor cells in hypertension-related cerebral small vessel disease. *Stroke* **43,** 256–258.
11. Rodríguez-Carrio, J., Alperi-López, M., López, P., Alonso-Castro, S., Ballina-García, F. J., Suárez, A. (2015) Angiogenic T cells are decreased in rheumatoid arthritis patients. *Ann. Rheum. Dis.* **74,** 921–927.
12. López, P., Rodríguez-Carrio, J., Martínez-Zapico, A., Caminal-Montero, L., Suarez, A. Senescent profile of angiogenic T cells from systemic lupus erythematosus patients. (2016). *J. Leukoc. Biol.* **99,** 405–412.

KEY WORDS:
angiogenesis · endothelial progenitor cells · vessel regression

Editorial: **Flt3 ligand—friend or foe?**

By Irina Voronov and Morris F. Manolson[1]

Faculty of Dentistry, University of Toronto, Ontario, Canada

RECEIVED SEPTEMBER 28, 2015; REVISED DECEMBER 11, 2015; ACCEPTED DECEMBER 12, 2015. DOI: 10.1189/jlb.3CE0915-445RR

▶ SEE CORRESPONDING ARTICLE ON PAGE 413

RA is an autoimmune inflammatory disease characterized by pain, periarticular inflammation, and bone erosion around the affected joints. It is unequivocal that osteoclasts are the cells responsible for the pathologic bone loss observed in RA patients [1].

Osteoclasts are the multinucleated cells formed by fusion of hematopoietic mononuclear precursors. In a healthy body, the amount of bone formation and resorption is perfectly balanced: osteoblasts, osteocytes, and stromal cells produce M-CSF and RANKL, factors necessary to promote osteoclastogenesis, as well as osteoprotegerin, a decoy receptor to sequester RANKL and therefore, to inhibit osteoclast formation. During a disease, numerous factors affect this balance, shifting it toward increased or decreased osteoclast activity. For example, during acute inflammation, macrophages and neutrophils secrete proinflammatory cytokines, such as IL-1 and TNF-α, which can then activate osteoclasts directly via up-regulation of the NF-κB signaling pathway and indirectly, by inducing osteoblasts and stromal cells to secrete higher levels of RANKL. As a result, osteoclastogenesis is up-regulated, and bone resorption is increased. In autoimmune disease, such as RA, in addition to macrophages, T cells, B cells, dendritic cells, and synovial fibroblasts are involved, contributing to the complexity of disease. Macrophages secrete proinflammatory cytokines and increase osteoclast formation directly and indirectly. B cells produce anticitrullinated protein antibodies, and these antibodies can directly induce osteoclastogenesis. T cells also play a significant part: Th17 cells produce IL-17 and RANKL and therefore, can affect osteoclast formation directly or indirectly by inducing synovial fibroblasts to secrete higher levels of RANKL. Th1 and Th2 cells secrete IFN-γ and IL-4, respectively, both known to be involved in negative regulation of osteoclastogenesis and therefore, to suppress osteoclast activation. Dendritic cells have also been implicated in RA: they have the potential to differentiate into osteoclasts under inflammatory conditions, further contributing to bone loss. In the paper by Svensson et al. [2], published in this issue of the *Journal of Leukocyte Biology*, the authors propose that Flt3L, a dendritic cell differentiation factor, is another factor involved in RA and serves as a negative regulator of osteoclastogenesis and a bone-protective factor.

Little is known about the role of Flt3L in RA. Two independent groups had shown that patients with RA have elevated levels of Flt3L in synovial fluid, as well as in serum [3, 4]. Ramos et al. [3] also demonstrated that peripheral blood monocytes not only appear to be the primary source of Flt3L in RA patients but also express the highest levels of the Flt3L receptor (Flt3/CD135), suggesting an autocrine regulation. Flt3L KO mice are protected from developing arthritis in a CIA model and have lower levels of proinflammatory cytokines, CII-specific IgG2a antibodies, and decreased synovial inflammation [5].

So, what is known about Flt3L and Flt3 signaling? Flt3 (CD135) is a receptor tyrosine kinase expressed in hematopoietic precursors and plays a role in hematopoietic expansion. Flt3L, the only ligand known

Abbreviations: CIA = collagen-induced arthritis, Flt3L = Fms-like tyrosine kinase 3 ligand, IRF8 = IFN regulatory factor 8, KO = knockout, mBSA = methylated BSA, RA = rheumatoid arthritis, RANKL = receptor activator of NF-κB ligand, TACE = TNF-α-converting enzyme, T_{reg} = regulatory T cell, WT = wild-type

1. Correspondence: University of Toronto, 124 Edward St., Toronto, Ontario, M5G 1G6, Canada. E-mail: m.manolson@utoronto.ca

to bind to Flt3, is expressed by a variety of tissues, including stromal fibroblasts and T lymphocytes, and exists in membrane-bound and soluble forms (reviewed in ref. [6]). TACE is the enzyme responsible for Flt3L shedding. As the expression levels of TACE are increased in the tissues of RA patients, this most likely contributes to the increased Flt3L levels in serum and synovial fluid of these patients. Flt3L binding to the receptor Flt3/CD135 causes dimerization and autophosphorylation of the receptor. Several downstream signaling pathways are activated, including PI3K/Akt/mTOR, JAK/STAT, and RAS/RAF/Erk pathways, and are involved in survival, proliferation, and differentiation of hematopoietic progenitor cells. Mice lacking Flt3 or Flt3L have reduced hematopoietic precursors and a defect in early B cell development. Furthermore, Flt3L KO mice have dramatically reduced dendritic cells [7], demonstrating that Flt3L is a crucial factor responsible for dendritic cell development in mouse bone marrow (**Fig. 1**).

Is there a connection between Flt3L and osteoclasts? Apparently, there is a direct connection. Lean et al. [8] showed that Flt3L can be used as an alternative to M-CSF to induce osteoclastogenesis in RANKL-treated bone marrow cells. These cells, although much fewer and considerably smaller compared with M-CSF and RANKL groups, were multinucleated, tartrate-resistant acid phosphatase positive, and capable of bone resorption. Furthermore, an injection of Flt3L directly into a mouse joint increased joint inflammation and bone erosion in a *Staphylococcus aureus*-derived peptidoglycan model of inflammatory arthritis [4], suggesting that Flt3L plays a proinflammatory role in this model. Another interesting phenomenon is the ability of dendritic cells to differentiate into osteoclasts and that this transdifferentiation is promoted by the RA microenvironment [9]. To summarize, Flt3L levels are increased in RA; Flt3L KO mice are protected from developing arthritis in a collagen-induced model; Flt3L is processed by TACE (a protease abundant in the RA milieu), involved in hematopoietic stem cell and dendritic cell differentiation and proliferation; and finally, Flt3L can directly promote osteoclastogenesis. All of these findings suggest that Flt3L has proinflammatory and pro-osteoclastogenic properties, further contributing to bone loss.

In the manuscript by Svensson et al. [2], the authors present data suggesting that Flt3L may play a protective role in bone, as Flt3L KO mice had lower trabecular bone mineral density, trabecular bone volume, thickness, and number, and Flt3L injections appeared to rescue this bone phenotype. Furthermore, with the use of an mBSA model of inflammatory arthritis, the authors showed that arthritic joints of KO mice had increased the number of osteoclasts in the periarticular zone compared with WT controls, whereas the treatment of KO animals with Flt3L reduced the number of osteoclasts. Another observation made by the authors was that mRNA expression of IRF8, a known negative regulator of osteoclast differentiation (reviewed in ref. [10]) was decreased in bones of Flt3L KO mice. Moreover, both in vivo injections of Flt3L and in vitro treatments increased IRF8 expression. As only gene expression was assessed, it is not clear if the IRF8 transcriptional activity was also up-regulated. The authors also assessed the presence of T_{reg} and Th17 cells in the bone marrow and in the spleen, as these cells have been shown to play a protective role or a proinflammatory role, respectively, in RA (reviewed in ref. [11]). Therefore, the T_{reg}/Th17 ratio represents the status of the immune homeostasis. Flt3L KO mice had a lower T_{reg}/Th17 ratio compared with WT, suggesting increased inflammatory response in the KO animals.

These findings contradict previous data, showing that Flt3L KO mice are protected from developing arthritis [5]. What could be the explanation for these conflicting results? One reason for these discrepancies could be the use of different animal models: mBSA versus the CIA model, the most commonly used mouse model of RA, which is characterized by B and T cell responses, as well as the presence of the anticitrullinated peptide antibodies. The mBSA animal model of inflammatory arthritis, although not without its advantages, is not an ideal model of RA, as it does not breach the immunologic tolerance as seen in humans. Therefore, the composition of the inflammatory infiltrate in the synovial cavity is different in mBSA-induced compared with the CIA mouse model. What are the other reasons? Unfortunately, it is hard to say, as the bone phenotype of Flt3L KO mice has not been studied. Svensson et al. [2] did show a decrease in several bone parameters; however, a detailed characterization of osteoclasts from Flt3 KO mice in vivo and in vitro is missing from both studies. A detailed analysis of the osteoclast phenotype could answer many questions. After all, these mice are reported to have reduced numbers, not only of dendritic cells but also neutrophils, NK cells, and monocytes [12] and therefore, a reduced pool of osteoclast precursors.

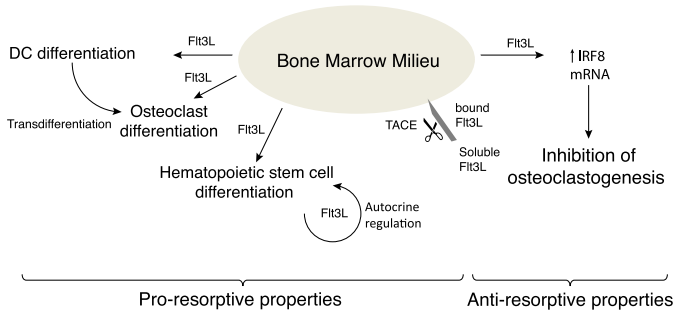

Figure 1. Flt3L—proresorptive or antiresorptive? There is more evidence suggesting that this ligand is proresorptive. Flt3L levels are increased in RA. TACE, the protease also abundant in RA, cleaves membrane-bound Flt3L to the soluble form. It is involved in hematopoietic stem cell differentiation; these cells not only produce Flt3L but also express the receptor Flt3, suggesting an autocrine regulation. Flt3L is particularly important for dendritic cell (DC) development, which in turn, is known to transdifferentiate into osteoclasts, the cells responsible for bone loss in RA. Finally, Flt3L can substitute for M-CSF and promote osteoclastogenesis directly, further contributing to bone loss. In contrast, the Flt3L KO mouse model has an increased osteoclast number and decreased IRF8 mRNA, suggesting an antiresorptive role for this cytokine.

So, is Flt3L a friend or a foe in RA? We still do not know. Clinical studies show that Flt3L levels are increased in RA patients but not in patients with gout [3], indicating that there is disease specificity rather than a response to inflammation. Further detailed characterization of Flt3L KO bone phenotype, examining osteoblasts and osteoclasts, in vivo and in vitro, would help clarify the role of Flt3L in autoimmune and inflammatory conditions.

REFERENCES

1. Sato, K., Takayanagi, H. (2006) Osteoclasts, rheumatoid arthritis, and osteoimmunology. *Curr. Opin. Rheumatol.* **18,** 419–426.
2. Svensson, M. N., Erlandsson, M. C., Jonsson, I. M., Andersson, K. M., Bokarewa, M. I. (2016) Impaired signaling through the Fms-like tyrosine kinase 3 receptor increases osteoclast formation and bone damage in arthritis. *J. Leukoc. Biol.* **99,** 413–423.
3. Ramos, M. I., Perez, S. G., Aarrass, S., Helder, B., Broekstra, P., Gerlag, D. M., Reedquist, K. A., Tak, P. P., Lebre, M. C. (2013) FMS-related tyrosine kinase 3 ligand (Flt3L)/CD135 axis in rheumatoid arthritis. *Arthritis Res. Ther.* **15,** R209.
4. Dehlin, M., Bokarewa, M., Rottapel, R., Foster, S. J., Magnusson, M., Dahlberg, L. E., Tarkowski, A. (2008) Intra-articular fms-like tyrosine kinase 3 ligand expression is a driving force in induction and progression of arthritis. *PLoS One* **3,** e3633.
5. Ramos, M. I., Karpus, O. N., Broekstra, P., Aarrass, S., Jacobsen, S. E., Tak, P. P., Lebre, M. C. (2015) Absence of Fms-like tyrosine kinase 3 ligand (Flt3L) signalling protects against collagen-induced arthritis. *Ann. Rheum. Dis.* **74,** 211–219.
6. Drexler, H. G., Quentmeier, H. (2004) FLT3: receptor and ligand. *Growth Factors* **22,** 71–73.
7. Lyman, S. D., Jacobsen, S. E. (1998) c-kit Ligand and Flt3 ligand: stem/progenitor cell factors with overlapping yet distinct activities. *Blood* **91,** 1101–1134.
8. Lean, J. M., Fuller, K., Chambers, T. J. (2001) FLT3 ligand can substitute for macrophage colony-stimulating factor in support of osteoclast differentiation and function. *Blood* **98,** 2707–2713.
9. Rivollier, A., Mazzorana, M., Tebib, J., Piperno, M., Aitsiselmi, T., Rabourdin-Combe, C., Jurdic, P., Servet-Delprat, C. (2004) Immature dendritic cell transdifferentiation into osteoclasts: a novel pathway sustained by the rheumatoid arthritis microenvironment. *Blood* **104,** 4029–4037.
10. Ivashkiv, L. B., Zhao, B., Park-Min, K. H., Takami, M. (2011) Feedback inhibition of osteoclastogenesis during inflammation by IL-10, M-CSF receptor shedding, and induction of IRF8. *Ann. N. Y. Acad. Sci.* **1237,** 88–94.
11. Komatsu, N., Takayanagi, H. (2015) Regulatory T cells in arthritis. *Prog. Mol. Biol. Transl. Sci.* **136,** 207–215.
12. McKenna, H. J., Stocking, K. L., Miller, R. E., Brasel, K., De Smedt, T., Maraskovsky, E., Maliszewski, C. R., Lynch, D. H., Smith, J., Pulendran, B., Roux, E. R., Teepe, M., Lyman, S. D., Peschon, J. J. (2000) Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood* **95,** 3489–3497.

KEY WORDS:
Rheumatoid Arthritis · osteoclasts · bone resorption · Fms-like tyrosine Kinase 3 ligand · CD135