The Etiology of Paget's Disease of Bone: Viral and Genetic Interactions

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Studies of the etiology of Paget's disease have focused separately on the viral and genetic components of the disease. In this issue of *Cell Metabolism*, Kurihara et al. (2011) join these components, reporting that sequestosome 1 mutation in patients and mice activates osteoclasts, while measles virus induces the phenotype of Paget's disease.

Paget's disease of bone is a chronic skeletal disorder found primarily in populations of European origin and usually diagnosed in older individuals (>50 years), although the slow progression of the disease and the often asymptomatic status of patients suggests the onset may be years or decades earlier. Bone resorption is the primary underlying abnormality, with osteolytic lesions the earliest radiologic findings observed. As the disease process advances, the osteolytic areas evolve into a patchy osteosclerotic character. In the later stages there is grossly osteosclerotic bone with chaotic structure, areas of secondary osteolytic fronts, and considerable enlargement and bowing of lower extremity bones. A characteristic finding in Paget's disease is the presence of nuclear inclusions (occasionally cytoplamic) resembling nucleocapsids of paramyxoviruses in osteoclasts (Rebel et al., 1974). Furthermore, Paget's disease occurs sporadically and in families with an autosomal dominant transmission pattern but with incomplete penetrance, due to mutations in the sequestosome 1 gene (Laurin et al., 2002). Therefore, the etiology of Paget's disease has focused on the viral component of paramyxoviruses, because of the finding of nucleocapsid-like structures in pagetic osteoclasts, and on the genetic component, because a subset of patients come from families with an autosomal dominant transmission of the disorder.

In this issue of *Cell Metabolism*, Roodman and colleagues, who defined the molecular characteristics of osteoclasts in Paget's disease and developed an animal model in transgenic mice utilizing measles virus nucleocapsid protein (MVNP) (Kurihara et al., 2006), collaborated with the French-Canadian investigators who first discovered sequestosome 1 mutations in Paget's disease to define the interactions between the viral and genetic influences on osteoclast formation (Kurihara et al., 2011), thereby uniting the two fields. Sequestosome 1 encodes for the p62 protein, which is a scaffold protein that plays an important role in RANKL signaling in osteoclasts. In this study, Kurihara et al. studied bone marrow specimens from patients with familial Paget's disease (with $p62^{P392L}$) from clinically involved and uninvolved sites or normal subjects. The effects of antisense-MVNP on in vitro osteoclast formation in subjects with and without MVNP expression in the marrow cells were determined. Patients with Paget's disease typically show numerous osteoclasts in Howship's lacunae, many of which are larger and contain many more nuclei than normal. In the patient specimens with MVNP expression, antisense MVNP significantly reduced osteoclast formation, number of nuclei per cell, TATA box-binding protein associated factor 12 (TAF-12) expression, 1,25 $(OH)_2D_3$-stimulated IL-6 production, and bone resorption, thus reversing the usual characteristics of osteoclasts generated from marrow specimens of Paget's disease patients. There was no effect of antisense-MVNP on the specimens of patients with negative MVNP or normal subjects who were also MVNP negative. However, both MVNP-positive and MVNP-negative cells were hyperresponsive to receptor activator of nuclear factor kappa-B ligand (RANKL) and formed more osteoclasts than cells from normal subjects. Nuclear number per osteoclast was similar after RANKL treatment in $p62^{P392L}$/MVNP-negative and normal marrow cultures but was significantly lower than in cultures from $p62^{P392L}$/MVNP-positive patients. The results indicate that the sequestosome 1 mutation sensitizes the osteoclast precursors to the stimulation of RANKL, while MVNP is responsible for most characteristics of osteoclasts in Paget's disease including increased number and nuclei, increased bone resorption capacity per osteoclast, increased TAF-12 expression, and increased responsiveness to RANKL, tumor necrosis factor, and 1,25 $(OH)_2D_3$.

To further explore the interactions of MVNP and the sequestosome 1 mutation, Kurihara and colleagues utilized a variety of transgenic mice to demonstrate that MVNP mice with or without $p62^{P394L}$ (the mouse equivalent of $p62^{P392L}$) develop pagetic osteoclasts and express high levels of IL-6 dependent on p38MAPK activation. When the MVNP mice were bred with IL-6 knockout mice, pagetic osteoclasts were absent and bone formation parameters were below those observed in wild-type mice. Mice bred to have MVNP and $p62^{P394L}$ had the most severe bone lesions typical of Paget's disease. The results of these studies suggest that MVNP alone can cause Paget's disease, but susceptibility and severity may be influenced by gene mutations or genetic variants in an individual (Figure 1). Genome-wide association studies have suggested that variants of other genes that influence osteoclast function may also predispose to the development of Paget's disease, but the functional relevance of these findings has yet to be established (Albagha et al., 2010).

Kurihara and colleagues have merged human and animal studies to provide

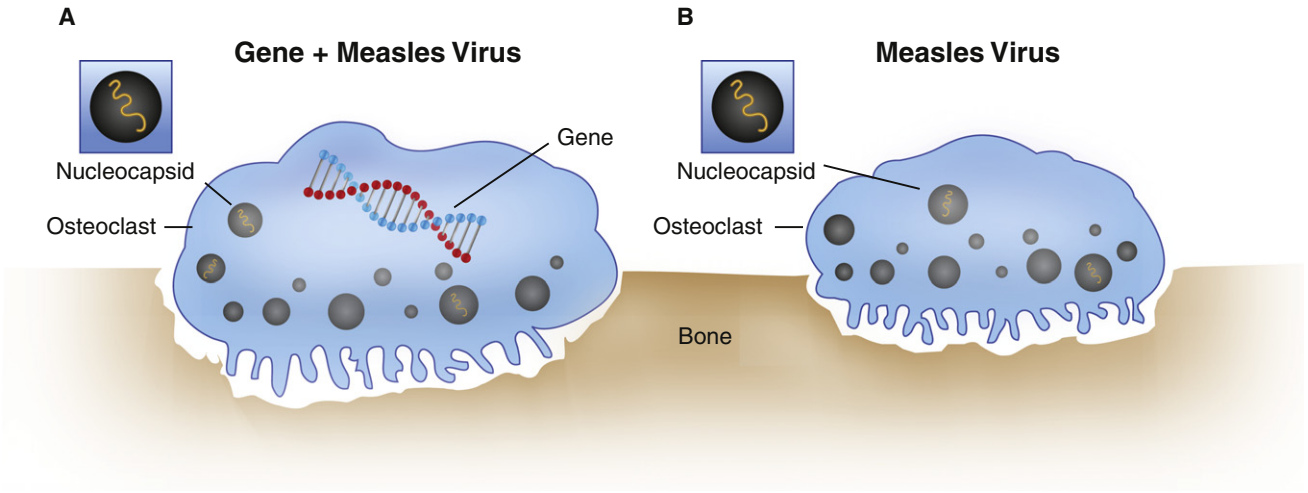

Figure 1. Model Depicting Osteoclast Activity in Paget's Disease
(A) A combination of a gene mutation and measles virus nucleocapsid protein produces enhanced osteoclastic activity.
(B) Measles virus nucleocapsid protein alone enhances osteoclastic activity, albeit to a lesser degree.

a better understanding of the pathogenesis of Paget's disease. Their data clearly support the clinical observations that a gene mutation may increase susceptibility to develop Paget's disease and may explain why patients with familial disease may have somewhat more skeletal lesions and be more symptomatic than sporadic patients. The viral data provide stronger evidence for the role of MV in Paget's disease and what mechanisms account for the effect of MV on osteoclasts and osteoblasts on this disorder. The finding that knockout of IL-6 in MVNP mice prevents the development of pagetic osteoclasts is not unexpected, but the reduction of bone formation parameters in these animals suggests that IL-6 plays a role in linking bone formation to bone resorption, a surprising observation.

A number of questions remain to be answered for a full understanding of Paget's disease. Although generally classified as a metabolic bone disorder, what accounts for the small numbers of affected bones in most patients? Can other paramyxoviruses induce a pagetic phenotype? Studies utilizing immunochemical and RNA techniques have suggested that not only MV (Rebel et al.1980; Reddy et al., 1995) but respiratory syncytial virus or canine distemper virus could be responsible for a slow virus infection analogous to subacute sclerosing panencephalitis which occurs in children following MV infection (Mills et al., 1981; Gordon et al., 1991), although other studies have failed to confirm these findings (Helfrich et al., 2000). Is there a genetic component to paramyxovirus persistence? Can a genetic mutation(s) and/or variant alone produce the pagetic phenotype? What role does genetics play in marrow stromal cell and osteoblast function? The answers to these questions would not only clarify the etiology of Paget's disease but would increase our understanding of bone biology.

REFERENCES

Albagha, O.M., Visconti, M.R., Alonso, N., Langston, A.L., Cundy, T., Dargie, R., Dunlop, M.G., Fraser, W.D., Hooper, M.J., Isaia, G., et al. (2010). Nat. Genet. *42*, 520–524.

Gordon, M.T., Anderson, D.C., and Sharpe, P.T. (1991). Bone *12*, 195–201.

Helfrich, M.H., Hobson, R.P., Grabowski, P.S., Zurbriggen, A., Cosby, S.L., Dickson, G.R., Fraser, W.D., Ooi, C.G., Selby, P.L., Crisp, A.J., et al. (2000). J. Bone Miner. Res. *15*, 2315–2329.

Kurihara, N., Zhou, H., Reddy, S.V., Garcia Palacios, V., Subler, M.A., Dempster, D.W., Windle, J.J., and Roodman, G.D. (2006). J. Bone Miner. Res. *21*, 446–455.

Kurihara, N., Hiruma, Y., Yamana, K., Michou, L., Rousseau, C., Morissette, J., Galson, D.L., Teramachi, J., Zhou, H., Dempster, D.W., Windle, J.J., Brown, J.P., and Roodman, G.D. (2011). Cell Metab. *13*, this issue, 23–34.

Laurin, N., Brown, J.P., Morissette, J., and Raymond, V. (2002). Am. J. Hum. Genet. *70*, 1582–1588.

Mills, B.G., Singer, F.R., Weiner, L.P., and Holst, P.A. (1981). Proc. Natl. Acad. Sci. USA *78*, 1209–1213.

Rebel, A., Malkani, K., and Basle, M. (1974). Nouv. Presse Med. *3*, 1299–1301.

Rebel, A., Basle, M., Pouplard, A., Kouyoumdjian, S., Filmon, R., and Lepatezour, A. (1980). Lancet *2*, 344–346.

Reddy, S.V., Singer, F.R., and Roodman, G.D. (1995). J. Clin. Endocrinol. Metab. *80*, 2108–2111.