

Interleukin 6, RANKL, and Osteoprotegerin Expression by Chikungunya Virus-Infected Human Osteoblasts

TO THE EDITOR—Chow et al [1] recently implicated interleukin 6 (IL-6) in the persistent arthralgia that occurs in some patients following infection with chikungunya virus (CHIKV). They observed that plasma IL-6 concentrations in patients with persistent arthralgia were higher than those in fully recovered patients; the significance of this observation is supported by the known role of IL-6 in causing joint pain [2]. A role for IL-6 in persistent arthralgia is further supported by the finding by Hoarau et al [3] that IL-6 is specifically expressed in the affected joint during chronic chikungunya disease. Nevertheless, the

plasma IL-6 concentration in patients with chronic disease is low (interquartile range, 4–40 pg/mL) and close to normal values [1].

IL-6 is expressed by a variety of cell types, including osteoblasts, and the low circulating levels suggest the joint as a potential source of this cytokine. IL-6 stimulates the release of RANKL [4] and inhibits the one of its decoy receptor osteoprotegerin (OPG) [5] by osteoblasts, therefore promoting osteoclastogenesis and bone resorption [6]. The RANKL/OPG ratio indeed drives osteoclastogenesis and osteoclast activation [7]. This raises the possibility that dysregulation of IL-6, RANKL, and OPG during CHIKV infection may contribute to joint pathology. In this context, we aimed to determine whether osteoblasts may be involved in CHIKV induced chronic rheumatic syndromes.

First, we tested whether primary human osteoblasts are susceptible to CHIKV infection in vitro. Osteoblast cultures were prepared from bone samples obtained from a healthy male subject during a knee operation for a cause unrelated to arthritis. The patient's medical history indicated no autoimmune disorders, metabolic diseases, intake of immune suppressant/stimulating drugs, or immunotherapy for 3 months before surgery. Bone fragments were cultured in α minimum essential medium supplemented with 10% fetal calf serum, 100 mM ascorbic acid, 20 mM HEPES, and 2 mM L-glutamine. After 2 weeks, confluent cells surrounding fragments were collected and subcultured. Osteoblast characterization by osteocalcin staining and measurement of alkaline phosphatase activity showed >98% purity [8]. Cell monolayers were infected with CHIKV at a multiplicity of infection of 0.1 for 1 hour at 37°C, washed, and fed with fresh media.

As shown in Figure 1A, CHIKV replicated in osteoblasts between days 1 and 20 after infection, at levels comparable to that described for macrophages [9]. The decrease in virus production with

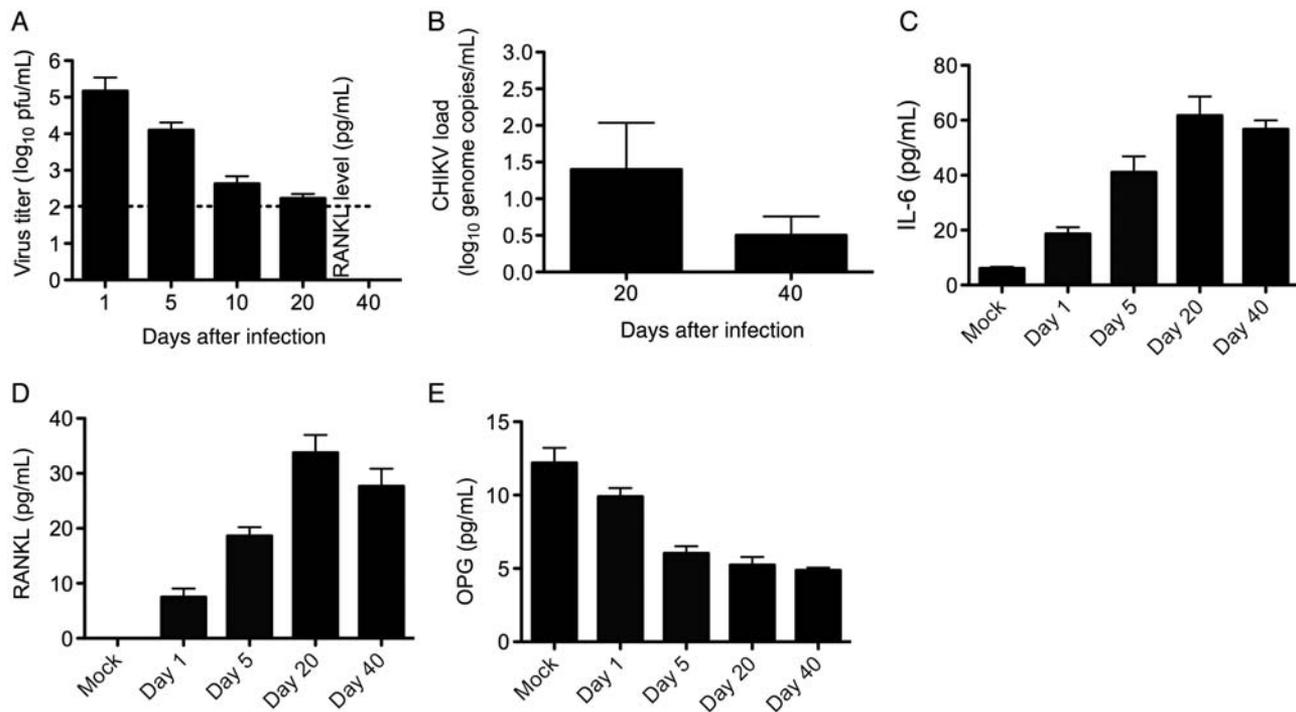


Figure 1. Effects of chikungunya virus (CHIKV) infection on human osteoblasts. Culture supernatant was collected at different times after infection of primary human osteoblasts by CHIKV (multiplicity of infection = 0.1). *A*, CHIKV titer in supernatants was quantified by plaque assay. pfu, plaque-forming units. *B*, CHIKV messenger RNA was quantified by reverse transcription–polymerase chain reaction. Results were always included in the linear range of amplification. *C–E*, Concentrations of interleukin 6 (IL-6; *C*), RANKL (*D*), and osteoprotegerin (OPG; *E*) in supernatants of mock-infected osteoblasts and CHIKV-infected osteoblasts collected at different times after infection. Cytokines were quantified by enzyme-linked immunosorbent assay.

time may be because about 90% of cells underwent cytopathic effect by day 5 after infection, after which the remaining cells grew to form a confluent monolayer with no further toxicity (data not shown). Despite this massive cell death, CHIKV was detectable by plaque assay, until day 20 after infection, and by reverse transcription polymerase chain reaction in cells, until day 40, when the cultures were stopped (Figure 1*B*).

To our knowledge, this is the first demonstration that osteoblasts are susceptible to CHIKV infection and could contribute, in addition to macrophages [3, 9], to viral persistence in the joint. Infection induced the expression of IL-6 and RANKL in osteoblast cultures with very similar kinetics that differed from that for virus replication (Figure 1*C* and 1*D*). The IL-6 and RANKL concentrations, assessed by enzyme-linked immunosorbent assay in supernatants, were

increased as early as day 1 after infection as compared to mock-infected cultures, continued to increase up to day 20, and were maintained at high levels at day 40. Alternatively, CHIKV infection decreased the constitutive expression of OPG by about 60%, with no return to preinfection levels (Figure 1*E*). This sustained induction of IL-6 and RANKL and repression of OPG expression by infection suggests the establishment of an independent autocrine loop. Indeed, osteoblasts express the IL-6 receptor and gp130, and an autocrine action of IL-6 on this cell type has been previously described. An autocrine mechanism may explain the maintenance of each IL-6–sustainable effect initiated by early replication. These data suggest that, in CHIKV-infected patients, an altered RANKL/OPG ratio may contribute to bone loss and to the occurrence of arthritis/arthralgia. IL-6 also induces the

expression of MCP-1/CCL-2, which attracts monocytes/macrophages to the site of inflammation [10], consistent with data from Hoarau et al, who described, in chronic arthritic syndrome, the expression of both IL-6 and MCP-1/CCL-2 together with CHIKV persistence in the joint and macrophage infiltration [3].

Our present data thus support the view that CHIKV infection of osteoblasts may initiate a vicious cycle in which IL-6 triggers its own expression and attracts monocytes/macrophages in an environment prone to osteoclast-like cell differentiation. This clearly supports the proposal by Chow et al that IL-6 is central to persistent arthralgia induced by CHIKV [1]. However, it would be of interest to know whether Chow and colleagues have measured RANKL and osteoprotegerin levels in their CHIKV patient cohorts, as these data will help further understand this pathogenic mechanism.

Notes

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References

1. Chow A, Her Z, Ong EK, et al. Persistent arthralgia induced by Chikungunya virus infection is associated with interleukin-6 and granulocyte macrophage colony-stimulating factor. *J Infect Dis* **2011**; 203:149–57.
2. Schaible HG, von Banchet GS, Boettger MK, et al. The role of proinflammatory cytokines in the generation and maintenance of joint pain. *Ann N Y Acad Sci* **2010**; 1193: 60–9.
3. Hoarau J, Bandjee M, Trotot P, et al. Persistent chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response. *J Immunol* **2010**; 184:5914–27.
4. Palmqvist P, Persson E, Conaway HH, Lerner UH. IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF-kappa B ligand, osteoprotegerin, and receptor activator of NF-kappa B in mouse calvariae. *J Immunol* **2002**; 169:3353–62.
5. Liu XH, Kirschenbaum A, Yao S, Levine AC. Cross-talk between the interleukin-6 and prostaglandin E(2) signaling systems results in enhancement of osteoclastogenesis through effects on the osteoprotegerin/receptor activator of nuclear factor- κ B (RANK) ligand/RANK system. *Endocrinology* **2005**; 146:1991–8.

6. Takahashi N, Maeda K, Ishihara A, Uehara S, Kobayashi Y. Regulatory mechanism of osteoclastogenesis by RANKL and Wnt signals. *Front Biosci* **2011**; 16:21–30.
7. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Spelsberg TC, Riggs BL. Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. *Endocrinology* **1999**; 140:4367–70.
8. Stephens SR, Hobbs C, Hutmacher DW, Bacic-Welsh D, Woodruff MA, Morrison NA. Myocyte enhancer factor 2c, an osteoblast transcription factor identified by dimethyl sulfoxide (DMSO)-enhanced mineralization. *J Biol Chem* **2011**; 286:30071–86.
9. Labadie K, Larcher T, Joubert C, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. *J Clin Invest* **2010**; 120:894–906.
10. Romano M, Sironi M, Toniatti C, et al. Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity* **1997**; 6:315–25.

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