Research shows bone loss causes joint pain from alphaviral diseases

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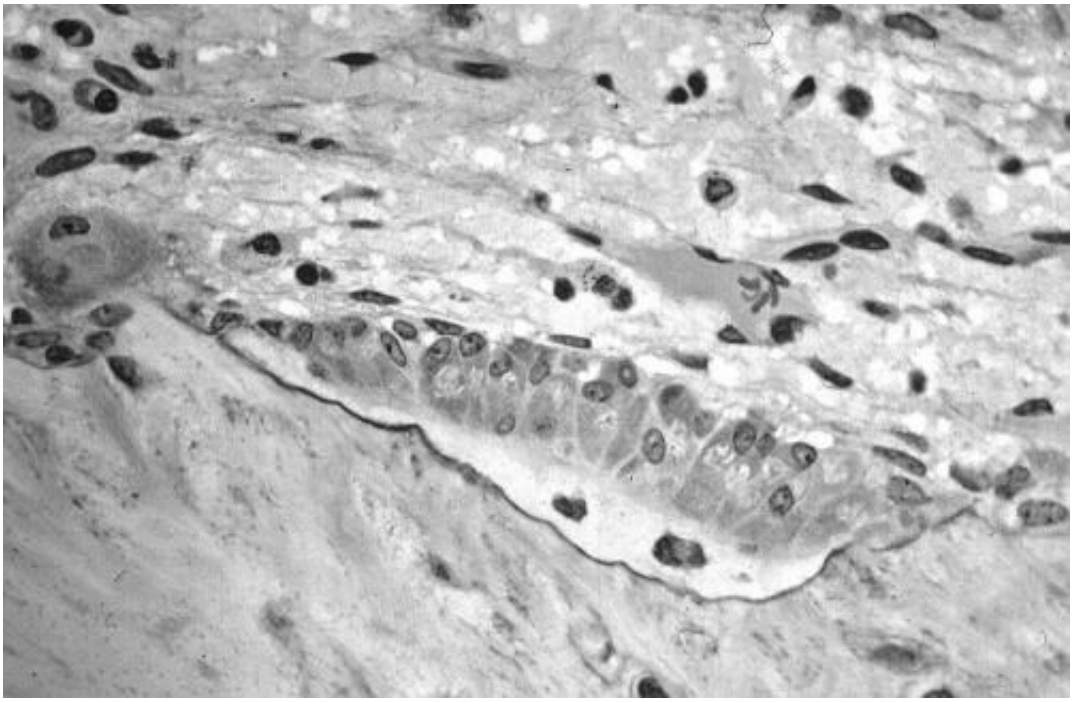

Osteoblasts actively synthesizing osteoid. Credit: Robert M. Hunt; Wikipedia.

(Medical Xpress)—Arthritogenic alphaviruses such as Ross River virus (RRV) cause disabling joint pain. To understand what causes this pain, Suresh Mahalingam of Griffith University in Australia and his team studied how RRV affects human and mouse osteoblasts. They found that RRV concentrates in osteoblasts, where it induces the production of inflammatory cytokines. This leads to increased bone loss, which causes pain. The research appears in the *Proceedings of the National Academy*

of Sciences.

RRV, sindbis virus (SINV) and chikungunya virus (CHIKV) belong to a group of alphaviruses that cause pain resembling the pain of rheumatoid arthritis. This pain can last for months after the viruses themselves are gone. Usually, doctors tell patients who have contracted arthritis-inducing alphaviruses to manage their pain by taking anti-inflammatory drugs like ibuprofen.

Recent research shows that patients with CHIKV develop bone lesions in their joints. Mahalingam and his colleagues wanted to see whether alphaviruses cause bone loss. They studied the effect of RRV on osteoblasts, cells involved in bone synthesis.

First, the researchers infected cultured human osteoblasts with RRV. This stimulated the production of the cytokines IL-6, CCL2 and IL-1β, which promote bone loss. The RRV-infected osteoblasts had higher RANKL levels and lower OPG levels than non-infected osteoblasts. An elevated RANKL/OPG ratio disturbs bone homeostasis, causing excessive loss of bone. When Mahalingam's team examined the synovial fluid of patients with RRV, they found these patients also had high RANKL/OPG ratios.

The researchers then studied RRV-infected mice. After killing the mice, they examined the leg and foot bones. They found that most of the virus was concentrated in osteoblasts, rather than in the marrow, reticulocytes or leukocytes. The virus remained in the osteoblasts for 21 days post infection, while it disappeared from the other parts of the bone after two days.

Microcomputed tomography of bones from RRV-infected mice showed clear evidence of bone loss in the vertebrae and tibial ephiphysis by the 15th day. When the team examined osteoblasts cultured from mice with

RRV, they found that, as with cultured human osteoblasts, RRV induced production of IL-6, CCL2 and IL-1β.

Mahalingam's team then tested whether an antibody that neutralizes IL-6 could prevent bone loss from RRV infection. They treated the hind limbs of RRV-infected mice with an IL-6 neutralizing antibody 10 days post infection, and found that the ratio of bone volume to tissue volume was preserved.

The research shows that IL-6 plays an important role in causing bone loss and the subsequent pain associated with arthritis-inducing alphaviruses like RRV. It suggests that patients would benefit by taking cytokine-inhibiting antibodies in the early stages of these diseases.

More information: Arthritogenic alphaviral infection perturbs osteoblast function and triggers pathologic bone loss, Weiqiang Chen, *PNAS*, DOI: 10.1073/pnas.1318859111

Abstract
Arthritogenic alphaviruses including Ross River virus (RRV), Sindbis virus, and chikungunya virus cause worldwide outbreaks of musculoskeletal disease. The ability of alphaviruses to induce bone pathologies remains poorly defined. Here we show that primary human osteoblasts (hOBs) can be productively infected by RRV. RRV-infected hOBs produced high levels of inflammatory cytokine including IL-6. The RANKL/OPG ratio was disrupted in the synovial fluid of RRV patients, and this was accompanied by an increase in serum Tartrate-resistant acid phosphatase 5b (TRAP5b) levels. Infection of bone cells with RRV was validated using an established RRV murine model. In wild-type mice, infectious virus was detected in the femur, tibia, patella, and foot, together with reduced bone volume in the tibial epiphysis and vertebrae detected by microcomputed tomographic (μCT) analysis. The RANKL/OPG ratio was also disrupted in mice infected with RRV; both

this effect and the bone loss were blocked by treatment with an IL-6 neutralizing antibody. Collectively, these findings provide previously unidentified evidence that alphavirus infection induces bone loss and that OBs are capable of producing proinflammatory mediators during alphavirus-induced arthralgia. The perturbed RANKL/OPG ratio in RRV-infected OBs may therefore contribute to bone loss in alphavirus infection.