A RANK insider resolving the enigma of the fever chart

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Mammals have evolved a complex system for controlling bone remodeling. Babies require calcium for healthy bones and they obtain it from their mother's milk. Nursing mothers release calcium from their bones. Surprisingly, however, the same system also plays a key part in the control of fever and of female body temperature. This finding is reported in a paper in this week's issue of *Nature* from Josef Penninger's group at the IMBA in Vienna, Austria.

The so-called RANK protein and the molecule that binds to it, the RANK ligand or RANKL, form a focus of the work of Josef Penninger, director of the Institute of Molecular Biotechnology (IMBA) in Vienna. In 1999 his group deleted the RANKL gene from mice and showed that the RANK/RANKL system was the "master regulator" governing bone loss (Kong et al. 1999 *Nature* 402, 304-309). The work provided the fist genetic proof for a completely new and rational treatment for osteoporosis, one of the most serious public health problems for older women. The results of phase III clinical trials for a human antibody to RANKL have recently been published (see Cummings et al. 2009, New Eng. J. Med. 361, 756-765) and, pending approval by the authorities, it is conceivable that this antibody will soon be made widely available for osteoporosis treatment.

Considering that such treatments might be of potential benefit to millions of patients, it is important to understand any possible side-effects and in this regard the old observation (see Kartsogiannis et al. 1999, *Bone* 25, 525-534) that RANKL is also expressed in the brain is

highly interesting. The function of RANK and RANKL in the brain was completely unknown and forms the basis of Penninger's latest work. To investigate it, Reiko Hanada - an endocrinologist and Postdoc in Penninger's group - injected RANKL into mice and rats, intending to look for effects on behaviour. As Penninger says, "in principle the injections could have resulted in changes to the animals' intelligence or memory or in subtle behavioural alterations that we could never have detected. But we were lucky. The results were dramatic and obvious - the animals stopped moving and developed really high temperatures."

That this was not mere coincidence was suggested by the observation that RANK and RANKL are not present in all areas of the brain. Rather, the proteins were found to be restricted to areas that other groups had previously implicated in the control of body temperature. And Penninger's group showed further that injections of RANKL triggered changes in areas of the brain known to be involved in the fever response, through which mammals increase their body temperature to fight infection. Unlike normal mice, mice that had been engineered to lack RANK in the brain did not respond to simulated infections by raising their body temperature although they appeared otherwise normal. Taken together, these results show that RANK and its ligand are involved in the regulation of the body's fever response to fight infections.

Because Penninger's group had previously shown that RANK and RANKL control the production of milk during pregnancy, it seemed possible that the system might also have an effect on the body temperature of females. Penninger freely concedes that "this was a hunch but one that seemed worth checking." And his speculation turned out to be correct: female - but not male - mice lacking RANK in the brain show a significant increase in body temperature compared with their littermates, at least during daylight hours. As a result, such female mice have much lower differences in body temperature between day and night. In a final experiment, this effect was revealed to be at least

partially the result of sex hormones released from the ovaries.

Because the experimental work was performed in mice and rats, it seemed extremely likely that the results would be relevant to other mammals, including man. Proof that this is the case arose from a chance lunchtime meeting at a conference, during which Penninger learned of a family whose children had defects in the RANK gene. As predicted by Penninger's work, these children showed much lower fever responses to infection. Even when they contracted pneumonia, their body temperatures scarcely rose.

Penninger's data link bone metabolism to the control of temperature during infection and, even less expectedly, to the gender-specific control of body temperature. Although any explanation for the difference between males and females is still speculation, Penninger notes that "the RANK/RANKL system is intricately involved with reproductive biology, transferring calcium from mothers' bones to their children. Perhaps the changes in female body temperature controlled by RANK and its ligand are also related in some way to reproduction or to human fertility." As an example, it is conceivable that the RANK/RANKL system may be responsible for the sudden bursts of high temperature associated with hormonal changes - and with osteoporosis - in older women.

The elegant experiments reported in the present paper were performed at the IMBA in close collaboration with groups at the Medical University of Vienna as well as in Berlin and in Japan - Penninger acknowledges in particular the contribution of Shuh Narumiya, "whose expertise on fever was crucial to our understanding". The work also demonstrates the importance of mouse genetics: the findings could not have arisen from work on isolated cell lines. Penninger himself retains a childlike sense of wonder that "one little mouse" - the RANKL deletion his group published a decade ago - "should have given rise both to a scientifically

based treatment for osteoporosis and to new and exciting speculations on human reproductive biology, with all their potential therapeutic implications."

Source: Research Institute of Molecular Pathology