OSTEOIMMUNOLOGY

NK cells: natural bone killers?

Natural killer (NK) cells contribute to the bone erosion characteristic of rheumatoid arthritis (RA) by inducing the differentiation of monocytes into osteoclasts, according to new research by Söderström *et al.* published in *Proceedings of the National Academy of Sciences*.

Although osteoclasts have been implicated as the cell type predominantly responsible for mediating periarticular bone loss in RA, the immunological events underlying their formation at these sites remain unclear. NK cells are known to be present in the inflamed synovium of patients with RA—representing up to 20% of all lymphocytes in the synovial fluid (SF)—from the early stages of the disease. In addition, a proportion of these SF NK cells are found side-by-side with monocytes, an arrangement that presumably enables cell–cell interactions. To answer the question of whether the interaction between NK cells and monocytes leads to the differentiation of monocytes into osteoclasts, the investigators experimented with cells isolated from the SF of patients with RA.

“We set up coculture protocols between highly enriched SF monocytes and SF NK cells using a culture medium known previously to facilitate NK cell survival and expansion,” explains lead author Kalle Söderström. “In this system, we tested whether a low number of NK cells and a high number of monocytes, close to the physiological ratio found in the inflamed joints of RA patients, would trigger monocyte differentiation towards osteoclasts.”

With an NK-cell:monocyte ratio of 1:10, *in vitro* cocultures were performed in the presence or absence of interleukin (IL)-15, a cytokine present in the RA synovium that is known to regulate the differentiation and activation of NK cells. When NK cells and autologous CD14⁺ monocytes were placed

in direct contact with each other in the presence of IL-15, the cultures produced large numbers of round, multinucleated cells that expressed the osteoclast marker tartrate-resistant acid phosphatase (TRAP). Staining confirmed that these cells also expressed markers associated with functional resorbing osteoclasts. Analysis of lacunar resorption on dentine slices confirmed that the cells were capable of eroding bone substrates.

A standard method of producing osteoclasts from peripheral blood (PB) monocytes *in vitro* involves two key cytokines involved in bone resorption, macrophage-colony stimulating factor (M-CSF) and receptor activator of NFκB ligand (RANKL). However, SF NK cells seem to be a more-efficient trigger for the formation of osteoclasts from PB monocytes: coculture with IL-15-activated SF NK cells produced more and larger TRAP⁺ osteoclasts than coculture with exogenous M-CSF and RANKL. “Our findings indicate that NK cells are very potent in driving the differentiation of monocytes into osteoclasts,” says Söderström. “The process is rapid and you need few NK cells to do the job.”

The authors demonstrated that SF NK cells express both M-CSF and RANKL, and, additionally, that prolonged coculture with IL-15 upregulated the expression of these factors. In further experiments, the neutralization of either M-CSF and RANKL suppressed SF NK cell-induced osteoclastogenesis.

SF NK cells also express CD56 and are phenotypically related to a minor subset of PB CD56^{bright} NK cells found in both patients with RA and healthy individuals. Upon stimulation with IL-15, PB CD56^{bright} NK cells excreted M-CSF at levels similar to those of SF NK cells, although in both these cell groups the excretion of soluble RANKL was induced only with the highest dose of IL-15 (100 ng/ml). Consistent with these

findings, the authors observed that PB CD56^{bright} NK cells from healthy individuals were more potent than CD56^{dim} NK cells (and PB T cells from healthy donors) in inducing the formation of osteoclasts from autologous PB monocytes.

To investigate the role of NK cells in osteoclastogenesis *in vivo*, the authors turned to a mouse model of RA, collagen-induced arthritis (CIA). They first confirmed the presence of RANKL⁺ NK cells in the joints of DBA/1 mice with CIA; notably, these cells were found next to sites of bone erosion. Next, specific depletion of NK cells *in vivo* by use of a polyclonal anti-asialo GM1 antibody (after immunization with type II collagen but before the onset of arthritis) was shown to dramatically reduce the signs of bone erosion.

Interestingly, inflammation, pannus formation and synovitis were also reduced in mice treated with the anti-asialo GM1 antibody, suggesting that NK cells have a pathogenic role not only in bone destruction but also in other manifestations of arthritis. Previous studies have shown that neutralization of RANKL in RA and in CIA reduces bone loss but with minimal effects on inflammation; the authors suggest that distinct NK cell subsets might be responsible for each of these processes, with RANKL⁺ NK cells being primarily involved in bone erosion. “We need to compare the efficacy of NK cells side-by-side with other SF cell subsets known to trigger osteoclastogenesis *in vitro*,” says Söderström. “Future research in mice may involve generating mice with RANKL^{-/-} NK cells.”

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