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COMMENTARY

Aminobisphosphonates, statins and the mevalonate pathway: a cross-road to fine-tune the activation of NK and Vγ9Vδ2 T cells

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Commentary on: Nussbaumer O, Gruenbacher G, Gander H, Thurnher M. DC-like cell-dependent activation of human natural killer cells by the bisphosphonate zoledronic acid is regulated by γδ T lymphocytes. Blood 2011; **118**(10):2743–2751.

Nussbaumer *et al.* have recently provided new mechanistic insights into the immune responses elicited by aminobisphosphonates (NBPs).[1] These drugs are commonly used to treat bone disease in solid cancer and multiple myeloma, and to prevent bone loss in osteoporosis.[2] Zoledronic acid (ZA), the most potent NBP currently available for clinical use, is a specific inhibitor of farnesyl pyrophosphate synthase (FPPS) in the mevalonate (Mev) pathway.[3] The Mev pathway is a highly conserved metabolic cascade that converts Mev into sterols, such as cholesterol, and nonsterol isoprenoids, such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate. Cholesterol is critical for the physicochemical properties of all eukaryotic cells, whereas isoprenoids are required for the isoprenylation and activation of small G-proteins, like Ras and Rho, that control cell proliferation, cytoskeleton remodelling, motility and angiogenesis. The metabolic consequences of ZA-induced FPPS inhibition are two-fold: (1) a decreased content of intracellular isoprenoids leading to dysfunctional G-proteins and alterations of their downstream signalling pathways;[4] and (2) an intracellular accumulation of isopentenyl pyrophosphate (IPP) leading to the formation of 1-adenosin-5′-yl ester 3-(3-methylbut-3-enyl) ester triphosphoric acid, a pro-apoptotic ATP analog generated by combination of IPP with AMP by aminoacyl-tRNA-synthetase.[5] Interestingly, IPP and similar compounds are also generated in the Mev and non-Mev pathways of bacteria and other pathogens during isoprenoid biosynthesis. These metabolites, collectively termed phosphoantigens, represent the natural ligands of Vγ9Vδ2 T cells, a unique subset of unconventional T cells deeply involved in innate immune responses against microbes, stressed cells and tumor cells.[6–9] A growing body of evidence indicates that Vγ9Vδ2 T cells are endowed with a functional plasticity that confers them a wide repertoire of immune functions ranging from effector functions to the positive or negative regulation of other immune subsets, including the capacity to act as unconventional antigen-presenting cells (APC).[10]

Several groups have shown that ZA-induced IPP accumulation in tumor cells or APC can be exploited to intentionally activate Vγ9Vδ2 T cells and trigger immune responses against tumor cells or infectious agents.[11–13] Different *ex vivo* and *in vivo* strategies are under clinical investigation to develop Vγ9Vδ2 T-cell-based adoptive immunotherapy interventions.[14] Interestingly enough, natural killer (NK) cells, another subset of innate effector cells, can also be activated by manipulation of the Mev pathway using statins.[15] The latter are specific inhibitors of hydroxymethylglutaryl coenzyme A reductase, the rate-limiting enzyme in the Mev pathway acting upstream to FPPS. The metabolic fallouts of statin- and ZA-induced Mev pathway inhibition are partially different: both agents generate intracellular isoprenoid deprivation, but the latter only induces IPP increase and activates Vγ9Vδ2 T cells. Altogether, these data point to the Mev pathway as a metabolic cross-road regulating the activation of both Vγ9Vδ2 T cells and NK cells.

Nussbaumer *et al.* provide an additional piece of evidence to dissect the cellular network linking NK cells and Vγ9Vδ2 T cells *via* the Mev pathway. The scenario is orchestrated by dendritic cell (DC)-like cells, a unique subset of CD14+ CD56+ APC displaying phenotypic and functional features similar to monocyte-derived DC generated *in vitro* with granulocyte macrophage colony-stimulating factor and type 1 interferon.[16] These cells have previously been shown to activate Vγ9Vδ2 T cells or NK cells depending on whether the Mev pathway is manipulated with statins or NBPs: ZA and interleukin (IL)-2 trigger Vγ9Vδ2 T-cell activation,[17] whereas statins and IL-2 trigger NK-cell activation.[15]

Nussbaumer *et al.* have recently provided new data against this dichotomy by showing that ZA and IL-2 can induce the concurrent activation of both NK cells and Vγ9Vδ2 T cells via different mechanisms.[1] NK cells are stimulated by IL-18 and IL-1β, which are produced by DC-like cells as a consequence of ZA-induced caspase-1 activation, whereas Vγ9Vδ2 T cells

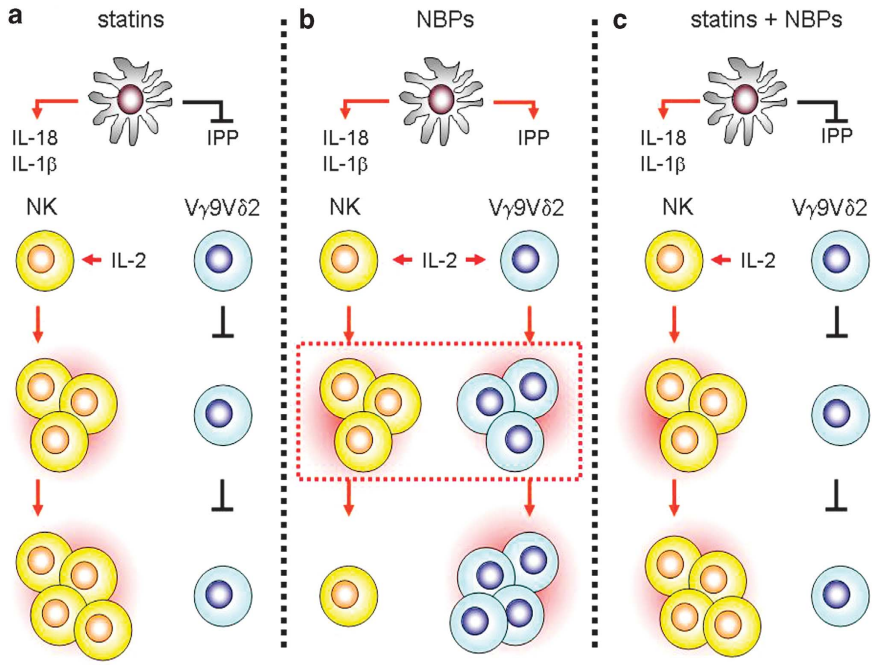

Figure 1 Effects of Mev pathway inhibition with statins (**a**), NBPs (**b**) or statins with NBPs (**c**) on NK cells and Vγ9Vδ2T cells. Scenario **b** likely represents the most tuneful immune response that can be evoked by Mev pathway manipulation. In this setting, there is a transient and concurrent activation of both NK cells and Vγ9Vδ2T cells that benefits both cell subsets (rectangle). Then, Vγ9Vδ2T cells take over and switch off NK-activation by deleting DC-like cells and interrupting IL-18 and IL-1β production. See also text for details.

are activated by ZA-induced IPP accumulation in DC-like cells. Even if these activations are initiated simultaneously to provide early and effective immune responses, Nussbaumer *et al.* suggest that they become mutually exclusive later on. Vγ9Vδ2 T cells are inclined to progressively take over NK cells as they switch off NK-cell activation by eliminating the major source of IL-18 and IL-1β, that is, DC-like cells. The authors suggest that Vγ9Vδ2 T cells are responsible for this elimination as NK-cell activation is kept on provided that Vγ9Vδ2 T cells are removed from the culture. We have also shown that the concurrent stimulation of Vγ9Vδ2 T cells with DC or tumor cells copulsed with ZA and statins promotes NK-cell proliferation in the absence of any activation of Vγ9Vδ2 T cells. Thus, we can envisage three different scenarios in which NK cells and Vγ9Vδ2 T cells behave in different ways depending on the drug used to manipulate the Mev pathway of DC-like cells (**Figure 1**). Scenario A represents the situation in which the Mev pathway is inhibited by statins only. Intracellular isoprenoid deprivation leads to caspase-1 activation and release of IL-18 and IL-1β, and NK-cell activation. Under these circumstances, there is no concurrent activation of Vγ9Vδ2 T cells because hydroxymethylglutaryl coenzyme A reductase is upstream to FPPS and there is no IPP accumulation. Scenario B is generated when DC-like cells are incubated with NBPs such as ZA. In this case, there is also isoprenoid deprivation leading to caspase-1 activation, IL-18 and IL-1β production, and NK-cell activation. However, the Mev pathway is inhibited downstream to FPPS, and therefore there is intracellular accumulation of IPP leading to the concurrent Vγ9Vδ2 T-cell activation. Several data indicate that Vγ9Vδ2 T-cell activation is not detrimental to NK cells whose immune performances are improved upon interaction with activated Vγ9Vδ2 T cells.[18] The rectangle shown in scenario B highlights this transient, but productive cellular cross-talk, which is finalized to mount a rapid and conclusive immune response. After boosting NK-cell activation, however, Vγ9Vδ2 T cells take command of the situation and switch off IL-18 and IL-1β production by eliminating DC-like cells. It is not unusual in the immune system that APC are eliminated by the very same cells that they triggered their activation earlier on. One possible reason is to avoid long-term and uncontrolled immune activation, which can lead to autoimmunity and/or inflammation.[19] We can speculate that Vγ9Vδ2 T cells are very well suited to this task because they can specifically target DC-like cells via TCR-dependent IPP recognition, whereas NK cells are devoid of this capacity. NK cells and Vγ9Vδ2 T cells have also to compete for cytokines such as IL-2, an essential growth factor for both NK and Vγ9Vδ2 T cells, and compete for the same ligands and counter-receptors on target cells as they are both equipped with very similar arrays of killer activatory and inhibitory receptors. Thus, it is possible that, after a rapid delivery of a potent and collaborative reaction, resources are rationalized to grant a prolonged and effective immune response. At this point, we do not know why Vγ9Vδ2 T cells are preferred to NK cells.

Scenario C mimics scenario A and also mimics what Nussembauer *et al.* have observed when Vγ9Vδ2 T cells are removed from the cultures. In the presence of NBPs and statins, DC-like cells survive longer, the production of IL-18 and IL-1β is maintained, and NK cells are activated and produce interferon-γ for a more prolonged period of time because Vγ9Vδ2 T cells are not activated and do not eliminate DC-like cells. An inappropriate and prolonged NK-cell activation can be responsible for local tissue damage, especially in those tissues like mineralized bone where NBPs can reach very high concentrations. The authors hypothesize that local inflammation sustained by NK cells can contribute to the osteonecrosis of the jaw, an intimidating and serious side effect observed in approximately 1–5% of

multiple myeloma and solid cancer patients receiving repeated doses of NBPs.[20] So far, it is unknown whether NK-cell activation of scenario C is different from that represented in scenario A in terms of duration, effectiveness and side effects. Future studies should evaluate whether NK-cell activation for clinical applications should be pursued using statins alone (scenario A) or statins and NBPs (scenario C). NBPs are pleiotropic drugs with biological activity ranging from the capacity to exert direct cytotoxic activity on tumor cells to the functional modulation of the tumor microenvironment. These properties are unlikely to have a major role if NBPs are used to activate Vγ9Vδ2 T cells *ex vivo*, but they can become very relevant when these agents are given *in vivo*, either alone or in association with IL-2 and statins.

In conclusion, Nussbaumer *et al.* have improved our knowledge about the cellular interactions regulating NK-cell and Vγ9Vδ2 T-cell activation and provided further evidence that the Mev pathway can be considered a metabolic cross-road to fine-tune the activation of these cell subsets. The important insights are: (1) NBPs can exert immune-modulatory activity in the absence of Vγ9Vδ2 T-cell activation; (2) both NBPs and statins promote caspase-1 activation as a consequence of intracellular isoprenoid deprivation; (3) caspase-1 activation drives the secretion of pro-inflammatory cytokines such as IL-18 and IL-1β, and promotes NK-cell activation; (4) the duration of NK-cell activation can become inappropriate in the absence of concurrent Vγ9Vδ2 T-cell activation. Based on these data, we have to reassess the clinical use of statins as anti-inflammatory agents in the light of their capacity to induce the secretion of IL-18 and IL-1β and long-term NK-cell activation in the absence of concurrent Vγ9Vδ2 T-cell activation. On the contrary, NBPs seem to have the capacity to generate a more tuneful immune response involving both NK cells and Vγ9Vδ2 T cells. It remains to be established which are the physiological and pathological settings in real life that recapitulate the immune responses evoked *in vitro* by Mev pathway inhibition with statins and NBPs.

Conflict of Interest

The author declares no conflict of interest.

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