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Induction of immune responses by β-tricalcium phosphate particles

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Calcium phosphates (CaP) are the main mineral constituents of bones and teeth. Various CaP with different structure and physicochemical properties are used in the health, medical and industrial fields. CaP ceramics including hydroxyapatite and beta-tricalcium phosphate (β-TCP) have been used as bone substitutes or coating materials of the implants in orthopedic and dental applications. CaP particles are also known to cause inflammatory responses, which are thought to be an unfavorable characteristic of prosthetic coating materials. On the other hand, this induction of immune responses by CaP may contribute to the treatments of infectious diseases and cancer. This study aimed to clarify the effect and mechanism of β-TCP for activation of immune system, and to explore the possibility of β-TCP as novel immunomodulator.

1. The *in vivo* effect of β-TCP on immune system.

Granulometry analysis of β-TCP revealed that particle size was 5.373 ± 0.342 μm (mean ± standard deviation) in diameter. *In vivo* biological effect of β-TCP was investigated by subcutaneously injecting particles into C57BL/6 mice. β-TCP induced an inflammatory reaction and promoted migration of a significant number of various immune cells including neutrophils, histiocytes (macrophages) and lymphocytes to the area surrounding the injection site. As a second *in vivo* test, β-TCP was used in combination with tumor antigen (ovalbumin protein) in E.G7-OVA syngeneic murine tumor model. When compared to a non-treated control group, a significant delay in tumor growth was observed in a group administered tumor antigen and β-TCP simultaneously. These findings showed that β-TCP induced activation of immune system *in vivo*. In addition, it was demonstrated that β-TCP enhanced the prophylactic anti-tumor effect caused by vaccination of tumor antigen, and showed that activation of innate immunity by β-TCP led to effective induction of adaptive immune responses.

2. The effect of β-TCP on activation of dendritic cells (DC) and macrophages (MΦ).

The effect of β-TCP on DC and MΦ *in vitro* were investigated to clarify possible mechanisms for the activation of immune system by β-TCP. Mouse bone marrow-derived DC and MΦ were incubated with β-TCP particles for 24 hours, and morphological change, surface marker expression, cytokine production were analyzed. Both DC and MΦ migrated to β-TCP particles, and most of the cells, especially MΦ, engulfed the particles. β-TCP increased the percentage of mature DC and MΦ,

and upregulated the expression of antigen-presenting MHC molecules, CD86, CD80 and CD40 in DC, and CD86 in MΦ, crucial costimulators in T cell activation. In DC, β-TCP increased the expression of CCR7, crucial for entry into the lymph nodes. The production of proinflammatory cytokine TNF-α was observed only in MΦ. In both DC and MΦ, β-TCP induced secretion of the chemokines CCL2, CCL3 and CXCL2, which mediate recruitment of immune cells from blood to peripheral tissue. Furthermore, endocytosis activity was significantly decreased in DC treated with β-TCP. These findings showed that β-TCP stimulated maturation and activation of antigen-presenting cells (APC) such as DC and MΦ, and induced secretion of cytokines and chemokines.

3. The effect of β-TCP on the NLRP3 inflammasome activation in DC and MΦ.

It was investigated whether β-TCP cause proinflammatory cytokine IL-1β secretion through activation of the NLRP3 inflammasome *in vitro*. LPS-primed mouse bone marrow-derived DC and MΦ, and PMA-differentiated human macrophage THP-1 cells were used. These cells incubated with β-TCP particles in the presence or absence of inhibitors of inflammasome activation, and then IL-1β secretion were determined. β-TCP induced IL-1β secretion in caspase-1-dependent manner. β-TCP-induced IL-1β secretion was significantly suppressed by inhibition of potassium efflux, phagocytosis and generation of reactive oxygen species (ROS). Furthermore, β-TCP-induced IL-1β secretion was significantly decreased in THP1-defASC and THP1-defNLRP3 cells, sublines derived from THP-1 cells deficient for ASC and NLRP3, respectively. These findings showed that β-TCP induced IL-1β secretion from DC and MΦ *via* the NLRP3 inflammasome activation.

In conclusion, this study indicated that β-TCP promoted maturation/activation of APC such as DC and MΦ, and led to activation of the NLRP3 inflammasome. Furthermore, innate immune reaction activated by β-TCP effectively enhanced antigen-specific immune responses. Thus, β-TCP is supposed to be one of the best candidates as novel immunomodulator used in health and medical fields.