

COMMENTARIES

Can Active Immunization Against Macrophage Migration Inhibitory Factor by DNA Vaccination Prevent Postmenopausal Osteoporosis?

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Commentary on: Onodera S, Oshima S, Nishihira J, Yasuda K, Tohyama H, Irie K, Koyama Y. Active immunization against macrophage migration inhibitory factor using a novel DNA vaccine prevents ovariectomy-induced bone loss in mice. *Vaccine*. 2008 Feb 6;26(6):829-36.

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine and glucocorticoid-induced immunomodulator released in response to a variety of inflammatory stimuli. MIF is an upstream regulator of inflammatory cytokines such as TNF- α and IL-1, and controls the magnitude of the immune and inflammatory response (1). Because estrogen suppresses the production of MIF via inhibition of the transcription of the MIF gene (2;3), Onodera *et al.* hypothesized that MIF plays an important role in the cytokine cascade in response to estrogen deficiency to cause bone loss. To address this issue, they previously demonstrated that mice deficient in the MIF gene were protected from ovariectomy-induced trabecular bone loss (4). In a report published in the February 6th edition of *Vaccine* (5), the authors now take a novel approach against estrogen deficiency-induced bone loss by actively immunizing against MIF by DNA vaccination.

Molecular targeted therapies have been developed for the treatment of various disorders. For the treatment of postmenopausal osteoporosis, passive immunization against molecules essential for osteoclastic bone resorption such as RANK ligand, and for osteoblastic bone formation such as sclerostin, are currently under clinical development. However, these passive immunization therapies are costly and may induce antibody-antibody responses, which can limit the long-term

efficacy of such treatment or can cause serious immunogenic responses. In the report by Onodera *et al.* (5), the authors introduced a DNA vaccine that elicits an autoantibody against MIF, a vaccine that previously exhibited a significant prophylactic effect against arthritis in murine models of arthritis (6). Using this DNA vaccination strategy, the researchers investigated whether active immunization against MIF could suppress ovariectomy-induced bone loss. Thus, the authors address two discrete issues in this paper: one is the role of MIF in the development of osteoporosis induced by estrogen deficiency, and the other is the technical development of DNA vaccination to neutralize MIF action.

The authors substituted the cDNA region that encodes the second loop region of murine MIF with the cDNA encoding a promiscuous T_H epitope from tetanus toxin (TTX) (7). The MIF/TTX plasmid DNA was injected into tibial muscles in between a pair of electrode needles, and gene transfer was performed by electroporation. Immunization with the immunologically active T_H-modified MIF-DNA vaccine bypassed the immunological tolerance of mice to the MIF self-protein. Because high titers of autoantibodies that cross-reacted with native MIF were detected four weeks after gene transfer, the authors ovariectomized these mice four weeks after DNA vaccination. Four weeks thereafter, the animals' distal femoral bones were analyzed by microCT and bone histomorphometry. Analysis by microCT

demonstrated that ovariectomy caused a reduction in BV/TV in control vaccine (CV)-injected mice, but not in MIF/TTX-injected mice. Bone histomorphometric analysis revealed that ovariectomy elevated bone formation parameters, OV/BV and OS/BS, as well as bone resorption parameters, N.Oc/B.Pm and Oc.S/BS, in CV-treated mice, whereas MIF/TTX-administered mice were resistant to these effects of ovariectomy.

There are some limitations in the study. First, bone histomorphometry failed to demonstrate significant changes in BV/TV by ovariectomy not only in MIF/TTX-treated mice, but also in CV-injected mice. In addition, it was not shown whether active immunization against MIF in fact suppressed bone turnover markers, which should be elevated by estrogen deficiency. Furthermore, although the authors' conclusion was based upon the assumption that DNA vaccination against MIF suppressed the increase in bone-resorbing cytokines after estrogen deficiency, the expression levels of cytokines known to be elevated in estrogen deficiency and to play an important role in the development of postmenopausal osteoporosis, such as IL-6, IL-1 and TNF- α , were not measured in these immunized mice. Nevertheless, the novel approach against estrogen deficiency-induced bone loss using a DNA vaccination strategy taken by Onodera *et al.* warrants further investigation as a potential new treatment modality for postmenopausal osteoporosis.

In order for active immunization using DNA vaccination against MIF to be considered for clinical use, several questions need to be answered. First, DNA vaccination has not been tested in humans, and it is essential to examine its safety, as well as to develop non-invasive measures for gene transfer in humans. Second, the influence of the suppression of various cytokine levels by MIF on immunologic protection against infectious or neoplastic diseases needs to be clarified. The time course of autoantibody titers and the necessity of re-vaccination after active immunization also need to be studied in order to achieve sustained suppression of MIF actions. Although

clarification of many issues is required before a DNA vaccination strategy can be considered for clinical use, the report by Onodera *et al.* is an important step towards the development of a new therapeutic approach for the treatment of osteoporosis.

Conflict of Interest: None reported.

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