

# The systemic skeletal effects of immunization

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## INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by synovial thickening, infiltration of inflammatory cells and destruction of the underlying bone.

Murine models of arthritis investigate:

1. pathogenic mechanism
2. potential therapeutic targets

Similar to human disease, animal models could be accompanied with systemic osteopenia which could affect quantification of the local bone loss induced by arthritis.

In the present study, we used the model of antigen induced arthritis (AIA), to assess the extent of osteopenia caused by immunization.

## OBJECTIVES & AIMS

- the objective of the study is to assess the effect of immunization protocol on the vertebral bone volume

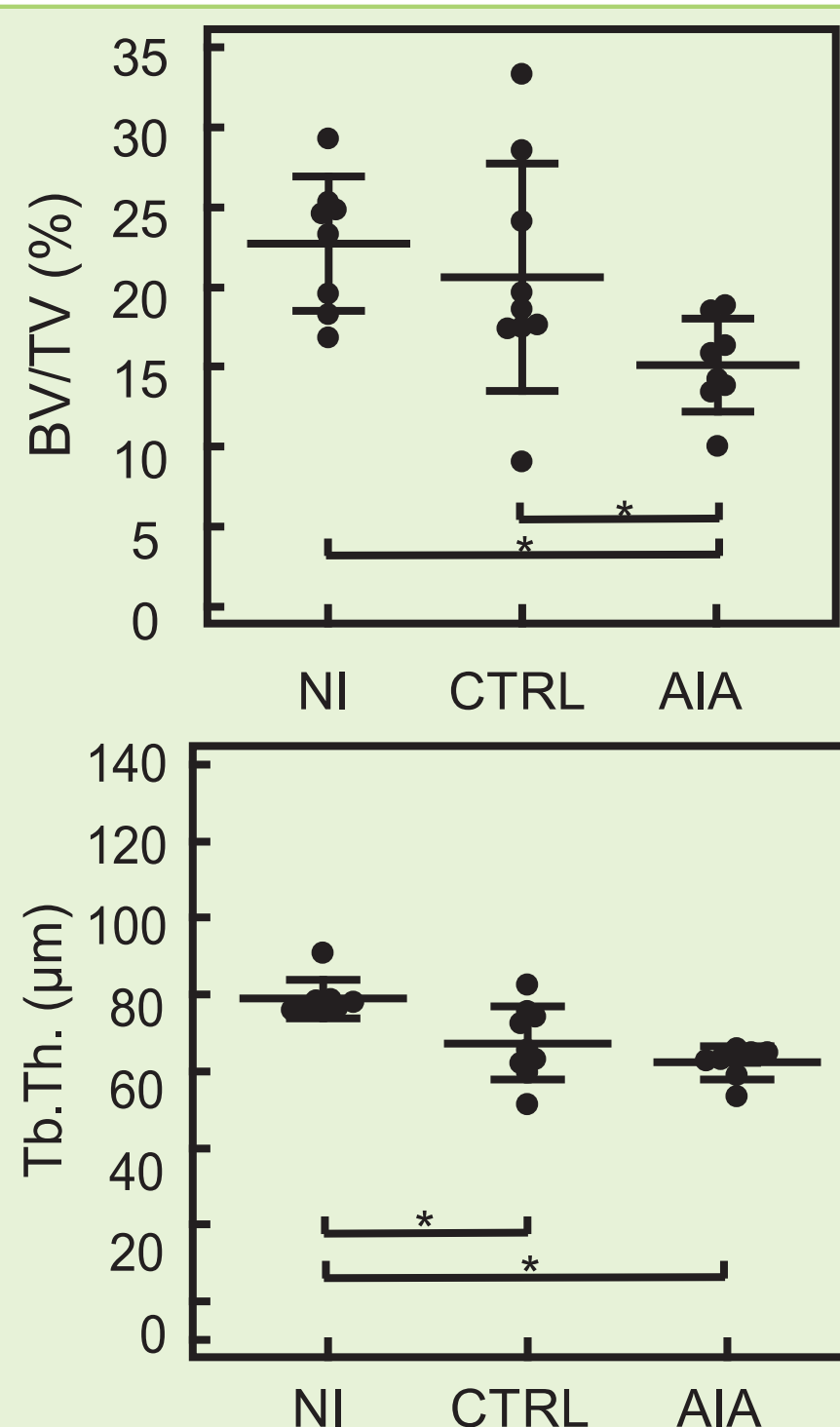
Specific aims of this study are:

1. to assess trabecular bone volume in second lumbar vertebrae by  $\mu$ CT
2. to estimate the effect of different concentrations of *M.tuberculosis* in immunization protocol on vertebral trabecular bone volume

## RESULTS

- trabecular bone volume (BV/TV) of the second lumbar vertebrae was significantly lower in immunized groups (ctrl and AIA) in comparison to non-immunized group ( $p < 0.05$ , Kruskal-Wallis test)

- there was no difference in BV/TV between AIA and ctrl mice, as well as between groups immunized with different concentrations of *M. Tuberculosis*

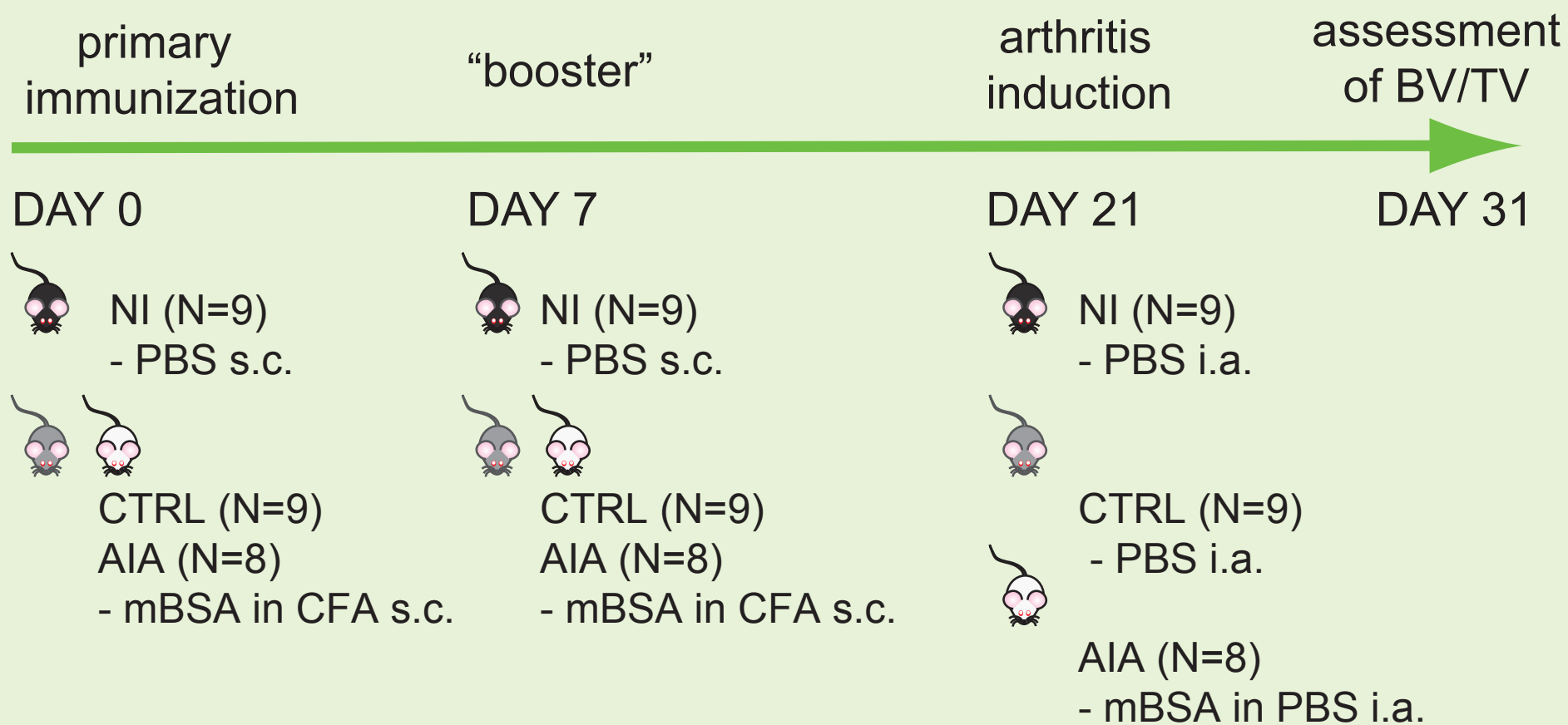


**Figure 3.** Micro-CT analysis of trabecular bone volume (BV/TV) and trabecular thickness (Tb.Th.) in second lumbar vertebrae (L2) from non-immunized mice (NI), immunized mice injected i.a. with PBS (CTRL), and mice with arthritis (AIA). Bone volume was significantly lower in AIA group in comparison with other groups. Trabecular thickness was significantly lower in both immunized groups in comparison to NI group.

\*statistically significant difference from NI mice,  $p < 0.05$ , Kruskal-Wallis test

## METHODS

- twelve-weeks old C57B6 female mice



- mice were immunized with methylated bovine serum albumin (mBSA) in complete Freund's adjuvant (CFA) injected subcutaneously (s.c.)

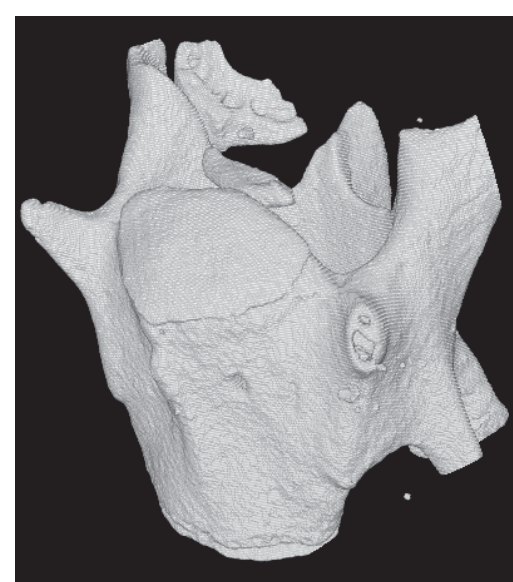
- on day 21, only AIA groups mice were intraarticular (i.a.) injected with mBSA in PBS in both knees, while control (CTRL) groups were injected only with PBS

- AIA and CTRL were subdivided in two groups:

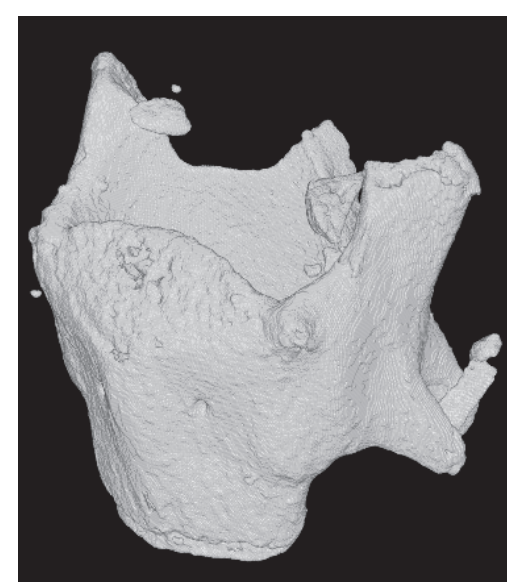
- A) group immunized with emulsion containing 2.0 mg/mL *M.tuberculosis*
- B) group immunized with emulsion containing 0.5 mg/mL *M.tuberculosis*

- NI group was injected with PBS both s.c. and i.a.

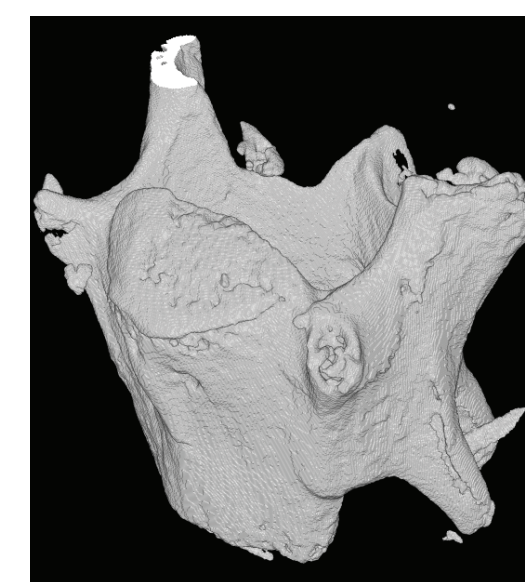
- trabecular bone volume (BV/TV) of the second lumbar vertebrae was assessed by  $\mu$ CT



NI



CTRL

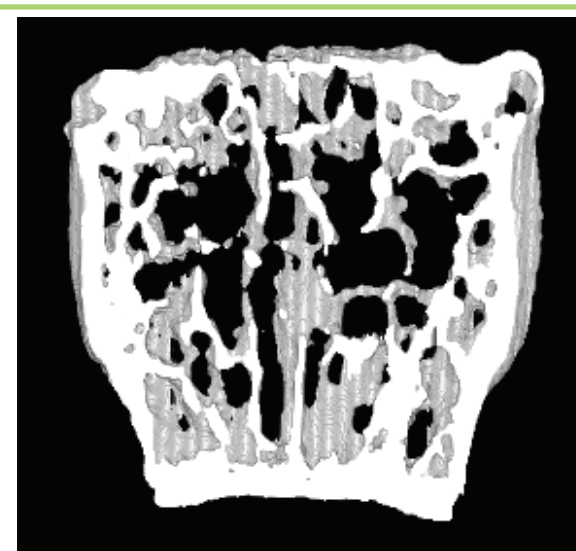


AIA

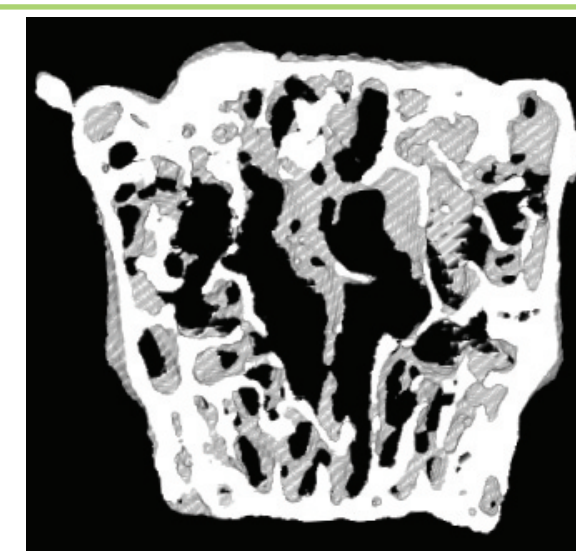
**Figure 1.** Representative 3D models of the second lumbar vertebrae (L2), from micro-CT reconstruction images from non-immunized mice (NI), immunized mice injected i.a. with PBS (CTRL), and mice with arthritis (AIA). Multiple bone erosions are visible on vertebrae from CTRL and AIA groups, while there are no similar changes in NI group.



NI



CTRL



AIA

**Figure 2.** Representative 3D sections of the second lumbar (L2) vertebral bodies, from micro-CT reconstruction images, from non-immunized mice (NI), immunized mice injected i.a. with PBS (CTRL), and mice with arthritis (AIA). Thinner trabeculae are visible on AIA and CTRL group in comparison with NI group.

## CONCLUSION

These findings suggest that even low-adjuvant immunization protocol induces systemic osteopenia which should be taken into account when assessing local bone loss in arthritis. Our preliminary data point that epiphyseal areas are less affected by immunization and might be most suitable to assess effects of local inflammation on bone.