Lysosomal Associated Membrane Protein-2 (LAMP-2) is Involved in Osteoblastic RANKL Signaling

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INTRODUCTION

Multinucleated osteoclasts are specialized cells with the capacity to resorb bone. To perform this activity, the osteoclast has a unique membrane area, the ruffled border, where protons are released to lower the pH. The membrane of the ruffled border is formed by fusion of lysosomal vacuoles and thus resembles the membrane of a lysosome. LAMP-1 and 2 constitute 50% of the total amount of proteins of the latter membrane. Since it is known that the expression of LAMP-2 is very high in the ruffled border membrane, we investigated whether this protein plays a role in the formation of osteoclasts and/or a role in resorption.

MATERIALS AND METHODS

Bone marrow cells and osteoblast-like cells were isolated from 6 week old wild type and LAMP-2 deficient male mice. The bone marrow cells were cultured with M-CSF and RANKL for 6 days or co-cultured with osteoblast-like cells for 21 days. Gene expression of RANKL was measured by qPCR. The presence of RANKL at the cell-membrane was investigated by FACS analysis.

RESULTS

In wild type osteoblasts LAMP-2 is highly expressed throughout the cell

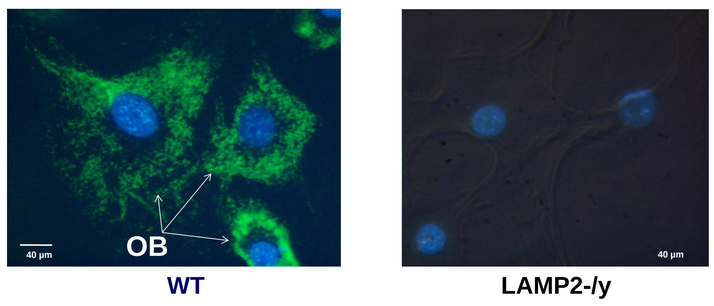


Fig. 1 LAMP-2 localization in wild type and LAMP-$2^{-/y}$ osteoblasts (OB). The green LAMP-2 label is present throughout the wild type cell and completely absent in the LAMP-$2^{-/y}$ cells. Nuclei were stained with DAPI (blue).

Osteoblasts of LAMP-2 deficient mice do not induce osteoclast formation

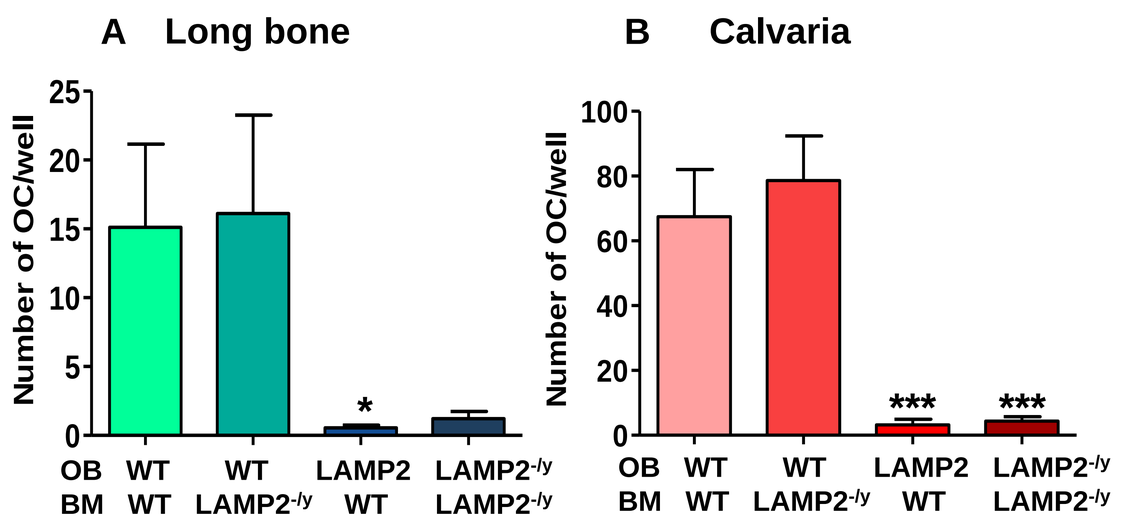
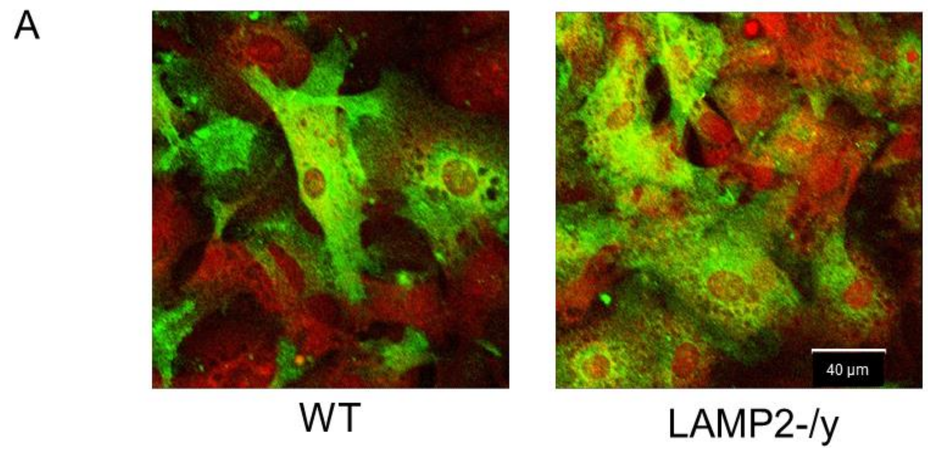


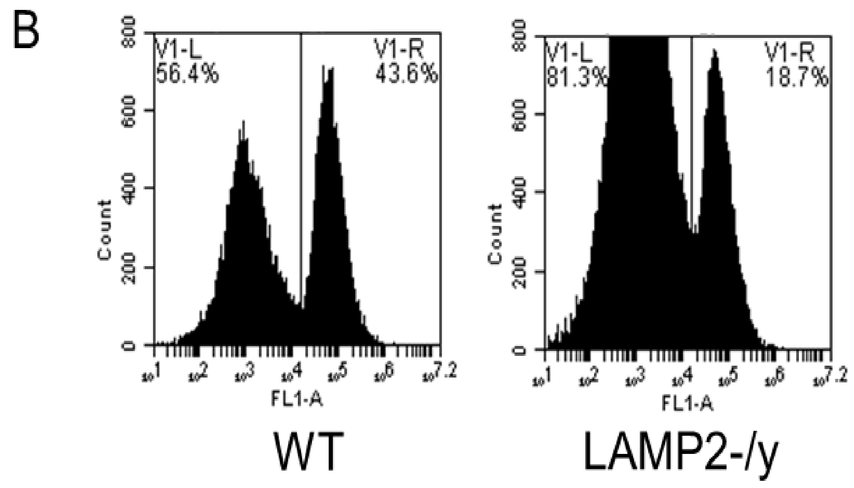
Fig. 2 Osteoblasts of LAMP-2 deficient mice do not induce osteoclast formation. Osteoblasts (OB) and bone marrow cells (BM) from long bone (A) and calvaria (B) of wild type (WT) and LAMP-$2^{-/y}$ mice were co-cultured for 10 days. Wild type osteoblasts induced the formation of multinucleated osteoclasts. This occurred with bone marrow cells of both genotypes (WT or LAMP-$2^{-/y}$). Hardly any osteoclasts were generated in the co-cultures with LAMP-$2^{-/y}$ osteoblasts (n= 9, *p< 0.05, ***p< 0.001.

RESULTS

Total RANKL labeling is comparable in LAMP-$2^{-/y}$ and in WT osteoblasts



Membrane bound RANKL is lower in LAMP-$2^{-/y}$ than in WT osteoblasts



OB	% unlabeled cells	% labeled cells	Mean fluoresence
WT	49.6 ± 6	51.6 ± 7	20.220
LAMP-2 -/y	80.8 ± 0.8	19.2 ± 0.8	3.193

Fig. 3 Immunolocalization of RANKL in wild-type and LAMP-$2^{-/y}$ osteoblasts. A. WT and LAMP-$2^{-/y}$ were labeled for RANKL and visualized with alexa-488 (green), the nuclei were stained with propidium iodide (red). About 50% of the osteoblasts from both, WT and LAMP-$2^{-/y}$ mice are intensely labeled for RANKL. B. FACS analysis showed that not all cells express RANKL on their membrane. 52% of WT osteoblasts were positive for membrane localized RANKL, whereas only 19% of the LAMP-$2^{-/y}$ osteoblasts expressed RANKL on their membrane. WT osteoblasts contained about a 6-fold higher level of membrane-bound RANKL compared to the LAMP-$2^{-/y}$ cells.

CONCLUSIONS

An intriguing finding of the present study was the involvement of LAMP-2 in osteoblast-mediated osteoclast formation. The osteoblasts from the deficient mice lacked the capacity to induce osteoclast formation. We found that deficient osteoblasts expressed 6-fold less RANKL on their plasma membrane, thus making it plausible that this caused the almost completely inhibited osteoclastogenesis.

No conflict of interest

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