

IL-1 β Differently Affects Osteoclastogenesis of Distinct Subsets of Osteoclast Precursors

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INTRODUCTION

Osteoclasts known as bone resorbing cells, are the culprits in many inflammation related bone diseases such as rheumatoid arthritis and periodontitis. Macrophage precursors follow successive stages of maturation from early blasts (CD31^{hi} Ly-6C⁻) to myeloid blasts (CD31⁺ Ly-6C⁺) into monocytes (CD31⁻ Ly-6C^{hi}). Each of these precursors has the potential to differentiate into multinucleated osteoclasts. It has been reported that these three subsets respond differently under physiological conditions. However their responses under inflammatory conditions is not known. One of the best known inflammatory cytokines is IL-1 β , which is shown to be the strongest inducer for bone resorption.

This study aimed to test the effect of IL-1 β on osteoclastogenesis of different mouse myeloid subsets, early blasts, myeloid blasts and monocytes. We analyzed the number of TRACP⁺ multinucleated cells, the level of bone resorption and gene expression.

METHODS

Bone marrow cells were isolated from 6-weeks-old male C57BL/6 mice. Cells were labeled with CD31 and Ly-6C for cell sorting. Three populations, early blasts, myeloid blasts, and monocytes, were achieved by flow cytometry, according to the expression level of CD31 and Ly-6C (Figure 1).

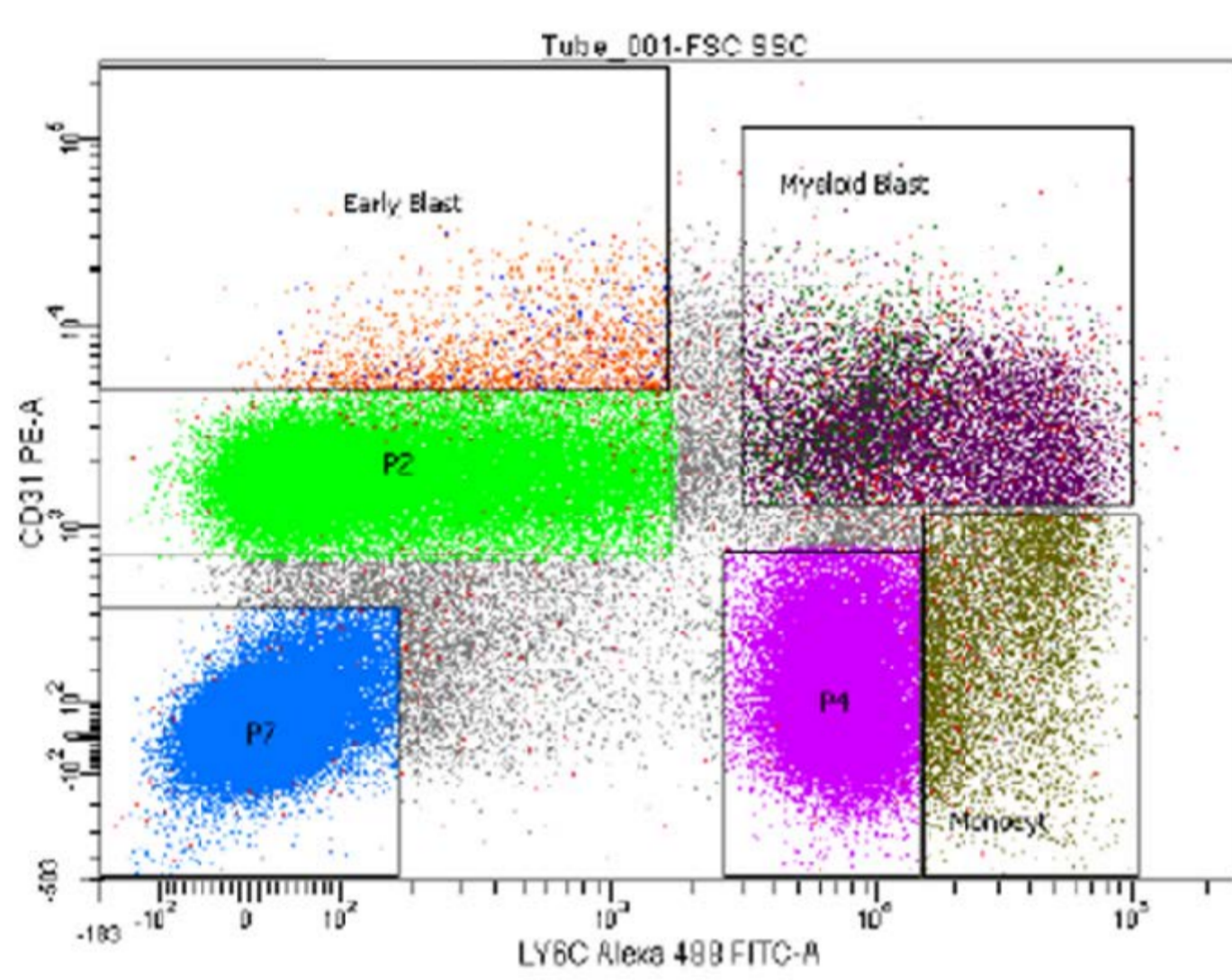


Fig 1. Flow cytometry gating for the three subsets.

Cells were cultured on plastic or on bone, in the presence of M-CSF (30ng/ml) and RANKL (20ng/ml) for 4-6 days, without or with IL-1 β (0.1, 1, 10 ng/ml). Cells were then fixed and stained for TRACP, and nuclei were counterstained by DAPI. Osteoclasts were identified as multinucleated TRACP⁺ cells using fluorescent microscope. The TRACP⁺ cells were counted and categorized in 4 different groups with 3-5, 6-10, 11-20 and >20 nuclei. Coomassie brilliant blue was used to visualize bone resorption. Pit percentage was analyzed by ImagePro. qPCR was performed to investigate gene expression with or without IL-1 β , both on plastic and on bone.

RESULTS

1. Osteoclasts generated by the three subsets showed differences in size and number.

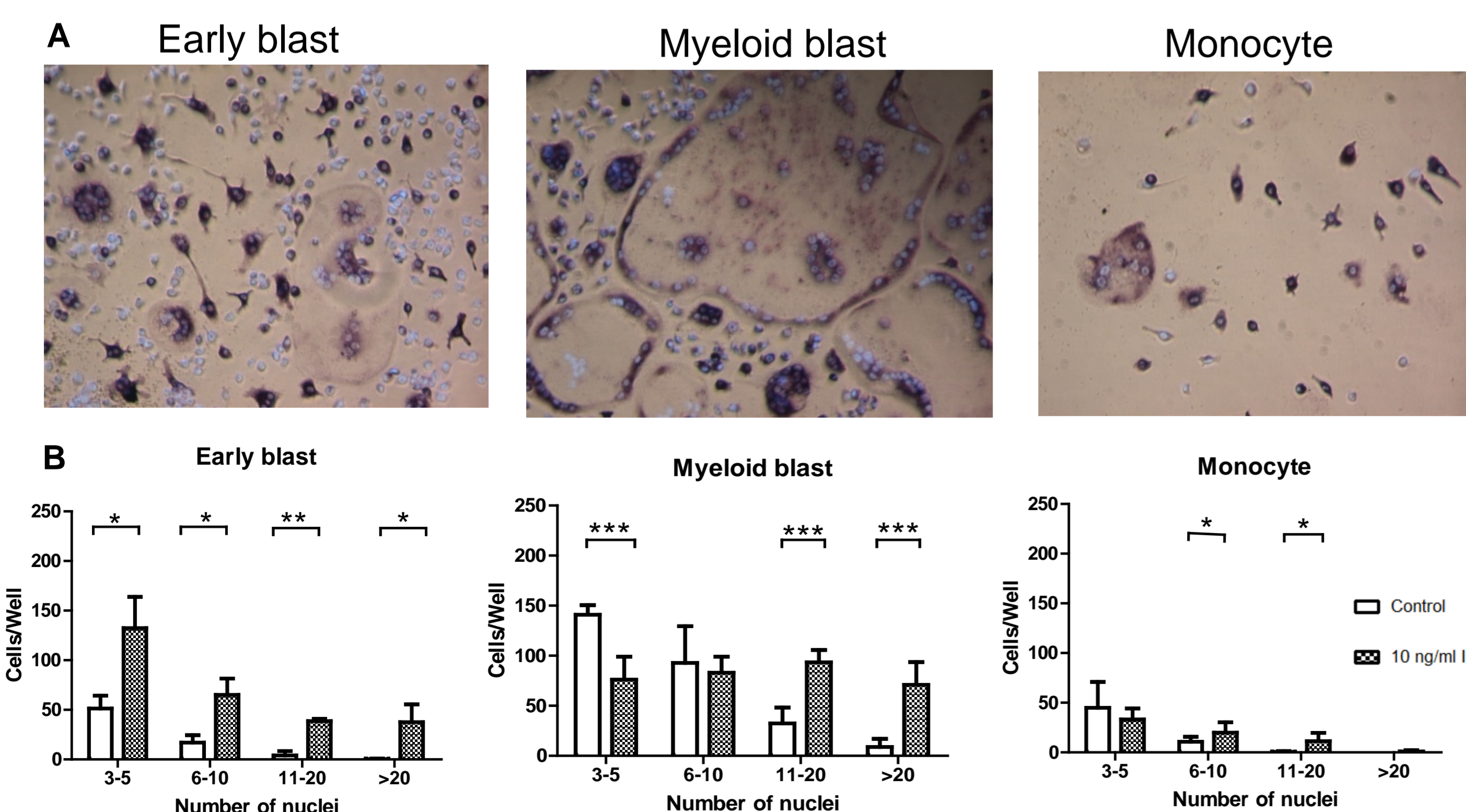


Fig 2. A. Morphology of osteoclasts observed by a combination of light and fluorescent microscopy. Osteoclasts were stained for TRACP activity⁺ (purple) and nuclei were stained blue. B. The number of TRACP⁺ multinucleated cells. (n=6, *p<0.05, **p<0.01, ***p<0.001).

RESULTS

IL-1 β stimulated osteoclastogenesis in all three subsets. In early blasts, the TRACP⁺ cells were mostly small (3-5 nuclei) cells while in myeloid blasts IL-1 β helped generating a high number of large osteoclasts (>10 nuclei).

2. IL-1 β significantly enhanced bone resorption in all three subsets, being highest for myeloid blasts.

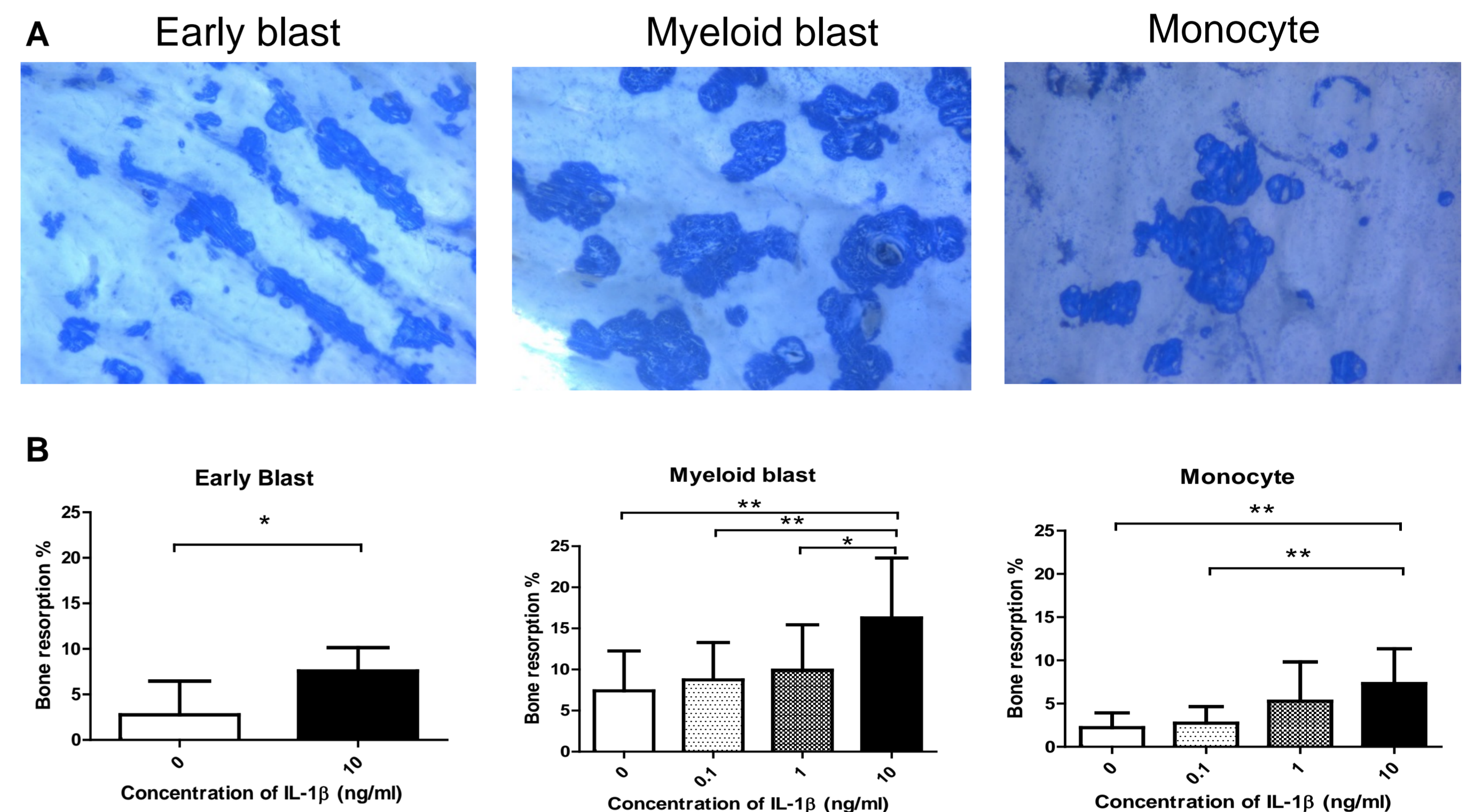


Fig 3. Bone resorption. A. Pit formation by three subsets in the presence of 10 ng/ml IL-1 β stained by Coomassie Brilliant Blue. B. Percentage of bone resorption under the influence of different concentrations of IL-1 β . (n=6, *p<0.05, **p<0.01, ***p<0.001).

3. Gene expression.

Gene expression of osteoclast markers TRACP and DC-STAMP was in agreement with the counting result: highest expression by myeloid blasts. The two IL-1 β receptors were differently expressed by the different subsets. This was particularly during the early time points, suggesting that early blasts were more sensitive to IL-1 β in early stage.

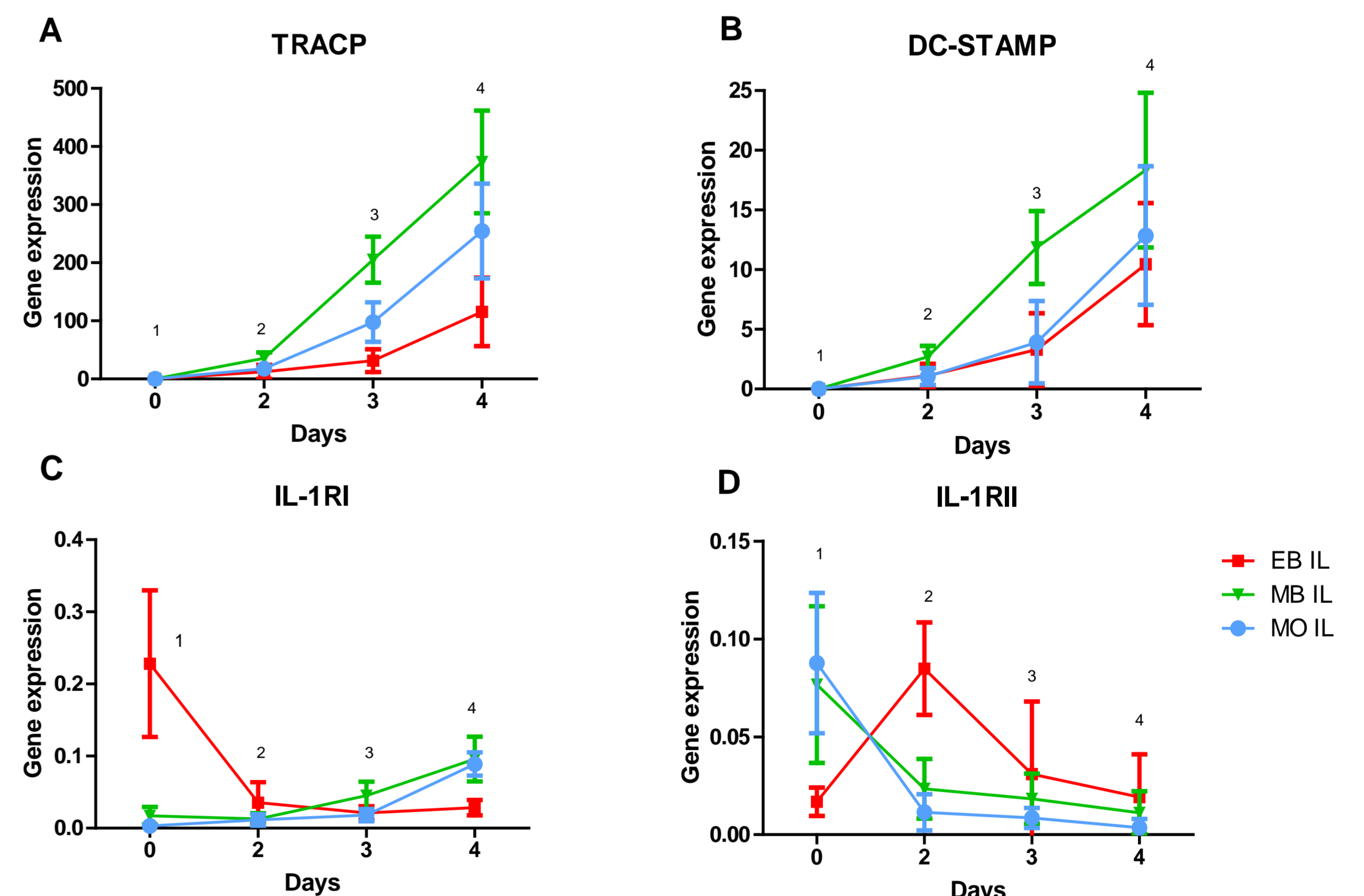


Fig 4. Gene expression. A. TRACP: 1. ns/*ns, 2. ***/ns/**, 3. ***/***/**, 4. ***/***/**. B. DC-STAMP: 1. ***/***/ns, 2. ***/ns/**, 3. ***/ns/**, 4. ns/ns/ns. C. IL-1RI: 1. ***/***/ns, 2. */*/ns, 3. ***/ns/**, 4. ***/***/ns. D. IL-1RII: 1. ***/***/ns, 2. ***/***/ns, 3. ns/ns/ns, 4. ns/ns/ns. (Significant differences were shown in sequence: EB vs MB/ EB vs MO/ MB vs MO. n=6, *p<0.05, **p<0.01, ***p<0.001).

CONCLUSIONS

IL-1 β up-regulates osteoclastogenesis and bone resorption. This was found for all three subsets of precursors analyzed. IL-1 β mainly increases the early blast derived osteoclasts in cell number while in myeloid blast culturing IL-1 β mainly increases the cell size. In the monocyte culture the most distinct feature is that it proliferates very low and slow therefore cannot generate many osteoclasts. This may suggest that monocytes are the physiological osteoclast precursors whereas early blasts and myeloid blasts are the cells responsible for the higher bone degradation at inflammatory sites.

The authors declare that there is no conflict of interest.

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