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TNF-α antagonist infliximab inhibits osteoclast formation of peripheral blood mononuclear cells but does not affect periodontal ligament fibroblast mediated osteoclast formation

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Introduction

The inflammatory cytokine tumor necrosis factor-alpha (TNF-α) is elevated in inflamed periodontal tissues and may contribute to periodontitis progression. TNF-α stimulates formation and activity of osteoclasts, the cells that cause alveolar bone degradation and subsequent tooth loss. We previously showed that TNF-α is elevated in co-cultures of periodontal ligament fibroblast (PDLF) and peripheral blood mononuclear cells (PBMC). Hence, TNF-α could be a determining factor in osteoclast formation in these cultures.

To assess the role of TNF-α in periodontitis associated osteoclast formation *in vitro*, osteoclast formation was analysed in the presence of the anti-TNF-α therapeutic agent infliximab in two culture systems: (i) PBMC in co-culture with periodontal ligament fibroblasts from controls and periodontitis patients, or (ii) with PBMC only.

Methods

- TNF-α ELISA:
Culture supernatants of the previous 4 days were obtained at 7, 14 and 21 days of co-cultures. The OptEIA TNF-α ELISA (BD Biosciences, San Jose, CA) was performed according to the manufacturer's protocol. All measurements below the detection limit of 5.6 pg/ml were regarded assigned 0.

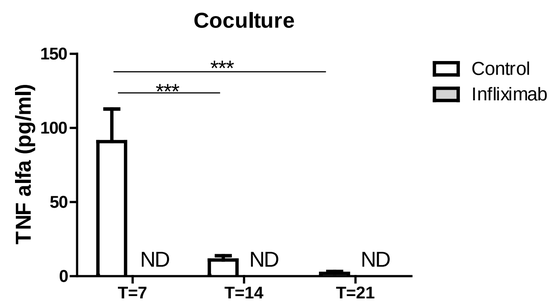
- Co-cultures:
Periodontal ligament fibroblasts (PDLF) were seeded in 48-well plates, 1.5 x 10^4 per well, for 24 hours. Subsequently, 0.5 x 10^6 Peripheral Blood Mononuclear Cells (PBMC) per well were seeded on top of the PDLFs (0.4 ml medium per well). Co-cultures were performed for additional 7, 14 and 21 days in DMEM with 10% FCS and 1% antibiotics. Culture medium was refreshed every 3 or 4 days. Infliximab (Trade name: Remicade; Centocor, Leiden, The Netherlands) was used in concentrations 0.1, 0.3, 1.0 and 10 µg/ml. The highest two concentrations were shown to severely inhibit synoviocyte mediated osteoclast formation.

- Cell cluster analysis:
Number and size were assessed using low magnification micrographs of high density PBMC cultures. Two standardized micrographs and four wells per condition were used from replicate experiments.

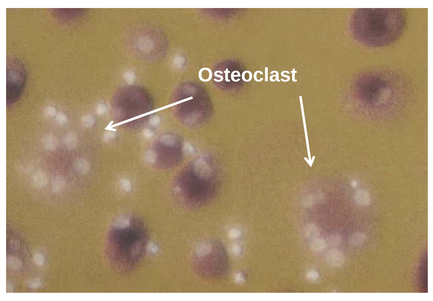
- High density PBMC cultures:
PBMC were seeded in 96 wells plates at a density of 0.5x10^6 cells per well and cultured in DMEM containing 10% FCS and 1% antibiotics and in the presence of vehicle (water), OPG, Infliximab, or heat inactivated Infliximab. Culture medium was refreshed every 3 or 4 days, cells were fixed after 21 days with 4% PBS buffered formaldehyde and stained for TRACP activity.

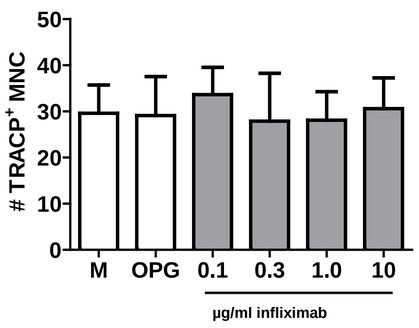
Results

High TNF-α expression early in PDLF-PBMC co-cultures.
PDLF-PBMC co-cultures were used to assess osteoclast formation in the absence or presence of infliximab. TNF-α was measured in the supernatant. Levels were high at day 7 and levels decreased significantly over time. The cytokine was not detectable when cells were cultured in the presence of infliximab.

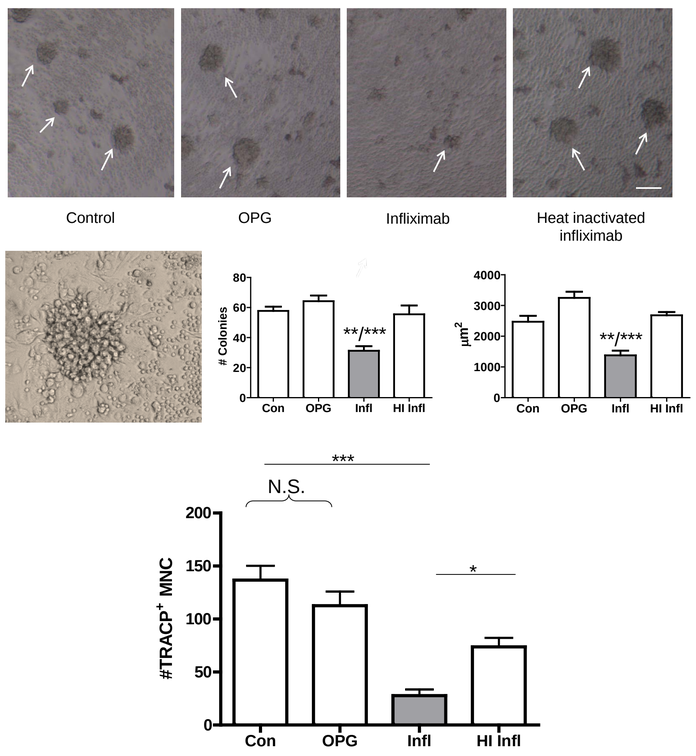


Infliximab or OPG does not affect the capacity of periodontal ligament fibroblasts to induce osteoclast formation.
The formation of TRACP+ multinucleated cells was assessed after 21 days of co-culture. OPG or Infliximab had no effect on osteoclast formation.





Infliximab inhibits formation of TRACP+ MNC in PBMC cultures.
To assess whether TNF-α plays a role PBMC cultures, PBMCs were cultured at high density without fibroblasts in the presence of OPG, infliximab or heat inactivated infliximab. Infliximab resulted in a reduced number and size of cell clusters and correlated with a decreased number of TRACP+ MNCs. OPG or heat inactivated infliximab did not affect cell cluster number, size or number of TRACP+ MNC that formed. These results imply that TNF-α rather than RANKL facilitates this type of osteoclast formation.



Conclusions

Our study shows that the contribution of TNF-α to osteoclast formation is cell system dependent.
Osteoclast formation in PDLF-PBMC co-cultures seems to be TNF-α (and RANK-L) independent, whereas blocking of the cytokine decreases osteoclast formation in PBMC cultures.
Possibly TNF-α contributes to PBMC induced osteoclast formation by establishing stronger cell-cell interactions that precede osteoclast formation.

No conflict of interest

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