Ex vivo study of IL-6 expression and function in immune cell subsets from human periapical lesions

Abstract

Aim

Even though IL-6 is a key inflammatory cytokine in periapical lesions (PLs), its function in stable periapical disease is unknown. Therefore, the aim of this study was to investigate the following: first, the *ex vivo* production of IL-6 by clinically different PLs; next, subsets of immune cells expressing IL-6 in PLs according to their inflammatory status and finally, modulatory effect of IL-6 on T-cell cytokine production in cell cultures.

Methodology

Inflammatory cells were isolated from a total of 95 human PLs. Detection of IL-6^{+} cells within the myeloid and lymphoid populations was performed by multicolour flow cytometry. ELISA and FlowCytomix Microbeads Assay were used to measure cytokine levels in culture supernatants. To study the role of IL-6 in PLs, mononuclear cells (MNC) from symptomatic (Sy) or asymptomatic (Asy) PLs were treated with IL-6 or Tocilizumab, an IL-6R blocking antibody. The differences between groups were tested by unpaired *t*-test, Mann–Whitney or Friedman tests.

Results

The levels of IL-6 in PL MNC culture supernatants were significantly higher compared with total PL cells and PL granulocytes ($p < .001$). MNC from Sy PLs produced significantly higher levels of IL-6 than MNC from Asy PLs ($p < .001$). Flow cytometry analysis showed that NKT cells, $CD8^{+}$ T cells and M2 macrophages (MØ), were dominant IL-6^{+} cells, in contrast to $CD4^{+}$ T cells. However, $CD8^{+}$ and $CD4^{+}$ T cells contributed the most to IL-6 production. IL-6^{hi} producing MNC cultures had higher levels of Th1 (IFN-γ), Th17 (IL-17A), Tfh (IL-21) and RANKL, whereas Th2 (IL-4) and Tregs cytokines (IL-10, TGF-β) were lower compared with IL-6^{low}-producing cultures. Exogenous IL-6 stimulated 17A, IL-21 and RANKL, independently of PL activation status, but decreased IFN-γ, IL-4 and IL-33 levels in IL-6^{hi}-producing cultures. Tocilizumab increased IL-10 and TGF-β in IL-6^{low}-producing cultures. All differences were $p < .05$.

Conclusions

Most immune cells from Sy PLs expressed higher levels of IL-6 compared with Asy PLs, which correlated with IL-6 production in culture. Analysis of cytokines suggested a dominant pro-inflammatory T-cell response and osteodestructive function of IL-6 in PLs judging by Th17/Tfh cell activation, Tregs inhibition and increased RANKL/OPG ratio. Excessive IL-6 production decreased Th1/Th2 responses.