World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:12, No:02, 2018

The Pro-Reparative Effect of Vasoactive Intestinal Peptide in Chronic Inflammatory Osteolytic Periapical Lesions

Authors : Michelle C. S. Azevedo, Priscila M. Colavite, Carolina F. Francisconi, Ana P. Trombone, Gustavo P. Garlet

Abstract : VIP (vasoactive intestinal peptide) know as a potential protective factor in the view of its marked immunosuppressive properties. In this work, we investigated a possible association of VIP with the clinical status of experimental periapical granulomas and the association with expression markers in the lesions potentially associated with periapical lesions pathogenesis. C57BL/6WT mice were treated or not with recombinant VIP. Animals with active/progressive (N=40), inactive/stable (N=70) periapical granulomas and controls (N=50) were anesthetized and the right mandibular first molar was surgically opened, allowing exposure of dental pulp. Endodontic pathogenic bacterial strains were inoculated: Porphyromonas gingivalis, Prevotella nigrescens, Actinomyces viscosus, and Fusobacterium nucleatum subsp. polymorphum. The cavity was not sealed after bacterial inoculation. During lesion development, animals were treated or not with recombinant VIP 3 days post infection. Animals were killed after 3, 7, 14, and 21 days of infection and the jaws were dissected. The extraction of total RNA from periodontal tissues was performed and the integrity of samples was checked. qPCR reaction using TaqMan chemistry with inventoried primers were performed in ViiA7 equipment. The results, depicted as the relative levels of gene expression, were calculated in reference to GAPDH and β-actin expression. Periodontal tissues from upper molars were vested and incubated supplemented RPMI, followed by processing with 0.05% DNase. Cell viability and couting were determined by Neubauer chamber analysis. For flow cytometry analysis, after cell counting the cells were stained with the optimal dilution of each antibody; (PE)-conjugated and (FITC)-conjugated antibodies against CD4, CD25, FOXP3, IL-4, IL-17 and IFN-γ antibodies, as well their respective isotype controls. Cells were analyzed by FACScan and CellQuest software. Results are presented as the number of cells in the periodontal tissues or the number of positive cells for each marker in the CD4+FOXp3+, CD4+IL-4+, CD4+IFNg+ and CD4+IL-17+ subpopulations. The levels mRNA were measured by qPCR. The VIP expression was predominated in inactive lesions, as well part of the clusters of cytokine/Th markers identified as protective factors and a negative correlation between VIP expression and lesion evolution was observed. A quantitative analysis of IL1β, IL17, TNF, IFN, MMP2, RANKL, OPG, IL10, TGFβ, CTLA4, COL5A1, CTGF, CXCL11, FGF7, ITGA4, ITGA5, SERP1 and VTN expression was measured in experimental periapical lesions treated with VIP 7 and 14 days after lesion induction and healthy animals. After 7 days, all targets presented a significate increase in comparison to untreated animals. About migration kinetics, profile of chemokine receptors expression of TCD4+ subsets and phenotypic analysis of Tregs, Th1, Th2 and Th17 cells during the course of experimental periodontal disease evaluated by flow cytometry and depicted as the number of positive cells for each marker. CD4+IFNg+ and CD4+FOXp3+ cells migration were significate increased 7 days post VIP treatment. CD4+IL17+ cells migration were significate increased 7 and 14 days post VIP treatment, CD4+IL4+ cells migration were significate increased 14 and 21 days post VIP treatment compared to the control group. In conclusion, our experimental data support VIP involvement in determining the inactivity of periapical lesions. Financial support: FAPESP #2015/25618-2.

Keywords : chronic inflammation, cytokines, osteolytic lesions, VIP (Vasoactive Intestinal Peptide)
Conference Title : ICI 2018 : International Conference on Immunology
Conference Location : London, United Kingdom
Conference Dates : February 15-16, 2018