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SOCS-3 Regulates Alveolar Bone Loss in Experimental Periodontitis

E. Papathanasiou[1,2,3], A. Kantarci[1], A. Konstantinidis[3], H. Gao[4], and T.E. Van Dyke[1]

Appendix

Methods

Animals. LysM-cre mice were obtained from The Jackson Laboratory, and their Cre recombinase expressions were under the control of endogenous Lyz2 promoter/enhancer elements. When LysM-cre mice were crossed with SOCS-3fl/fl mice (The Jackson Laboratory), Cre-mediated recombination resulted in deletion of SOCS-3 (suppressor of cytokine signaling 3) in myeloid cell lineage.

To induce periodontal disease, *Porphyromonas gingivalis* (strain A7436) was cultured on BD Trypticase Soy Agar, with 5% Sheep Blood (TSA II) plates. Plates were incubated for 3 d at 37 °C in an anaerobic chamber (80% N_2, 15% CO_2, 5% H_2; Airgas). Colonies were randomly selected and anaerobically cultured overnight at 37 °C in Schaedler's broth supplemented with vitamin K and hemin. Animals were given sulfamethoxazole/trimethoprim, 10 mL per pint in deionized water, ad libitum for 10 d, followed by a 4-d antibiotic-free period.

Micro–computed tomography analysis of alveolar bone. After proper image orientation, a region of interest (ROI) via a digital box with fixed size (4,660 × 1,000 × 2,010 μm) was positioned identically for all samples to allow standardized comparisons between samples. The width of the ROI was dictated by the height of contour of the molars at the cementoenamel junction. Height of the ROI was measured from molar cusp tips to the crest of alveolar bone. Depth was measured from the buccal to the palatal aspect of the scanned sample.

Histology. Left hemisected maxillae were fixed in 10% neutral buffered formalin for 48 h and demineralized in 10% EDTA (0.1M Tris, pH 6.95) on a rotator at room temperature. EDTA solution was replaced every 48 to 72 h for 3 wk. After decalcification, tissues were rinsed with running water for 30 min and submerged in ethanol. Specimens were paraffin embedded, with thin sections (5 μm) cut along the molars in a mesiodistal plane and stained with either hematoxylin and eosin or TRAP (tartrate-resistant acid phosphatase). All images were captured at ×100 and × 400 magnification by means of a digital microscope (Zeiss Axio Observer A1) equipped with a digital camera (AxioCam HRc r1.6) and saved as JPEG files.

Immunohistochemistry: RANKL and OPG. Sections (5 μm) were deparaffinized and rehydrated. Antigen retrieval was performed with 10mM Tris 1mM EDTA buffer, pH 9.0, in a microwave oven at 50% power (1 min) and 20% power (10 min). After cooled to room temperature, slides were washed with phosphate-buffered saline (PBS; 10 min). Endogenous peroxidase was blocked with 1% hydrogen peroxide solution in methanol for 30 min, protected from light, and rinsed in PBS twice. Slides were immersed in a 1.5% goat serum albumin/PBS solution for 20 min to block nonspecific binding and incubated overnight at 4 °C with the primary antibodies diluted in 1.5% goat serum albumin: anti-RANKL (polyclonal rabbit antibody, ab9957, Abcam; 1:2,000 dilution) or anti-OPG (polyclonal rabbit antibody, ab183910, Abcam; 1:1,000 dilution). Negative controls were incubated in the absence of primary antibody. After reaching room temperature, slides were washed twice with PBS and incubated with biotinylated secondary antibody (goat anti-rabbit immunoglobulin G-B and rabbit anti-goat immunoglobulin G-B, Abcam; 1:200 dilution) for 30 min at room temperature and rinsed in PBS. Streptavidin-biotin-peroxidase (ABC Kit, Vecstain; Vector Laboratories Inc) was added for 30 min. Slides were washed in PBS and the reaction visualized with chromogenic 3,3′-diaminobenzidine tetrahydrochloride hydrate (Sigma-Aldrich) in PBS with 3% hydrogen peroxide for 4 min, counterstained with Harris hematoxylin for 10 s, washed in running tap water for 2 min, immersed in dH_20 for 30 s, dehydrated, and coverslipped with Permount (Thermo Fisher Scientific).

[1]Department of Applied Oral Sciences, Center for Periodontology, Forsyth Institute, Cambridge, MA, USA
[2]Department of Periodontology, Tufts University School of Dental Medicine, Boston, MA, USA
[3]Department of Preventive Dentistry, Periodontology and Implant Biology, Dental School, Aristotle University of Thessaloniki, Thessaloniki, Greece
[4]Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Corresponding Author:
T.E. Van Dyke, Vice President for Clinical and Translational Research Chair, Department of Applied Oral Sciences, Center for Periodontology, 245 First Street, Cambridge, MA 02142, USA.
Email: tvandyke@forsyth.org