Maresin 1 Promotes Wound Healing and Socket Bone Regeneration for Alveolar Ridge Preservation

Chin-Wei Wang, Shan-Huey Yu, Tobias Fretwurst, Lena Larsson, James V. Sugai, Jonathan Oh, Kristen Lehner, Qiming Jin, William V. Giannobile

Appendix

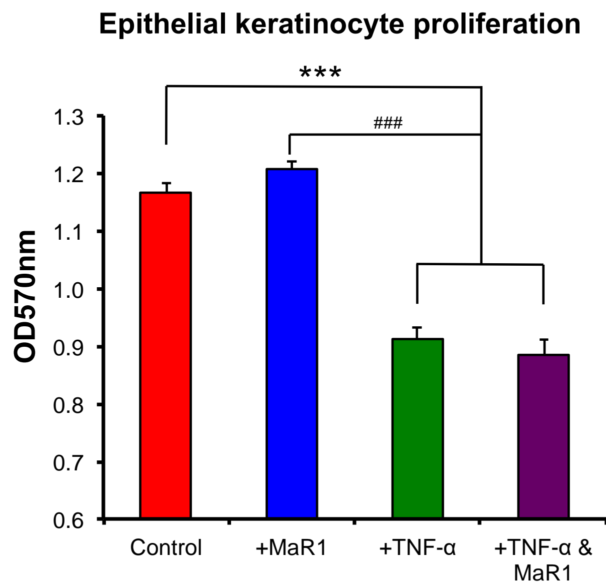
MATERIALS & METHODS

Human Keratinocyte Proliferation Assay

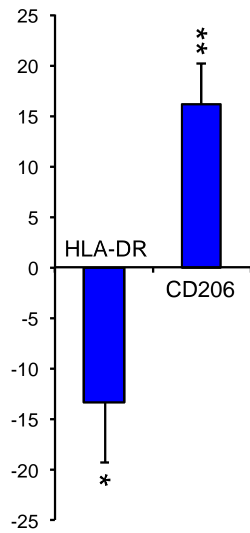
A human keratinocyte cell line, NOK-SI (kindly donated by R. Castilho lab), was cultured and maintained in Dulbecco's Modified Eagle Medium (Invitrogen, USA) supplemented with 10% fetal bovine serum (Hyclone, PA, USA) and 1% penicillin-streptomycin (Invitrogen). Approximately 9 x 10^3 cells were seeded in a 48-well plate overnight, then either 1nM recombinant human TNF-α (R&D Systems, MN, USA), 10 nM MaR1 (Cayman Chemical, MI, USA), or media alone was added and replenished everyday. An MTT (3-[4,5-di-methylthiazol-2yl]-2,5-diphenyl-tetrazolium bromide) cell viability assay (R&D Systems) was performed following the manufacturer's protocol on day 3 to compare the NOK-SI cell proliferation.

Macrophage phenotype analysis

Human peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient Ficoll-Histopaque isolation. Isolated PBMCs were purified by adhesion to tissue culture plates for 60 min. These cells were then cultured for 7 days in RPMI 1640 medium supplemented with 10% FCS and 20 ng/ml GM-CSF similar protocol as previous published (Dalli et al., 2013). Cells were then incubated with 10 nM of MaR1 or vehicle (PBS) for 6 h at 37°C. At the end of the incubation, the cells were detached, and surface expression of HLA-DR (clone G64-6; BD Biosciences, San Jose, CA, USA) and CD206 (clone19.2; BD Biosciences) was determined by flow cytometry as described previously (Dalli et al., 2013).



Appendix Figure 1. TNF-α inhibits human epithelial keratinocyte proliferation while MaR1 has limited effects. Human epithelial keratinocytes (NOK-SI) were incubated with 10nM MaR1 or/and 1nM TNF-α for 3 days and their proliferation was assessed by MTT Assay. N=6. Results are presented as mean ± SEM, ***$p < 0.001$ vs. control; $^{###}p<0.001$ vs. MaR1 after Bonferroni correction.



Appendix Figure 2. MaR1 regulates macrophage phenotype polarization. GM-CSF cultured primary macrophages were incubated with 10nM MaR1 for 6 hours and the expression of surface markers HLA-DR and CD206 were assessed with flow cytometry. n=3 separate donor and cell preparation. Results are presented as mean ± SEM, *p< 0.05, **p< 0.01 vs. control.