

ODONTOBLASTS SENSE PATHOGENS AND TRIGGER AN IMMUNE RESPONSE IN THE HUMAN DENTAL PULP.

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INTRODUCTION: The human tooth is the target of a substantial number of oral bacterial agents which are responsible for the development of carious lesions. These agents induce demineralization of enamel that normally constitutes an impermeable barrier protecting the underlying dentin and pulp tissues from the oral environment. When the enamel barrier is disrupted, dentin becomes exposed to the oral environment and is degraded by Gram-positive bacteria, including *Streptococcus*, *Lactobacillus* and *Actinomyces* spp. that largely dominate the dentin carious lesion microflora. Bacterial penetration into the disrupted dentin leads to the development of inflammatory and immune events in the underlying dental pulp, the molecular and cellular determinants of which remain largely unknown.

RESULTS: In this study we evidenced by real-time PCR that *in vitro* differentiated odontoblasts expressed genes encoding the pattern recognition molecules TLR1-6 and 9, but not TLR7, 8 and 10. Expression levels of TLR2, 3, 5 and 9 were significantly increased when odontoblasts were stimulated by lipoteichoic acid (LTA), a component of Gram-positive bacteria sensed by TLR2. TLR2 increase was confirmed at the protein level by flow cytometry. Immunostaining showed the localization of TLR2 in the cell membrane of LTA-stimulated odontoblasts, whereas TLR2 was not detected in unstimulated cells. Translocation of the NF- κ B transcription factor from the cytoplasm to the nucleus confirmed TLR signalling pathway activation. Gene array and real-time PCR analyses demonstrated that odontoblasts expressed several chemokine-related genes among which CCL2, CCL7, CXCL2 and CXCL10 were up-regulated by LTA. Antibody array analysis revealed a higher level of CCL2 and CXCL10 in culture

supernatants from LTA-stimulated odontoblasts than in controls. These supernatants augmented immature dendritic cell migration *in vitro*. Immunohistochemical analysis of human teeth demonstrated that CCL2 was expressed *in vivo* by odontoblasts and blood vessels present under active carious lesions, but not in healthy dental pulps. Finally, real-time PCR analysis revealed that gene expression of major dentin matrix components (type I collagen, dentin sialophosphoprotein) and TGF- β 1 was down-regulated in odontoblasts *in vitro* by LTA.

DISCUSSION & CONCLUSIONS: Together these data suggest that odontoblasts activated by LTA are able to initiate an immune response by secreting chemokines that recruit immature dendritic cells, while down-regulating their specialized functions of dentin matrix synthesis and mineralization. These results support a role for odontoblasts in the sensing of pathogens that enter dentin tubules during the carious process and in the triggering of immune events within the pulp tissue.

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