Effect of Inflammatory Cytokines on DNA Methylation and Demethylation

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INTRODUCTION

Periodontitis is a common cause of tooth loss and contributes to other common conditions. It affects 46% of adult Americans, 8.9% of them severely. Bacteria are essential for initiation, but host factors are crucial for development of chronic inflammation. An imbalance exists between inflammatory and anti-inflammatory cytokines, and increased levels of IL-1, IL-4 and Th2-driven gingival fibroblasts to produce inflammatory mediators including prostaglandin E2 (PGE2) and matrix metalloproteinases (MMPs), which contribute to continuing inflammation and destruction of tissues supporting teeth.

Periodontitis causes a chronic, low-grade systemic inflammation that contributes to development of other conditions, including cardiovascular disease, preterm low birth weight, diabetes and several types of cancer.

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Here we show that both inflammatory cytokine IL-1 and inflammatory mediator PGE2 inhibit expression of the de novo methyltransferase DNMT3a as well as Ten Eleven Translocation 1 (TET1) in human gingival fibroblasts derived from patients with periodontitis, and present evidence that the effects of IL-1 may be mediated at least partly through the COX-PGE2 pathway. The results so far are consistent with the idea that chronic exposure to fibroblasts to inflammatory signaling can result in changes in DNA methylation/demethylation. These changes could result in global and/or gene-specific changes which over time might contribute to the persistence of inflammation.

METHODS

Cell culture - Human gingival tissue samples are obtained with informed consent from patients receiving treatment for periodontitis under a protocol approved by PCOM’s IRB (protocol hydrogen-4/05-014). The gingival tissue is processed by enzymatic dispersion to produce primary cultures. Cells are maintained in Eagles Minimal Essential Medium (EMEM) supplemented with 10% fetal bovine serum and antibiotics/antimycotics (penicillin, streptomycin, amphotericin, G418/BLCL). Cells between passages 3 and 5 are used for experiments.

RNA Isolation and Analysis - Total RNA was isolated from cells untreated (controls) or treated with IL-1 (10 ng/mL) or L-1 (10 ng/mL), NS-398 (1 µM) of PGE2 (1-100 µM) for the indicated times. Two µg RNA was converted to cDNA using the High Capacity kits (Applied Biosystems) and probes for detection of DNMT1, DNMT3a, TET1 and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH; Applied Biosystems).

RESULTS & DISCUSSION

Figure 3. PGE2 causes dose-dependent inhibition of DNMT1, DNMT3a and TET1 mRNA in HGF - Total RNA was isolated from HGF cell cultures treated for 24 hours with IL-1 (10 ng/mL), NS-398 (1 µM) or L-1 + NS-398. DNMT1, DNMT3a and TET1 mRNA levels were quantified by real-time PCR, normalized to levels of GAPDH mRNA, and expressed relative to levels in the untreated controls harvested at time 0. The graph represents data from 5 independent experiments using HGF cultures derived from samples from 5 different individuals, average ± SEM. Statistical significance was determined using One Way ANOVA with post-hoc Bonferroni. *p<0.05.

Figure 4. IL-1 inhibition of DNMT3a expression may be partially reversed by inhibition of COX-2 - Total RNA was isolated from HGF cell cultures treated for 24 hours with IL-1 (10 ng/mL), NS-398 (1 µM) or IL-1 + NS-398. DNMT1, DNMT3a and TET1 mRNA levels were quantified by real-time PCR, normalized to levels of GAPDH mRNA, and expressed relative to levels in the untreated control. The graph represents data from 4 independent experiments using HGF derived from 4 different individuals, average ± SEM. Statistical significance was determined using One Way ANOVA with post-hoc Bonferroni. *p<0.05.

CONCLUSION

• These data provide evidence that both IL-1 and PGE2 affect expression of the de novo DNA methyltransferase DNMT3a and the demethylating enzyme TET1.

• Treatment of HGF cultures with PGE2 caused a dose-dependent decrease in mRNA levels of DNMT1, DNMT3a and TET1 as compared to untreated controls.

• DNMT3a expression was inhibited by IL-1 regardless of when the control sample was isolated, but DNMT1 was induced by IL-1 only as compared to a time zero control. This suggests that expression of DNMT1 is affected by time of culture, but not by IL-1 per se.

• Global levels of DNA methylation increased slightly over 72 hour treatment with IL-1 as compared to a time zero control. This is consistent with the changes observed in DNMT1 expression over time, and most likely do not reflect changes brought about by IL-1.

• Although inhibition of COX-2 with NS-398 seemed to partially reverse the effects of IL-1 on expression of DNMT3a and TET1, this effect is not statistically significant. It is unclear at this time whether the effects of IL-1 might be mediated at least partly by increased PGE2 production.

• Even modest changes in expression of these enzymes, if sustained over time, might be expected to have significant effects on gene expression patterns.

• Further experiments are needed to determine:
  • whether changes in DNMT3a and TET1 mRNA levels in response to IL-1 and PGE2 are reflected in changes in levels of protein and/or enzymatic activity.
  • whether any resulting gene specific changes in DNA methylation can be identified.
  • molecular mechanisms involved.

REFERENCES


