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Expression of LBP, CD14 and TLR 2/4 in Human Chronic Periodontitis

L.J. Jin[1]*, L. Ren[1], W.K. Leung[1] & R.P. Darveau[2]

[1]The University of Hong Kong, Hong Kong; [2]University of Washington, Seattle, USA

INTRODUCTION

- Periodontal pathogenesis is characterized by LPS activation of a cascade of pro-inflammatory cytokines and mediators from host cells via pattern recognition receptors including LPS-binding protein (LBP), CD14 and Toll-like receptor family (Schumann et al. 1990; Wright et al. 1990; Jin & Darveau 2001; Vivier & Malissen 2005).
- In addition to mediating cellular response to LPS, LBP and CD14 also act in cellular clearance of LPS through neutralization and inactivation of LPS (Tapping et al. 1999; Dixon et al. 2004).
- Although increasing *in vitro* studies have investigated the interactions of LBP, CD14 and TLRs with LPS, no *in vivo* study assessed the interrelationship of these innate recognition sensors and their association with periodontal conditions.
- We recently for the first time showed the local expression of LBP in gingival tissues and that both mCD14 and LBP peptide expression levels in periodontally healthy subjects were significantly higher than in patients with chronic periodontitis (Jin et al. 2004; Ren et al. 2004).

AIMS

- The present study further investigated the interrelationship of LBP and CD14 expression in human gingiva as well as the co-expression of TLR 2 and -4 in association with periodontal health and disease.

SUBJECTS & METHODS

- **Subjects:**
  - 43 adults (47.9±3.7 yrs) with untreated advanced chronic periodontitis.
  - Presenting unresolved lesions following initial periodontal treatment.
  - 15 periodontally healthy subjects (23.4±3.6 yrs) as controls.

  All subjects fulfill the following criteria:
  - No systemic diseases.
  - No prior periodontal treatment and use of immunosuppressive drugs.
  - No antibiotics or anti-inflammatory drugs in preceding 6 months.
  - Written and oral informed consents obtained.
- **Examination & collection of samples:**
  - Parameters include PI, BOP, PD and PAL by Florida Probe
  - Standard radiographic examination
  - The biopsies collected during periodontal surgery (Figs. 1 & 2) from both patients and healthy controls include:

Patients: 1) Periodontal pocket tissues (PoTs)
PD ≥ 6mm, AL ≥ 5mm, BOP(+)
2) Clinically healthy tissues (HT-Ps)
PD ≤ 3mm, AL < 2mm, BOP(-)
Healthy control subjects:
3) Periodontally healthy tissues (HT-Cs)

Fig. 1

- **Immunohistochemistry**
  - Tissue fixation and embedding.
  - Serial sections in 4μm thick.
  - Primary mAbs: mouse anti-human LBP (Biometec Ltd, Germany), mCD14 and CD1a (Neomarkers Inc, USA), CD68 (Dako, Denmark), and TLR-2/4 (Imgenex Co. USA).
  - CD68 and CD1a were co-detected with mCD14
  - (+) expression was analysed by using an image analysis system and presented as (+)Area%.

Fig. 2

- **RT-PCR**
  - The LBP and CD14 mRNAs were detected by RT-PCR.
- **Statistical analysis**
  - Chi-square test and ANOVA were used as appropriate to determine the significance of the differences in data sets between controls and patients. Spearman rank correlation analysis was performed to determine the correlation of detection frequency and expression levels of LBP with mCD14.

RESULTS

- No significant correlation was found between the expression patterns and levels of LBP and mCD14 with age in both patient and control groups. The co-expression of LBP and mCD14 (Fig. 3) is detected in overall 89% of the samples from patients (PoTs and HT-Ps), 91% in PoTs and 85% in HT-Ps, as well as in 100% of HT-Cs.
- LBP and mCD14 peptides expression levels were significantly higher in healthy controls than in patients. An overall positive correlation existed between LBP and mCD14 in both detection expression ($r=0.608$, $p<0.001$) and expression levels ($r=0.304$, $p<0.05$).

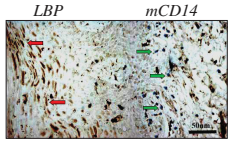

Fig. 3

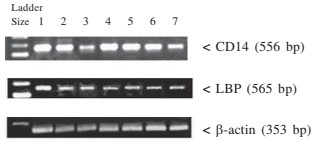

Fig. 4

- CD14 mRNA was detected in all categories of samples, while LBP message was detected in over 50% of various categories of gingivaltissues (Fig. 4). LBP and mCD14 mRNAs were co-detected in 55% of PoTs, 55% of HT-Ps and 75% of HT-Cs. No significant difference was found in detection frequency among them.
- In PoTs, TLR-2 expressed in both pocket epithelia and connective tissues (Figs. 5B & C) where it mainly expressed on macrophages-like cells (Fig. 5D). While TLR-4 was mainly detected in connective tissues (Fig. 5G).
- In HT-Ps, weak TLR-2 expression was found in gingival epithelia (Figs. 5E & F), and no TLR-4 expression was detected (Fig. 5H).
- In HT-Cs, the expression pattern of TLR-2 and -4 was similar to that of HT-Ps. Fig. 5A: (-) control.
- CD68-labelled macrophages were found in the connective tissues of PoTs, while they were sparsely seen in connective tissues of HT Ps and HT-Cs. CD1a-labelled dendritic cells were consistently detected in the suprabasal layer of oral gingival epithelium in all categories of gingival biopsies.

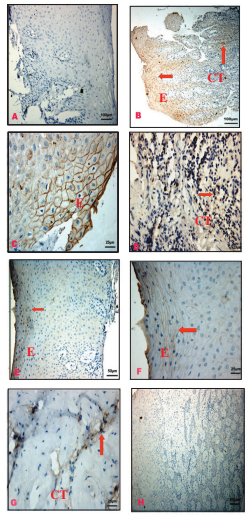

Fig. 5

- In PoTs, mCD14 (green arrow) was co-detected on CD68-labelled macrophages (red arrow) in the connective tissues beneath pocket epithelium (Fig. 6A) and on CD1a-labelled dendritic cells (red arrow) in the pocket epithelium and connective tissues interface (Fig. 6B). No similar expression could be detected in HT-Ps and HT-Cs.

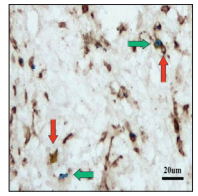

Fig. 6A

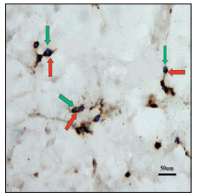

Fig. 6B

CONCLUSIONS

- *In vivo* expression of LBP and mCD14 may be interrelated.
- Inappropriate expression of LBP and mCD14, coupled with altered expression profiles of TLR-2 and -4, may be related to periodontal pathogenesis.

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E-mail: ljjin@hkusua.hku.hk