Research session | Modulation of host response elements in periodontitis

O030

PD-L1 up-regulation and signaling in carcinoma cells by PD-L1 up-regulation and signaling in carcinoma cells by P. gingivalis cell wall components

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Background & Aim: The immune-regulatory programmed death-ligand 1 (PD-L1) acts as a co-signaling molecule in cell-mediated immune response. It mediates regulation of T cell activation and tolerance and down-regulates T cell function and survival. Up-regulation of PD-L1 on host cells may contribute to chronicity of inflammation. Tumor-associated PD-L1 expression has been linked to immune-evasive mechanisms by which tumor cells escape immune surveillance. Porphyromonas gingivalis (P. gingivalis), a keystone pathogen in periodontitis, invades host cells and expresses several virulence factors. The aim of this study was to investigate the signalling pathway of the PD-L1 induction in epithelial cells by membrane fractions and cell wall components of P. gingivalis.

Methods: Human squamous carcinoma cells stimulated with isolated P. gingivalis membranes and cell wall components. Chemical inhibitors were used to identify possible members of the signalling pathway mediating the PD-L1 up-regulation. The CRISPR/Cas9 method was applied to generate Myd88 and RIP2 knock-out cells. PD-L1 protein expression was quantified by Western blot analysis.

Results: Up-regulation of PD-L1 induced by P. gingivalis membranes could be blocked effectively using gefitinib, an inhibitor of the receptor-interacting serine/threonine-protein kinase 2 (RIP2), RIP2, component of the signaling cascades of innate and adaptive immune response, is recruited by NOD1 and NOD2 after activation and oligomerization. RIP2 mediates up-regulation of PD-L1 through activation of nuclear factor kappa B (NF-kB). The NF-kB inhibitor BAY11 - 7082 (2.5 μM) reduced the expression by 64.4 ± 23%, gefitinib caused a reduction of PD-L1 expression by 57.6 ± 22% (2.5 μM) and 75.1 ± 23% (10 μM). Knock-out of MyD88 stimulation demonstrated that PD-L1 up-regulation was MyD88-independent while RIP2 knockout prevented PD-L1 upregulation after stimulation with P. gingivalis membranes.

Conclusion: We provide evidence that membrane proteins of P. gingivalis up-regulate the immune-regulatory receptor PD-L1 using a pathway that includes essential components of the NOD1 and NOD2 signaling cascade.

O031

Impact of periodontitis on gingival crevicular fluid levels of pro-inflammatory cytokines in smokers

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Background & Aim: The aim of this study was to investigate the pro-inflammatory cytokine expression profiles in gingival crevicular fluid (GCF) in patients with advanced chronic periodontitis and to assess the impact of smoking on local levels of pro-inflammatory cytokines.

Methods: Thirty patients with chronic periodontitis (CP; 20 smokers and 10 non-smokers) and 20 periodontally healthy subjects (10 smokers and 10 non-smokers) were recruited. Clinical parameters including the plaque index (PI), the gingival index (GI), and the bleeding on probing (BOP) were recorded on the day of GCF sampling. Interleukin-1β (IL-1β), Interleukin-2 (IL-2), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-12 (IL-12), Interleukin-16 (IL-16), and Tumor necrosis factor-α (TNF-α) were measured in GCF samples using a multiplex immunoassay.

Results: IL-1β and IL-8 levels were significantly higher (p < 0.01), while IL-2, IL-6 (p < 0.01) and TNF-α levels (p < 0.01) were significantly lower in GCF from patients with CP regardless of smoking status. IL-8 and IL-16 levels were observed to be negatively correlated with smoking regardless of periodontal status (p < 0.05).

Conclusion: Our results suggest that the impact of inflammation on local cytokine levels of the diseased sites is more prominent than the effect of smoking.

O032

Identification of periodontal pathogens and inflammatory cytokines in obese subjects with chronic periodontitis

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Background & Aim: Obesity is a suggested putative risk factor for development and progression of chronic periodontitis (CP). This study aims to identify and quantify subgingival periodontal pathogenic and inflammatory cytokines in obese subjects with CP and to correlate their presence with clinical parameters.

Methods: Based on body mass index criteria (obese ≥ 30 kg/m²; non obese <25 kg/m²), CP subjects were assigned into obese (n = 147) and non-obese (n = 134) groups. Visible plaque index (VPI), Gingival bleeding index (GBI), Probing pocket depth (PPD) and Clinical attachment loss (CAL) were recorded. Subgingival plaque and blood was sampled from all subjects. Real time PCR (qPCR) was used to determine prevalence and quantity of Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Prevotella intermedia (Pi) and Aggregatibacter actinomycetemcomitans (Aa). Quantification of resistin, Tumour necrosis factor alpha (TNF-α) and Interleukin (IL)-6 and IL-17 concentrations were performed by ELISA.

Results: Obese group had higher mean VPI and GBI but lower mean PPD and CAL than non-obese group (p < 0.05). Frequency of detection for resistin and IL-6 was higher in non-obese group as compared to obese group (p < 0.001) while TNF-α was higher in obese group. Total amounts of resistin and IL-6 were significantly higher in obese group (28.99 ± 33.87 pg/ml, 70.87 ± 31.15 pg/ml) than non-obese group (19.07 ± 24.1 pg/ml, 27.71 ± 26.99 pg/ml) (p = 0.001). There was no difference in frequency of detection of periodontal-pathogens between both groups. Pg and Tf showed higher mean counts in obese subjects (p < 0.001) while Aa had higher counts in non-obese subjects (p < 0.05). Among obese subjects, weak correlation was detected between mean PPD and
Conclusion: Obese-CP subjects had higher mean counts of Pg and Tf and serum resistin and IL-6 levels than non-obese subjects. Weak positive correlation exists between mean PPD and Tf in obese-CP subjects.

O033

Antimicrobial peptides (AMPs) genes expression in moderate to severe chronic generalized periodontitis patients; a pilot study

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Background & Aim: Despite all our efforts, periodontal diseases still affect nearly 60% of adults in Europe (König et al., 2010). Actually, the pathogenesis of these diseases is based on an inadequate immune-inflammatory response of periodontal tissues against stimuli related to the development and maturation of the oral biofilm (Page and Kornman, 1997). Antimicrobial Peptides (AMPs) are known to be involved in the early innate immune response against bacterial challenge thanks to their antibacterial and immunomodulatory properties. Recently, forty-five antimicrobial peptides (AMPs) have been identified in gingival crevicular fluid, periodontal tissues and saliva (Gorr and Abdolhosseini 2011). The objective of this pilot case-control clinical trial was to study the expression of genes encoding AMPs by RT-qPCR in patients with moderate to severe generalized chronic periodontitis compared to healthy patients from a simple method of sampling commonly used clinically: the gingival smear.

Methods: This study included twenty-three subjects (twelve patients with moderate or severe generalized chronic periodontitis and eleven healthy patients as control group). AMPs gene expressions were assayed using RT-qPCR from periodontal pocket smears.

Results: Among the forty-five AMPs known to be present within the oral sphere, 15 could be detected in periodontal pocket smears using RT-qPCR. The results of this pilot study exhibited significantly difference between healthy and affected groups for three of these AMPs: lysozyme C (LyzC), α-defensin 4 (DEFA4), histatin 3 (HTN3). The relative expression of DEFA4 appeared to be a protective factor against periodontitis.

Conclusion: These results support the hypothesis of the involvement of AMPs in periodontal physiopathology. These AMPs implication could lead to better understanding of periodontal pathogenesis or even innovative therapeutic targets.

O034

Stress-related hormones in association to periodontal condition in adolescents – results of the epidemiologic LIFE child study

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Background & Aim: Aim of this study was the investigation of the association between blood levels of stress-related hormones, nutritional status and early signs of periodontal disease in children and adolescents.

Methods: Within the LIFE Child study, 498 adolescents (10 to 18 years) were included. Early signs of periodontal inflammation were measured by probing pocket depth (PPD) at six index teeth. Oral hygiene and presence of orthodontic appliance (OA) were recorded. Additionally, in 98 subjects, chairside active matrix metalloproteinase-8 (aMMP-8) test was performed. Blood levels of stress-related hormones (cortisol, dehydroepiandrosterone-sulfate [DHEA-S]) and, supplementary, interleukin-6 (IL-6), C-reactive protein (CRP), insulin and HbA1c were measured. Nutritional status was recorded by the body-mass-index-standard-deviation-score (BMI-SDS).

Results: Higher DHEA-S and BMI-SDS were found to be associated with positive aMMP-8 result even after adjusting for age and gender (p = 0.027, pBMI = 0.026). However, no statistically significant associations between stress-related hormones (cortisol and DHEA-S) and presence of maximum PPD >3 mm were found (p > 0.05). In ROC analyses, areas under the curves were small for all investigated parameters (AUC<0.63). Significant associations of positive aMMP-8 test result, IL-6, insulin as well as BMI-SDS and maximum PPD >3 mm could be shown (p < 0.05). The percentage of OA (n = 473) was significantly higher in the PPD >3 mm group (p = 0.037). DHEA-S concentration was higher in males and with increasing age.

Conclusion: The results reveal the possibility of a weak association between early signs of periodontal disease in adolescents and stress-related hormones, e.g. DHEA-S, as well as nutritional status. Accordingly, the observed differences were too small to be clinically relevant and should be confirmed by investigating high-risk groups. DHEA-S and BMI-SDS seem to be appropriate to detect possible associations with periodontal condition.

O035

Effect of nonsurgical periodontal treatment on gingival crevicular fluid and salivary YKL-40 and IL-6 levels in chronic periodontitis patients

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Background & Aim: YKL-40, a new acute phase protein, associated with inflammation, endothelial dysfunction and tissue remodeling which is released by a variety of cells. The aim of present study was to evaluate gingival crevicular fluid (GCF) and saliva YKL-40 and interleukin (IL)-6 levels in patients with generalized chronic periodontitis (CP) following nonsurgical periodontal treatment.

Methods: A total of 52 subjects (26 chronic periodontitis; 26 periodontally healthy) were included to our study. Clinical periodontal measurements were recorded; GCF and saliva samples were obtained at baseline from all participants and at 1 and 3 months following periodontal therapy from CP patients. GCF and saliva YKL-40 and IL-6 levels were analyzed by enzyme-linked immunosorbent assay. Data were analyzed statistically using nonparametric tests.

Results: GCF and saliva YKL-40 and IL-6 levels were significantly higher in CP patients at baseline compared to healthy controls (p < 0.001). All clinical parameters showed significant reduction following nonsurgical periodontal therapy (p < 0.001).