Sex Differences in the Immune Response to Chronic Periodontitis

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ABSTRACT

Introduction: Chronic Periodontitis (ChP) is an inflammatory condition that results from oral dysbiosis and host immune dysfunction. Epidemiologic evidence has shown that men are more susceptible to ChP than women. Despite gender differences in behavior and socioeconomic status influencing oral health, the biological sex-associated immunological mechanisms underlying the pathogenesis of ChP are unclear. The objective of this study is to identify sex differences in the immune responses to chronic periodontitis.

Methods: We used a high-parameter mass cytometry immunoassay to perform an in-depth single-cell proteomic analysis of the peripheral blood immune responses in 14 ChP patients (6 males and 8 females) and 14 healthy control subjects (6 males and 8 females). Preliminary analysis was performed on 1) male vs. female in the control group, 2) male vs. female in the ChP group, 3) control vs. ChP in males, 4) control vs. ChP in females. Over 520 immune features representing the relative distribution of innate and adaptive immune cell subsets as well as their endogenous or stimulated intracellular functional responses to *Porphyromonas gingivalis*-derived lipopolysaccharide (LPS), TNF-α, IFN-α, and a cocktail of IL-2, -4, and -6, and GM-CSF were studied.

Results: We found 16 features in control subjects and 7 in ChP patients that were significantly different between males and females. Specifically, endogenous phosphorylated P38, a component of the MyD88 pathway, in neutrophils was higher in females compared to males in healthy control subjects, which was consistent with previously described sexual dimorphism in the innate immune responses. The analysis also revealed exaggerated proinflammatory response to LPS in circulating neutrophils and monocytes from male patients with ChP, but not female patients with ChP.

Conclusion: Our preliminary findings demonstrate the possibility of a sex-specific immune dysfunction associated with ChP that can be detected in the systemic circulation. Future studies in a larger cohort are needed to validate our results.

INTRODUCTION

- Of the 115 million people affected, epidemiological and clinical studies show **a biased prevalence of chronic periodontitis(ChP) in males, accounting for 57% and females 39%.** This difference may suggest a sexual dimorphism in ChP pathogenesis and elucidate a novel component in disease etiology.
- **Sex differences in immune responses have been observed throughout the whole life span (Figure 1).** These sex differences in immune responses result in differential susceptibility of males and females to infectious diseases, as well as the outcome of treatment.
- **The pathogenesis of periodontitis is the interaction between dysbiosis and host immune responses (Figure 2).** Dysbiotic microbial communities of keystone pathogens and pathobionts are thought to exhibit synergistic virulence whereby not only can they endure the host response but can also thrive by exploiting tissue-destructive inflammation, which fuels a self-feeding cycle of escalating dysbiosis and inflammatory bone loss, potentially leading to tooth loss and systemic complications.
- Although there are sex differences in immune responses, how do the differences affect the pathogenesis of periodontitis is still unknown.
- **Objective:** to investigate whether a sex-specific immune dysfunction associated with ChP can be detected in systemic circulation.

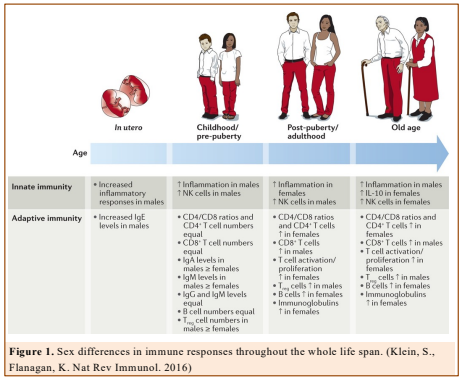


Figure 1. Sex differences in immune responses throughout the whole life span. (Klein, S., Flanagan, K. Nat Rev Immunol. 2016)

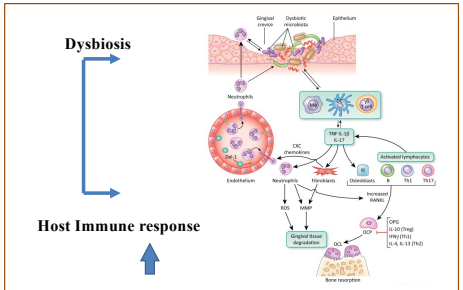
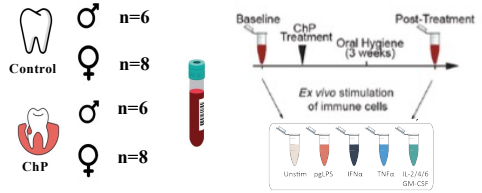


Figure2. Pathogenesis of periodontitis is the interaction between dysbiosis and host immune responses (Hajishengallis G. Trends Immunol. 2014)

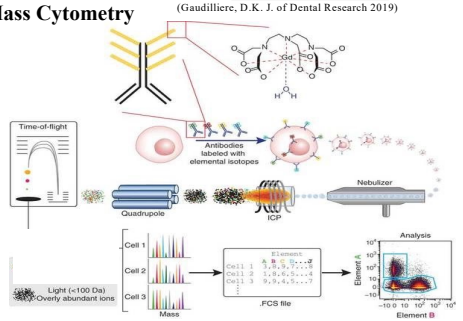
METHODS

A. Patient recruitment and sample collection



(Gaudilliere, D.K. J. of Dental Research 2019)

B. Mass Cytometry



(Modified from Bendall , Nat Biotechnol. 2012)

C. Antibody Panel

Phenotype				Function	
Antigen	CyTOF Channel	Antigen	CyTOF Channel	Antigen	CyTOF Channel
CD235ab	In113	TCRγδ	Sm152	pCREB	Sm149
CD61	In113	CD33	Gd158	pSTAT5	Nd150
CD45	In115	Tbet	Gd160	pP38	Eu151
CD66	La139	FoxP3	Dy162	pSTAT1	Eu153
CD7	Pr141	CD16	Ho165	pSTAT3	Sm154
CD19	Nd142	CD25	Tm169	prpS6	Gd155
CD45RA	Nd143	CD3	Er170	pMAPKAPK	Tb159
CD11b	Nd144	CD15	Yb172	IκB	Dy164
CD4	Nd145	HLA-DR	Yb174	pNFκB	Er166
CD8a	Nd146	CD14	Yb175	pERK1/2	Er167
CD11c	Sm147	CD56	Yb176		
CD123	Nd148				

RESULTS

- We compared all 520 immune features immune between Control and ChP using the cell-signaling Elastic Net (csEN) algorithm. The box plot (**Figure 3**) shows that this predictive model separates patients with ChP from control (P-Value=1.67E-4), suggesting profound systemic immune dysfunction in ChP patients.
- In the heatmap (**Figure 4**) with unsupervised clustering algorithm, samples of male were separated from samples of female.
- We found 16 features in control subjects and 7 in ChP patients that were significantly different between males and females (**Figure 5**).
  - The analysis also revealed exaggerated proinflammatory response to LPS in circulating neutrophils and monocytes from male patients with ChP, but not female patients with ChP.
  - Endogenous pP38 in neutrophils was higher in females compared to males in healthy control subjects, which was consistent with previously described sexual dimorphism in the innate immune responses.

RESULTS

Systemic Immune Dysfunction in ChP

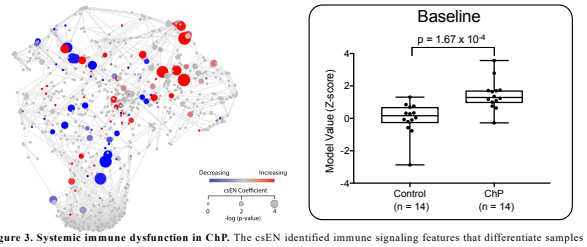


Figure 3. Systemic immune dysfunction in ChP. The csEN identified immune signaling features that differentiate samples from patients with ChP and those from controls at baseline. Each node is colored by model coefficient. Red and blue indicate features elevated and decreased in samples from patients at baseline, respectively. Node size is proportional to the P value of the difference between patient and control samples (Wilcoxon rank sum test). (Right panel) For each of the 28 patients, a unique model value from the csEN is represented as a z score in the box plot showing that the model significantly differentiates the patients with ChP (n = 14) from the controls (n = 14). Values are presented as median, interquartile range, and range. (Gaudilliere, D.K. J. of Dental Research 2019).

Heatmap of All Immune Features and Samples

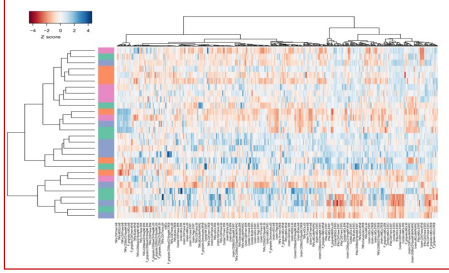


Figure 4. Sex differences in immune features. We visualized all the immune features in a heatmap, in which each column is an immune feature. And, we used an unsupervised clustering algorithm to group samples with similar immune responses together. Samples of male (colored by orange and pink) were clustered seperately from samples of female (colored by green and blue).

Sex-specific Immune Dysfunction associated with ChP

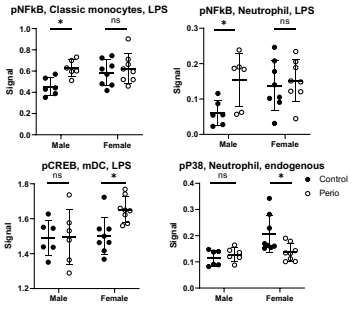


Figure 5. Sex-specific immune dysfunction associated with ChP . We found 16 features in control subjects and 7 in ChP patients that were significantly different between males and females.

The signal level of pNFkB in classic monocytes and neutrophils were only higher in male patients with periodontitis, revealing an exaggerated proinflammatory response to LPS in circulating neutrophils and monocytes from male patients with ChP, but not female patients with ChP.

The signal level of pCREB in myeloid dendritic cell and pP38 in neutrophil were only changed in females, suggesting the sexual dimorphism in the innate immune responses.

Endogenous pP38 in neutrophils was higher in females compared to males in healthy control subjects, which was consistent with previously described sexual dimorphism in the innate immune responses.

CONCLUSION

- ❑ **Our preliminary findings demonstrate the possibility of a sex-specific immune dysfunction associated with ChP that can be detected in the systemic circulation.**
- ❑ **Future studies in a larger cohort are needed to validate our results.**