Systemic Immunological Consequences of Chronic Periodontitis

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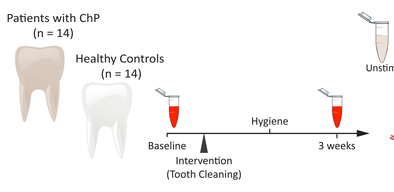
ABSTRACT

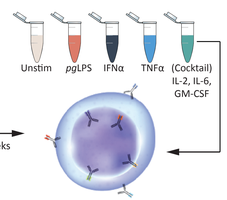
Chronic Periodontitis (ChP) is a prevalent inflammatory disease affecting 46% of the US population. ChP produces a profound local inflammatory response to dysbiotic oral microbiota that leads to destruction of alveolar bone and tooth loss. ChP is also associated with systemic illnesses including cardiovascular diseases, malignancies, and adverse pregnancy outcomes. However, the mechanisms underlying these adverse health outcomes are poorly understood. We used a highly multiplex mass cytometry immunoassay to perform an in-depth analysis of the systemic consequences of ChP in patients, before and after periodontal treatment in this prospective cohort study. A high-dimensional analysis of intracellular signaling networks revealed immune system-wide dysfunctions differentiating patients with ChP from healthy controls. Notably, we observed exaggerated pro-inflammatory responses to P. gingivalis-derived lipopolysaccharide in circulating neutrophils and monocytes from patients with ChP. Simultaneously, natural killer cell responses to inflammatory cytokines were attenuated. Importantly, the immune alterations associated with ChP were no longer detectable three weeks after periodontal treatment. Our findings demarcate systemic and cell-specific immune dysfunctions in patients with ChP which can be temporarily reversed by the local treatment of ChP.

STUDY DESIGN

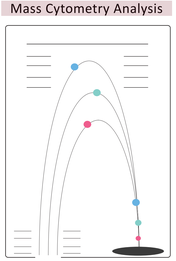
Patient recruitment and sample collection



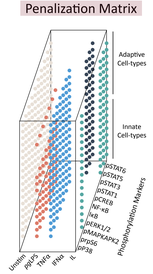
Ex vivo stimulation of immune cells with cytokines



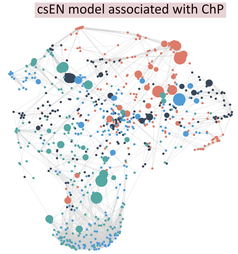
Mass Cytometry Analysis



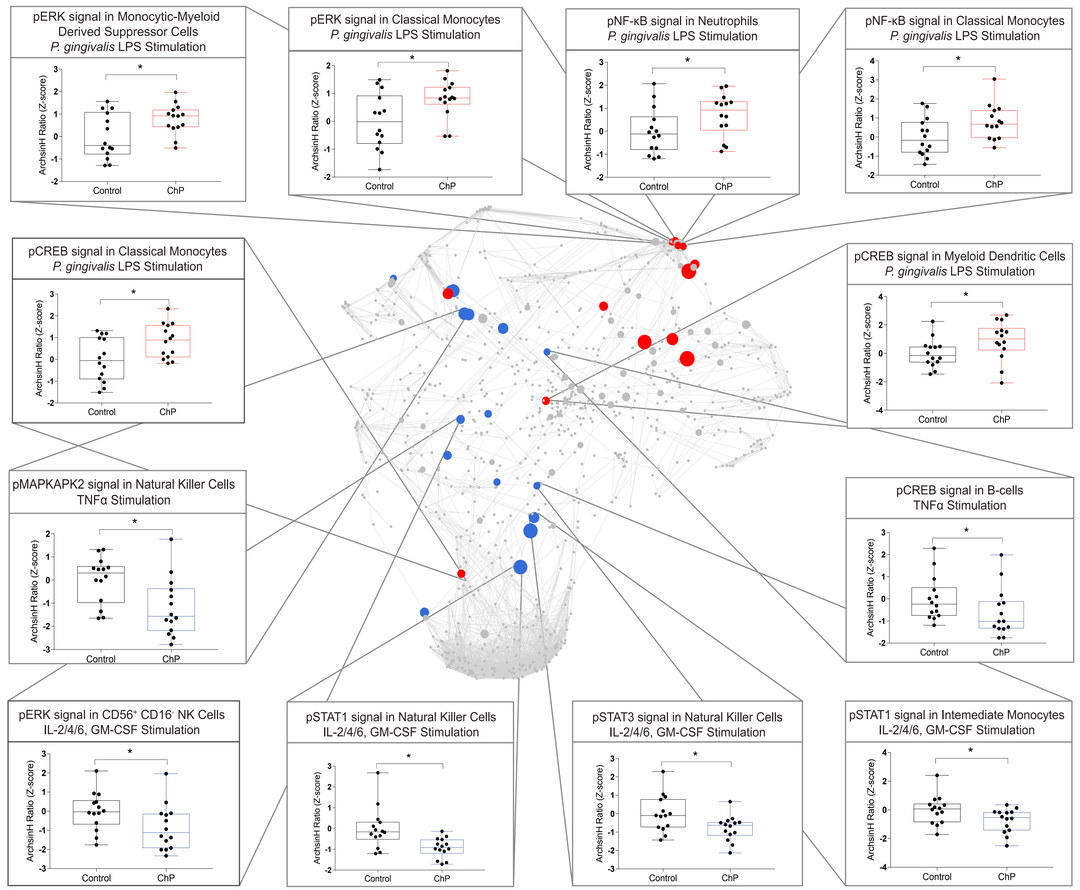
Penalization Matrix



csEN model associated with ChP



csEN model identifies systemic immune alterations associated with ChP



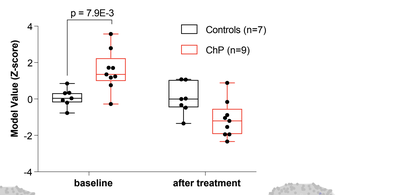
(Center) Baseline immune network with the 17 canonical features selected by the csEN model, labeled on the correlation network, that differ between ChP patients and controls. (Periphery) Box plots depicting 12 of the canonical csEN model components (Archsinh ratio of the stimulated samples compared to unstimulated samples, z-scored).

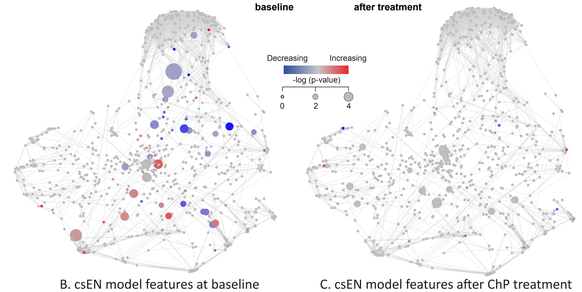
Demographic	Metric	Patients with ChP (14)	Healthy Controls (14)	Test
Age	Median (Range)	40.5 (29-61)	36.5 (26-57)	t-test p = 0.12
Sex	Male (n) Female (n)	6 8	6 8	chi-square p = 1.00
Race/Ethnicity n (%)	African American Asian Caucasian Latino	2 (14%) 3 (21%) 0 (0%) 9 (64%)	2 (14%) 6 (43%) 3 (21%) 3 (21%)	chi-square p = 0.072
Body Mass Index	Mean (Range)	28.9 (19-40)	24.6 (19-31)	t-test p = 0.07
Comorbidities	Anemia Hypertension Morphea Thyroid Disease	1 1 0 1	0 0 1 0	

Clinical Feature	Metric	Patients with ChP (14)
Periodontal Classification	Stage III (n) Stage IV (n)	10 4
Deepest Periodontal Pocket (mm)	Mean (SD)	7.60 (1.12)
Largest Clinical Attachment Loss (mm)	Mean (SD)	8.42 (1.73)
Number of pockets ≥ 5mm	Median (Range)	36 (7-84)
Number of teeth with furcation involvement	Median (Range)	0 (0-2)
Number of sites with radiographic calculus	Median (Range)	8 (2-27)

RESULTS

A. csEN model values before and after ChP treatment



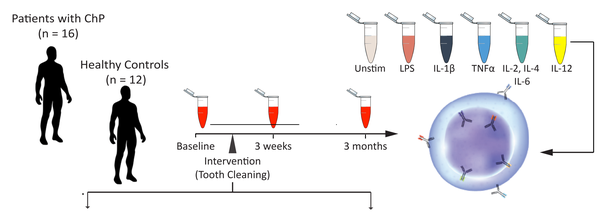


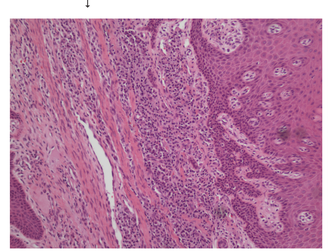
B. csEN model features at baseline

C. csEN model features after ChP treatment

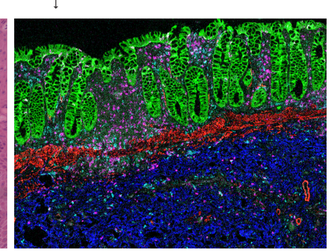
The csEN values are increased in samples from patients with ChP before treatment compared to controls (9 patients, 7 controls, Wilcoxon rank-sum test p=7.9E-3) but not after treatment. Graphical representation of csEN model component values overlaid on the immune signaling network for the patients who underwent scaling and root planing (n=9) or prophylactic cleaning (n=7). Nodes are colored by the median difference in signaling activity (arcsinh transform) between patients with ChP and controls. The size of the nodes varies according to the p-value (Wilcoxon rank-sum test, one-tailed).

PHASE II





H&E stain of gingival tissue
Courtesy of **Dr. Xiaoyuan Han, PhD**



Imaging mass cytometry
Image of colon tissue
courtesy of **Dr. Kristen Rumer Md, PhD**
red: alpha smooth muscle actin; green:E-cadherin;
magenta: CD8; blue:collagen I; cyan:CD163 (macrophage)