The Role of CD5 in the Pathogenesis of Periodontal Disease

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Abstract

Periodontal disease is a chronic inflammatory oral disease initiated by *P. gingivalis*, a gram-negative bacterium, within the periodontal pockets that surround teeth. CD4+ T helper cells (Th) play a key role in the oral adaptive immune response. However, hyperactive Th cells produce inflammatory cytokines that accelerate alveolar bone loss and soft tissue damage. CD5 is a coreceptor found on T cells that inhibits T cell activation and limits effector function. We hypothesize that CD5 knockout (CD5KO) T cells have increased activation and effector function compared to wildtype when exposed to *P. gingivalis* LPS and that CD5 is a potential drug target to reduce inflammation in periodontitis. Using flow cytometry, we observed an increase in early and late T cell activation markers (CD25 and CD69) in CD5KO T cells co-cultured with gingival or oral mucosal epithelial cells stimulated with *P. gingivalis* LPS. We also analyzed the gene transcription and protein expression of key cytokines from T cells that contribute to inflammation and alveolar bone loss using RT-qPCR and ELISpot analysis. Our results demonstrate that CD5 plays a prominent role in periodontal disease. This research will provide valuable insights for novel treatments for periodontitis.

Characteristics of Periodontal Disease

Periodontal disease is a specific oral inflammatory disease initiated by *P. gingivalis*, a gram-negative bacterium that stimulates a chronic immune response in the oral tissue [1]. Interactions between *P. gingivalis* bacteria and oral mucosa and gingival epithelium cells lead to the release of cytokines into the periodontal pocket that recruit T cells to initiate an immune response [2]. In the case of periodontal disease, T cell function, differentiation, and proliferation exaggerate the immune response and lead to an increased inflammatory response and accelerated bone decay.

Figure 1: In a 2009-2014 study done in the American population, data showed that half of American adults 30 years or older suffered from a gum disease (mild, moderate, or severe periodontitis) and that 7.8 percent of those cases were issues of severe periodontitis [3]. Periodontal disease also has a widespread presence globally. In 2016,the World Health Organization (WHO) reported that over half of the global population suffered from oral diseases caused by tobacco and/or poor oral hygiene [4,5].

T cell function and the CD5 Co-receptor

Our hypothesis is that T cell activation is regulated by CD5, which affects the inflammatory response and pathogenesis of periodontal disease.

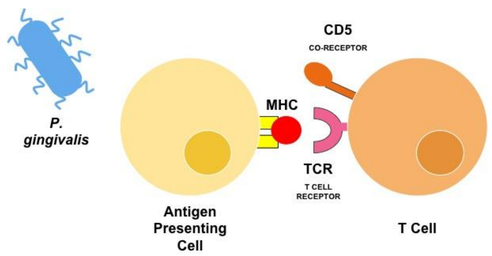

Figure 2: T cell function is regulated and initiated by T cell signaling receptors (TCRs) and co-receptors found on the surface of T cells that inhibit or enhance the T cell immune response. CD5 is a co-receptor found on B cells and T cells. Previous studies have shown that CD5 + B cells play a significant role in the overactive oral immune response characteristic of periodontal disease and we aim to determine the role of CD5 in the pathogenesis of periodontal disease [6]. To our knowledge, no studies have been performed to analyze the function of CD5 in the T cell immune response. We therefore are seeking to characterize the role of CD5 in T cell activation and in the initiation of a chronic inflammatory response.

Materials and Methods

1. Oral Mucosa and Gingival Epithelial Cell Lines
2. Cell Lines were exposed to LPS from *P. gingivalis* (LPS-PG) and LPS from *E. coli* (LPS-EC) for 24 or 48 hours
3. Splenocytes from CD5KO and CD5 WT mice were extracted and then extra T cells were co-cultured with the Oral Mucosa and Gingival Epithelial Cell Lines
4. Differences in the T cell activation were measured after 24 hours and 48 hours by analyzing expression of CD69 and CD25 activation markers by flow cytometry
5.RNA analysis/ELISpot Cytokine Assay
6.T cells were isolated from the splenocytes of CD5KO and WT mice and stimulated with *P. gingivalis* for ELISpot plating and cytokine antibody binding.

T cell Activation Markers

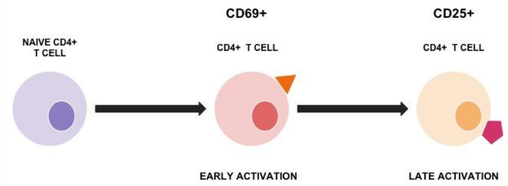

Figure 3: We measured differences in activation marker expression using flow cytometry after oral mucosa and gingival epithelial cell lines were exposed to *P. gingivalis-LPS* and *E.coli-LPS* and co-cultured with T cells from CD5KO and WT mice.

CD69 (Early Activation Marker)

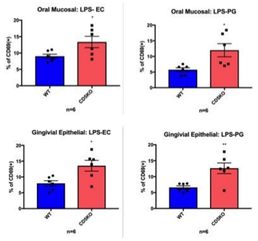

Figure 4: CD69, an early T cell activation marker, is upregulated in CD5KO cells after 24 hours, This data indicates that there is an increase in T cell activation in CD5KO cells, supporting our hypothesis that T cell activation is regulated by CD5.

CD25 (Late Activation Marker)

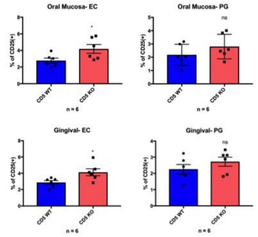

Figure 5: CD25 an late T cell activation marker, is upregulated in CD5KO cells after 48 hours, This data indicates that there is an increase in T cell activation in CD5KO cells, supporting our hypothesis that T cell activation is regulated by CD5.

Cytokine Differences between CD5KO and WT T cells

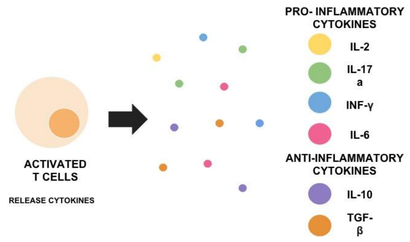

Table. Differentially expressed genes in CD5KO CD4+ T cells		
Gene Name	**Fold Change**	**Description**
IL-10	7.41	Anti-inflammatory cytokine
IFN-gamma	3.39	Produced by Th1 cells; differentiation of CTLs; Macrophage activation
TGF-beta 3	2.60	Anti-inflammatory; Immunoregulation
RANKL	1.96	Osteoclast Differentiation
Colony stimulating factor 1	6.28	Osteoclast Differentiation
interleukin 21	7.46	Th17 differentiation

Figure 6: RNA sequencing data shows that inflammatory cytokines are upregulated in CD5KO T cells. The next figure presents data from a preliminary ELISpot assay to measure cytokine production (IL-2, IL-17A, IFN-gamma, IL-10, TGF-beta1, and IL-6) which are linked to inflammation and periodontal disease as well as T cell differentiation.

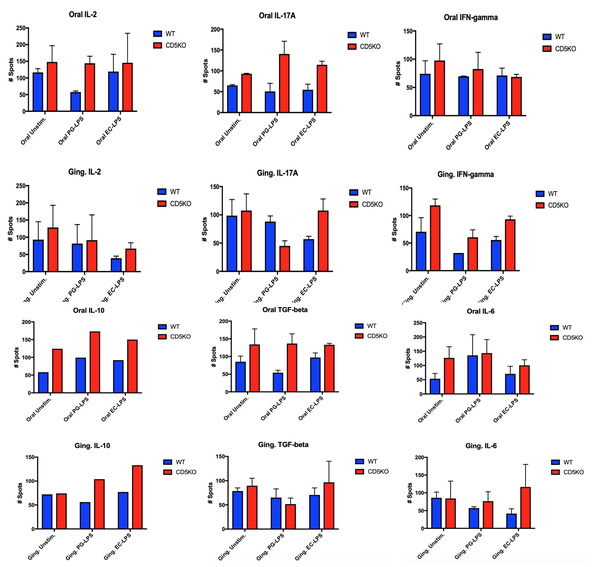

Figure 7: Preliminary Data using two replicates.. CD5KO T cells are shown to increase T cell activation even at basal level in a periodontal environment. These pro-inflammatory and anti-inflammatory cytokines are upregulated in comparison to WT cells.

Future Directions

We will further test our hypothesis by continuing our analysis of cytokine production differences between WT and CD5KO T cells by completing our last replicate.

Recent RNAseq data comparing transcriptome differences between naive CD5KO and WT cells show increased transcription of RANKL and M-CSF in CD5KO cells. In future experiments we will also measure WT and CD5KO T cell RANKL production and M-CSF, both contributing factors to osteoclast differentiation and alveolar bone decay in CD5 periodontal disease.

Additionally we will measure T cell migration to better understand the activation and immune response properties that differ between WT and CD5KO T cells and to further investigate the role of CD5 in the inflammatory response associated with periodontal disease.

Conclusions

CD5 plays a role in the inflammatory response associated with periodontal disease

Upregulation of CD69 and CD65T cell activation markers in CD5KO T cells co-cultured with gingival and oral mucosal epithelial cells and stimulated by *P. gingivalis* provide evidence that CD5 plays a role in the chronic inflammation characteristic to periodontal Disease. Preliminary inflammatory cytokine as well as RNA sequencing and Bioinformaticss analysis show that cytokine production could differ between CD5KO and WT T cells in periodontal Disease. These findings support our hypothesis.

Our findings will help us to develop novel treatments and avenues to mitigate and reduce the inflammatory immune response and alveolar bone decay characteristic of periodontal disease. Studies show that periodontal disease is also strongly correlated with the presence of other severe chronic inflammatory conditions, including cardiovascular disease, diabetes, and arthritis [7]. Although the exact mechanism of interaction between these similarly characterized diseases is currently unclear, understanding more about the pathogenesis of periodontal disease can help construct the molecular narrative about chronic inflammatory disease and lead to increased research opportunities and treatments.

References

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Contributions

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