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BSP Frank Ashley Report

Introduction

This summer I undertook a research-based elective at the Rheumatology Unit, Department of Medicine, Karolinska Institute in Stockholm, Sweden, with Dr Karin Lundberg's group, investigating a possible aetiological link between the periodontal disease-associated bacterium *Porphyromonas gingivalis* and rheumatoid arthritis (RA). I also visited Dr Tulay Linderbeg's research group in the periodontology department at Karolinska University Dental Hospital.

Background

Periodontitis is a chronic inflammatory disease that has been linked to other systemic health problems such as RA, (Winning and Linden, 2015; Hajishengallis, G. 2015). RA is a chronic inflammatory autoimmune disease that primarily affects the joints and many RA patients present with circulating anti-citrullinated peptide antibodies, (ACPAs), which are a diagnostic indicator. Citrulline is an amino acid that can replace the amino acid arginine in a process called citrullination. Citrullination is a part of normal physiology in cells, but has also been linked to cell death and inflammation including the rheumatoid joint. Protein citrullination is induced by a family of enzymes termed PADs, (peptidyl arginine deaminases). The precise details of RA pathogenesis are incompletely understood. Nonetheless, it is known that in RA there is citrullination of connective tissue peptides in the joints. This is followed by influx of ACPAs, and immune complexes into the joints, and inflammation and tissue destruction.

In RA, the peptides that become citrullinated and targets of ACPAs are not fully understood. Potential candidate peptides include fibrinogen, vimentin, alpha enolase and histones (Wegner et al., 2010). The site of peptide citrullination is also incompletely understood and the lungs and oral mucosa have been implicated. Periodontitis is thought to cause citrullination of host and bacterial peptides, which may be associated with the formation of these ACPAs in RA patients, (Hajishengallis, G. 2015; Potempa, Mydel and Koziel, 2017). Furthermore, *P. gingivalis,* a bacterium closely associated with chronic periodontitis*,* is the only known microorganism to express a PAD enzyme, (known as P.PAD) (McGraw et al., 1999).

It has been hypothesised that P.PAD may citrullinate bacterial peptide, and this may initiate an ACPA response to the citrullinated bacteria peptides. Some of the bacterial peptides resemble human peptides, and it has also been suggested that the antibodies may undergo mutations, which result in the antibodies becoming reactive against host peptide. In genetically predisposed individuals, this may cause antibodies to target the inflamed joint where host citrullinated peptides are found. Interestingly, patients with RA have shown an antibody response to a citrullinated peptide of the P.PAD enzyme of *P. gingivalis,* 'Citrullinated *P. gingivalis* PPAD' (CPP3) (Khlarlamova, 2018).

Aims

My elective involved performing ELISAs to test for reactivity with the following citrullinated antigens in serum samples from patients with chronic periodontitis (PD) and healthy controls (non-PD):

1. CCP2: Synthetic citrullinated peptides used in the screening test to diagnose ACPA+ RA
2. Citrullinated alpha enolase (CEP-1) and citrullinated histone (CitHis4 31-50): potential ACPA targets.
3. Arginine alpha enolase (REP-1): To compare reactivity compared with citrullinated version CEP-1.

Methods

I used a direct ELISA technique to measure antibody concentrations in serum by adding patient serum to plastic wells coated with target antigen (e.g. citrullinated peptide). Incubation gives time for citrulline specific antibodies to bind to coated citrullinated peptide present. A detector antibody is then added, which binds to the serum antibodies that have bound to the citrullinated peptide coating. Substrate (TMB), is added, causing the detector antibody to undergo a colour change. Increased colour change correlates with increased detector antibody present, which in turn means increased levels of serum antibodies specific for the citrullinated peptide. The colour change can be quantified by reading the optical density and the more colour change, the higher concentration of antibodies present. The ELISA technique is illustrated in figure 1.

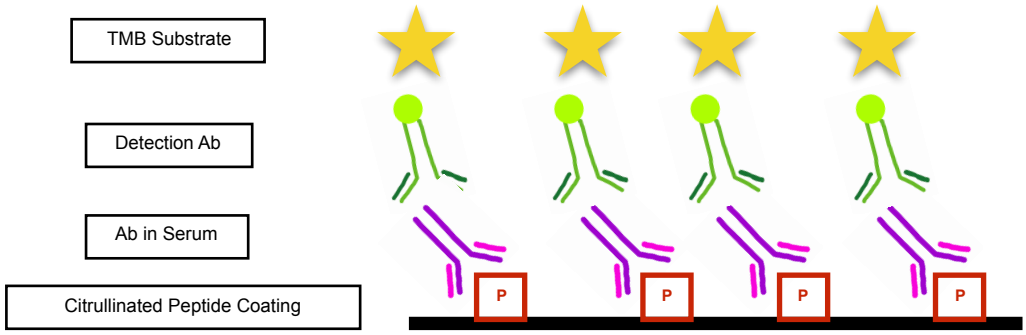

Figure 1: Stages of ELISA technique

Results

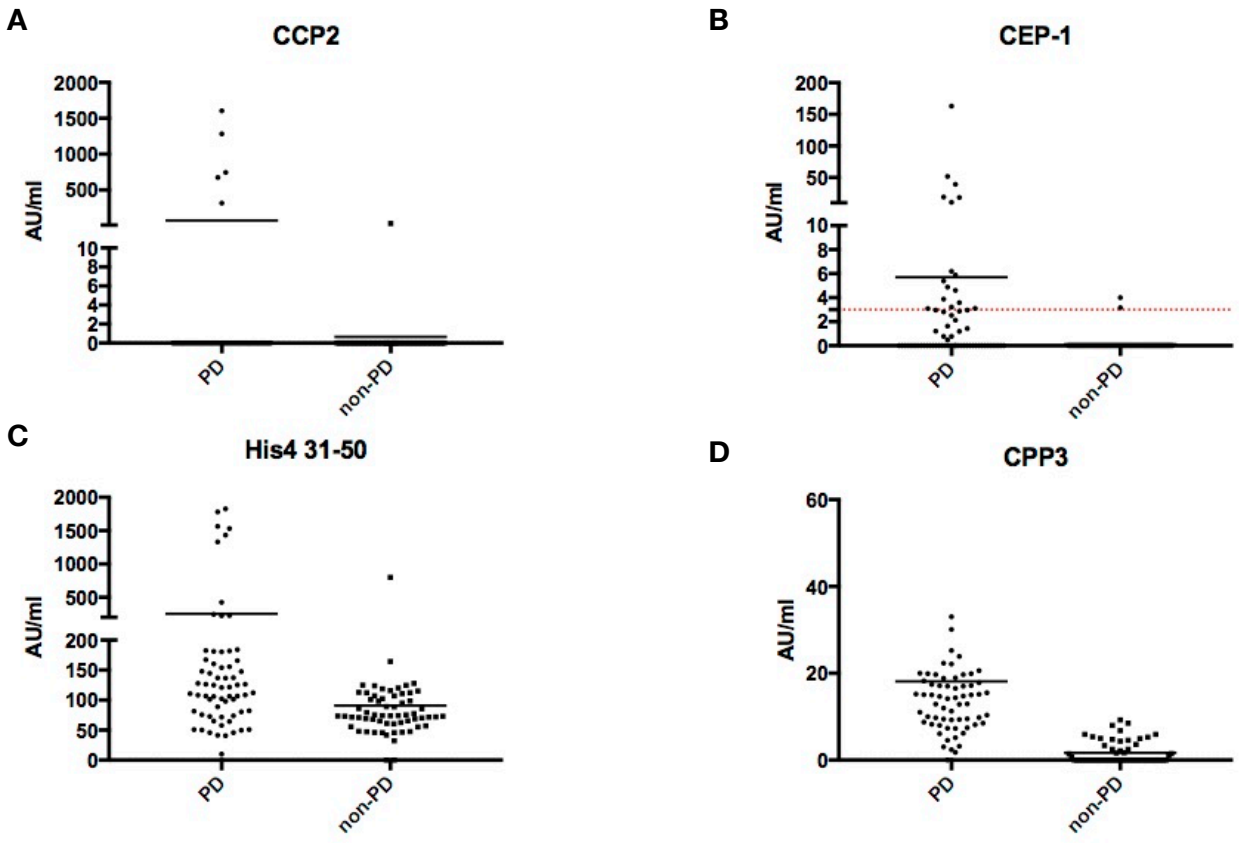

Figure 2: ELISAs were carried out to determine assay results showing elevated ACPA levels in systemically healthy patients with periodontitis (PD), compared to periodontally healthy controls (non-PD). Data are shown for CCP2 IgG (A), CEP-1 IgG (B), CitHis4 31-50 IgG (C) and CPP3 IgG (D). Serum samples from n=64 PD patients and n=56 non-PD controls were analysed at 1:10 dilution (CCP2), or at 1:100 dilution. The dotted line in C indicates cut-off for positivity, set based on n=120 non-RA population controls. Each dot represents 1 patient sample and the bar indicates mean value. Y-axes show antibody levels as AU/ml values, calculated based on standard curves.

There appeared to be increased serum antibody against CCP2, CEP-1 and CitHis431-50 in PD patients compared with non- PD patients and therefore shows ACPAs are detected in systemically healthy patients with periodontitis (Figure 2A, B, C). Experimental data obtained prior to my project demonstrated an increased antibody response for CPP3 in PD patients compared with non PD controls (Figure 2D) (Khlarlamova, 2018).

Next, the serum reactivity against citrullinated compared with native (non citrullinated) peptides was investigated. A previous experiment conducted shows the increased reactivity against CPP3 compared with arginine-containing control peptide RPP3 in PD patients (Figure 3A) (Khlarlamova, 2018). Figure 3B shows the CEP-1 antibody response was largely citrulline-specific in periodontitis patients, as the antibody response against the arginine-containing control peptide REP-1 was weak, when compared to the CEP-1 antibody response.

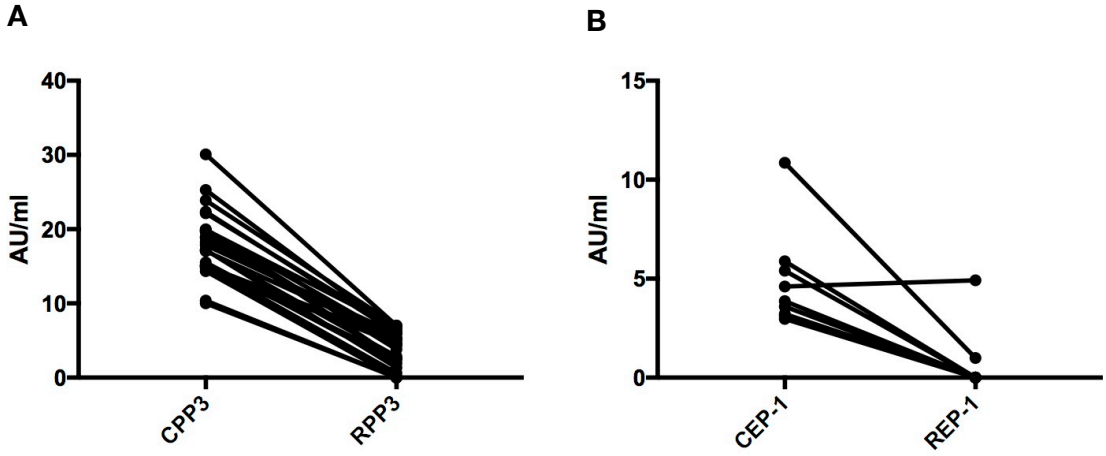

Figure 3: ELISA assay results showing citrulline- specific ACPA responses against CPP3 and CEP-1 in systemically healthy patients with periodontitis. Low antibody reactivity was recorded against the arginine-containing control peptides RPP3 (A), and REP-1 (B). Serum samples from n=64 PD patients were analysed for CPP3 and RPP3 reactivity at 1:100 dilution; serum samples from n=31 PD patients were analysed for CEP-1 and REP-1 reactivity at 1:100 dilution. Each dot represents 1 sample. Y-axes show antibody levels as AU/ml values, calculated based on standard curves.

Conclusion

Taken together, these results show a trend towards more reactivity against citrullinated human and bacterial antigens in periodontitis patients, compared to periodontally healthy controls. Moreover, the antibody responses appear to be citrulline-specific, as reactivity against the arginine-containing control peptides was low.

However, this pilot study must be repeated with increased number of samples and appropriate statistical tests performed to confirm any significance. Long term clinical implications for this research include helping to design pre-clinical intervention strategies to eliminate oral pathogens and treat periodontal disease in

individuals at risk, or in the early stages of RA, and to determine whether this can help reduce the disease process of both periodontitis and RA. For example, future studies could investigated the potential use of antibodies against CPP3 as a serological biomarker for the identification of PD patients at increased risk of developing RA.

Reflection

Upon reflection, I feel privileged to have had such an amazing opportunity to work in this exciting research environment. Performing experiments, participating in discussions and working with many different people opened my eyes to many different types of critical thinking, interpretations and analysis of research, as well as increasing my breadth and depth of knowledge of periodontal disease and rheumatology.

Of note, I was lucky enough to be given free attendance to a rheumatology away day whilst working in the lab. This was a conference where PhD students and post docs presented their ongoing research in the field of rheumatology. Throughout the day, hearing about the complex links between chronic inflammatory disease personally resonated with me and I believe that in the future, the field of periodontology will have increased collaboration with medical colleagues as these links become clearer.

It was particularly enjoyable visiting the Dental Hospital and Dr Tulay Lindberg. I had the opportunity to discuss and meet with staff and students from the periodontology research department. One of the students was starting an epidemiological project investigating periodontitis and rheumatoid arthritis and it was interesting to hear about another type of research into periodontal disease other than lab based.

I feel that this elective has inspired me to continue to pursue a career combining academia and clinical dentistry. Next academic year, I have decided to undertake a special study module in clinical research to continue developing my skills further.

References

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