Abstracts

Abstract-English

Periodontal Disease (PD) refers to a combination of inflammatory processes occurring in the gingival tissues surrounding the teeth, as a result of bacterial accumulations known as dental plaque. A staggering number of 50 % of the worldwide population has gingivitis (inflammation without any bone loss) qualifying this disease to constitute a global health problem. Numerous scientific studies underline that PD is clinically associated with the development and progression of Rheumatoid arthritis (RA) as well as other comorbidities. Mechanistically this link may be due to the activity of Peptidyl Arginine Deaminase (PPAD) expressed by a major periodontal pathogen, *Porphyromonas gingivalis*. PPAD preferentially citrullinates C-terminal arginine residues on peptides and proteins, thus creating neo-epitopes on host proteins. This process may lead to generation of anti-citrullinated protein antibodies (ACPA) and consequently to development of early onset RA.

The primary objective of this PhD project was focused on developing an effective inhibitory formulation against PPAD that would represent an important tool in our efforts to dissect the biology of this clinically relevant protein. As it is challenging to measure the mass increase of only +0.984016 between the non-citrullinated substrates and citrullinated products an activity assay for quantifying PPADs activity *in-vitro* was constructed. A novel purification methodology for the isolation of chicken polyclonal antibodies (IgY) was established and the antibodies raised against PPAD antigen were purified. Inhibitory potential of the obtained affinity purified IgY was evaluated using the constructed assay and showed 70 % inhibition of PPAD's activity *in-vitro*. The activity assay and the chicken polyclonal anti-PPAD antibody represent convenient tools that can be further utilized in studies on the biology of the PPAD enzyme as well as its connection to other diseases.

Additionally, a platform for the discovery of chicken single chain Fv fragments has been established. By using molecular cloning techniques, a chicken scFv phage display library has been constructed from hens immunized with PPAD antigen. Through panning and screening procedures a single binder has been identified, representing a great promise for the generation of the first monoclonal antibody against PPAD.

A recently discovered enzyme Karilysin (KLY18) from an oral pathogen *Tannerella forsythia* has also been associated with the development of pathological processes during periodontal infections. Chicken polyclonal antibodies have been developed against Karilysin and their diagnostic potential has been evaluated via construction of an immunological assay for quantification of the enzyme's concentration in biological samples. Significantly higher Karilysin concentrations were found in PD patient group when compared with the control group, thus qualifying the anti-KLY18 antibodies as a useful diagnostic reagent for assessing the presence of PD in clinical settings.