New Insights in Autoimmune Polyendocrine Syndromes 1 and 2

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Nicolas Kluger defended his PhD thesis on February 27, 2015 at the University of Helsinki. His main supervisor was Professor Annamari Ranki, Department of Dermatology, Venereology and Allergology, University of Helsinki and Helsinki University Central Hospital. The other supervisor was Associate Professor Camilla Schalin-Jäntti, Division of Endocrinology Department of Medicine Helsinki University Central Hospital. The Opponent was Professor Mikael Knip, Department of Paediatrics Helsinki University Central Hospital, Helsinki, Finland. The thesis is available at http://urn.fi/URN:ISBN:978-951-51-0832-6.

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, OMIM 240300) is a rare autosomal recessive disorder caused by mutations in the autoimmune regulator (*AIRE*) gene located on chromosome 21 (21q22.3). *AIRE* deficiency causes a loss in central immune tolerance, leading to the failure to eliminate autoreactive T cells in the thymus and allowing their escape to the periphery. Because of a founder effect, APECED is particularly prevalent in Finland (1/25,000) but is observed worldwide with variable prevalence. APECED patients are susceptible to mucocutaneous candidiasis and multiple endocrine autoimmune diseases such as primary hypoparathyroidism, adrenal insufficiency, primary hypogonadism, type 1 diabetes, hypothyroidism, and hypophysitis. They may also develop additional nonendocrine autoimmune diseases, such as alopecia areata/totalis, vitiligo, gastro-intestinal (GI) diseases, keratitis or tubulointerstitial nephritis (TIN). In addition, the patients typically develop a variety of serum tissue-specific autoantibodies, which are predictive of the development of autoimmune disease and anticytokine antibodies such as those against type I interferons and Th17-related interleukin IL-17 and IL-22.

The aim of this thesis was to study such manifestations of APECED that have not been well characterized before and also, to study health-related quality of life (HRQoL) among Finnish APECED and Addison s disease/APS2 patients.

We evaluated the clinical GI features and searched for novel markers of GI dysfunction in a Finnish cohort of 31 APECED patients. The main upper GI symptoms were dysphagia and retrosternal pain (45%) and the lower GI symptoms were constipation (48%), diarrhoea (45%), and malabsorption (16%). Previously, L-amino-acid decarboxylase (AADC) and tryptophan hydroxylase type 1 (TPH-1) antibodies have been demonstrated in APECED. AADC antibodies were found in 51% and TPH-1 antibodies in 39% of all patients. Also, a T-cell response to AADC was detected in 43%. One third of the patients had autoimmune enteropathy (AIE)-related 75 kDa antigen (AIE-75, 33%) and villin (29%) autoantibodies, and antibodies against brush borders and Paneth cells were detected in 29% and 20%, respectively. Mucosal intestinal IL-17 expression was decreased or negative in 77% of the intestinal samples. Duodenal chromogranin A and serotonin expression was absent or decreased in 50% and 66% of the patients, respectively. Of the clinical symptoms, constipation correlated with negative serotonin staining ($p<0.05$) and with AADC antibodies ($p=0.019$). Importantly, we found a correlation between autoantibodies against AADC, which is critical for serotonin and DOPA synthesis, and constipation. Constipation was also associated with a lack of serotonin expression in the enteroendocrine cells (EECs). Paneth cells were lacking in the duodenum in 20% of our intestinal samples, even though this was not associated with GI symptoms. In this Finnish APECED patient cohort, 17% (5/30) had moderate-to-severe

Fig. 1. Nicolas Kluger (*left*) defended his PhD thesis on autoimmune polyendocrine syndromes 1 and 2 in Helsinki in February 2015. Professor Mikael Knip was the Opponent (*2nd right*) and Professor Annamari Ranki acted as Custos and Supervisor (*2nd left*).together with Professor Camilla Schallin-Jäntti (*right*)

renal failure, including 10% (3/30) with TIN requiring transplantation, haemodialysis or immunosuppressive treatment. However, the latter did not seem to be efficient in controlling disease progression. All 3 patients with TIN had circulating antibodies against the distal part of the nephron, as did 30% of all cohort cases. The pathogenic relevance of such circulating antibodies is still unclear.

The immunological basis of hypoparathyroidism in APECED was explored by studying circulating calcium-sensing receptor (CaSR) and NALP5 antibodies. Although they were detected in 16 of 44 (36%) and 13 of 44 (30%) patients, respectively, we failed to find any clinically relevant statistical association. These APECED patients did not present circulating antibodies for other autoimmune diseases such as rheumatoid arthritis, celiac disease, bullous pemphigoid or pemphigus vulgaris. Some patients had antinuclear antibodies at a low-titre without clinical significance. Secondly, we evaluated the HRQoL among Finnish APECED and Addison s disease/APS2 patients and sought to determine which factors may predict a possible impairment.

Using HRQoL questionnaires for APECED (SF-36) and Addison s disease/APS2 patients (SF-36, 15D), we indeed observed impaired HRQoL. For the APECED patients, general health, emotional well-being and energy/vitality were the most diminished aspects of HRQoL. Among the patients with Addison's disease/APS2, compared to a large control population, physical or emotional role functioning, energy/vitality and general health were most affected. Discomfort and symptoms, vitality, and sexual activity were the most affected dimensions of the 15D scores. Affiliation with a patients' association, female gender, the presence of non-APS2 inflammatory comorbidities, lower educational level and a longer disease duration were independent predictors of impaired HRQoL in these patients.

Taken together, the results of this thesis show that APECED patients are genetically prone to develop autoantibodies to a multitude of tissue antigens but are still tolerant to some common autoantigens. The true clinical and biological relevance of these circulating autoantibodies has not yet been elucidated, and it is possible that they are only a reflection of T-cell-mediated immunity. They may, however, have a cumulative effect and clinical disease may arise only in patients with a combination of circulating antibodies, as seen in diabetes type 1. This may explain why we failed to find any association between any single type of antibody and a given symptom. For the lower GI track manifestations, we hypothesise a cumulative effect of the autoimmunity directed against both the enteroendocrine cells and the Paneth cells, leading to a dysfunction in both the secretion of serotonin in the gut and the secretion of antimicrobial defensins. Such a disturbance would have an effect on the gut microbiota.

The question of whether the neutralising antibodies against cytokines may have a paradoxical protective effect is open to debate. Lastly, despite having a high number of manifestations, patients with APECED seem to cope with their disease. Patients with Addison's disease have significantly impaired HRQoL compared to the general population.