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Re: evaluation of Mgr. Zuzana Paračková' PhD thesis:
Immune system dysregulation in type 1 diabetes"

Type 1 diabetes (T1D), due to its high incidence and far reaching socio-economic consequences, is one of the most studied autoimmune diseases worldwide. The aetiology of T1D is multifactorial and clearly involve genetic and environmental factors which somehow intersect and trigger the onset of disease. However, despite the thousands of publically available scientific articles related to this topic, the precise causes and mechanisms still remain to be elucidated. Unfortunately, this situation is mirrored by the failure of all immunotherapeutic approaches that have been utilized to provide an effective and widely applicable cure for T1D patients.

In this context, the thesis of Mgr. Paračková represents a successful attempt to analyze the contribution of innate and adaptive immune mechanisms and its cellular elements involved in the onset and maintenance of T1D. This specifically concerns the involvement of neutrophils, lymphocytes as well as dendritic and myeloid cells in the aetiology of T1D. Clearly, reading the thesis and the battery of accompanying primary papers which were published in prestigous international journals such as *Frontiers in Immunology, Scientific Reports, Journal of Autoimmunity, Immunology Letters,* on which Mgr. Paračková is the first author, it is clear that the the body of work chosen for these studies are unique, translational and in many aspects highly innovative. Collectively, the results of these studies has moved us one step closer to a better understanding of the potential causes of T1D. In my opinion, among the papers which are directly related to the main topic of this thesis, the manuscript which describes the potential role of neutrophil extracellular traps (NETs) in the aetiology of T1D clearly stands out. This work, in the midst of some controversies regarding the dysregulation of NET formation in T1D patients, showed that NETs could be one of the proximal events which lead to or contribute to the onset of T1D. While time is needed to validate the importance of this result for clinical practise, there is no doubt that this work represents the frontline research in the field of T1D. It is also important to emphasize that the stimulating environment and highly productive laboratory of Prof. Šedivá, the author's mentor, certainly played an indispensible role in this success.

By the same token, the wide-spectral scope of Mgr. Paračková thesis, which is strongly supported by experimental data that was obtained through a variety of methodological approaches demonstrates her intellectual capacity, hard work ethic, and practical skills. The

thesis is well written. It is presented in a shorten version with the abstract and literature overview, which is further split into two major blocks with several sub-chapters. Overall, the reading of this chapter is smooth and informative. 234 primary and review papers were used as literature sources and the history as well as the newest discoveries in the field of T1D are concisely yet comprehensively documented and described in the way which doesn't lack attractiveness and excitement. The chapter "Aims of the thesis" briefly introduces the main aims of this study and is followed by the list of Mgr. Paračková' original published works. This portfolio consists of an impressive 16 publications. The first eight are directly linked to the presented thesis while six of those publications are of the first author. The main topics of remaining eight publications deal with immunodeficiences and recent COVID19 research, of which four of them Mgr. Paračková is the first author. The major achievements of the former eight studies are briefly summarized in a separate chapter "Results" and are complemented with clearly stated conclusions. I consider the end result of Mgr. Paračková PhD study to be outstanding and unique. I believe that such achievement in this highly competitive field of research deserve a full admiration from her colleagues and scientific community.

Since I feel that conclusions of presented studies are very important and strong, I would like to use this opportunity to ask the following questions which largely concern the biological aspects of NETs in the aetiology of T1D:

1. **Neutrophils and NET formation**. Your proposal that NETs are involved in T1D pathogenesis is very provocative and intriguing. However, it is still not clear why T1D patients suffer from tangible neutropenia. Can the phenomenon of neutropenia itself account for different NET quality in T1D versus HDs? If it is safe to assume that each neutrophil contains the same amount of DNA and associated histones, then why would the quantity of DNA-histone complexes and other components differ in NETs from T1D and HD neutrophils? Can this quantitative difference account for a dramatic alteration in the immunogenicity of NETs? Can posttranslational modifications of NETs play a role? Are the kinetics of NET formation in neutrophils from T1D same as in HDs?

2. **NETs, Islet antigens and their presentations.** In your paper published in *Front Immunol (2020)* you suggested that NETs could contain islet antigens which via their presentation on DCs can elicit T and B cell specific responses. How would these antigens from pancreatic β-cells become associated with NETs? Apoptotic β-cells should be phagocytosed by DCs. Can neutrophils do the same? Is there any solid evidence for this? Could a triple β-cell-DC-neutrophil interaction be involved? Can NETs function as a costimulatory signal via TLR9 on DCs which phagocytosed apoptotic β-cells? Contradictory to your proposed model, the observed higher concentration of DNaseI in the serum in T1D patients suggests that the immunogenic effect of NETs is time-limited. How do you reconcile this conundrum? Last, but not least, why is it that physiological death of pancreatic β-cells provokes the recruitment of neutrophils to pancreas and their local netosis before the onset of T1D? Could you provide an illustration of your general model highlighting how NETs contribute to the onset and/or maintenance of T1D?

3. **Monocytes and DNA recognition.** Your own analysis of moDCs from T1D and HD showed that they differ in the expression profile of more that 1500 genes even in the unstimulated state. The literature which you have cited in your work refers to the fact that differentially expressed genes (DEGs) can be revealed also between T1D and HD blood monocytes from which moDCs are derived by *in vitro* culture. How overlapping are these two sets of DEGs? How long before the onset of T1D can these monocyte-related DEGs be detectable? Is the upregulation of gene expression of IFI16, DHX36, cGAS and STING after CpG treatment dependent on TLR9?

4. **Mouse model of NETs in T1D.** Is the NOD mouse model suitable for the study of the role of NETs in the aetiology of T1D? Interestingly, TLR9 $^{-/-}$ NOD is resistant to T1D onset (*Diabetologia, 2018, 61:2333*). Do you plan to use this mouse model in attempts to elucidate the role of NETs in T1D aetiology?

Based on the presented review above, I consider this thesis to be excellent, well written, and scientifically insightful. Without hesitation, I recommend the presented thesis of Mgr. Paračková to be accepted as the fulfilment of the requirements for the PhD degree.

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