I N S E R M U 1149

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Paris, August 8th, 2022

Object: Review of PhD manuscript of Ewa Maria Sroka

The PhD manuscript of Ewa Stroka is entitled “The role of alternative sources of antigen peptides for the major histocompatibility complex class I in the formation of immune responses and immune tolerance” and written in English. After a comprehensive abstract and a list of abbreviations, the manuscript starts with the introduction chapter (40 pages) that I strongly enjoyed. The introduction exposes in a very concise and clear way the main features of antigen presentation starting from the structure of MHC complex, followed by the description of the molecular mechanisms leading to the generation of MHC ligands. Almost 200 references are cited during the introduction chapter, covering in a historical manner the topic of antigen presentation, from the first discovery of MHC to the most recent advances in the field. The critical point of view on the published reports and the didactic way of presenting them are a strong demonstration of the scientific maturity of the PhD candidate.

The aims of the study are well explained. Briefly, the main goal of the PhD study was to evaluate the role of intron derived antigen peptides in MHC class I antigen presentation and subsequent CD8 T cell activation. The model for intron derived antigenic peptides consists in the introduction of SIINFEKL sequence in the intron 2 of beta globin gene, named hereafter “beta-globin-SL8”.

The work is structured in 2 aims: 1/ *in vitro* analysis of the SIINFEKL antigen production and presentation via MHC class I using “beta-globin-SL8” construct expressed in cell lines and 2/ in vivo assays of SIINFEKL antigen presentation using a “beta-globin-SL8” transgenic mouse in which the SIINFEKL coding

sequence has been introduced in the intron 2 of beta-globin gene. This mouse model, generated during the PhD training is named HBB.

Following the objective description, the manuscript presents the material and methods section (16 pages) and the experimental results (40 pages). It is important to mention that the manuscript does not include the draft of a scientific article, but the methods and the results are written by the candidate in an exhaustive and comprehensive way, dedicated to the thesis manuscript. This is remarkable.

I do not insist on the material and methods section, I just mention that it is well written and with enough details, allowing the reproduction of the experiments performed. In contrast, I would like to comment on the experimental results, which are of high quality and very interesting and original.

The *in vitro* experiments demonstrate that the "beta-globin-SL8" construct is well expressed when transfected in H1299 cell line and moreover it is able to generate the SIINFEKL peptide that is loaded on MHC class I and able to activate OT1 transgenic T cells.

The in vivo experiments using HBB transgenic mice are extremely original and the obtained results are unexpected and very interesting. The transgenic mice do not show any phenotypic abnormalities, the immune cell populations are normal and the mice do not show anemia. The transgenic modification does not affect the beta-globin levels and the "beta-globin-SL8" mRNA is detected in the bone marrow, blood and spleen. As expected, the transgene mRNA is not expressed by the mature erythrocytes, but probably by erythroblasts. The most original and important finding of this part is that the transgene expression induces a robust immunotolerance in the HBB mice. This is a major finding with important consequences for the field of anti-tumor immunity, but also for example in the field of gene therapy, when the immune tolerance is essential to avoid immune response against the transgene.

The last sentence of the PhD manuscript is" I hope the work presented here will be taken forward as well as be a source of scientific inspirations…".

Indeed, this work is a great source of scientific inspiration by opening several new scientific questions, which are excellently approached by Ewa Sroka in the discussion chapter, evidence of her scientific maturity.

To conclude, I appreciate the quality of scientific writing, the rigor of experiments presented, the originality of the results obtained and the critical point of view of the candidate on both her personal results and the published reports. The discussion section presents very well the questions remaining open, such as, for example, the nature of cell population that presents *in vivo* SIINFEKL peptide and induces immune tolerance.

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I am entirely in favor of the defense of this thesis and I believe that the candidate merits to obtain the title of doctor with distinction.
The results of this PhD training have been submitted for publication and are in positive revision to PNAS. Considering their quality, their novelty and their potential for design of new therapeutic strategies for breaking immune tolerance in oncology or for establishing immune tolerance in gene therapy, I believe that the scientific article will be published in a high impact journal.
In addition to the manuscript in revision, Ewa Sroka is co-author of other 5 scientific articles and has 12 presentations at scientific meetings.

I remain available for further enquiries if needed.

Yours sincerely,

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