

CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRALOVE

Department: Pharmacology and Toxicology Master's degree program in Pharmacy

Opponent's review of Master's thesis

Student's name: Anna Orbisová

Mentor of the thesis: Prof. PharmDr. František Štaud, Ph.D.

Year of the thesis
defense: 2023

Consultant of the thesis: Dr. Cilia Abad, Ph.D.

Opponent of the thesis: PharmDr. Eduard Jirkovský, Ph.D.

Title of the thesis:

Effect of inflammation on placental metabolism of tryptophan

Formal comments: number of pages: 52, number of figures: 14, number of tables: 1, number of references: 102.

Type of work: Experimental work

- a) The aim of the thesis is: Fulfilled
- b) Language and graphic level: Excellent
- c) Processing of the theory: Excellent
- d) Methods description: Excellent
- e) Results description: Excellent
- f) Discussion and conclusions: Excellent

I recommend Diploma thesis for the recognition as Rigorous thesis .

Opponent's comments:

Reviewed thesis describes changes in tryptophan metabolism in human placenta explants influenced by maternal inflammation models simulates bacterial (LPS) and viral infection (Poly I:C). The work is excellent in all formal aspects, using appropriate and well described methodological approaches and results are clearly expressed. The literature overview and discussion use relevant and up-to-date references. Although the number of references used is huge (102) some basic aspects would be better to further express for the reader to more clarify the background. I have also some comments to the methodology. These comments do not lower the value of the work, the work is excellent piece of work and I fully recommend it for the defense and recognition as the Rigorous thesis.

Questions:

- 1) In the literature background is not fully explained why tryptophan is so important for (maternal) immune reaction. What exactly is tryptophan used for there?
- 2) The distinctive inflammatory milieu (p. 17, ch. 3.3, 1st para.) – what means TH1 and TH2 in description of proinflammatory and anti-inflammatory state (resp.)? Is it only abbreviation?

- 3) Which methods did you participate by your hand? The methods section is brilliant as part of future research paper with many co-authors, but for your diploma thesis I would appreciate any explanation of your contribution on this great teamwork (with all respect to your effort and contribution on the work).
- 4) Is there any other difference between THP1 and THP2 except their predominant localization? THP2 should be in mainly in brain (as you described) and you found it in placenta. Are there any differences e.g. in enzymatic kinetics? From my point of view, THP2 responded more like THP1.
- 5) How could you explain the difference between increased expression of THP1 and THP2 detected by qPCR (Fig. 11 and 12) with decreased amount of THP1 transcripts detected by ddPCR? There is different sample size ($n \geq 8$ vs. $n = 5$). If they all are the same "patients", how did you avoid a bias, and why did you statistically compared whole data set (all groups) by Kruskal-Wallis test in Figs. 11 and 12, while in Fig. 14 were compared just selected pair of groups used by Mann-Whitney?
- 6) You did not present immunoblots for relative quantification of proteins used in Fig. 14.

Evaluation of Master's thesis: Excellent

Recommendations for the thesis defense: Recommended

In Hradec Kralove 15.9.2023

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Opponent's signature