Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

OPPONENT'S REVIEW ON DIPLOMA THESIS

Name of the student: Bc. Simona Fišerová, BSc.

Thesis title: The effect of *Ixodes ricinus* tick serpin on the cytotoxic function of natural killer cells

Supervisor: Mgr. Jaroslava Lieskovská, Ph.D.

Referee: RNDr. Jan Ženka, CSc
Referee`s affiliation: Faculty of Science, University of South Bohemia in České Budějovice

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	3
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	3
Formal requirements – points in total		20
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	2

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	3
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Practical requirements – points in total		26
POINTS IN TOTAL (MAX/AWARDED)	48	46

Comments of the reviewer on the student and the thesis:

Presented thesis is high quality well written study. All appointed aims were fulfilled.

Introduction is well arranged and transparent. However I will appreciate more detailed characteristics of NK serine proteases. I noted small mistake (species name with capital letter) as well.

Methods were well chosen. In this part I will recommend to attach list of compounds used.

Results. I appreciated careful optimization and testing of calcein-AM method. Small note - It will be better to include column with unstimulated NK cells in Fig. 12. Fig 13 can be used as well to show exactly difference observed.

Discussion. This part is critically and thoroughly written.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

Regarding Calcein-AM release method: It is not clear, if commercial kit was used. Some reference are in Fig 4 only, nevertheless origin of this kit is not mentioned.

LAL assay – it is not clear, if kit was used (producer). If not, citation is required

Isolation of NK cells from spleen – I will appreciate citation of method used. Purity determination will appreciated as well

Producer of IFN-gamma kit is missing.

Centrifugation – use always „g" instead of RPM

Relatively big background in calcein-AM release method is not optimal. 51chromium release method seems to be gold standard. Do you can recommend another method to replace this method based on radioactivity measurement?

Which methods could you recommend for preparation of better purified NK cells?

Why you suppose that IL-2 would activate mainly NK cells?

Which activated cells produce IFN-gamma?

It was shown that NK cells recognize and kill cells infected by TBEV What is the role of CTL?

How to explain the lack of the effect of IRS-4 on NK cytotoxic activity? Problem with penetration to

lytic synapse?

Did you tested IRS-4 based stimulation of IFN-gamma production in absence of IL-2?

Stimulation of IFN-gamma by IRS-4 is not expected but very interesting. Do you have further plans in this area? I will very appreciate to be informed about progress.

Conclusion:

In conclusion,

I r e c o m m e n d

the thesis for the defense and I suggest the grade 1

In České Budějovice **30.6.2020**

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signature