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Examiner´s Report on PhD Thesis

Candidate: **MUDr. Mouhammed O. Abuattiech**

Title of thesis: **Elements of Immune Fitness**

Supervisor: **prof. RNDr. Jan Krejsek, CSc**.

External examiner: **LTC. assoc. prof. RNDr. Zuzana Kročová, Ph.D.**

A. Selection of thesis theme

Present thesis deals with the influence of lymphocyte receptor diversity on the adaptive immunity functions. Since the effect of TCR and BCR diversity on effective immune response has not yet been elucidated an aim of thesis is very topical.

B. The experimental methods and the results

To solve a selected topic, MUDr. Abuattiech established 5 specific aims.

For solution of specific aim 1, 2 and 3, the candidate determined TCR V^{β} diversity, CDR3 size spectratyping of TCR V^{β}, T cell priming to Pan DR reactive epitope (PADRE) peptide, then he provided analysis of activated cells, T cell proliferation assay, delayed-type hypersensitivity assay, rejection of skin grafts and *Pneumocytis murina* infection. Range and application above mentioned methods was definitely well-founded because MUDr. Abuattiech achieved **the results** that clarify **specific aims 1, 2 and 3**:

1/ Decreased numbers of T cells or contraction of TCR diversity are not associated with immunodeficiency.

2/ The mice with contracted diversity of the T cell repertoire have relatively normal cell-mediated immunity.

3/ Adaptation of T cell compartment may be contributed by the adaption of “memory-like properties”.

4/ T cell functions on murine model are independent on B cells or immunoglobulins.

5/ Severe contraction of TCR repertoire do not cause increased *Pneumocytis* load in QM mice (they have reduced B cell diversity)

To reach the results that should make the Aim 4 and 5 clear, MUDr. Abuattiech provided thymectomy by surgery or by sham-surgery, Ig gene analysis, TRECS (T cell receptor excision circles), TCR beta chain diversity analysis, delayed type hypersensitivity assay, and then he measured surface markers by flow cytometry and immunoglobulins by ELISA and ELISASPOT. Using these methods he obtained the results that fully answered on the questions done in the **Aim 4 and 5**.

6/ The thymus contributes to priming T cell responses and to affinity maturation of antibodies.

7/ Thymectomy do not decrease T cell numbers and does not contract TCR diversity.

8/ Thymectomy does not impair primed T cell responses, enhance B cell memory and maintains "normal" T cell memory but impairs cellular immunity to minor antigens.

9/ Production of long lived antibody secreting cells is not defective in athymic mouse.

10/ The interdependence between BCR affinity and B cell presentation to T cells exist. T cell help limits the expansion and differentiation of B cells in the germinal center independently on BCR engagement. On the other hand enhancing B cell antigen presentation by GC B cells do not induce antibody affinity maturation thus B cell fate is provided by combined signals.

C. Format of thesis

Thesis is written in English without grammar and formal mistakes. Complex phenomena, relationships and connections are made readable and in understandable written form. The biggest benefit of this thesis, for me as reader, is in very well done composition that helped me understand what and why the author solved. Obtained results were drew up in two manuscripts and published in the journals with high impact factors (J Immunol IF 5.859, Eur J Immunol IF 5.103). Number of citation of these two papers reached number 18.

D. Comments and questions

I found only few mistakes and discrepancies that did not make any impact on quality of the work:

- ✓ Some abbreviations mentioned in text are missing in the list of abbreviations (p. 17, NP-specific antibody secreting cells, p.55 H&E).
- ✓ The format of references in text is not uniform (example – p.22, line 3 and line 22) .

- ✓ Description of the figures would contain complete information about the figure but results should not be described (p. 54, p. 65).
- ✓ Under the Figure 5 is written: “Statistical analysis was done with a long-rank test” but I did not find any statistical data.
- ✓ Format of standard deviation is not uniform (p. 53, 57).
- ✓ Quality of some graphs is not good, most probably because they were copied from original articles (p.61, 62, 64, 66, 67, 68, and 69). I would recommend to insert copy of paper to the thesis or to make original graphs.

I would like to have two minor and two main questions:

- ✓ In the Fig. 7 (p.58), there is shown the detection of *P. murina* by real time RT-PCR. How do you explain the presence of *P. murina* in QM mice treated with saline?
- ✓ Figure 16, p. 68. Why did you measure NP-specific IgM and IgG1 only at athymic and shamed-operated mice and not at wild type C57Bl/6 mice?
- ✓ You quote Delves and Roitt who define innate immunity (p. 12): “Innate immunity is characterized by having a preset very standardized method of responding to offending agent that is recognized in antigen-independent manner”. Antigens (toxins, bacteria, viruses, and other foreign substances) are defined as substances that stimulate production of antibodies, and I think that innate immunity is indispensably included in this process. Can you tell me your opinion?
- ✓ It was thought that quality of cell-mediated immunity depends on diversity T cell receptors. Do you have any idea how your results can influence the strategy in immunization of individuals with thymic defect?

E. Conclusion and recommendation

MUDr. Abuattiech fulfilled all five designated aims and I definitely recommended his thesis for defense.

Hradec Králové 24th July 2013

LTC. assoc. prof. RNDr. Zuzana Kročová, Ph.D.