UNIVERSITY OF CALIFORNIA, DAVIS

BERKELEY • DAVIS • IRVINE • LOS ANGELES • MERCED • RIVERSIDE • SAN DIEGO • SAN FRANCISCO • SANTA BARBARA • SANTA CRUZ

CHARLES L. BEVINS, M.D., Ph.D.
DISTINGUISHED PROFESSOR OF MICROBIOLOGY AND IMMUNOLOGY
SCHOOL OF MEDICINE
TUPPER HALL Rm. 3146
DAVIS, CALIFORNIA 95616

June 3, 2023

doc. RNDr. Magdalena Krulova, Ph.D., FRSC
Chair of the examination board
Department of Doctoral Studies
Charles University
Albertov 6, 128 00
Praha 2, CZ

RE: PhD Thesis Report, Mr. Tomas BRABEC

Dear Dr. Krulova,

It is with pleasure that I provide a report on my assessment of the doctoral thesis submitted by Mr. Tomas Brabec to the Faculty of Science of Charles University in partial fulfilment of the requirements of the degree of Doctor of Philosophy in the Study Programme Immunology.

My own research program is focused on mechanisms of innate immune function at mucosal surfaces of humans and other mammals. This perspective enables me to appreciate the significance of Mr. Brabec's studies, but I declare no conflict of interest or undue bias.

The thesis, entitled "**Immune-epithelial interactions in the multilayered model of intestinal homeostasis**", includes an Abstract, Introduction (provides background and context for the dissertation research), Current State of Knowledge (a literary overview to provide mechanistic insights into the processes which establish and maintain homeostasis in the intestine), Thesis Aims (five questions to advance understanding of intestinal homeostasis), Results (six primary research publications in excellent journals that Mr. Brabec has co-authored during his Ph.D. studies, including one as lead author in *Mucosal Immunology*), Discussion and Conclusions, and Literature Citations. The Thesis includes four figures (in addition to those in the publications) - two illustrating single-cell sequence analysis and two providing schematic overview of key concepts.

The scope and depth of the Introduction and Current State of Knowledge provide sufficient background to enable a clear understanding of the study rationale and appreciation of the strong premise for these investigations, as well as an integration of the forthcoming results from Mr. Brabec's primary investigations into the emerging principles of the field. The Thesis Aims are logical and clearly stated. The Results include six primary publications, one primary publication currently *in press*, and one review. The Discussion provides a summary and perspective, as well as some ideas for future investigation.

Questions for further consideration and comment

1) On page 9, a widely held opinion that is seldom disputed is stated, "The primary function of the immune system is to protect the organism from invading pathogens." Based on his studies of invertebrates Tomas Bosch has posed a provocative dissenting idea that "immune systems

evolved because of the need to control the resident beneficial microbes." Please comment briefly on this idea.

> [Bosch, TCG, (2013). Cnidarian-microbe interactions and the origin of innate immunity in metazoans. Annu Rev Microbiol 67: 499-518. {PMID: 23808329}]

2) In the *Introduction*, a summarizing comment that helps frame the investigations in this thesis states, "Thus, immune homeostasis in the intestine, which contains the largest reservoir of foreign antigens coming from harmless sources on top of intestinal self-antigens, is arguably one of the most complex topics in the immunology field. While most food antigens are localized there, the intestine also contains the largest variety of commensal microbiota, unmatched anywhere else in the body." Ideas regarding triggers of adaptive immune responses include Charles Janeway theory, often referred to as the "immunologist's dirty little secret", and Polly Matzinger's "danger theory." With regards to mounting an immune response to food antigens, please very briefly comment on the possible merits of these alternative ideas.

> [*vis a vis* Table 1, Pradeu, T and Cooper, EL, (2012). The danger theory: 20 years later. Front Immunol **3**: 287. {PMID: 23060876}]

3) On multiple occasions (p25,30,31,32,33,37), reference is made to, "homeostatic proinflammatory immune responses in the intestine." Pathologists historically refer to inflammation in terms of four cardinal features (heat, swelling, redness and pain) and describe inflammation in terms of the local leukocyte infiltration. Some of the homeostatic immune responses discussed in the thesis may not induce inflammation *per se* and could be considered "pre-inflammatory" rather than proinflammatory. Please briefly comment.

4) Please comment briefly on whether the immune responses elicited by colonization by Segmented Filamentous Bacteria (SFB, *Candidatus* Savagella) as discussed (p26-30) are exclusively host beneficial or possibly of some benefit to the microbe. Also, can you briefly speculate on how IgA might limit SFB growth?

5) On page 25, it is stated that "While commensal microbes pose no threat under homeostatic conditions, they can overgrow and cause severe pathology when not kept in check." The implication here seems to be that numbers of microbes is the key, if not sole factor. Is taxonomic composition of the microbial population also a factor for homeostasis vs. pathology?

Minor Points

Typographical error, p21 line 10 (catabolism).
Typographical error, p26 line 3 (capitalize Gram [referring to Professor Hans Christian Gram]).
Typographical error, p31 line 7 (capitalize Clostridia).

OVERALL ASSESSMENT

Intestinal homeostasis is vitally important to health and well-being of the host, but the underlying mechanisms of this complex topic are incompletely understood. Immune tolerance is a central component of intestinal homeostasis, and deficits in tolerance to self-antigens, food antigens and microbiota-derived antigens can lead to pathologies such as autoimmune disease, food allergies and inflammatory bowel disease, respectively. A key emerging theme in the field is the importance of epithelial-immune cell interaction.

The data included in this dissertation constitutes distinct and valuable contributions to knowledge in the field of immunology. In the investigation principally led by Mr. Brabec, interleukin-17-mediated stimulation of Paneth cell antimicrobial functions is discovered as an important mechanism of immune-mediated control of the intestinal microbiota, with perturbations of this

axis resulting in susceptibility to inflammatory intestinal pathology. The dissertation is lucid and well written and is essentially free from errors of style and grammar.

Based on my participation in over sixty PhD dissertation committees at the University of Pennsylvania and the University of California, I conclude that this is an excellent dissertation and would be recommended for defense and approval at both universities. The thesis represents a distinct contribution to the Mr. Brabec's field of research, and it is essentially acceptable as it stands.

In conclusion, I hope that my opinions on this dissertation are helpful. I am happy to provide further comment, should clarification be necessary. I look forward to the oral examination and participating remotely.

With best regards,

Charles L. Bevins MD, PhD
Distinguished Professor