**Reviewers Report on PhD Thesis: Tomas Brabec**

**Summary:**
This thesis details a series of studies by the candidate that address the question of immune control of tolerance to both self-antigens and exogenous challenges from the commensal microbiota. The thesis details the current state of the art in the field of immune tolerance and suppression and details a wide array of mechanisms through which immune signals dictate homeostatic control of the microbiota and prevent inappropriate inflammation driven by gut-dwelling microbes. The main body of work primarily driven by the candidate details cytokine-mediated control of Paneth cell responses to prevent microbially driven inflammation, which was published as a first author paper in *Mucosal Immunology* in 2023.

Overall the thesis is well written, clearly structured and demonstrates knowledge and understanding of the specifics of his project-associated work. The oral exam should explore the broader understanding of mucosal immunology and capacity for critical thinking, with questions selected from those below.

**General Questions:**

- In your own words, what are the main advances or key findings you have made in your PhD when taking together your thesis work as a whole?

- You have described different mechanisms through which T cells are induced into a state of tolerance. Mechanistically what happens to the T cell when it is deleted, becomes anergic or is suppressed?

- Why do you think it's advantageous for the immune system to have multiple mechanisms to induce immune tolerance? Why is central tolerance not sufficient?

- In addition to self, and commensal microbes, you discuss food as a challenge that must be tolerated: How else might food and nutrition influence the immune system in the gut and beyond?

- Significant energy is utilized into preventing inflammation against commensal microbes? Giving specific examples/details, can you explain the benefits of tolerating a gut-resident microbiota to the host?

- Commensal specific T cells are not "deleted" by central or peripheral tolerogenic mechanisms. Why do you think we retain these cells given their potential to drive inflammation and tissue damage in the gut?

- How do AMPs including Reg3 and alpha defensins act to modulate bacterial growth?

- What do you consider to be the most important open questions in regards to our understanding of immune tolerance? What would be the first experiment(s) you would design to begin to address that question?

**Thesis – specific questions**

- Regulatory T cells are discussed as having GATA-3+ and RORgt+ subsets. How do these transcription factors confer functionality on Treg to aid their specific roles?

- Multiple cell types (myeloid cells, Tregs, MHCII+ RORgt+ APCs) can suppress inflammation. Can you give an example of how these cells act together (i.e. in a tripartite circuit) to suppress inflammation?

- SFB drives a type 3 immune response in a manner dependent upon attachment to the epithelium. What is known about how attachment itself drives this effect mechanistically?

- What are the transcriptional networks that decide if an intestinal crypt stem cell becomes a goblet cell, Paneth cell, M cell or enterocyte etc?

- What do you think the function of AIRE is in peripheral eTACs, given the apparent lack of TRA expression?

**Questions on Brabec et al Mucosal Immunology 2023**

- What are the ligands for IL-17RA? Can you rule out an effect of cytokines other than IL-17A in driving PC function? How might you design experiments to distinguish between ligands if you had unlimited money/time?

- Do you think Ileal PC are intrinsically different? Or are ileal IL-17RA-dependent signatures simply due to localization of Th17/type 3 lymphocytes to this part of the gut? In the latter case, what signals cause these cells to enrich in the ileum in particular?

- **Fig 1b/S2d:** Is IL-17RA restricted to PC, or could other IEC/stem cell subsets signal via IL-17RA. How might you further rule out upstream effects in your mouse model - including on thymic selection or eTACs?

- **Fig 1c:** IL-17A/F induces a 1.5 fold increase in *Defa20* gene expression – how might you test whether that is a *biologically meaningful* increase?

- **Fig 1h:** Ileal PC numbers are reduced. How might you design experiments to differentiate a developmental effect due to a lack of PC development/maturation due to effects at level of stem cell, versus an activation of mature Paneth cells via IL-17RA?

- **Fig 2a+b**: Whole ileal transcriptome reveals significant gene changes in IL-17RA$^{dPC}$ mice that would be expected to be lymphocyte associated? What might these gene changes tell us about how PC communicate with the immune system?

- Are tertiary lymphoid structures e.g. cryptopatches, effected in IL-17RA$^{dPC}$ mice?

- **Sup Fig 3**: The nature of the bacteria that change in IL-17RA$^{dPC}$ mice litters varied. How might you get a better understanding of ***how*** the microbiota is functinally altered as a community (as opposed to what specific taxa change).

- **Fig 3:** If microbiota transfer into DSS mice drives inflammatory disease but cannot be attributed to a specific set of bugs (Sup Fig 8) then how might the IL-17RA$^{dPC}$ mice microbiota be driving inflammation? Why does the WT microbiota not induce inflammation in this setting?

- **Fig 3:** Changes in ileal inflammation following DSS were inferred to correlate with type 1 interferon production. How does type 1 interferon act to protect against ileal pathology?

- **Figure 3C**: i.p. of IL-17A/F is sufficient to protect IL-10KO mice from inflammation driven by a microbiota from IL-17RA deficient animals. Why do you think endogenous IL-17 responses in the recipient KO animal are not sufficient to protect in this scenario? How might IL-17A/F function independently of it's effects on PC (e.g. systemically)?

- IL-22 – Reg3 axis is presumably intact in these animals? What are the specific effects of Reg3 vs alpha defensins in microbial regulation? What might be the advantage of having two distinct type 3 cytokine cues to induce each arm of this AMP response?

**Recommendation:**
It is my opinion that this written thesis reaches the standards appropriate for award of the degree of PhD.

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