Distinct characteristics of novel immunoregulatory canine non-conventional TCRαβ⁺CD4⁻CD8α⁻ double-negative T cells

Laura Karwig¹, Peter F. Moore², Gottfried Alber¹, Maria Eschke¹

¹ Institute of Immunology, Molecular Pathogenesis, Center for Biotechnology and Biomedicine, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany
² Department of Veterinary Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA, United States

Introduction
Potential immunosuppressive capacity of canine non-conventional TCRαβ⁺CD4⁻CD8α⁻ double-negative (dn) T cells

- Conventional CD4⁺FoxP3⁺ regulatory T cells (Treg) are crucial for controlling immune responses, thereby maintaining homeostasis and self-tolerance.¹
- Recently, within the substantial population of non-conventional TCRαβ⁺CD4⁻CD8α⁻ double-negative (dn) T cells of dogs, a novel FoxP3⁺ Treg-like subset was described.²
- Similar to conventional Treg cells, canine Treg-like dn T cells are characterized by high expression of the surface molecule CD25.²

Aim of the study
- Study the putative regulatory role of canine non-conventional FoxP3⁺ and FoxP3⁻ TCRαβ⁻ dn T cells by a functional in-vitro assay.

Methods & Results

Transwell™ suppression assay: How do they suppress?

1. Isolation of different effector T cell (Teff) populations from peripheral blood mononuclear cells and analysis of FoxP3 expression

- The sorted Teff populations were cultured in a Transwell™ system that only allows soluble factors to pass through. Where indicated, CD4⁻CD25⁺ cells were added to allow for secretion of inhibitory soluble factors by Teff.

- dnCD25⁺ and CD4⁻CD25⁺ Teff cells are able to mediate suppression in a cell-cell contact-independent manner.

- In contrast to CD4⁺CD25⁺ T eff, they do not require signals from CD4⁺CD25⁻ T cells to secrete inhibitory soluble factors.

2. Suppressive effect of Teff on the proliferation of mitogen-stimulated responder T cells (Tresp)?

- dnCD25⁺ Teff suppress the mitogen (ConA)-driven proliferation of Tresp to a similar extent as conventional CD4⁺CD25⁺ Treg cells.
- They also moderate suppressive effects by dnCD25⁻ Teff (but not CD4⁺CD25⁻ Teff).

3. Cell-cell contact dependency?

- Addition of a neutralizing anti-IL-10 antibody in comparison to a non-blocking isotype control antibody in the Transwell™ system.

4. IL-10 dependency?

- Secretion of IL-10 by canine dnCD25⁺ and CD4⁺CD25⁻ Teff is necessary to induce suppression in the Transwell™ system.

Summary and conclusion

Our results demonstrate that canine non-conventional TCRαβ⁻ dn T cells contain unique and potent suppressive subpopulations in vitro. This is of high relevance, given the immunotherapeutic potential of manipulating regulatory T cell responses in vivo.

References

¹ Thornton et al., J Exp Med. 1998
² Rabiger et al., Front Immunol, 2019
³ Mair et al., Vet Immunol Immunopathol, 2019
⁴ Vöell et al., Eur J Immunol, 2011
⁵ Zhang et al., J Mol Med, 2001
⁶ Pinheiro et al., Immunology, 2010