Abstract N04: The CCL2/CCR4 Axis in Regulatory T-cell Trafficking to Canine Glioma - A Novel Therapeutic Target

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BACKGROUND
Canine gliomas share many similarities with their human counterparts, including recruitment of immunosuppressive regulatory T-cells (Tregs) that inhibit host immune responses. We have shown previously the importance of the chemokine CCL2 and its high-affinity receptor CCR4 in recruitment of Tregs in a murine orthotopic glioma model and elevated CCL2 in canine high grade gliomas. However, mechanisms responsible for Treg recruitment in canine glioma are poorly defined.

HYPOTHESES
1. Canine Tregs possess canonical Treg markers and can be isolated for downstream assays
2. Glioma-derived CCL2 is a potent chemoattractant for canine Tregs signaling via CCR4

ANIMALS
Four healthy dogs.

METHODS
In vitro, mechanistic study. Canine Tregs were characterized by flow cytometry using canonical markers CD3, CD4, CD25, FOXP3, and CCR4 and sorted using fluorescent-activated cell sorting. CCL2 mRNA expression was assessed via RTqPCR in four canine glioma cell lines.
(1110, 0514, J3T-Bg, G06A). Treg migratory capacities were assessed using a Boyden chamber assay. Anti-CCL2 antibody and a CCR4 inhibitor (C021) were used in abrogation studies.

**RESULTS**
The canine CD4+CD25high T-cell population expressed canonical Treg markers FOXP3 and CCR4. Canine Treg migration was enhanced by CCL2 and glioma cell line-derived supernatant. Blockade of the CCL2/CCR4 axis abrogated migration of Tregs. CCL2 mRNA was expressed in all cell lines and expression increased when exposed to Tregs but not to conventional CD4+ T-cells.

**CONCLUSION**
CD25high expression is critical for isolation of canine Tregs. Our study validated CCL2/CCR4 as a bi-directional Treg-glioma signaling axis and represents a rational target for immunotherapy in clinical patients.

**Title:** CCL2/CCR4 Axis in Regulatory T-cell Trafficking to Canine Glioma – A Novel Therapeutic Target

![Figure 1. Glioma-derived CCL2 is a potent chemoattractant for canine Tregs signalling via CCR4](image-url)