Title of the project:
CTLA-4 induced signal transduction in CD8 T-cell differentiation: Implications for tumour-checkpoint therapy

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Summary of the project:
Tc17 cells are known for low IFN-γ and granzyme B expression, resulting in diminished cytotoxicity. Adoptive transfer into Ag-bearing hosts converts Tc17 cells partially towards IFN-γ producing phenotype still retaining some Tc17 characteristics like increased persistence of survival. Nevertheless molecular mechanisms involved in Tc17 lineage plasticity and stability are yet to be identified. CTLA-4, a homologue to primary co-stimulatory molecule CD28 abrogates T-cell responses by cell intrinsic and extrinsic mechanisms. Studies have shown that CTLA-4 diminishes cytotoxicity of Tc1 cells by selective downregulation of granzyme B, Eomes, and IFN-γ production, but its impact on Tc17 cell differentiation and plasticity is not known.

Introduction:
Optimal T cell activation requires signals via the T cell receptor (TCR) and co-stimulatory molecules. CD28 is the primary co-stimulatory molecule that binds to the B7 ligand molecules and augments and sustains T cells responses. CTLA-4, a homologue to CD28 also recognizes and binds to B7 ligand molecules at a higher affinity than CD28 (1,2). Even though CTLA-4 is known as a negative regulator of T-cell activation, very little is known about its role in regulation of functional responses in T cells.

Results and discussion:
CTLA-4$^{+/+}$ Tc17 cells produce more IL-17 than CTLA-4$^{-/-}$ Tc17 cells
To evaluate the impact of CTLA-4 on the generation of Tc17 cells, naive CTLA-4$^{+/+}$ and $^{-/-}$ CD8+ OT.1 T cells were stimulated with $OVA_{257–264}$ in the presence of Tc17 inducing cytokines. Cytokine expressions were then measured on day3 and 4 when CTLA-4 is expressed on the surface of T cell at a maximum level. CTLA-4$^{+/+}$ Tc17 cells showed more intracellular IL-17 than CTLA-4$^{-/-}$ Tc17 cells,

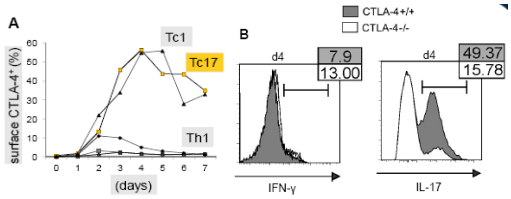

Figure 1: *CTLA-4+/+ and -/- CD8+ T-cells were cultured in Tc1/Tc17 conditions. (A) Surface expression of CTLA-4 and (B) Intracellular levels of IL-17 and IFN-γ were measured by FC.*

with a continuous increase from day 2 to the maximum on day 4 after primary stimulation. The distinct difference in IL-17 production was also consistent in co-cultures experiments (equal amounts of CTLA-4$^{+/+}$ and $^{-/-}$ OT.1 CD8+T cells stimulated with APC), indicating a cell-intrinsic effect of CTLA-4 that enhances differentiation of theTc17 program (3).

CTLA-4$^{-/-}$ Tc17 cells efficiently control the tumor growth compared to CTLA-4$^{+/+}$ Tc17 cells.
To determine the effect of *in vitro* differentiated CTLA-4$^{+/+}$ and $^{-/-}$ Tc17 cells in controlling tumour progression an adoptive transfer model was used where in vitro cultured CTLA-4$^{+/+}$ and $^{-/-}$ Tc17 cells were adoptively transferred into tumour bearing mice and tumour progression was then measured. Tumour progression was significantly reduced in mice that received CTLA-4$^{-/-}$ Tc17 cells whereas reduction of tumour progression was very less or completely absent in mice that received CTLA-4$^{+/+}$ Tc17 cells. As in vitro re-stimulated CTLA-4$^{+/+}$ and $^{-/-}$ Tc17 cells show different Tc1 cytokine expression, lineage plasticity might be the crucial factor in controlling the functionality of CTLA-4$^{+/+}$ and $^{-/-}$ Tc17 cells (4). So lineage plasticity of adoptively transferred CTLA-4$^{+/+}$ and $^{-/-}$ Tc17 cells were analysed and an enhanced expression of Tc1 characteristics i.e. enhanced IFN-γ/TNF-α^{high} producers were observed in CTLA-4$^{-/-}$ Tc17 cells which would correlate well with the functionality of CTLA-4$^{-/-}$ Tc17 cells in controlling tumour progression (5).

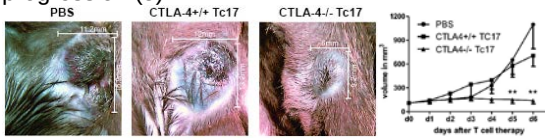

Figure 2: *Recipient mice were s.c injected with B16-OVA melanoma cells. 12 days later when a visible tumor is grown, the recipient mice were transferred i.v. with 3 day cultured CTLA-4+/+ and CTLA-4-/- Tc17 cells, considering that time point as day 0 tumor growth was measured.*

Perspectives:
The data demonstrates a cell-intrinsic effect of CTLA-4 that enhances the differentiation of Tc17 cells and role of CTLA-4 in reducing the susceptibility for Tc17 lineage plasticity. Determining the downstream signalling events involved in CTLA-4 mediated Tc17 differentiation and lineage plasticity repression gives an insight into a detailed mechanism of Ipilimumab, a CTLA-4 Mab used in tumour therapy.

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