University of Fribourg / Faculty of Science and Medicine / Department of Oncology, Microbiology and Immunology

Nucleofection-based CRISPR-Cas9 gene editing of naive and *in vitro*-activated primary mouse CD8$^+$ T lymphocytes

Petra Pfenninger

Nucleofection-based CRISPR-Cas9 gene editing emerged as a powerful method for fast and precise inactivation of target genes to study their function in basic immunological research. Here, we tested various nucleofection conditions to achieve the highest cell survival and knockout efficiency while preserving functionality of naive and *in vitro*-activated primary mouse CD8$^+$ T lymphocytes. Nucleofection of naive CD8$^+$ T lymphocytes did not alter their antiviral immune competence *in vivo*, whereas *in vitro*-preactivated CD8$^+$ T lymphocytes showed a moderately diminished *in vivo* cell survival ability after nucleofection. In summary, CRISPR-Cas9 gene editing of naive and *in vitro*-activated primary mouse CD8$^+$ T lymphocytes efficiently generates mutant cells without degeneration of their *in vivo* function.
Rate of protein decay after gene inactivation depended on the half-life of the respective protein.
In two additional side projects, we used the optimized nucleofection condition for naive CD8$^+$ T lymphocytes to inactivate gene encoding intracellular protein non-muscle myosin II and chemokine receptor CCR7. Here, nucleofection-based CRISPR-Cas9 gene editing was proven to be a fast method to precisely inactivate a target gene in primary mouse CD8$^+$ T lymphocytes.
In the second main project, we investigated whether CD8$^+$ T lymphocytes complete their effector cell differentiation at the priming site or whether they adapt their differentiation in response to additional signals they receive in the periphery. We showed that generation of terminally differentiated effector cells persists in the periphery, which is mediated by integrin α4. However, integrin α4 signal on effector CD8$^+$ T lymphocytes was dispensable.

Jury:
Prof. Dr. Jens V. Stein (thesis supervisor)
Prof. Dr. Doron Merkler (external co-examiner)
Prof. Dr. Michael Walch (internal co-examiner)
Prof. Dr. Curzio Rüegg (president of the jury)