ENGLISH SUMMARY

When a pathogen successfully breaches a tissue barrier, professional antigen presenting cells get activated and present pathogen-derived antigens to naïve $CD8^+$ T cells in the draining lymph node (dLN). Upon cognate antigen recognition and reception of co-stimulatory signals and cytokines, antigen-specific naïve $CD8^+$ T cells become activated, clonally expand and acquire effector functions, allowing the resulting cell pool to eliminate the pathogen at the inflamed site. Once the pathogen has been cleared, the majority of the 'effector phase' T cells dies due to apoptosis, however, a small number of $CD8^+$ T cells survives long-term. These memory $CD8^+$ T cells persist independent of the presence of antigen, and provide a rapid proliferative and cytotoxic response upon reinfection. Interestingly, work over the past decade has established that memory $CD8^+$ T cells do not only persist systemically, but also at sites of former pathogen entry. The latter tissue-resident memory $CD8^+$ T cells (T_{RM}) have permanently abandoned the circulation and can be found in nearly all tissue sites in mice and humans, such as the skin and lung epithelium. Importantly, $CD8^+$ T_{RM} are superior in controlling local reinfections as compared to their circulating counterparts. Because of their prominent role in providing tissue protection, knowledge on how these populations arise and how they mediate such protection *in situ* is of particular value for the treatment of local diseases.

In this work, I study the formation and function of $CD8^+$ T_{RM}. I start off by discussing which genetic technologies can be used to study the various steps in the 'life of a T cell' (chapter 2-3). Subsequently, by using the skin as a model system, I investigate how naïve $CD8^+$ T cells differentiate into T_{RM} (chapter 4-5) and examine how these cells exert their protective function with the tissue (chapter 6-8).

Technologies to study the 'life of a T cell'

In **chapter 2**, we review how gene-expression and signaling reporters can be used to study T cell activation, function and differentiation. We conclude that, provided careful reporter design and data interpretation is performed, genetically encoded reporter systems provide a powerful tool to study the various aspects of T cell behavior in a relatively unperturbed setting.

In order to track or manipulate the behavior of T cells *in vivo*, biomedical researchers often make use of reporter or modifier proteins. However, upon introduction of cells expressing these exogenous proteins into immunocompetent animals, immune rejection can occur. In **chapter 3**, we develop a novel genetically engineered mouse model ('Tol') that is immunologically tolerant to a series of fluorescent reporter and modifier proteins. This strain can be used as a recipient for cells expressing the included exogenous genes and provides a methodology by which novel poly-tolerant mouse models can be generated.

The formation of skin-resident memory CD8$^+$ T cells

In **chapter 4**, we discuss the current knowledge on the formation of tissue-resident memory CD8$^+$ T cells. Here, we outline the external factors that steer naïve CD8$^+$ T cells onto the road of tissue-specific T cell memory. In particular, we show that T_{RM} formation is not only dependent on factors provided at the inflamed tissue site, but highlight that also signals present in the dLN during the naïve cell stage and during T cell priming contribute to this process. In order to study the origin of skin-T_{RM}, in **chapter 5**, we barcode-label individual naïve CD8$^+$ T cells and track their clonal output upon DNA vaccination by single cell lineage tracing. In this work, we show that the capacity to form T_{RM} is instilled in CD8$^+$ T cells before the cells enter the affected tissue and that this capacity remains fixed after a secondary immune challenge.

Protective function of skin-resident memory CD8$^+$ T cells

In **chapter 6**, we investigate the mechanisms by which CD8$^+$ T_{RM} mediate protection in murine skin. We demonstrate that upon activation by cognate antigen, CD8$^+$ T_{RM} rapidly instruct nearby and distant skin cells to enter into an anti-viral and anti-microbial state, a process which is largely mediated via the pro-inflammatory cytokine IFNγ. Moreover, this tissue-wide conditioning by activated CD8$^+$ T_{RM} protects the skin from a secondary infection with an antigenically unrelated (opportunistic) pathogen. This work demonstrates that the antigen-specific activation of a few CD8$^+$ T_{RM} induces a broad-spectrum protective response in a large number of surrounding cells.

In order to monitor the dynamic behavior of CD8$^+$ T_{RM} and other immune cells *in situ* in human tissue, we have developed a novel *ex vivo* imaging technology. In **chapter 7**, a step-by-step protocol is described to label and track these cells in either fresh murine or human skin biopsies. In **chapter 8,** we apply this technology to investigate the migratory behavior of tissue-resident memory CD8$^+$ T cells and other immune cells in healthy human skin. Here, we demonstrate that CD8$^+$ T_{RM} actively patrol the epidermal and dermal skin tissue. In the epidermis, CD8$^+$ T cells migrate in the basal layer below a pool of sessile Langerhans cells. In the dermal compartment, CD8$^+$ T cells move in collagen type I-rich and -poor areas, and along dermal vessels. The *ex vivo* imaging technology described in this work allows for the real-time study of CD8$^+$ T cells and other immune cells in healthy human skin, but also provides a platform to study these cells in skin disease.

Conclusion and future perspectives

In this thesis, I have provided insight in how CD8$^+$ skin-T_{RM} populations are formed, how they protect against local reinfection and how they behave in healthy human skin. Firstly, together with my colleagues, I demonstrate that CD8$^+$ T_{RM} are not only distinct from systemic memory T cell subsets in terms of gene- and protein expression, but also by descent. Second, this works shows that CD8$^+$ skin-T_{RM} act as local sentinels that trigger a rapid and tissue-wide

immune response upon foreign antigen recognition. Third, I demonstrate that tissue patrol is a property of $CD8^+$ T_{RM} in healthy human skin. In addition, in collaboration with my co-workers, I have generated two novel technologies (i.e. the 'Tol' mouse strain and the *ex vivo* imaging technique) that can be used as such or adapted by other biomedical researchers in order to investigate key aspects of (T) cell behavior and skin biology. Understanding the signals that drive $CD8^+$ T_{RM} formation, and insights in the function of these cells within both healthy and diseased skin, will be essential to allow the manipulation of these populations for therapeutic benefit.