The Control of Mononuclear Phagocytes over Primary and Secondary $CD8^+$ T cell Responses to Virus Infection of the Skin

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1 ABSTRACT

1.1 Abstract (English)

Cytotoxic T lymphocytes (CTL) are effector cells of the adaptive immune system, that are specialized for the defense against intracellular pathogens such as viruses. To fulfil that task, pathogen-specific CTL need to be activated by certain stimuli provided through the interaction with Mononuclear phagocytes (MNP). In this thesis the relevance of MNP is addressed in the control of primary CTL responses upon intradermal immunization with Vaccinia virus as well as of the secondary response in the skin, that is mediated by local antiviral $CD8^+$ memory T cells.

First, we study the response to acute Vaccinia virus infection, that is mediated by synergizing innate and adaptive immune cells. Over the course of infection, we identified massive changes in the composition of the MNP network within the infected skin and identified subsets dependent on CCR2- and/or FLT3- signaling for their residency in skin. We demonstrate that antigen presentation and co-stimulatory molecule expression on CCR2 dependent cells controls the sustained antiviral response, the differentiation of CTL into memory precursor cells and the establishment of $CD8^+$ tissue resident memory T cells (T_{RM}) in the skin.

In a second study, we addressed how $CD8^+$ T_{RM} in skin can be antigen-specifically recalled to fight reinfection *in situ*. Located in the uppermost layer of the skin, keratinocytes are the target cells of skin-tropic viruses, thus infected keratinocytes might be the first site where antigen becomes visible to skin resident $CD8^+$ T_{RM}. Our experiments revealed that antigen derived from keratinocytes is cross-presented by Langerhans cells (LC) for $CD8^+$ T_{RM} recall, thus LC are crucial in the protection against reinfection of the skin.

1.2 Zusammenfassung (deutsch)

Zytotoxische T-Lymphozyten (CTL) sind Effektor-Zellen des adaptiven Immunsystems, die auf die Abwehr von intrazellulären Krankheitserregern wie Viren spezialisiert sind. Um diese Aufgabe zu erfüllen, bedarf es der gezielten Aktivierung von antiviralen CTL durch bestimmte Stimuli, welche durch die Interaktion mit mononukleärer Phagozyten (MNP) vermittelt werden. In dieser Dissertation wird die Bedeutung von MNP für die Kontrolle von primären CTL-Antwort nach intradermaler Immunisierung mit dem Vaccinia-Virus sowie der sekundären durch antivirale Gedächtniszellen vermittelten Immunantwort in der Haut untersucht.

Zunächst wurde die akute Immunreaktion auf eine Vaccinia-Virus-Infektion der Haut untersucht, diese Reaktion setzt sich zusammen aus der Aktivierung und Rekrutierung von angeborenen sowie adaptiven Immunzellen. Im Verlauf einer akuten Vaccinia-Virus-Infektion der Haut haben wir massive Veränderungen in der Zusammensetzung des MNP-Netzwerks der infizierten Haut beobachtet und Untergruppen identifiziert, deren Lokalisation in der Haut jeweils von CCR2- und/oder FLT3-Signalen abhängig sind. Wir konnten zeigen, dass die Antigenpräsentation und die Expression von ko-stimulierenden Molekülen auf CCR2-abhängigen Zellen die anhaltende antivirale Antwort, die Differenzierung von CTL zu Gedächtnisvorläuferzellen und die Etablierung von $CD8^+$ gewebes-residenten Gedächtnis-T-Zellen (T_{RM}) in der Haut kontrollieren.

In einer zweiten Studie untersuchten wir, wie persistierenden $CD8^+$ T_{RM} in der Haut antigen-spezifisch erneut aktiviert werden und so eine Reinfektion im Gewebe bekämpfen. In der obersten Schicht der Haut gelegen, sind Keratinozyten die Zielzellen für hauttropische Viren. Infizierte Keratinozyten könnten daher der erste Ort sein, an dem virales Antigen für die in der Haut ansässigen $CD8^+$ T_{RM} sichtbar wird. Unsere Experimente zeigen, dass von Keratinozyten stammendes Antigen von Langerhans-Zellen (LC) kreuzpräsentiert wird und dann antigenspezifisch $CD8^+$ gewebsresidenten Gedächtniszellen aktivieren können. Daher sind LC entscheidend für den Schutz vor erneuter Infektion der Haut.

2 INTRODUCTION

2.1 The Immune system

The immune system is a complex network of effector molecules, specialized cells, tissues and organs designed to protect organisms from pathogens, such as viruses, bacteria, uni- and multicellular eukaryotes. In addition to its vital role in defending against microbial invaders, the immune system also initiates defense mechanisms against abnormal cells within the body that have become malignant or infected. The immune system faces the challenging task of distinguishing between the harmful and the harmless to prevent damage and, subsequently, to ensure the organism's survival.

2.1.1 Hematopoiesis

The cells of the mammalian immune system originate after embryogenesis from pluripotent hematopoietic stem cells (HSC) at regular intervals (Wilson and Trumpp, 2006; Morrison and Scadden, 2014). In contrast to other immune cells, only Langerhans cells and several tissue macrophages predominantly originate from early progenitors of the yolk sac or fetal liver and once resident in the tissue they self-renew locally (Chorro et al., 2009). The common stem cell of all other immune cells, the HSC, reside in the bone marrow and can differentiate into common progenitors of the myeloid and the lymphoid lineage (Kondo, 2010). The common lymphoid progenitor serves as a precursor for B cells, T cells, Natural Killer cells (NK cells) or Innate Lymphoid cells (ILC) and contribute to plasmacytoid dendritic cells (pDC); in contrast, granulocytes, monocytes, macrophages, mast cells and most dendritic cells (DC) are part of the myeloid lineage. The myeloid lineage includes also non-classical immune cells like platelets, known primarily for contributing to hemostasis e.g. in wound healing, and erythrocytes, which are responsible for oxygen delivery. (Murphy et al., 2009; Poltorak and Schraml, 2015)

2.1.2 Innate and Adaptive Immunity

The immune system comprises two complementary branches with distinct tasks and properties: the innate and the adaptive immune system (Medzhitov and Janeway, 2000). Both systems work together synergistically to mount efficient and enduring defense against invading pathogens.

The innate immune system is responsible for immediate unspecific responses, whereas adaptive immunity mediates long-lasting specialized responses. This collaboration involves utilizing both the humoral and cellular components of the immune system (Hoebe et al., 2004; Iwasaki and Medzhitov, 2010). The innate immune system is described as the first line of defense as it is activated immediately after infection (Murphy et al., 2009). Thus, innate effector mechanisms curtail the infection until adaptive immune cells are matured. Innate immune cells recognize conserved molecular patterns of microbes by germline-encoded, so-called pattern recognition receptors (PRR) located on their surface or intracellularly (Kimbrell and Beutler, 2001; Uematsu and Akira, 2008). Once a microbe is detected, host defense pathways are activated by regulating the expression of various immune response genes and initiating enzyme cascades (Iwasaki and Medzhitov, 2010; Medzhitov and Janeway Jr., 2000). Innate immune cells include phagocytes, which seek, engulf and destroy pathogens. Phagocytes can be divided in macrophages and granulocytes. The family of granulocytes comprise of neutrophilic, basophilic and eosinophilic granulocytes. All granulocytes in common is the possession of cytotoxic granules, which they release to fight microbes. Neutrophils are the most abundant and earliest cell infiltrating inflamed tissue immediately at the start of an immune response. Neutrophils have the special ability to form neutrophil extracellular traps (NETs) and reactive oxygen species to catch and kill microbes directly and attract other immune cells (Burn et al., 2021). Together with anti-microbial peptides, the complement system and phagocytes take direct effect within minutes and up to hours after infection. In addition, innate immune cells can recruit and activate adaptive immune cells, contributing to controlling the spread of the pathogen.

Adaptive immune responses take longer to develop compared to innate immune responses. It typically takes around four days for a sufficient number of adaptive immune cells, i.e. lymphocyte clones, to be generated and differentiated into effector cells (Bonilla and Oettgen, 2010). Only this mature effector cell state permits adaptive immune cells to participate actively in host defense. At first glance, this process may appear inefficient. However, the adaptive response is tailored, highly effective and provides long-term protection. Once the adaptive immune cells have matured and successfully fought pathogen invasion, most undergo apoptosis, but some are retained and have the ability to mount rapid and robust secondary responses. This memory capability of the adaptive immune system to react faster and more effectively upon re-exposure to the same pathogen was initially described by Edward Jenner in 1798 during his pioneering work on smallpox vaccination (Brunham and Coombs, 1998). The key mediators of the adaptive immune system, responsible for a memory response adapted to the pathogen, are lymphocytes. These lymphocytes are further divided into B cells and T cells.

This thesis addresses the question of how mononuclear phagocytes or keratinocytes modulate $CD8^+$ T cell-mediated primary and secondary immune responses of the skin. Accordingly, the mononuclear phagocyte system, the structure of the skin compartment and the development and function of $CD8^+$ T cells will be described in depth in the following.

2.2 The Mononuclear phagocyte system

2.2.1 The History of the Mononuclear phagocyte system

Already in 1883, Metchnikoff discovered the significance of phagocytosis, which play an important role in host defense as they engulf whole microbes or particulate material and were later found to be required for processing pathogenic material for being utilizable for lymphocytes (Gordon, 2016). Phagocytic active cells are called phagocytes. Based on the morphology of the phagocyte's nucleus, they are divided into polymorphonuclear phagocytes, i.e. granulocytes with multi-lobed nuclei, and mononuclear phagocytes having a round nucleus. Van Furth et al. introduced the concept of the mononuclear phagocyte system, as a network of monocytes, macrophages and precursor cells spanning the whole body, stating that all macrophages within tissues are derived from blood monocytes (van Furth et al., 1972). After the discovery of dendritic cells by Steinman and Cohn (Steinman and Cohn, 1973), they were also integrated into van Furth's mononuclear phagocyte (MNP) system (van Furth, 1981). All of such cells of the MNP system belong to cells of the myeloid lineage, which generally have a high turnover rate, unlike lymphoid cells, and can be quickly mobilized after pathogen detection. However, the initial theory of monocytes as the transient precursor reservoir for mononuclear phagocytes in tissues was later found to be only valid during inflammation or in epithelia exposed to microbiota (Ginhoux and Jung, 2014), when Ly-$6C^{high}$ monocytes differentiate into infiltrating macrophages or DC. Differently, under homeostatic conditions, most tissue-resident macrophages in adults are independent of monocytes (Hashimoto et al., 2013; Yona et al., 2013). Today's definition of MNP system includes monocytes, macrophages, DC and all precursor cells, these cells contribute to tissue integrity, innate immunity, pathogen clearance and the induction of adaptive immune responses (Merad et al., 2013; Ginhoux and Jung, 2014; Varol et al., 2015). MNP have been well defined across tissues and species under steady state conditions, though during inflammation, it is still an ongoing challenge to phenotypically dissect subsets of the MNP compartment, in particular the separation of cDC and other MNP. Reasons for that is that MNP possess a high phenotypical and functional plasticity, that have led to inconsistencies in nomenclature among previous studies in this

field. Thus, a revised definition of MNP system suggested a classification of cells based on ontogeny rather than on location, function and phenotype (Guilliams et al., 2014; Jenkins and Hume, 2014). In this thesis, MNP of the skin during viral infection were characterized phenotypically and functionally based on ontogeny for this purpose.

2.2.2 Inflammatory and Patrolling Monocytes

Monocytes arise from myeloid precursor cells in the fetal liver during embryonic hematopoiesis (Hoeffel et al., 2015). In adults, monocyte reservoirs are the bone marrow (van Furth and Cohn, 1968) the blood and the spleen (Swirski et al., 2009), from there they are mobilized upon tissue injury. In mice, two principal subsets of monocytes with distinct migratory properties and functions were identified: the inflammatory monocytes (Ly-6C^{high}) and patrolling monocytes (Ly-6C^{low}) (Geissmann et al., 2003). In general, inflammatory monocytes (Ly-6C^{high}) express CCR2, which allows them to respond readily to inflammatory chemokines such as CCL2 and CCL7. The release of CCL2 by inflamed tissues triggers the egress of inflammatory monocytes (Ly-6C^{high}) from the bone marrow, enabling circulation through the bloodstream (Auffray et al., 2007; Serbina et al., 2008). Within blood, circulating inflammatory monocytes (Ly-6C^{high}) are short-lived, but these circulating monocytes can extravasate by active recruitment along chemokine gradients produced by inflamed peripheral tissues (Boring et al., 1997; Palframan et al., 2001). Pro-inflammatory cytokines and microbial products released from inflamed tissues can initiate an ‘emergency’ myelopoiesis in the bone marrow, when cells at various stages of the myeloid life cycle have the potential to be released from the bone marrow, reflected by the heterogeneity of monocytes and related cells observed in circulation and peripheral tissues, where they can differentiate into monocyte-derived phagocytes, such as monocyte-derived macrophages or DC, both differing from tissue-resident macrophages (Tsou et al., 2007; Auffray et al., 2009; Ng et al., 2023). The counterparts of inflammatory monocytes are patrolling monocytes (Ly-6C^{low}), which live longer than inflammatory monocytes (Ly-6C^{high}). In homeostasis, patrolling monocytes (Ly-6C^{low}) function as differentiated blood-resident macrophages by continuously patrolling the vasculature (Auffray et al., 2007; Carlin et al., 2013). They contribute to tissue repair by engulfing debris and the recruitment of other immune cells during inflammatory conditions (Thomas et al., 2015).

2.2.3 Infiltrating and Tissue-resident Macrophages

The name macrophage is Greek and means ‘big eater’, which is what they do: They remove apoptotic cells, debris and microbes by phagocytosis. Macrophages move within tissues by amoeboid

locomotion, are distributed across tissues and express typical surface markers such as F4/80, CD64 and CD11b. Two subsets of macrophages are distinguished based on their origin. Infiltrating macrophages develop upon inflammatory stimuli from circulating inflammatory monocytes (Ly6^{high}), whereas tissue-resident macrophages develop independently from circulating monocytes; tissue-resident macrophages reside in tissues early in life in two distinct waves from yolk sack and fetal liver (Ginhoux and Jung, 2014). In adults, these long-living tissue-resident macrophages renew themselves by low-level local proliferation *in situ*, unlike other myeloid cells, which primarily originate from the bone marrow (Ginhoux and Guilliams, 2016).

2.2.4 Monocyte-derived Cells

While early studies have suggested, that monocytes can differentiate into inflammation induced DC, more recent work revealed that pre-cDC are precursors of these DC. Early *in vitro* studies describe monocytes from blood differentiating in presence of growth factors into a DC-like phenotype(Inaba et al., 1992; Sallusto and Lanzavecchia, 1994; Jonuleit et al., 1997). *In vivo*, Ly-6C^{high} monocytes were described to upregulate Major-Histo-Compability Complex (MHC) II and CD11c expression and migrate to lymph nodes to induce CD4 responses or prime CD8 T cells in non-lymphoid tissues (León and Ardavín, 2008; Wakim et al., 2008; Nakano et al., 2009; Aldridge et al., 2009). These finding lead to the assumption, that these cells can orchestrate adaptive immune responses depending on the immune microenvironment and external stimuli (Murphy et al., 2009). More recently an international group of scientists led by Bart Lambrecht discovered a novel type of antigen-presenting cell, the inflammatory cDC2, which holds a phenotype reminiscent of monocyte derived Cells (CD11b^{+} sirp1α^{+} CD64^{+}), but derives from blood pre-cDC. They demonstrated that monocyte-derived Cells (moC) exist across tissues but do not present antigens, indicating that moC as potent inducers of immune responses described in previous studies might be a case of mistaken identity, since moC fractions might have been contaminated by the recently identified inflammatory cDC2. This inflammatory cDC2 emerges after inflammation in peripheral tissues, hold migratory capacities and combines features of monocytes, macrophages and conventional DC, to induce optimal immune responses to respiratory virus infections, house dust mite induced asthma, human adjuvant system AS01 and mycobacterial infection of the skin (Bosteels et al., 2020b; a). However, the function of both cells, namely monocyte-derived Cells and inflammatory cDC2, during viral infection of the skin remains elusive.

2.2.5 Dendritic Cells

Dendritic cells (DC) were first described in the spleen of mice by Steinman and Cohn (Steinman and Cohn, 1973). Under the light microscope, they observed cells with features distinct from typical macrophages and monocytes: a type of cell forming many dendrites (Steinman and Cohn, 1973; Steinman et al., 1979). This initial observation sparked the discovery of DC in nearly all lymphoid and non-lymphoid tissues; however, an enrichment of DC was found in compartments that face the external environment, i.e. epithelial tissues such as the skin (Alvarez et al., 2008). In mice, DC are defined as MHC-II$^+$ CD11c$^+$ cells (Metlay et al., 1990; Nussenzweig et al., 1981; Steinman et al., 1979). As the sentinels of the immune system, DC usually represent a rare cell population compared to other cell types. However, their low number is compensated by the fact that a single DC can interact with up to 500 T cells per hour (Bousso and Robey, 2003), making them to potent inducers of immune responses. DC are specialized in antigen processing and presentation after they have scavenge dead cells, pathogens, and other debris. DC are equipped with various intracellular and extracellular receptors that allow them to sense the presence of pathogens or inflammatory cell death. After exogenous or endogenous material is ingested and specific receptors are stimulated, innate and adaptive immune responses can be induced (Gordon, 2002). In detail, the cells get activated through the recognition of either 1) pathogen-associated molecule patterns (PAMPs) by pattern recognition receptors (PRRs), or 2) the Fc part of antibodies decorating the pathogens' surface by Fc receptors. Once activated by these innate receptors, the APC, harboring foreign material, can mature and migrate to the draining lymph nodes. For example, skin draining lymph nodes filter lymph draining from the skin, collect antigens from distal sites and provide a platform to bring rare cell populations in close proximity. Inside these secondary lymphoid organs, APC present the pathogen-derived peptides on MHC to naïve lymphocytes. Among all APC, DC exhibit the most comprehensive ability to prime naïve T cells because even without T helper cells they can directly activate naïve T Lymphocytes, which highlights their role as a key link between innate and adaptive immunity, as described in the work of Paul Ehrlich and Ilya Metchnikov (Nobel Prize in Physiology or Medicine in 1908). Based on ontogeny, gene expression profiles, and subsequent surface molecules, DC can be discriminated from monocytes and macrophages (Malissen et al., 2014). DC show a very heterogeneous range of phenotypes and function depending on the tissue localization and the presence or absence of inflammation. In general, DC comprise of three groups: plasmacytoid DC (pDC), conventional DC (cDC), and eventually monocyte-derived cell (MC). In the skin, information about the unique function of distinct DC subpopulations is still limited, in particular under inflammatory conditions.

2.3 The Skin is a Barrier Tissue

The skin acts as a protective barrier, shielding the body from external threats. The prevention of water loss, thermoregulation and protection against invading pathogens are the main tasks of the skin. Protection mechanisms of the skin include the mechanical barrier function, the secretion of chemicals and a dense network of cutaneous immune cells providing immune surveillance. For the latter, various immune cell populations reside in the skin tissue. Each population fulfils its unique function while collaborating closely within the tissue and the systemic network of the body.

The skin is an epithelial tissue structured in anatomically distinct layers, which are traversed by hair follicles, sebaceous glands, nerve fibers (Cohen et al., 2020), blood and lymphatic vessels (Tripp et al., 2008). Each layer of the skin has its specific predominant structural cell that builds as a bulk of cells the stratified appearance of the tissue. The outermost layer is the epidermis, which is separated by the basement membrane from the subjacent dermis. The dermis is connected to the subcutaneous fatty layer, also known as hypodermis or subcutis.

2.3.1 The Epidermis

The epidermis lacks nerve fibers and vessels in contrast to the dermis. The most abundant cell type of the epidermis is the keratinocyte. Within the epidermal layer of skin keratinocytes are arranged in four strata, representing progressive stages of differentiation. Mitotically active keratinocytes are located close to the basement membrane. From there, they differentiate over time into corneocytes until they reach the outermost stratum of stratified keratinocytes. This layer of cornified, highly compressed cells prevents water loss from the body (Chambers and Vukmanovic-Stejic, 2020). Keratinocytes are involved in sensing pathogens and danger signals (Heath and Carbone, 2013) by expressing several PPRs such as TLR 1-6 and 9 (Di Meglio et al., 2011). Upon infection, keratinocytes can secrete inflammatory cytokines, chemokines, and antimicrobial peptides (AMPs) to initiate defense mechanisms. Keratinocytes were also found to be able to respond to T helper cell 1 derived cytokines (Albanesi et al., 2001) and regulate the recruitment of LC (Malissen et al., 2014).

Besides keratinocytes other cells are embedded within the epidermal strata, these are melanocytes, Langerhans cells (LC) (Merad et al., 2008), tissue-resident memory CD8 T cells (Gebhardt et al., 2009, 2011), murine $\gamma\delta$ T cells (Nielsen et al., 2017) and innate lymphoid cells (ILC) (Kobayashi et al., 2020). Melanocytes produce UV radiation absorbing Melanin, which prevents UV rays from penetrating deeper layers and thus protects from DNA damage and reactive oxygen species.

LC are the sentinel and the only mononuclear phagocyte of the healthy epidermis, they contribute to both immunity and tolerance. Murine LC originate from monocytes derived from the fetal liver, with a minor contribution from myeloid cells of the yolk sac derived (Hoeffel et al., 2012). Before birth, these progenitors seed the skin, where LC maintain themselves *in situ* and express the surface markers Langerin (CD24), CD11c, MHC-II and EpCAM. LC self-renew *in situ* and are resistant to high doses of ionized radiation due to the expression of the cell cycle inhibitor Cdkn1a (Price et al., 2015), thus they remain of host origin in bone marrow chimeric mice (Merad et al., 2002). In contrast, other dermal DC undergo a constant turnover by getting replaced by circulating BM-derived precursors and are thus dependent on radio-sensitive HSC. In mice, tissue-resident $CD8^+$ memory T cells lodged in the epidermal layer are the first line of defense against reinfection by recognizing peptide antigens (Topham and Reilly, 2018). These cells are supplemented in monitoring the epidermis by $\gamma\delta$ T cells that sense stress-induced molecules and non-peptide antigens.

2.3.2 The Dermis

The dermis is traversed by vessels and nerve fibers, rich in immune cells and the predominant structural cell is the fibroblast. The hypodermis, on the other hand, is composed mainly of adipocytes and connective tissue (Watt, 2014). Different populations of myeloid and lymphoid immune cell populations are located in the dermis. In contrast to the epidermis, the dermal mononuclear phagocyte network is more heterogenous, comprising different subsets of dendritic cells (DC) and macrophages (Tamoutounour et al., 2013). Furthermore, the dermal layer contains innate lymphoid cells, NK cells, CD8 and CD4 T cells, B cells, granulocytes, mast cells and monocytes. Immune cells can also traffic through the tissue, which results in highly dynamic changes in the composition of cell populations in the dermis, especially when it comes to inflammation (Pasparakis et al., 2014).

Dermal cDC and moC have a short lifespan under steady state conditions, they are continually replaced by extravasating bone marrow derived precursors. Their differential dependence on FLT3 and CCR2 is commonly utilized in mouse models to investigate the role of the different DC subsets in the formation of immune responses. The generation of cDC from pre-cDC depends on the interaction of FMS-related tyrosine kinase 3 (FLT3) and its ligand (Guilliams et al., 2016), whereas the migration of moC precursor, meaning Ly-$6C^{high}$ monocytes, from bone marrow into the skin depends on CCR2 signaling (Domínguez and Ardavín, 2010; Serbina et al., 2008). moCs were suggested to function like DC (Tacke et al. 2007), due to their ability to induce adaptive immunity, such as activation of $CD8^+$ memory cells (Wakim et

al., 2008), and a similar transcriptomic profile as CD11b^{+} cDC (Tamoutounour et al., 2013). However, their involvement in the induction of adaptive immunity is controversial.

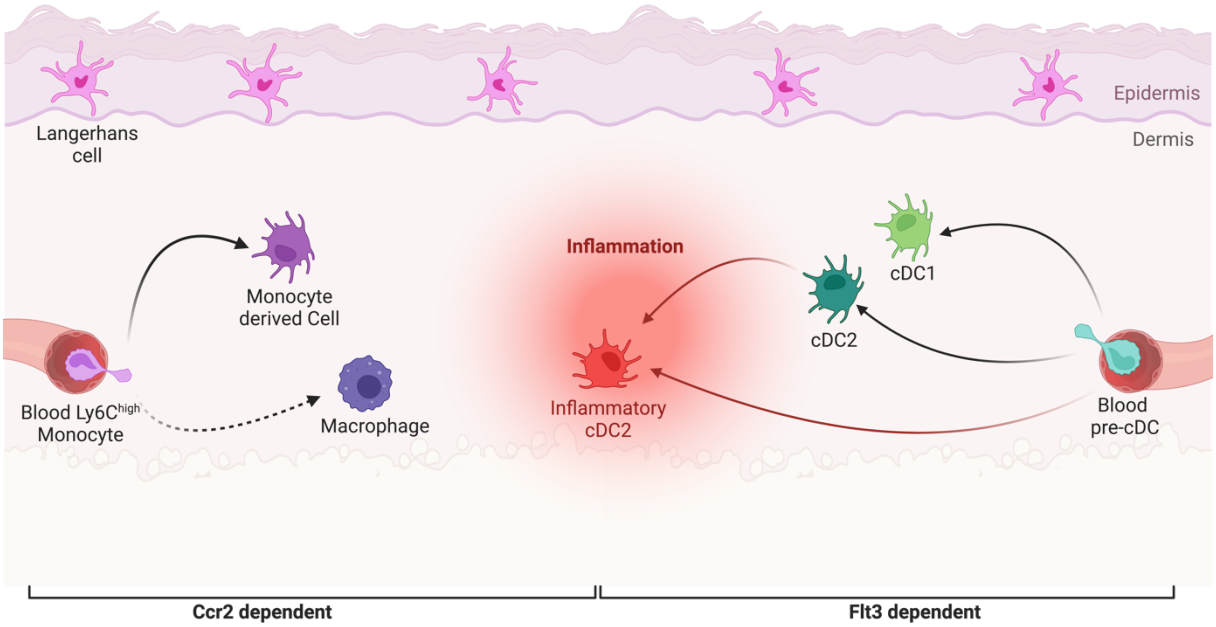

Figure 1. Schematic Representation of mononuclear Phagocytes resident in the murine Skin
The skin is divided into the epidermis and the dermis. Among the mononuclear phagocytes only Langerhans cells reside in the epidermis, all other mononuclear phagocytes belong to the dermal compartment. Tissue macrophages and Langerhans cells originate from embryonic progenitors and both are capable of self-renew. The dermis is traversed by blood vessels, that allows the recruitment of blood-born cells in the skin tissue. Blood Ly-6C^{high} monocytes extravasate into the dermis in an CC-chemokine receptor 2 (CCR2)-dependent manner, where they differentiate in dermal monocyte-derived cells and probably also in Macrophages (indicated by the dashed arrow). Similarly, blood-derived pre-cDC precursors enter the dermis in a Fms-related tyrosine kinase 3 (FLT3)-dependent manner and reside in the dermis as differentiated Conventional dendritic cells (cDC). cDC2 or pre-cDC2 have the capability to adopt a hybrid inflammatory cDC2 phenotype dependent on CCR2-signalling, which exhibit shared characteristics in terms of phenotype, gene expression, and function with both cDC and monocyte-derived cells. Created with Biorender.com.

In contrast to moC, cDC express the surface marker CD26 and undergo homeostatic maturation under steady-state conditions to continuously deliver self-antigen to T cell zones of draining lymph nodes for the establishment of peripheral tolerance to prevent responses of auto-reactive T cells (Probst et al., 2003; Sporri and Reis e, 2005; Waithman et al., 2007). After activation in response to innate stimuli cDC in the skin undergo terminal differentiation, up-regulate the expression of co-stimulatory molecules and migrate through afferent lymphatic vessels (Tripp et al., 2008) to skin-draining lymph nodes, where they promote clonal expansion of naïve antigen-specific T cells and orchestrate T cell effector function (Clausen and Stoitzner, 2015). Dermal cDC can be further divided into two subsets: cDC1 and cDC2 (Malissen et al., 2014; Henri et al., 2010). cDC1 were found to have a pivotal role in cross-presentation of antigen (Bedoui et al., 2009; Henri et al., 2010) and are identified as Langerin(CD24)$^{+}$ XCR1^{+} DC in the murine dermis. Activator protein-1 (AP-1) family transcription factor basic leucine zipper transcriptional factor ATF-like 3 (BATF3), together with Interferon regulatory factor

8 (IRF8) controls their late development. cDC2 are dependent on Interferon regulatory factor 4 (IRF4) and form a specific population of mouse dermal DC expressing CD11b$^+$ sirp1α^+.

Recently a subset similar to the phenotype of CD11b$^+$ cDC2 has been described in the dLN and infected tissues. This subset, termed inflammatory (infl.) cDC2 (CD11b$^+$ IRF4$^+$) displays a hybrid phenotype of cDC1, monocytic and cDC2, which is reflected by the expression of CD64, Mar-1 and intracellular IRF8 expression. Through the gained Fc receptor expression infl. cDC2 can internalize immune complexes to boost MHC-II restricted CD4$^+$ T cell responses and can induce CD8$^+$ T cell responses (Bosteels et al., 2020b). Upon activation, antigen bearing cDC migrate in an CCR7 dependent manner to the dLN, where they can prime naïve T cells (Hintzen et al., 2006; Vermaelen et al., 2000).

2.4 The antiviral CD8$^+$ T cell Response

2.4.1 Pox virus Infection of the Skin

The virus *Variola major* is a DNA virus of the orthopox family and is the causative agent of small pox. Due to its high contagiousness it probably killed more people than any other disease in human history. In the twentieth century alone smallpox has been responsible for 400 million deaths (Parker et al., 2014). Edward Jenner proved that cowpox can protect against small pox infection, which sparked the brilliant concept of using vaccination as a prophylaxis against transmittable diseases. Finally, a worldwide vaccination program using *vaccinia virus* (VACV) as a small pox vaccine led to the certified eradication in 1980 (Hammarlund et al., 2003; Stewart and Devlin, 2006). Immunization with VACV results in an acute infection in human as well as mice, that provokes the development of an adaptive immune response. Experimentally intradermal VACV infection of the skin is induced by the application of virus into the cutaneous tissue at the murine ear pinnae. Due to the local virus replication in cells of the skin, disease progression becomes evident locally: the clinical picture is characterized from 4 days post infection (dpi) on by cutaneous lesions and tissue thickening, that is caused by massive cell infiltration (Tscharke and Smith, 1999). The tissue infiltrating cells comprises first subsets of Ly-6C$^+$ and Ly-6G$^+$ phagocytes, and is followed a few days later by T cell infiltration. With increasing numbers of T cells the viral titer is decreasing (Fischer et al., 2011), which reflects directly the important role of cytotoxic CD8$^+$ T cells in the progress of virus clearance. Importantly, in the protection against VACV reinfection the T cell response plays a pivotal role, rather than neutralizing antibody production (Liu et

al., 2010). After infection clearance the majority of antiviral T cells are resident in skin (Jiang et al., 2012), we investigated how these antiviral $CD8^+$ T cell are formed and how they get recalled.

2.4.2 Origin and Maturation of $CD8^+$ T lymphocytes

Bone marrow-derived lymphocytes express a single kind of structurally unique receptors, in $\alpha\beta$ T cells these receptors consist of an α chain assembled with a β chain and can recognize protein derived antigen. Through recombination (Grawunder et al., 1998) and the imprecise joining of an array of gen segments coding for the two chains, the repertoire of antigen receptors among T lymphocytes is tremendous (Jeske et al., 1984). This increases the probability to recognize certain antigens (Medzhitov and Janeway, 2000).

Immature T lymphocytes leave the bone marrow and colonize the thymus to undergo T cell receptor (TCR) rearrangement and maturation, including positive and negative selection (Takahama, 2006). In the thymus only T lymphocytes that express a TCR capable of recognizing antigens on self-MHC are positively selected; this process is also called self-restriction (Zinkernagel and Doherty, 1997). Depending on their ability to recognize either MHC-I or MHC-II, double positive T lymphocytes ($CD4^+$ $CD8^+$) differentiate either in single positive CD8 (if recognizing MHC-I) or single positive CD4 (if recognizing MHC-II) T cells respectively (Carpenter and Bosselut, 2010). In the second step, which is called negative selection, auto-reactive T cells are eliminated. For the purpose of central tolerance, T cells holding a TCR with no or low affinity to self-antigen survive (Sprent et al., 1988; Starr et al., 2003). After thymic selection the remaining T cells can identify foreign antigens on self MHC, egress the primary lymphoid organ as mature but naïve T cells and thus enter circulation to encounter their cognate antigen.

2.4.3 The Formation of an anti-viral CTL mediated Adaptive Immune Response

Viruses attach to cells and replicate inside the host cells by using its cellular pathways for the synthesis of new viral particles (Modrow et al., 2013), by doing so they also feed viral material into the host's protein recycling machinery. Inside the host's cells non-functional, misfolded endogenous proteins, as well as viral proteins during infections, are ubiquitinated and sub-sequentially degraded by proteasomes into peptides to recycle amino acids and thus prevent intracellular protein aggregation. Linearized fragments, with between 8 to 11 amino acids in size, derived of formerly intracellular proteins are loaded on MHC-I molecules. Due to two distinct processes for antigen loading on MHC,

intracellular antigens are in principle loaded on MHC-I, whereas extracellular antigens are associated with MHC-II. $CD8^+$ T cells exclusively recognize MHC-I restricted antigens, while $CD4^+$ T cells are dependent on antigens presented on MHC-II molecules. Antigen loading is followed by presenting the antigen-MHC complex on the cell's surface. From the site of infection antigen-loaded APCs migrate to the draining lymph nodes or the spleen. The lymph nodes collect antigen draining through the lymph from the periphery, whereas the spleen is specialized to present blood born antigens. Priming of naïve T cells predominantly occurs in these two secondary lymphoid organs, namely lymph nodes and spleen (Abbas Abul K. et al., 2014). The antigen-MHC complex is recognized by naïve T cells through TCR ligation. If additional accessory signals such as co-stimulation and cytokines are delivered, naïve T cells are activated and differentiate into effector T lymphocytes, this process is called T cell priming. Depending on the stimulus, APC modulate the outcome of lymphocyte-mediated immune responses through either surface expression of activating or inhibiting co-stimulators as well as the secretion of cytokines or chemokines. Thus, APC such as dendritic cells (DC) play a key role in orchestrating T cell responses, because they provide all of these three signals for T cell activation. DC are most efficient in priming of T cells and thus hold specializations for antigen processing, presentation and T cell activation, such as constitutive expression of co-stimulatory molecules and MHC-II.

Priming of T cells is followed by a process called clonal selection, a term established by Frank Macfarlane Burnet (Burnet, 1976). This process ensures that only adaptive immune cells are activated, which are antigen specific. Only these specific cells can undergo clonal expansion by autocrine IL-2 signaling and consequentially can differentiate into effector cells to form a specific defense against a certain pathogen. Cytotoxic T Lymphocytes (CTL) are effector T cells derived from $CD8^+$ $\alpha\beta$ T cells. Once activated, effector T cells express activation specific surface molecules, such as CD44. They enter the blood circulation and traffic to inflamed non-lymphoid tissues, such as epithelial surfaces, where immune responses are required. Depending on the target tissue, effector T cells express certain addressins and selectins (e.g. CD62L for the egress in lymphoid tissues) to find their way throughout the body. The recognition of cognate antigens on the MHC-I complex of infected cells occurs through the TCR on the CTL's surface and takes place at the site of infection, where the subsequent killing of the target cells is initiated. CTL induce apoptosis in target cells via two alternative ways: through the usage of Fas/Fas-ligand interaction or by unidirectional release of cytotoxic substances such as Granzyme B and Perforin. Additionally, CTL produce antiviral cytokines such as IFNγ, TNFα and IL-2, which are also essential for the resolution of infections. In viral infections IFNγ was found to be the most important cytokine, since it induces the upregulation of an array of genes, whose expression is responsible for diverse antiviral responses for example through IFITM3 (Ariotti et al., 2014). For instance, IFNγ upregulates the expression of MHC molecules, which in turn boosts antigen

presentation and thus T effector cell election (Zhou, 2009; Früh and Yang, 1999). Through the removal of pathogenic material and infected cells, replicative viral particles are eliminated and the infection is cleared. The absence of pathogen derived antigen and concurrent danger stimuli, approximately 14 days after infection, leads to the contraction of the number of CTLs. Most CTLs undergo apoptosis, but a small fraction of T cells persist and become long-living memory T cells. (Abbas Abul K. et al., 2014).

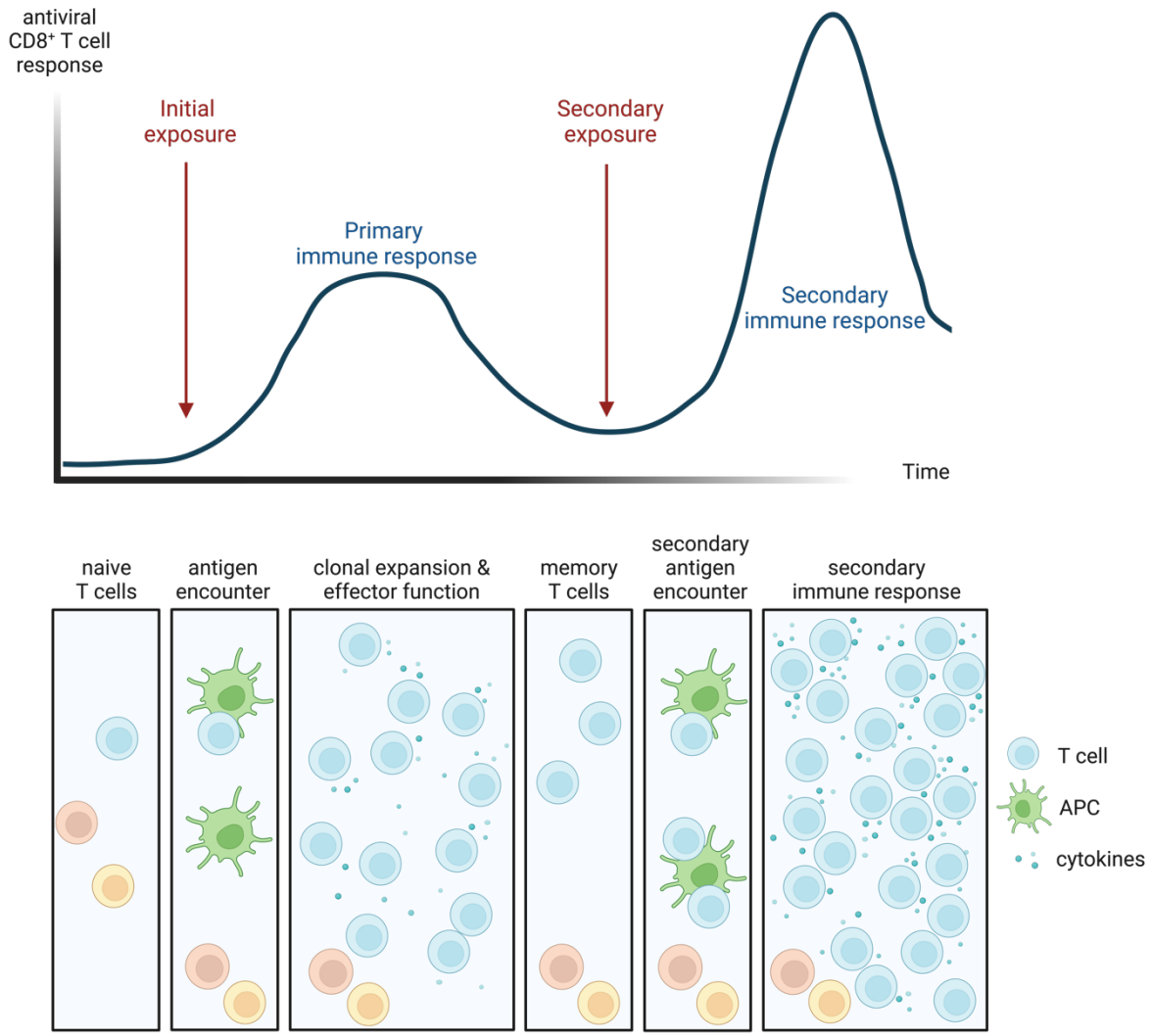

Figure 2. The Course of a primary and secondary antiviral CD8⁺ T cell mediated adaptive Immune Response
After virus infection naive CD8⁺ T cells encounter viral antigen and get primed by antigen-presenting cells (APC) in secondary lymphoid organs. Priming induces proliferation and the differentiation into cytokine producing cytotoxic T lymphocyte (CTL), which can eliminate the virus. Upon virus clearance the CTL response contracts, but a small number of memory lymphocytes persists to ensure long term immunity to the target virus. Upon reinfection the antigen specific recall of memory T cells is induce through the encounter of cognate antigen, which is presented on an APC. The recall of memory T cells leads to an enhanced response and thus to a more rapid virus clearance in comparison to the primary immune response. Created with Biorender.com.

2.4.4 $CD8^+$ T cell derived immunological Memory

Memory T cells account for the highly efficient long-term immunity against local and systemic reinfection (Abbas Abul K. et al., 2014). These cells are characterized by their ability to respond most rapidly to cogante antigen encounter and their prolonged survival (Cui and Kaech, 2010). The longevity of memory T cells is programmed during the conversion from effector to memory T cell, when IL-7 signaling induces low level proliferation and the expression of anti-apoptotic proteins. Compared to naïve T cells, persisting memory cells mount an enhanced response to secondary antigen reencounter, leading to an accelerated clearance of a homologous reinfection (Ochsenbein et al., 2000; Wherry et al., 2003a).

Memory T cells are heterogeneous in their tissue localization, their ability to migrate to different compartments and accordingly in their expression pattern of adhesion molecules and chemokine receptors required for homing. In the early stages of memory T cell research two lineages were described in blood (Sallusto et al., 1999). Memory T lymphocytes expressing the receptors CCR7, CD127 and CD62L for the homing to secondary lymphoid organs (SLOs) were termed central memory T cells (T_{CM}). It was proposed, that those provide a pool of cells for recall responses by giving rise to a second wave of effector T cells, which traffic to SLO, where they get access to antigens presented by APC. Besides T_{CM} another population was identified, which not only recirculates from blood to SLO, but also egresses blood or lymphatics to enter peripheral tissues, e.g. skin. These cells were termed effector memory T cells (T_{EM}) and can be distinguished from T_{CM} by the absence of CCR7 as well as lack of CD62L on their surface (Sallusto et al., 1999) combined with heightened levels of effector molecule expression (Rosato et al., 2017). In the periphery T_{EM} respond to antigenic stimulation by rapidly producing effector cytokines to initiate immune responses.

Soon the model of two memory T cell populations underwent revision, when non-circulating memory T cells were observed in the periphery and highly enriched at sites of previous infection. Thus the idea of memory T cells residing in the periphery, remaining there permanently without recirculation through lymphatics or blood, arose (Masopust et al., 2001). Indeed, these non-circulating were identified at epithelial tissues such as the skin or mucosa and called tissue resident memory T cells (T_{RM}) (Gebhardt et al., 2009; Jiang et al., 2012; Mackay et al., 2015; Steinert et al., 2015). In skin, the composition of the local pool of T_{RM} is shaped by cross-competition of local antigen, which fine-tunes the repertoire of antigen specific memory cells (Muschaweckh et al., 2016; Khan et al., 2016). Thus, TCR stimulation of antigen-specific $CD8^+$ T cells at the site of infection controls T_{RM} development. T_{RM} are kept resident in skin because they express the C-type lectin receptor, CD69 and the alpha subunit of the Eβ7 integrin, CD103 (Gebhardt and Mackay, 2012). The interaction of T_{RM} with E-cadherin of

epithelial cells mediates retention and survival of this subpopulation of memory T cells in the skin (Carbone et al., 2013). It was found, that this third population of memory $CD8^+$ T cells is crucial for surveying and mounting rapid immune responses *in situ* upon reinfection (Schenkel and Masopust, 2014). T_{RM} cells are lodged at the frontline sites of infection, in order to respond immediately. In case of reencounter of their cognate antigen T_{RM} produce rapidly IFNγ, that leads to the upregulation of the addressin VCAM-1, which recruits leukocytes into non-lymphoid tissues (Schenkel et al., 2014a). Thus, T_{RM} provide rapid immunity against local attacks, surpassing the protection mediated by their circulating counterparts (T_{CM}) or antibodies, which was shown upon viral challenge with HSV-1 and vaccinia virus in mice (Liu et al., 2006; Gebhardt et al., 2009; Jiang et al., 2012) as well as in human disease (Clark et al., 2012). Nevertheless, the precise mechanism of how T_{RM} are reactivated and which cells are involved in that process remain largely unresolved.

2.4.5 The Recall of $CD8^+$ Memory T cells

In general, $CD8^+$ T cell responses to intracellular pathogens are initiated by TCR stimulation by cognate antigen bound to MHC-I of an antigen-presenting cell. Since the fast majority of cells (except red blood cells) express MHC-I molecules, any cell can in principle act as an APC to provide the antigenic stimuli for activation. Furthermore, non-professional APC, which normally do not express MHC-II, were found to respond to conditions like inflammation, infection, stress or tissue injury, with the upregulation of MHC-I and -II expression (Hershberg et al., 1997), increase in antigen and inflammatory cytokines and the release of damage-associated-molecular patterns (DAMP), IL-33, ATP, etc. (Arosa et al., 2017). T cells located in the same area, such as T_{RM}, sense those danger-signals through the expression of the antigen specific TCR, killer Ig-like receptor, leukocyte Ig-like receptor, NKG2A, DAMP receptors, IL-33 receptors, purinergic receptors, and others (Arosa et al., 2017). Naïve T cells require a tightly controlled activation mediated through a multi-signal process mediated by bone-marrow derived APC (Sigal et al., 1999; Sigal and Rock, 2000; Lenz et al., 2000). In contrast memory cells hold a more efficient TCR signaling machinery than naive T cells (Bachmann et al., 1999) and in their activation speed might take priority over control mechanisms, as persisting memory cells have already proven to be effective.

It was suggested that T_{RM}, unlike naïve T cells, might be directly activated by TCR stimulation through nearby infected nonprofessional antigen-presenting cells, allowing the initiation of an immediate immune response at the site of antigen encounter (Crowe et al., 2003). However, despite some evidence the formal proof for the functional relevance of a non-pAPC for memory recall responses remains elusive. More recent studies revealed, that the recall of $CD8^+$ memory T cells requires CD28 co-stimulation for their optimal expansion and cytolytic function (Borowski et al., 2007), this co-

stimulation is provided by DC (Zammit et al., 2005). Interestingly, through restoration of IL-2 signaling (Fuse et al., 2008) or by CD137 stimulation (Bertram et al., 2004) the dependence on CD28 co-stimulation for the activation of memory T cells, but not naïve T cells, can be overcome. However, the crucial role for DC for the initiation of a secondary response to pathogen infection was shown *in vivo*: By utilizing bone marrow chimeras expressing either appropriate MHC molecules on the radioresistant or the radiosensitive compartment, bone marrow derived DC were found to be required for the secondary immune response to influenza virus infection of the lung (Castiglioni et al., 2008). Similarly, in the case of HSV-1 infection of the skin the recall response by central memory T cells (T_{CM}) was dependent on radiosensitive DC, but not on radioresistant LC (Belz et al., 2006). Importantly, in the latter study the conclusions were drawn on numbers of antigen specific T cells in spleen 8-10 days after infection and might thus be only valid for memory T cells present in spleen, such as T_{CM}, indicating that the recall of memory $CD8^+$ T cells might be more complex. Another study using VSV re-challenge brought the evidence, that the requirements for the recall of memory $CD8^+$ T cells are memory subset specific. This study found that the depletion of $CD11c^+$ DC affect $CD62L^{high}$ memory $CD8^+$ T cells, which home to the lymph node, more than their $CD62L^{low}$ counterparts (Zammit et al., 2005). Thus, the recall of memory T cells in non-lymphoid organs, i.e. T_{RM}, might be exempted from being dependent on DC.

Notably, early studies were primarily concerned with understanding the requirements for activating circulating memory T cells, thus the subset of non-circulating memory T cells had not been extensively studied, that changed with the discovery of distinct memory T cell subsets (Sallusto et al., 1999; Schenkel and Masopust, 2014). For example, studies analyzing parameters such as T cell expansion, disease scores, weight loss, survival, and viral titers after HSV re-infection concluded that DC are important for $CD8^+$ T_{RM} recall in the skin (Wakim et al., 2008) and the female reproductive tract (Shin et al., 2016). Importantly, all of such readouts were assessed many days after reinfection, when it is already difficult du distinguish between the engagement of incoming circulating memory T cells and resident memory T cells within the tissue. To address this caveat, when investigating the requirements for secondary immune responses, it is advisable to asses T_{RM} recall either directly by quantification of TCR-stimulation or IFNγ production of T_{RM}, or to determine parameters downstream of T_{RM} mediated protection in the very early phase after reinfection, before T_{CM} infiltrate the tissue.

A very elegant study on the recall of memory T cells following reinfection of the lung by Influenza A virus measured TCR stimulation directly by using a Nur77 reporter system. They identified the $CD11c^+$ $XCR1^+$ APC as the required cell for the reactivation of $CD8^+$ memory T cells in the lymph node, while the cellular requirements for the recall of lung $CD8^+$ T_{RM} are less stringent, diverse APC, including hematopoietic and non-hematopoietic cells, imparts distinct functional responses (Low et al., 2020).

The revisited concept arose, that dependent on the anatomical niche and subset of memory T cell distinct stimuli for reactivation might be required. In skin the cellular requirements for the recall of $CD8^+$ T_{RM} remain largely undefined and are subject of this thesis.

2.5 Objective of this thesis

CTL mediate immunity against intracellular microbes like viruses. CTL priming and differentiation control allows viral clearance, since activated CTL kill infected cells and recruit other immune cells. Professional APC of the MNP family are required for CTL priming and differentiation, however structural cells might be also able to impact an immune response.

Especially during viral skin infection, the network of MNP undergoes significant changes in terms of cell number and phenotype, highlighting the importance of their adaptation to the current skin condition. At the same time, the heterogeneity in MNP is the reason for the difficulty in identifying those cells when studied during inflammation. To address this, we described the MNP network during viral infection in the skin based on surface marker expression and ontogeny. Next, we investigate the functional specialization of MNP subsets in the CTL response to viral skin infection. Utilizing bone marrow chimeric mice allowed us to specifically impair intrinsic features of MNP subsets such as antigen presentation, co-stimulation, transmission of T cell help and migration. We monitored the effects of MNP in the control of the formation of CTL responses and immunological memory development *in vivo* upon Vaccinia virus infection of the skin.

Tissue-resident memory $CD8^+$ T cells (T_{RM}) are responsible for maintaining local immune surveillance within the tissue and initiating the most immediate immune responses upon reinfection. However, the precise mechanism of how $CD8^+$ T_{RM} are reactivated and which cells are involved in that process remains unresolved. To address this, antigen expression in keratinocytes was induced in transgenic mice, and the recall response by activated antigen-specific T_{RM} in the skin was examined. The respective engagement of cells for antigen presentation to T_{RM} was assessed, considering the functional role of keratinocytes and also of professional APC in this process.

3 MATERIAL

3.1 Instruments

Table 1. Instruments

Instrument	Type	Supplier
Agitator	VOS16	VWR
Caliper	209-658	Mitutoyo
Cell counting chamber	Neubauer Zählkammer improved	Assistant
Centrifuge	Heraeus Multifuge 3L	Thermo Scientific
Flow Cytometer 3 Laser	Canto	Becton Dickinson
Flow Cytometer 5 Laser	Symphony	Becton Dickinson
Fluorescence activated cell sorter	AriaII	Beston Dickinson
Forceps (curved)	Dumont #7 (11271-30)	Fine Science Tools
Gel documentation system	Gel Doc XR System	Bio-Rad
Gel electrophoresis chamber	several	Bio-Rad
Glas homogenisator 2 mL	TT57.1	Roth
Incubator	CO_2-Incubator	Sanyo
Laboratory scale	AE 100	Mettler
Laboratory scale	LP2200P	Sartourius
Laminar flow	MSC-Advantage	Thermo
Light microscope	CKX31	Olympus
Micro centrifuge	5415 D	Eppendorf
Micro pipette	10 µL, 20 µL, 200 µL, 1000 µL	Eppendorf
Multichannel pipette	50 µL, 200 µL	Eppendorf
PCR cycler	peqstar	Peqlab
pH Meter	pH 537	WTW
Pipette boy	several	Integra Biosciences
Power supply unit	EPS 3501 XL	BioRad
Scissors	91460-11	Fine Science Tools
Teflon pestel for 2 mL reservoir	TT63.1	Roth
Thermo shaker	TS-100	Peqlab

Instrument	Type	Supplier
Tissue homogenizer	VOS-16	VWR
Vibrating platform shaker	Titramax 100	Heidolph
Vortexer	Vortex Genie 2	Bender&Hobein AG

3.2 Consumption Items

Table 2. Consumption Items

Item	Type	Supplier
Cell culture flask	75 cm^2, 175 cm^2	Greiner
Cell strainer	70 µm pore size	Greiner
Disposable nitrile / latex gloves	-	Sempercare
Eppendorf cups	0.5 mL, 1.5 mL, 2.0 mL	Greiner
FACS tubes	5.0 mL	Sarstedt
Falcon	15 mL, 50 mL	Greiner
Glass capillary	-	Roth
Injection needle	Gauge 27 ½ inch	B. Braun
PCR tube plate & lids	96 well á 0.2 mL	Peqlab
Pipette tips with/without filter	10 µL, 20 µL, 200 µL, 1000 µL	Nerbe plus/Eppendorf
Serological pipette	5 mL, 10 mL, 25 mL	Greiner
Syringe	1 mL, 5 mL, 10 mL	B. Braun
Multi well plate	6 well, 24 well, 96 well (Flatbottom, U or V shape)	Greiner

3.3 Chemicals

Table 3. Chemicals

Reagent	Supplier
4-Hydroxytamoxifen (≥ 70% Z isomer)	Sigma-Aldrich
Acetic acid	Sigma-Aldrich

Reagent	Supplier
Agarose	AppliChem
Ammoniumchloride (NH4Cl)	Roth
Anthralin formulation (Dithranol in vaseline)	Langguth group, Mainz
Condensed Milk (sugared)	Nestle
Cremor baslis	Langguth group, Mainz
Crystal violet	Roth
Dimethyl sulfoxide (DMSO)	Roth
Diphtheria toxin	Sigma
dNTPs (100mM)	Thermo Fisher Scientific
Ethanol 100%	AppliChem
Ethanol 96% (EtOH)	Roth
Ethidium bromid (EtBr)	Roth
Ethylenediaminetetraacetic acid (EDTA)	Roth
Fingolimod (FYT720)	Sigma
Forene® (Isoflurane)	Abbott
Green go Taq Flexi Buffer (5x)	Promega
HEPES	Roth
IMI-Sol formulation (5% imiquimod (w/w))	Langguth group, Mainz
Ketamine	Inresa
Magnesiumchloride (25mM)	Promega
Monosodiumphosphate (NaH2PO4)	Roth
Optiprep (60%)	Sigma
Pluoronic F68 10%	Gibco
Roti® Histofix 4%	Roth
Xylazine (Rompun)	Bayer
Potassium chloride (KCl)	Roth
Potassiumbicarbonate (KHCO3)	Merck
Sodiumazide (NaN3)	Roth
Sodiumchloride (NaCl)	Roth
TE buffer	Sigma Aldrich
Trypan-blue	Sigma-Aldrich
Tween-20	Roth

Reagent	Supplier
Xylene cyanol	Sigma

3.4 Buffer and Solutions

Table 4. Buffer and Solutions

Name	Composition
ACP lysing buffer Ammonium-Chloride-Potassium (ACP)	0.1 mM EDTA 150 mM NH_4Cl 1 mM $KHCO_3$ in ddH_2O pH 7,3
Crystal violet staining buffer	0.2 % (w/v) crystal violet in ddH_2O
DNA blue solution	500 mg Xylene cyanol in 1 L H_2O
FACS buffer	1 % BSA 20 mM EDTA 0.02 % Sodiumazide in PBS
Fixation buffer	2 % Roti® Histofix in PBS
HEPES buffered saline	50mM HEPES 150mM NaCl in ddH_2O pH 7.4
ketamine/xylazine	14.3 % ketamine 5.7 % xylazine in PBS
PBS buffer (10x) Phosphate buffered Saline (PBS)	1.4 M NaCl 0.1 M NaH_2PO_4 in ddH_2O pH 7,2
Proteinase K activation buffer	3 mM Calcium chloride 10 mM Tris-HCl 50 mM KCl (pH 8) in ddH_2O
SDQ buffer	50 mM KCl 10 mM Tris-HCl (pH 9.0) 0.45% Nonidet P-40 0.45% Tween-20

Name	Composition
	0.1 mg/mL Proteinase K in ddH_2O
TAE-buffer (50x)	2 M Tris-HCl 1 M Acetic acid 50 mM EDTA in ddH_2O
Trypan blue solution	0.05% (w/v) Trypan blue 140 mM NaCl 10 mM NaH_2PO_4 in ddH_2O

3.5 Oligonucleotides

Oligonucleotides for PCR were designed with the Geneious software, synthesized and solved in TE buffer at a concentration of 100 µM by Sigma-Aldrich. The primers were then stored at -20 °C in 10 µL aliquots (KO: knock out; WT: wildtype; fwd: forward, rev: reverse).

Table 5. Oligonucleotides for Genotyping

Genotype	Sequence from 5' to 3'	
Cre	fwd	GGACATGTTCAGGGATCGCCAGGCG
	rev	GCATAACCAGTGAAACAGCATTGCTG
$CCR2^{-/-}$	WT fwd	CACAGCATGAACAATAGCCAAG
	KO fwd	CCTTCTATCGCCTTCTTGACG
	Common rev	CCACAGAATCAAAGGAAATGG
$FLT3^{-/-}$	Common fwd	TCCACGTTGTTCCCTCTACC
	WT rev	TATGTGGGCAATTTGGCTCT
	KO rev	TGATCTCGTCGTGACCCAT
Langerin-DTR	WT fwd	GAATGACAGATCTGGCCTGAGCTCG
	KO fwd	TTCCAGCAGCTAGCCCTCTCCGAA
	Common rev	GTAGCTTTTATATGGTCAGCCAAGG
ST33	fwd	CCTCACATCATTGATGAGG
	rev	CACGCGGGCGTACATGG

3.6 PCR-Programs and Products

Table 6. PCR-Programs and Products for Genotyping

Genotype	PCR program	Product sizes
Cre	94 °C 30 s 65 °C 30 s 72 °C 60 s 30x 72 °C 5 min	Transgene: 350 bp WT: -
$CCR2^{-/-}$	94 °C 30 s 59 °C 30 s 72 °C 40 s 35x 72 °C 5 min	Transgene: 390 bp WT: 494 bp
$FLT3^{-/-}$	95 °C 30 s 59 °C 30 s 72 °C 40 s 35x 72 °C 5 min	Transgene: 700 bp WT: 250 bp
Langerin-DTR	94 °C 30 s 60 °C 60 s 72 °C 60 s 35x 72 °C 5 min	Transgene: 866 bp WT: 329 bp
ST33	94 °C 30 s 60 °C 30 s 72 °C 30 s 35x 72 °C 5 min	Transgene: 250 bp WT: -

3.7 Peptides

Table 7. Peptides

Name	Origin	Aminoacid sequence	Supplier
GP_{33-41} Peptide	Glycoprotein 2 of the Lymphocytic Chorio-meningitis-Virus (LCMV)	KAVNFATM	Proteogenix, France

3.8 Media

Table 8. Media

Name	Supplier
DMEM Dulbecco's Modified Eagle Medium	Gibco
HBSS Hanks Balanced Salt Solution	Gibco
RPMI-1640 Roswell Park Memorial Institute (*RPMI*) *1640* Medium	Sigma-Aldrich

3.9 Supplements for Buffer and Media

Table 9. Supplements for Buffer and Media

Name	Supplier
Bovines Serum Albumin (BSA)	PAA
Foetal Bovines Serum (FBS)	Gibco
L-Glutamine (Q)	Sigma-Aldrich
Penicillin-Streptomycin	Sigma Aldrich
Sodium pyruvate 100 mM	Gibco
β-Mercapto-Ethanol	Sigma

3.10 Composition of Media

Table 10. Composition of Media

Name	Composition
DMEM 10%	10% FCS 1% L-Glutamine 1% Sodium pyruvate 1% Penicillin-Streptomycin 50 µM β-Mercapto-Ethanol in DMEM
RPMI-1640 10%	10% FCS 1% L-Glutamine

Name	Composition
	1% Sodium pyruvate 1% Penicillin-Streptomycin 50 µM β-Mercapto-Ethanol in RPMI-1640

3.11 Enzymes

Table 11. Enzymes

Name	Reconstitution buffer	Concentration	Supplier
Collagenase IV	RPMI-1640 0%	batch specific	Worthington
DNase I Deoxyribonuclease I	Nuclease free ddH_2O	> 2,000 U/mg	Roche
Dispase® II	HEPES buffered saline	> 0.8 U/mg	Roche
Go Taq® Polymerase	-	5 U/µL	Promega
Proteinase K	Proteinase K activation buffer	600 U/mL	Serva
Trypsin-EDTA	-	1x	Sigma-Aldrich

3.12 Antibodies for Flow Cytometry

Since all experiments were performed in mice, the antibodies used for flow cytometry were specific for mouse antigens. To avoid antibody binding to Fc receptors the Fc-receptor-Blocks (rat anti-mouse CD16/32 clone 2.4G2; 1: 100 of 0.75 mg/mL, own production; rat anti-mouse Fc-γRIV block, clone E9E; 1:1250 of 10 mg/mL, Falk Nimmerjahn group) was used before adding staining antibodies.

Table 12. Antibodies for Flow Cytometry

Specificity	Fluorochrome	Clone	Supplier	Dilution
CD4	PE-Cy5	RM 4-5	Biolegend	1:400
	APC-Cy7	RM 4-4	Biolegend	1:400
CD8	Pacific Blue	53-6.7	Biolegend	1:400
	PE-Dazzle	53-6.7	Biolegend	1:400
	APC-Cy7	53-6.7	Biolegend	1:400

Specificity	Fluorochrome	Clone	Supplier	Dilution
CD11b	BV421	M1/70	BioLegend	1:500
	BV650	M1/70	BioLegend	1:500
	BV605	M1/70	BD	1:500
CD11c	APC-R700	N418	BD	1:500
CD19	PE-Cy5	1D3	BioLegend	1:500
	APC-Cy7	1D3	Biolegend	1:500
CD24	FITC	M1/69	Biolegend	1:1,000
	APC	M1/69	BioLegend	1:1,000
	BUV395	M1/69	BD	1:1,000
CD26	PE	H194-122	Biolegend	1:200
CD31	Alexa647	BM8	Biolegend	1:200
	PE-Cy7	BM8	Biolegend	1:200
CD34	R781	RAM34	BD	1:200
CD44	APC-R700	IM7	BioLegend	1:300
CD45.1	FITC	A20	BioLegend	1:500
CD45.2	BV421	104	Biolegend	1:150
CD49f	PE	GoH3	Biolegend	1:500
CD62L	PE-Cy7	MEL-14	BioLegend	1:500
CD64	BUV737	X54-5/7.1	BD	1:500
CD69	APC	H1.2F3	BioLegend	1:400
CD90.1	BV711	OX-7	BioLegend	1:1,000
CD90.2	APC	145-2C11	Biolegend	1:300
CD103	BV510	M290	BioLegend	1:200
	BV510	2E7	BioLegend	1:150
	BV711	M290	BioLegend	1:200
EpCAM(CD326)	APC	G8.8	Biolegend	1:2000
	PE-Dazzle	G8.8	Biolegend	1:2000
Fc-εRIa	PE-Dazzle	Mar-1	Biolegend	1:500
H-2D^{b}	PE	28-14-8	Biolegend	1:300
	FITC	28-14-8	Biolegend	1:300
H-2K^{b}	PE	AF6-88.5	Biolegend	1:300
	FITC	AF6-88.5	Biolegend	
Ly6-C	BV570	HK1.4	Biolegend	1:500

Specificity	Fluorochrome	Clone	Supplier	Dilution
Ly6-G	BV750	1A8	BD	1:500
MHC-II (I-A/I-E)	PE-Cy5	M5/114.152	BioLegend	1:1,000
	BV510	M5/114.152	Biolegend	1:2,000
	BV785	M5/114.152	BD	1:2,000
NK1.1	PE-Cy5	PK136	BioLegend	1:500
	APC-Cy7	PK136	BioLegend	1:500
pan45	BV785	30-F11	BioLegend	1:1,000
	BUV805	30-F11	BD	1:1,000
PDCA1	BV711	927	BD	1:500
Sca-1	BV421	D7	Biolegend	1:200
Siglec-F	BV480	E50-2440	BD	1:500
Sirp1α	PE	P84	Biolegend	1:300
	PE-Cy7	P84	Biolegend	1:300
XCR1	BV650	ZET	Biolegend	1:300

3.13 Tetramers for Flow Cytometry

Table 13. Tetramers for Flow Cytometry

Specificity	Fluorochrome	Supplier	Dilution
TCR specific for LCMV $B8R_{20-27}$	APC	own production	1:100
TCR specific for LCMV $GP_{33–41}$	BB515	own production	1:100

3.14 Small Molecule Dyes for Flow Cytometry

Table 14. Small molecule dyes for Flow Cytometry

Name	Specificity	Emission maxima	Supplier	Dilution
7-AAD Non-fixable viability dye	dead cells	647 nm	Biolegend	1:100
eFlour® 780 Fixable viability dye	dead cells	780 nm	eBiosciences	1:1000

3.15 Viruses

Vaccinia virus (VACV)

The recombinant Vaccinia virus (rVACV-G2) has the sequence coding for the glycoprotein 2 (G2) of LCMV WE inserted into the thymidine kinase locus of the parental strain Vaccinia virus Western Reserve (VACV-WR) (Hany et al., 1989). The Glycoprotein 2 contains the peptide $GP_{33–41}$, which is commonly used as a model antigen to investigate antigen specific immune responses e.g. following viral infection of the skin. The recombinant Vaccinia virus rVACV-G2 was originally obtained from Dr. D. H. L. Bishop (Oxford University, United Kingdom) and propagated on BSC-40 cells. Virus titer was determined by standard plaque assay. All procedures with infectious agents were done under safety level 2 conditions.

3.16 Cell Lines

BSC-40

BSC-40 cells belong to an epithelial cell line, which was originally obtained from the kidney of African green monkey (*Cercopithecus aethiops*). Cells were purchased from ATCC (ATCC® CRL-2761TM) and maintained as adherent monolayer cultures in DMEM 10%. Due to the cell lines' susceptibility for vaccinia virus, cells were infected for virus propagation or plaque assay.

3.17 Strains of Mice

All mice were on a C57BL/6J background, were bred and maintained under specific pathogen-free conditions at the Translational Animal Research Center of the University of Mainz, Germany. They were supplied with drinking water and food *ad libitum*. Animal procedures were conducted following institutional guidelines and with permission of the State of Rhineland Palatinate. For terminal sampling mice were euthanized by a lethal dose of CO_2.

B6.CD45.1 (B6.SJL-Ptprca Pepcb/BoyJ) and **B6.PL-Thy1a/CyJ** mice carry the congenic markers CD45.1 on all lymphocytes or CD90.1 on all T cells respectively. Both congenic strains of mice allow

identification of transferred cells as well as their successors by flow cytometry, since the cells of C57BL/6J mice are characterized by the expression of the congenic markers Ly5.2 on lymphocytes and Thy1.2 on T cells.

CCR2$^{-/-}$ (B6.129S4-CCR2^{tm1Ifc}/J) mice lack the C-C chemokine receptor type 2 (CCR2 or CD192), in these mice monocytes are no longer able to leave the bone marrow in order to infiltrate peripheral tissues. In skin these mice lack monocyte derived cells and inflammatory cDC2, all other cells of the local DC network develop normally (Boring et al., 1997; Bosteels et al., 2020a).

CCR7$^{-/-}$ (B6.129P2(C)-CCR7^{tm1Rfor}/J) mice lack the C-C chemokine receptor type 7 (CCR7), in these mice lymphocytes and Dendritic cells are not able to migrate into secondary lymphoid organs (Boring et al., 1997; Höpken et al., 2004).

CD40$^{-/-}$ (B6.129P2-Cd40^{tm1Kik}/J) mice are deficient of the co-stimulatory molecule CD40, which results in impaired humoral (Kawabe et al., 1994) and cell-mediated immune responses (Grewal and Flavell, 1997). CD40 is mostly expressed on B cells, DC and myeloid cells (van Kooten and Banchereau, 2000) and interacts with CD40L on activated T cells, leading to an licensed state of APC (Lanzavecchia, 1998) including the upregulation of cytokines such as IL-12, antigen-presenting molecules, costimulatory and adhesion molecules (Vonderheide, 2018).

CD70$^{-/-}$ (B6.Cg-Cd70$^{tm1.1Jda}$/J) mice lack the co-stimulatory molecule CD70. The ligand of CD70 is CD27, and is expressed on naïve T cells and upregulated after activation. CD70 deficiency in primary immune responses lead to an impaired antigen specific CD8^{+} T cell response and viral clearance to lymphocytic choriomeningitis virus (LCMV) (Munitic et al., 2013).

CD80$^{-/-}$ x CD86$^{-/-}$ (B6.129S4-Cd80^{tm1Shr}Cd86^{tm2Shr}/J) mice lack the co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2), upon stimulation both molecules can be rapidly upregulated on DC and regulates through the CD28/CTLA-4 expression on T cells activation or tolerance (Borriello et al., 1997; Sharpe and Freeman, 2002).

FLT3$^{-/-}$ (B6.129-FLT3^{tm1Dgg}/J) mice lack the FMS-like tyrosine kinase 3 (FLT3), which is in wildtype individuals expressed on early hematopoietic progenitor cells leading to differentiation into conventional DC. In FLT3$^{-/-}$ mice numbers of conventional DC are massively reduced in skin and other organs (Guilliams et al., 2016), whereas the development of monocyte derived cells remain unaffected (Mackarehtschian et al., 1995).

H-2Db$^{-/-}$ (B6.129P2-H-2Db^{tm1Bpe}) mice lack a functional gene for the MHC-class I haplotype H-2D^{b} (Vugmeyster et al., 1998), in those mice the H-2D^{b} restricted GP_{33-41} peptide presentation is disabled due to the lack of the specific presenting MHC class I molecule.

IFNγ-EYFP reporter mice (B6.129S4-IFN$\gamma^{tm3.1Lky}$/J) hold the 'interferon-gamma reporter with endogenous polyA transcript' (GREAT) allele, based on EYFP expression IFNγ producing cells can be identified using e.g. flow cytometry.

K14DIETER double transgenic mice Tg(KRT14-cre/ERT)20Efu/J x Tg(ACTB-GP33/NP396/bGal497/EGFP)69Gkl) allow tamoxifen-inducible presentation of three LCMV-derived CTL epitopes (GP_{33-41}/Db, GP_{34-41}/Kb and $NP_{396-404}$/Db) and one β-galactosidase-derived CTL epitope (β-$Gal_{497-504}$/Kb) by Cytokeratin 14 expressing cells, similar to the inducible expression of these epitopes by CD11c^{+} cells in DIETER mice (Probst et al., 2003).

Langerin-DTR (B6.Cd207^{tm1}(DTR/EGFP)Bjec) mice harbor a targeted insertion of the human Diphtheria toxin receptor (DTR) fused to eGFP in the Langerin locus and allow the conditional depletion of Langerin expressing cells by the injection of Diphtheria-toxin (DT) (Bennett et al., 2005).

Nur77-EGFP reporter mice (C57BL/6-Tg(Nr4a1-EGFP/cre)^{820Khog}/J) reporter mice express EGFP under control of the Nr4a1 (Nur77) promoter, The expression level of EGFP correlates with the strength of TCR stimulation following cognate antigen binding (Moran et al., 2011).

K14R26R-EYFP (Tg(KRT14-cre/ERT)20Efu/J x B6.129X1-Gt(ROSA)26Sor$^{tm1(EYFP)Cos}$/J) double transgenic reporter mice allow the tamoxifen-inducible expression of EYFP in Cytokeratin 14 expressing cells (Srinivas et al., 2001).

TCR327 (B6;D2-Tg(TcrLCMV)^{327Sdz}/J) mice carry the P14 transgenic T cell receptor that recognizes the LCMV-glycoprotein-derived epitope GP_{33-41} in the context of H-2D^{b} (Pircher et al., 1989), were bred to B6.Thy1.1 congenic mice.

4 METHODS

4.1 Genotyping of transgenic Mice

4.1.1 DNA Isolation from Biopsies

Biopsies of mice were taken either through removal of *phalanx distalis* from pups or through punching of the ear skin from weaned mice, simultaneously mice were marked using an unique identification mark for each individual per cage. The tissue biopsy was incubated in 500 µL SDQ-buffer containing 1.2 U/mL of proteinase K for 2 h at 65°C. The enzyme was then inactivated at 95°C for 20 min.

4.1.2 PCR and Gel-electrophoresis

The DNA solution of the biopsy was mixed with 250 µL DNA-blue solution, 1 µL of this mix was used for PCR to determine the genotype of the individual mouse by using transgene or wildtype specific primers. The composition of PCR mix is shown in Table 15. The PCR mix was filled up with ddH_2O to a total volume of 20 µL.

Table 15. Composition of PCR mix for Genotyping of Mice

Reagent	Volume in µL	Final concentration
5x Green GoTaq Flexi buffer	2.0	1x
MgCl2 solution (25mM)	1.2	1.5 mM
PCR Nucleotid Mix (each 10mM)	0.4	0.2 mM
Primer (each 100µM)	0.1	0.5 mM
GoTaq DNA Polymerase (5U/µL)	0.1	0.5 U
DNA solution isolated from biopsy	1.0	-

The PCR program was designed based on the length of the possible PCR-products and the melting temperature of primers. The sequences of transgene specific primer pairs and respective PCR programs are listed under the section ‘Material’. After completion of the PCR program the PCR product was analyzed by agarose gel electrophoresis (1% agarose gel, electrophoresis at 180 V for 20 min). Based on the respective length of the PCR product the genotype of each mouse was determined.

4.2 Virologic Methods

4.2.1 Propagation of Vaccinia Virus on BSC40 cells in Culture

Vaccinia virus (VACV) was cultivated on BSC-40 cells. The medium of a 175 cm^2 flask of semiconfluent BSC-40 cells was aspirated and replaced with 5 mL containing 5 x 10^5 PFU of VACV in DMEM 10%. The flask was incubated under gentle rocking for 1 h at RT. Then 20 mL DMEM 10% was added and put at 37°C for 36 to 48 h. When most cells showed cytopathic effects, cells and supernatant were collected in a 50 mL tube. After centrifugation (700 rcf at 4°C for 5 min) the supernatant was collected by aspiration, the pellet was resuspended in 2 mL of supernatant, aliquoted at 1 mL per tube and sonicated by ultrasound 5 times for 10 s with cooling on ice in between sonication periods. Tubes were then centrifuged at 16,000 rcf for 5 min at 4°C. The supernatant was then split into smaller aliquots of 100 µL, which were then stored at -80°C until usage for infections.

4.2.2 Vaccinia Virus Infection of the murine Skin

For viral skin infection mice were anaesthetized with ketamine/xylazine. Then 2 x 10^6 pfu of rVACV-G2 virus in 10 µL DMEM Medium were topically applied to the skin of the dorsal part of the ear pinna. Virus was introduced into the tissue by poking through the skin for 25 times using a 27 ½ G injection needle.

4.2.3 Determination of Vaccinia Virus Titer

Virus titer was determined of solutions from either newly generated virus stocks or the supernatant of homogenized skin tissue samples. BSC-40 cells were seeded in 24 well plates at 2 x 10^5 cells per well. After 24 h of incubation the medium was aspirated and replaced by 200 µL of serially diluted virus solutions (from undiluted to 1:100,000 dilution in 6 steps) in DMEM 10%. For viral adsorption, dishes were incubated for 1 h at 37°C. Then 1 mL DMEM 10% was added to each well and incubated further at 37°C until cytolytic effects arose as plaques in the monolayer (usually between 24 - 48 h of incubation). Then the medium was aspirated from the monolayer of cells and replaced by 1 mL of crystal violet solution per well. After 2 h the staining solution was aspirated, the wells were rinsed with distilled water and patted dry. Plaques were counted and the titer of the virus was calculated for each sample.

4.3 Experimental Applications at the murine Skin *in vivo*

4.3.1 Measurement of Ear Skin Thickness

Directly after euthanizing of mice the thickness of ear skin was measured using a caliper. The mean of at least three measurements at different areas of the tissue was calculated.

4.3.2 Topical 4OHT Treatment

For topical 4-hydroxy-tamoxifen (4OHT) treatment, anesthetized mice (ketamine/xylazine) were treated topically on the dorsal and ventral part of ear skin with 2.5 to 20 µg of 4OHT in a solution of ethanol, water and DMSO (48%/35%/17%) in a volume of 20 µl per ear. Mice were kept isolated for 4 h of incubation. Then the treated skin was cleaned with EtOH/water and the mice were placed back in the original cages.

4.3.3 Topical IMQ Treatment

For topical treatment with imiquimod (IMQ), mice were anesthetized (ketamine/xylazine) and treated on the dorsal and ventral part of ear skin with 50 mg IMI-Sol per ear (5% imiquimod (w/w), manufactured by the group of Prof. Peter Langguth (Johannes Gutenberg-University Mainz, Germany) per ear (Lopez et al., 2017).

4.3.4 Transcutaneous Immunization

Transcutaneous Immunization (TCI) of mice was conducted through the dithranol-imiquimod based vaccination (DIVA) protocol (Sohl et al., 2022), the formulations for topical treatment of skin were manufactured by the group of Prof. Peter Langguth (Johannes Gutenberg-University Mainz, Germany). Mice were anesthetized (ketamine/xylazine) prior to each treatment. Per ear the skin was topically treated with 50 mg dithranol in Vaseline. One day later the skin was treated topically with 50 mg IMI-Sol (5% imiquimod (w/w)) per ear, followed by the topical application of 100 µg of GP_{33-41} peptide (Proteogenix, France) in *cremor basalis* per ear. The development of an antigen-specific T cell immune response following TCI was monitored by tetramer-staining of $CD8^+$ T cells in the blood of treated mice.

4.4 Experimental systemic Applications *in vivo*

4.4.1 Adoptive Transfer of TCR327 CD8$^+$ T cells

Splenocytes were isolated by smashing the spleen through a 70 µm cell strainer. 1 x 10^6 splenocytes, containing approximately 1 x 10^5 TCR327$^+$ naïve CD8 T cells, in 100 µL HBBS were injected i.v. in the tail vein of each recipient mouse 1 day before viral infection.

4.4.2 Diphtheria-toxin Treatment

For depletion of DTR expressing Langerin$^+$ cells from Langerin-DTR mice, mice were injected twice with 500 ng Diphtheria toxin in 100 µl PBS i.v. in the tail vein at the indicated timepoints.

4.4.3 Generation of Bone marrow chimeric Mice

Bone marrow of donor mice was isolated by flushing tibiae and femora with HBSS. 5 x 10^6 bone marrow cells in 100 µL HBBS were injected i.v. in the tail vein of each age- and sex-matched recipient mouse, that had been lethally irradiated before with 9.5 Grey using a ^{137}Cesium source. Each reconstituted mouse received daily approximately 1 mg of sulfadoxin and 0.2 mg of trimethoprim (Borgal) via the drinking water for 3 weeks. Reconstitution of mixed bone marrow chimeric mice was monitored in blood based on the frequency of CD8$^+$ T cells originating from the transferred bone marrow. Before conducting experiments with those mice, they were rested for 8 weeks after transplantation.

4.4.4 Intravascular Labeling of CD8$^+$ T cells

For *in vivo* labeling of CD8$^+$ T cells the anti-CD8β antibody coupled to the fluorochrome PE was diluted in PBS. Each mouse received 0.8 µg of antibody in 100 µL of PBS i.v. in the tail vein and was euthanized 5 min later for analysis as described before (Low et al., 2020).

4.4.5 Oral Administration of Fingolimod

Fingolimod (FYT720) was diluted in a solution of DMSO / 0.9% NaCl and sugared condensed milk (2%/18%/80%). To each mouse 20 µg of Fingolimod in 100 µL milk mixture was fed through micropipette-guided drug administration (Scarborough et al., 2020) every second day.

4.5 Isolation of murine Cells

4.5.1 Sampling and Processing of Blood

Blood was collected in 1 mL FACS buffer after puncturing the submandibular vein using a steal lancet. 5 mL of ACP buffer was added. After 10 min of incubation at room temperature erythrocytes were lysed, then the cell suspension was centrifuged (300 rcf, 5 min, 4°C). The pellet was resuspended in PBS until further processing.

4.5.2 T cell Isolation from Skin draining Lymph Nodes or Spleen

For T cell isolation from skin draining lymph nodes or spleen, the tissue was smashed through a 70 µm cell strainer, centrifuged (300 rcf, 5 min, 4°C) and the pellet was resuspended in PBS until further processing.

4.5.3 Lymphocyte Isolation from Skin draining Lymph Nodes

For lymphocyte isolation from skin draining lymph nodes, the tissue was cut in small pieces. Followed by incubation in RPMI-1640 (without FCS) in presence of 800 U/mL collagenase type IV, and 50 U/mL DNase I for 30 min at 37°C while shaking. Afterwards, 0.5 M Na-EDTA was added to a final concentration of 10 mM and incubated further for 10 min. Cells suspensions were filtered via a 70 µm cell strainer, centrifuged (300 rcf, 5 min, 4°C) and the pellet was resuspended in PBS until further processing.

4.5.4 Generation of Single Cell Suspensions from whole Ear Skin

For DC as well as T cell isolation from ear skin, the ear was split in its dorsal and ventral part and then cut in small pieces. For the isolation of T cells, the homogenized tissue was incubated in RPMI-1640 (without FCS) with 800 U/ ml collagenase type IV (Worthington) and 50 U/mL DNase I for 90 min at 37°C while shaking. For the isolation of DC, the homogenized tissue was incubated in RPMI-1640 (without FCS) with 800 U/ ml collagenase type IV (Worthington), 1 mg/mL Dispase II (Roche) and 50 U/mL DNase I for 90 min at 37°C while shaking. After enzymatic digestion, 0.5 M Na-EDTA was added to a final concentration of 10 mM and incubated further for 10 min. Cells suspensions were filtered via a 70 µm cell strainer, centrifuged (300 rcf, 5 min, 4°C) and the pellet was resuspended in PBS until further processing.

4.5.5 Generation of Single Cell Suspensions from Epidermis or Dermis of the Ear Skin

For the separation of epidermis from dermis the ear skin was split in its dorsal and ventral part. With the dermal part facing down, each sample was put for 45 min at 37°C on the surface of digesting

solution of 2.5 mg/mL Dispase II and 10 µg/mL DNase I in PBS, then the epidermal layer was peeled off the dermal layer (Kitano and Okada, 1983). Each layer was cut in small pieces. Followed by incubation in RPMI-1640 (without FCS) in presence of 800 U/mL collagenase type IV, and 50 U/mL DNase I for 90 min at 37°C while shaking. After enzymatic digestion, 0.5 M Na-EDTA was added to a final concentration of 10 mM and incubated further for 10 min. Cell suspensions were filtered through a 70 µm cell strainer, centrifuged (300 rcf, 5 min, 4°C) and the pellet was resuspended in PBS until further processing.

4.5.6 Isolation of migratory Cells from the Epidermis

Ears of mice were cut off after euthanisia, rinsed in 70% ethanol and air-dried for 5 min. The ear skin was split in its dorsal and ventral part. Dorsal ear halves with the dermal part facing down, was put for 45 min at 37°C on the surface of digesting solution of 2.5 mg/mL Dispase II and 10 µg/mL DNase I in PBS, after incubation then the epidermal layer was peeled off the dermal layer (Kitano and Okada, 1983). Each epidermal sheet was then cultured with the dermal part facing down floating on 1.5 mL of complete RPMI-1640 10% in one well of a 24 well plate at 37°C (Weinlich et al., 1998). After 48 h of culture the emigrated cells on the bottom of each well were harvested. Cells were filtered via a 70 µm cell strainer, centrifuged (300 rcf, 5 min, 4°C) and the pellet was resuspended in PBS until further processing. This Protocol was adapted from the Patrizia Stoizner lab (Innsbruck, Austria).

4.6 Purification of murine Cells

4.6.1 Enrichment of Langerhans cells from the epidermal migratory Cell Fraction

For the enrichment of Langerhans cells, a density gradient was prepared. Into a 15 mL falcon, 3 mL of 11,6 % Opti Prep in HBSS was put underneath 2 mL of HBSS containing 1% Phenol red. After epidermal explant culture the emigrated cells from ten murine ears were pooled, resuspended into 2 mL of 15% Opti Prep in HBSS and put underneath the prepared two layers of gradient into the falcon. The falcon was centrifuged at 600 rcf for 15 min at room temperature without brake. The enriched fraction of Langerhans cells was aspirated carefully at the interphase between the layer of HBSS containing 1% Phenol red and the layer of 11,6% Opti Prep. Cells were washed once with PBS, centrifuged (300 rcf, 5 min, 4°C) and then the pellet was resuspended in PBS until further processing.

4.6.2 Purification of Langerhans cells using FACS

For further purification of Langerhans cells among the migratory cells from the epidermis they were sorted based on Langerhans specific surface marker expression (fluorescence activated cell sorting,

FACS). For this purpose, cell suspensions were centrifuged at 300 rcf for 5 min at 4°C, the pellet was resuspended in a mix of Fc-receptor blocks (2.4G2 and E9E) and fluorescently labeled antibodies in FACS buffer and incubated for 30 min at 4°C. After one washing step with FACS buffer, the cells were stained for viability using 7-AAD in FACS buffer. Living, lin^-, $CD45^+$, $MHC\text{-}II^+$, $EpCAM^+$ cells were FACS sorted using an BD AriaII equipped with five lasers (375 nm, 405 nm, 488 nm, 532 nm and 633 nm) with a 100 µm Nozzle and a reduced flow pressure of 15 psi. The obtained cells of interested were then once washed with RPMI-1640 10% and used in serial dilutions for co-culture with naïve T cells.

4.6.3. Magnetic Separation of $CD8^+$ T cells from Spleen

$CD8^+$ T cells were purified from the spleen of one naïve Nur77-eGFPx327TCRxThy1.1 mouse by magnetic cell separation using anti-CD8α microbeads with LS columns.

4.7 Assays and Flow Cytometry

4.7.1 Nur77-EGFPx327TCRxThy1.1 Co-Culture Assay

5×10^3 $CD8^+$ T cells per well were added to serial dilutions of antigen presenting cells in V-bottom 96-well plates. After 16 h of co-culture, the TCR stimulation of Nur77-EGFPxTCR327 T cells was determined flow cytometrically based on their Nur77-EGFP expression.

4.7.2 Immunostaining of Cells and Analysis by Flow Cytometry

Cells were stained for viability for 5 min at 4°C using the fixable live dead stain efl780 in PBS. Following one washing step with FACS buffer, the Fc-receptor blocks (2.4G2 and E9E) in FACS were applied to each sample and incubated for 10 min at 4°C. Then fluorescently labeled antibodies in FACS buffer were added and incubated for 30 min at 4°C, except antibodies staining for chemokine receptors were incubated for 30 min at 37°C. Following three washing steps with FACS buffer, surface stained cells were fixed for 10 min at 4°C in 2% Histofix in PBS. All centrifugation steps for washing occurred at 300 rcf for 5 min at 4°C. Fluorescence of single cells was quantified on the following flow cytometers: BD Canto equipped with three lasers (405 nm, 488 nm and 640 nm) or on a BD Symphony with five lasers (355 nm, 405 nm, 488 nm, 561 nm and 640 nm).

4.8 Computational Analysis of Flow Cytometry Data

Data were analyzed with FlowJo 10.8.2 (Becton Dickinson & Company). Data sets were cleaned up and either gated manually or by the application of FlowJo Plugins such as FlowSOM, tsne, UMAP on

multiparametric data sets. The advantages and disadvantages of manual gating versus algorithm supported analysis of multiparametric data obtained from flow cytometry on the example of mononuclear phagocyte subsets of the murine skin can be found in our recent paper (Probst et al., 2022).

4.9 Statistical Analysis

Statistical analysis was performed with Prism 9 (version 9.5.0, GraphPad). Statistical tests for significance were used as indicated. Depending on the experimental groups and distribution of data either two-way Anova test, students or non-parametrical t-test was used to calculate statistical significance of data.

5 RESULTS

5.1 The Control of cytotoxic T cell Responses in the Skin by CCR2 dependent Phagocytes

5.1.1 Research Objective

Cytotoxic $CD8^+$ T lymphocytes (CTL) play a critical role in the immune defense against viruses that infect cells of the skin, such as Vaccinia virus, a virus of the poxvirus family. Mononuclear phagocytes (MNP) act as professional antigen presenting cells (APC), that are important for the induction of antiviral $CD8^+$ T cell responses. The following section deals with the question, how MNP control CTL responses to a poxvirus infection of the skin and instruct the development of skin resident memory T cells (T_{RM}). Firstly, we characterized the cells of the MNP compartment of healthy and viral infected skin based on surface marker expression using flow cytometry (5.1.2). Secondly, we generated for the characterization and discrimination of MNP populations mouse models based on distinct ontogenies of subsets and assessed the targeted subsets of MNP in these models (5.1.3). Thirdly, we generated mouse models disabling distinct features, such as antigen presentation, co-stimulation, transmission of T cell help and migration, specifically in CCR2-dependent cells and monitored the effect on the CTL response to viral infection of the skin (5.1.4).

5.1.2 Changes within the cutaneous MNP Network in the Course of Pox Virus Skin Infection

In the healthy skin the mononuclear phagocyte compartment is comprised of five subsets of cells. While the Langerhans cells are located in the epidermis, there are four subsets resident in the dermis: cDC1, cDC2, monocyte derived cells (moC) and macrophages (MΦ). The composition of the MNP network in skin and subset specific characteristics change massively after pathogen associated stimuli, such as infection related tissue injury or innate immune activation. During cutaneous pox viral infection, the virus titer in skin reaches its maximum between 3 to 8 days post infection (dpi), after 8 dpi the virus titer drops until the number of infectious particles are not detectable anymore at 20 dpi (Figure 4a). Interestingly, the highest frequency of antiviral T cells among all $CD8^+$ T cells was detected between 8-12 dpi in the blood (Figure 8a,c) and both the start of increasing T cell numbers and the maximum number of phagocytes infiltrating the infected skin coincide at 5 dpi (Fischer et al., 2011). These infiltrating phagocytes may control antiviral $CD8^+$ T cell responses to infection in the skin. To investigate the impact of phagocytes on CTL responses, first gradual changes within the phagocyte network of the skin following viral infection were characterized by flow cytometry. For this purpose, the murine skin was dissociated using digestion enzymes to obtain a single cell suspension. The single

cell suspension was then stained with a set of monoclonal antibodies coupled to fluorochromes, enabling the identification of MNP in the skin based on surface marker expression by flow cytometry. For the identification of DC subsets in the healthy murine skin gating strategies for the delineation of subsets of the conventional DC family (Guilliams et al., 2016) or of monocyte derived cells (Tamoutounour et al., 2013) have been well established, but they fail to identify these subsets in the context of infection. To identify both, the cDC and cells of monocytic origin simultaneously, we generated an optimized multicolor staining panel. The inclusion of markers for innate immune cells, invading peripheral tissues under inflammatory conditions, such as neutrophils and eosinophils, was added to the staining panel to discriminate them from cells of the MNP compartment, allowing the characterization of immune cells in the healthy, as well as infected skin of mice. Moreover, the panel can be used for manual gating, but is also suitable to perform data analysis based on unsupervised clustering algorithm (Probst et al., 2022).

Subsequently this panel was used to phenotypically characterize the MNP compartment in the infected skin over the course of the infection. Ears from mice at steady state and after 1, 3 or 5 dpi were collected and processed to single cell suspensions for the analysis by flow cytometry. First unspecific binding of staining antibodies on Fc-Receptors of phagocytes was prevented by blocking of Fc-Receptors (Fc-γRIIb, Fc-γRIII, Fc-γRIV), followed by antibody based staining and the measurement of cells. For the analysis of flow cytometry data from all events debris, doublets, dead cells (viability dye$^+$) and stromal cells (pan45$^-$) were removed. Among the remaining pan45$^+$ cells T cells (CD90.2), B cells (CD19) and NK cells (NK1.1) were excluded (Figure 3a) and the pan45$^+$ lin$^-$ cells were further analyzed by the expression of 14 additional surface markers: Pdca1, CD64, Fc-εR1α, EpCAM, Xcr1, CD24, CD26, CD11c, Ly-6G, Ly-6C, Siglec-F, Sirp1α, MHC-II and CD11b. This lineage specific marker sets allow the identification of polynuclear and mononuclear phagocytes in skin such as monocytes (CD11b$^+$ Ly-6C$^+$), neutrophils (CD11b$^+$ Ly-6G$^+$), eosinophils (CD11b$^+$ SiglecF$^+$), mast cells (CD11b$^-$ Fc-εR1α^+), LC (CD11c$^+$ MHC-II$^+$ CD26$^-$ CD24$^+$ EpCAM$^+$), MΦ (MHC-II$^{low\text{-}high}$ CD26$^-$ CD11b$^+$ CD64$^+$), cDC (CD26$^+$ CD11c$^+$ MHC-II$^+$), which can then be subdivided through subset specific markers: CD24$^+$XCR1$^+$ for cDC1 and CD24$^-$sirp1α^+ for cDC2 (Figure 3b). We applied computer-aided approaches to visualize and characterize these pan45$^+$ lin$^-$ population based on their similarity in the expression of the 14 additional markers of the panel, which had not been used in the pre-gating of the pan45$^+$ lin$^-$ population. After collection of flow cytometry data from cells recovered from the skin of mice, all events were gated on pan45$^+$ lin$^-$ cells and the pan45$^+$ lin$^-$ cell fraction of all samples from skin tissue of 0-5 dpi were merged for further analysis. Based on this processed data, subsets of cells that share similar characteristics were identified with the help of the unsupervised clustering tool Flow-Self-Organizing-Maps (FlowSOM) and the dimensionality reduction algorithm Uniform Manifold Approximation and Projection (UMAP).

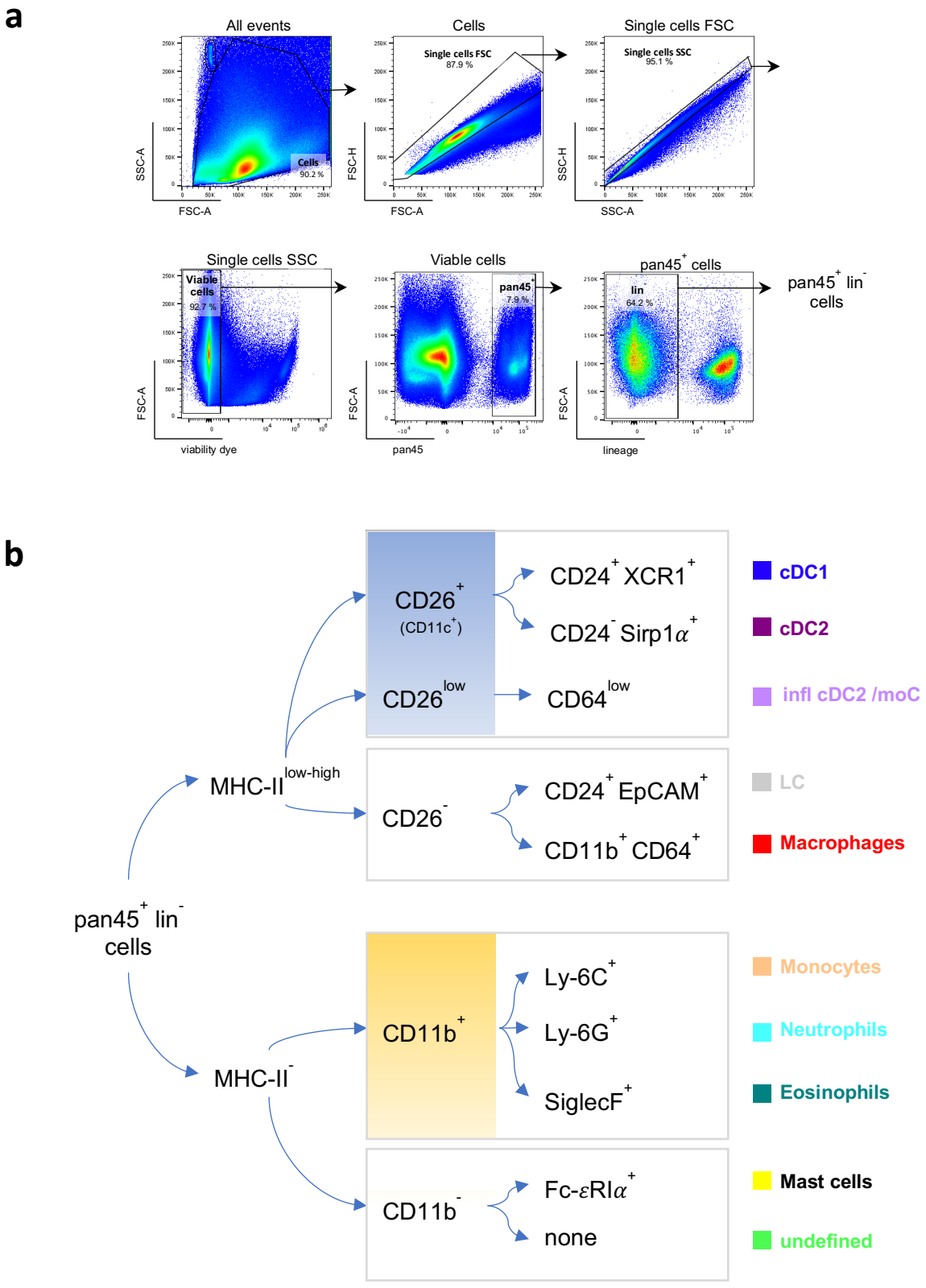

Figure 3. Identification of Phagocytes from murine Skin

*Ear skin tissue was enzymatically digested to generate a single-cell suspension, which was stained with antibodies for analysis by flow cytometry based on surface marker expression. (**a**) Representative gating of flow cytometry data from skin cells of two ears from healthy mice for the identification of pan45⁺ lin⁻ cells is shown. From all events debris, doublets, dead cells (efl780⁺) and stromal cells (CD45⁻) were excluded. Lineage negative cells, i.e. T cells (CD90.2), NK cells (NK1.1) and B cells (CD19), were removed from the remaining hematopoetic-derived cells (pan45⁺). (**b**) The identified pan45⁺ lin- cells were then further analysed based on the expression of 14 markers to determine phagocyte subsets. Phagocyte subsets in the murine skin were identified based on subset specific surface marker expression as schematically represented in the flow chart.*

FlowSOM is a clustering technique for the analysis of multiparametric flow cytometry data using self-organizing maps to obtain an overview of marker expression and detect subsets that could be overlooked using other methods (Van Gassen et al., 2015). Using the FlowSOM algorithm 10 distinct populations were identified in the dataset. Cells of each population (Pop0-9) have a similar surface marker expression pattern in common during the investigated first 5 days of viral skin infection (Figure 4b). For each identified Flow-SOM population, the expression level of the markers is represented by the color-code ranging from high expression in red to low expression in dark blue in the heatmap. Based on the characteristic surface marker expression, we assigned cell types to the FlowSOM populations. Through lineage specific marker expression specific cell types were identified (Figure 3b, Figure 4b). For example, LC could be clearly assigned to this cluster based on the high expression in the surface markers CD24 and EpCAM. The discerned populations by FlowSOM were used to annotate areas of the UMAP plot by color code. UMAP is a dimensionality reduction algorithm used to analyze complex data, such as the multiple parameters measured in flow cytometry. The multidimensional dataset is reduced onto a two-dimensional space and each cell is plotted as one dot. In such a 2D plot (UMAP plot) the similarity of cells in their marker expression pattern is represented through their relative proximity to each other (McInnes et al., 2018) (Figure 4c). Cells with similar characteristics are plotted close together, while cells with diverging expression patterns are plotted far apart. For example, LC and cDC1 share the expression of MHC-II, CD11c and CD24, thus cells of those populations cluster close to each other in the UMAP plot. In contrast cells belonging to the neutrophil populations represent a distinct cluster in the UMAP plot due to their exclusive expression of Ly-6G, while having only little expression of other markers in common with other cells. Examining the cell populations detected through FlowSOM during the initial five days of infection unveiled a rapid reduction of Langerhans cells and cDC and a very strong increase of monocytes, followed by an increase of neutrophilic granulocytes within the pan45^{+} lin^{-} cell compartment of the skin (Figure 4d).

Noteworthy, the assignment of cell types based on the marker expression pattern from the FlowSOM analysis was not clear for most populations expressing Sirp1α, with the exception of LC, which express simultaneously CD24 and EpCAM. The delineation of other Sirp1α^{+} subsets based on the analyzed set of markers was more difficult, these include cDC2 (Pop8), monocyte derived cells or inflammatory cDC2 (Pop6). While the residency in skin of monocyte derived cells and inflammatory cDC2 depend on CCR2/CCL2 interaction. The subset of cDC2 depends on the FLT3/FLT3L interaction. To obtain a more precise characterization of these cells, we investigated their respective reliance on the chemokine receptor CCR2 and the receptor tyrosin kinase FLT3 through our mixed bone marrow chimeric model.

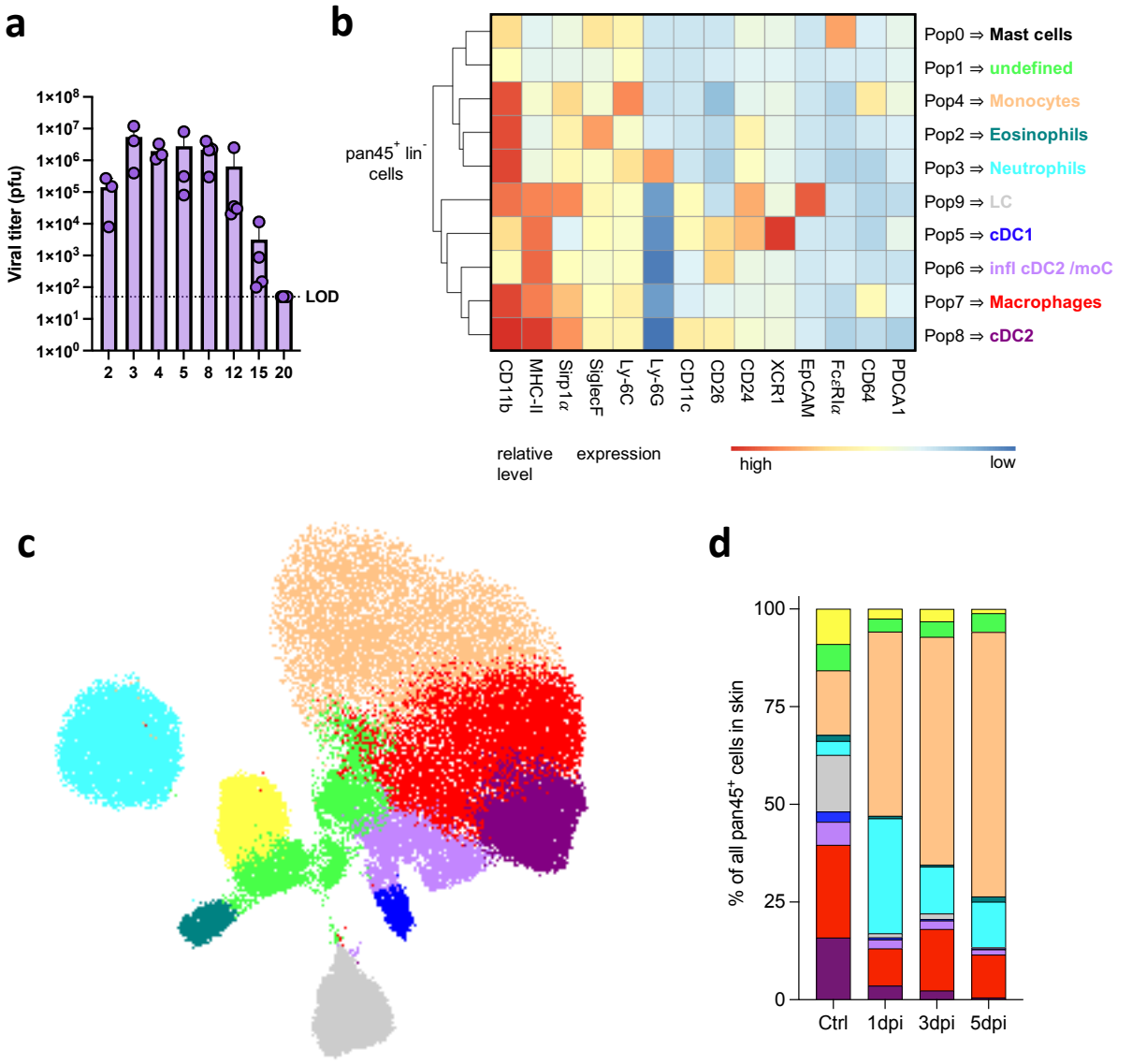

Figure 4. High dimensional Flow Cytometry of MNP in Pox Virus infected murine Skin

*Wt + wt bone marrow chimeric mice were infected on the ear with recombinant vaccinia virus (rVACV-G2) and the ears were collected at indicated days post infection (dpi) for the determination of virus titer and composition of phagocytes within the tissue. (**a**) Virus titter in infected ear skin of mice from 2-20dpi with n=3-4 was determined by plaque assay. (**b,c,d**) Relative surface marker expression of cells from ear skin tissue at 0-5dpi was assessed using flow cytometry. After manual gating on no debris, singlets, viable (efl780-) and pan45⁺ cells, 240,000 lineage negative (CD90.2⁻, NK1.1⁻, CD19⁻) cells from the skin of n=7 mice were analyzed by unsupervised clustering FlowSOM using the FlowJo plugin (14 parameter, 10 metacluster). All surface markers except those already used in the pregating (viability, pan45, lineage) were included in the analysis. Based on the relative expression of surface markers (x-Axis) shown in the FlowSOM heatmap, each FlowSOM population (Pop0-9) was manually assigned to a certain cell type shown on the right (**b**). Those ten cell populations were then used for color coded annotation of the dimension reduced UMAP plot of the same dataset (**c**). The frequency of the 10 identified FlowSOM populations among all pan45⁺ cells in the ear skin at individual dpi is shown (**d**).*

5.1.3 Ontogeny based Analysis of MNP of the Skin during Pox Virus Infection

Discrete receptor-ligand interactions or transcription factors are crucial for the residency of MNP subsets in the skin. The depletion of these crucial factors, e.g. in knock out mice, prevents the positioning of certain MNP subsets in skin, thus this ontogeny-based approach is used to characterize CCR2- or FLT3 dependent cells. Target cells were characterized, first based on surface marker expression, and then their functional role in the antiviral CTL response was analyzed. Utilizing mixed bone marrow chimera allowed the modulation of the immune system to study CCR2- or FLT3 dependent cells. CCR2 or FLT3 are required for the development of certain MNP subsets in skin. CCR2 expression is crucial for chemotaxis of monocytes from the bone marrow into blood circulation as well as the extravasation from blood into inflamed sites, where they differentiate into CCR2 dependent MNP (Auffray et al., 2007; Serbina et al., 2008). This quick reaction to form CCR2-dependend cells on demand, is in particular important during inflammation (Tamoutounour et al., 2013). In CCR2 deficient mice, monocytes are no longer able to leave the bone marrow and thus cannot access the blood or infiltrate peripheral tissues (Boring et al., 1997). While CCR2 deficiency affects cells of monocytic origin, the development of classical DC relies on signals mediated by FLT3. Mice deficient in FLT3 lack classical DC (Mackarehtschian et al., 1995; Guilliams et al., 2016).

Mixed bone marrow chimeric mice were generated to characterize CCR2 as well as FLT3 dependent cells in skin in the course of viral infection of the tissue. Lethally irradiated recipients were reconstituted with a mixture of wt and CCR2 knockout ($CCR2^{-/-}$) bone marrow or a mixture of wt and FLT3 knockout ($FLT3^{-/-}$) bone marrow. After 6-8 weeks, all hematopoietic cells undergo complete replacement by the descendants of the injected cells. As a result, the immune cell network of bone marrow chimeric mice is comprised of three compartments, cells of each compartment can be identified based on congenic marker expression (CD45.1, CD45.1/2, CD45.2): cells derived from the wt bone marrow (CD45.1/2), from the knock out bone marrow (CD45.2), and radioresistant cells remaining from the recipient (CD45.1) (Figure 5a). In these ‘3 in 1’ bone marrow chimeric mice CCR2 dependent cells (or FLT3 dependent cells) can be identified. By comparing the MNP subsets derived from the CCR2 (or FLT3) deficient progenitors versus cells from the wt compartment, cells of each compartment are identified based on compartment specific congenic marker (CD45.1, CD45.1/2, CD45.2) expression determined by flow cytometry. For each compartment of the mBMC a UMAP plot from the merged $pan45^{+}$ $lineage^{-}$ cells recovered from skin of $CCR2^{-/-}$ + wt (Figure 5b) or $FLT3^{-/-}$ + wt (Figure 5c) mBMC at 0-5 dpi was generated. Each plot was annotated through color coding of cell types, which were manually assigned based on the identified FlowSOM populations with common surface marker expression patterns (according to Figure 3b).

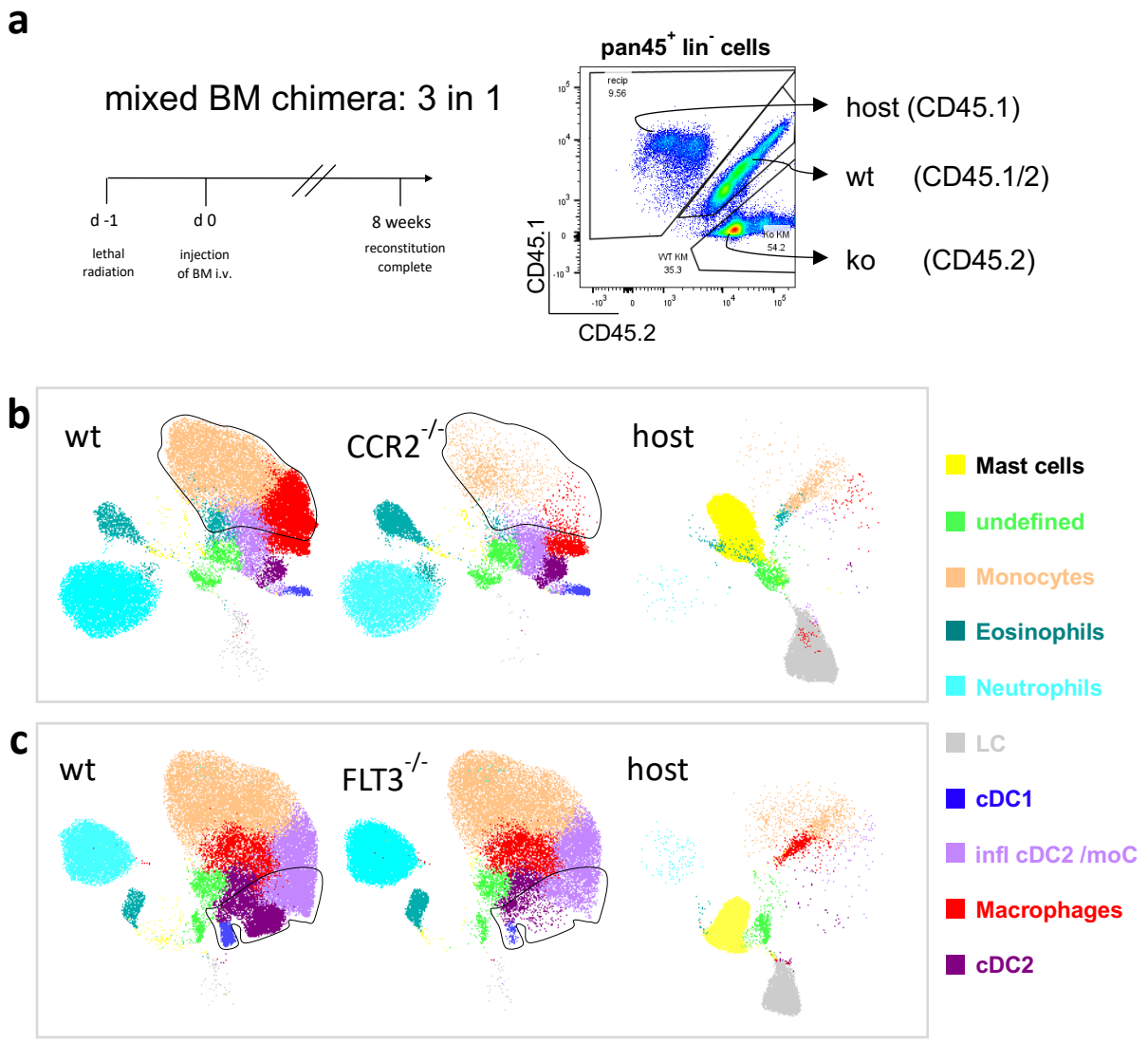

Figure 5. Identification of Phagocytes in infected murine Skin based on CCR2 and FLT3 Dependency

*$CCR2^{-/-}$ + wt or $FLT3^{-/-}$ + wt bone marrow (BM) chimeric mice were infected on the ear with recombinant vaccinia virus (rVACV-G2) and cells from ear skin were collected at 0,1,3,5 dpi to determine the composition of phagocytes within the tissue by flow cytometry. (**a**) A mixture of bone marrow of knockout (CD45.2) and wt (CD45.1/2) mice was transferred into lethally radiated host mice (CD45.1). After reconstitution $pan45^+$ lin^- cells of mixed BM chimera comprise of three compartments, distinguishable by their differential expression of CD45.1 and CD45.2, allowing to study those cells in their dependence on CCR2 or FLT3. (**b-c**) Relative surface marker expression of cells from ear skin tissue at 0-5dpi was assessed using flow cytometry. After manual gating on no debris, singlets, viable ($efl780^-$) and $pan45^+$ cells, 240,000 lineage negative ($CD90.2^-$, $NK1.1^-$, $CD19^-$) cells from the skin of n=7 mice were analyzed by unsupervised clustering FlowSOM using the FlowJo plugin (14 parameter, 10 metacluster). All surface markers except those already used in the pre-gating (viability, pan45, lineage) were included in the analysis. Based on the relative expression of surface markers, each FlowSOM population (Pop0-9) was manually assigned to a certain cell type. Those ten cell populations were then used for color coded annotation of the dimension reduced UMAP plot of the same dataset. Projection of the FlowSOM clusters shown in Figure 6 on the UMAP plot of the wt (CD45.1/2), knockout (CD45.2), and the radioresistant host (CD45.1/2) compartment of the skin from $CCR2^{-/-}$ + wt (**b**) or $FLT3^{-/-}$ + wt (**c**) BM chimera.*

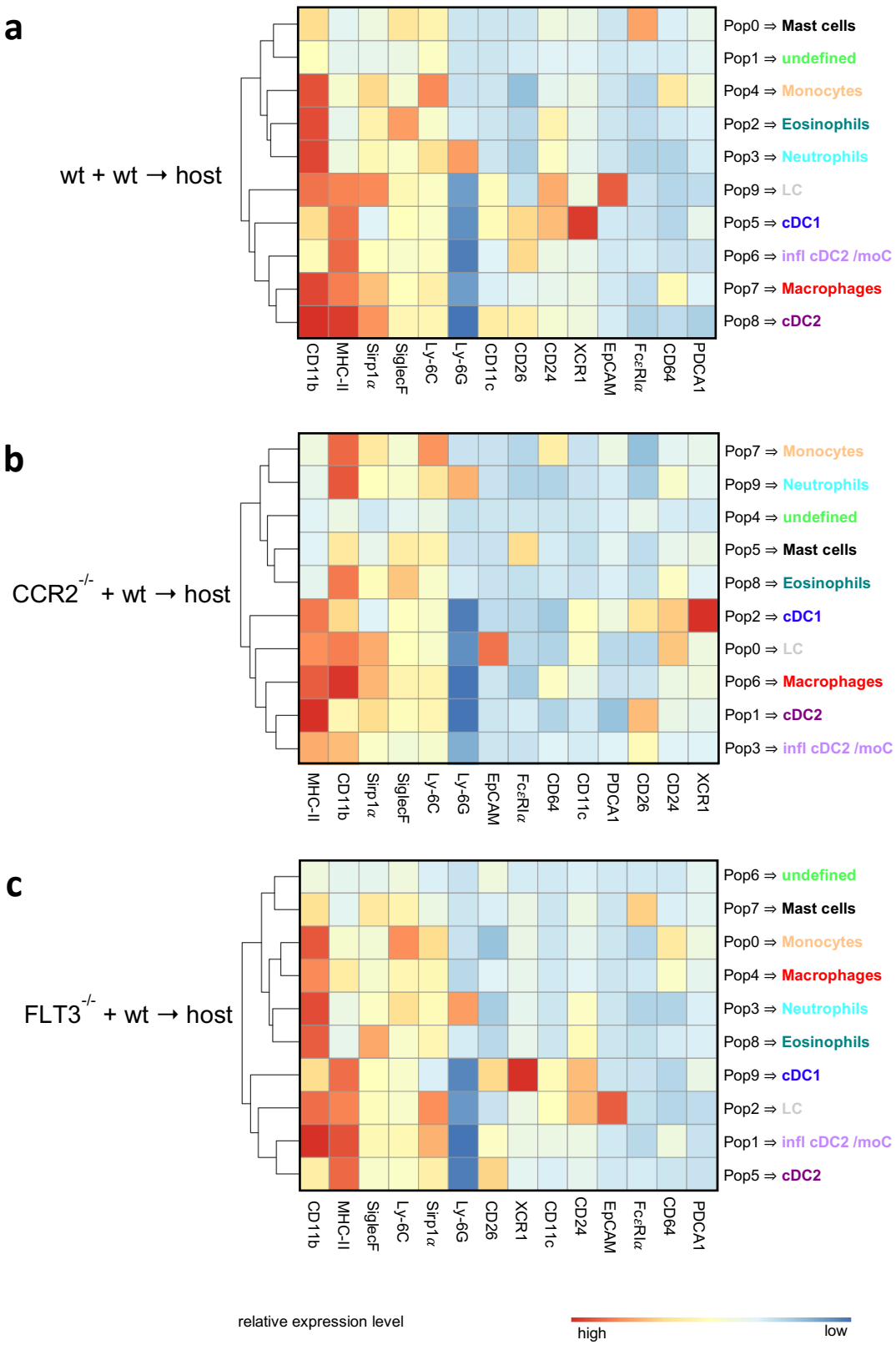

Figure 6. The Cell Populations from unsupervised Clustering are consistent between three different Types of BM-Chimera
*Wt + wt, CCR2$^{-/-}$ + wt or FLT3$^{-/-}$ + wt bone marrow (BM) chimeric mice were infected on the ear with recombinant vaccinia virus (rVACV-G2) at 0,1,3,5 dpi to determine the composition of phagocytes within the tissue by flow cytometry. Relative surface marker expression of cells from ear skin tissue was assessed using flow cytometry. After manual gating on no debris, singlets, viable (efl780^{-}) and pan45^{+}cells, 240,000 lineage negative (CD90.2^{-}, NK1.1^{-}, CD19^{-}) cells from the skin of n=7 mice were analyzed by unsupervised clustering FlowSOM using the FlowJo plugin (14 parameter, 10 metacluster). All surface markers except those already used in the pre-gating (viability, pan45, lineage) were included in the analysis. Based on the relative expression of surface markers (x-Axis) shown in the FlowSOM heatmap of pan45^{+}lin^{-} cells from the skin of Wt + wt (**a**), CCR2$^{-/-}$ + wt (**b**) or FLT3$^{-/-}$ + wt (**c**) bone marrow chimeric mice, each FlowSOM population (Pop0-9) was manually assigned to a certain cell type indicated on the right.*

In general, the cell types of the infected skin visualized by FlowSOM populations (Figure 6) and the UMAP plots (Figure 4,5) are similar and consequently consistent among the analysed three different types of mBMC (wt + wt; CCR2$^{-/-}$ + wt; FLT3$^{-/-}$ + wt). Mast cells, Langerhans cells and some cells of an undefined population were found to be host derived, whereas all other identified populations belong either to the wt or one of the knockout compartments. In the compartment of cells originating from the CCR2 deficient (CCR2$^{-/-}$) progenitor, less monocytes, macrophages, and cells possessing an inflammatory cDC2 and/or monocyte derived cell like phenotype have been identified (Figure 5b). Conversely, the compartment of cells derived from FLT3 deficient (FLT3$^{-/-}$) progenitors showed a reduction of the FLT3-dependent subsets of cDC1 and cDC2 (Figure 5c). Interestingly, the same population of cells possessing an inflammatory cDC2 (infl. cDC2) and/or monocyte derived cell (moC) like phenotype, which was already found to be reduced by CCR2 deficiency (Figure 5b), was also partially reduced by FLT3 deficiency (Figure 5c). However, the reduction of the infl. cDC2/moC population by CCR2 deficiency affect cells close to cells of monocytic origin such as monocytes (orange) and Macrophages (red). In contrast the FLT3 deficiency resulted in a reduction of a cell cluster close to the cDC2 population, indicating that the infl. cDC2/moC population consists of CCR2 and FLT3 dependent cells or cells which depend on CCR2 as well as FLT3 (Figure 5).

5.1.4 Antigen Presentation, Co-Stimulation, T cell help and Migration by CCR2 dependent MNP

To investigate the functional role of CCR2-dependent cells in the formation of an antiviral CD8$^+$ T cells response to skin infection a set of mixed bone marrow chimera was generated. The relevance of antigen presentation, co-stimulation through CD40, CD70, CD80, CD86 and migration to the draining lymph node by CCR7 expression on CCR2-dependent cells was analyzed in the context of viral skin infection based on the antigen specific CD8$^+$ T cell response in blood and their ability to persist as immunological memory cells in skin and secondary lymphoid organs. For the purpose of investigating the role of antigen presentation by CCR2 dependent cells for the formation of an antiviral CTL response mBMC were generated. Lethally irradiated mice were reconstituted with a mixture of CCR2$^{-/-}$ and H-2D$^{b-/-}$ bone marrow to generate a situation, where CCR2 dependent cells are disabled to present H-2D^b restricted antigens on their surface. As a control, irradiated mice were injected with a mixture of WT and H-2D$^{b-/-}$ bone marrow. After complete reconstitution mBMC were adoptively transferred with naïve 327TCR$^+$ CD90.1$^+$ CD8$^+$ T cells allowing the tracking of the CTL response against the H-2D^b restricted GP$_{33-41}$ peptide. One day later mBMC were infected with recombinant Vaccinia virus (rVACV-G2), expressing recombinantly the H-2D^b restricted GP$_{33-41}$ and natively the immune dominant H-2K^b restricted antigen B8R$_{20-27}$. Over the course of infection at 8, 12, 20, 30 dpi, the frequency of antigen specific T cells among CD8$^+$ T cells in the blood was assessed using flow cytometry (gating strategy

shown in Figure 7). Adoptively transferred 327TCR^{+} CD8^{+} T cells are identified based on CD90.1 expression. They hold a TCR specific for H-2D^{b} restricted GP$_{33\text{-}41}$ antigen and are thus uniformly positively stained for the GP$_{33\text{-}41}$ tetramer.

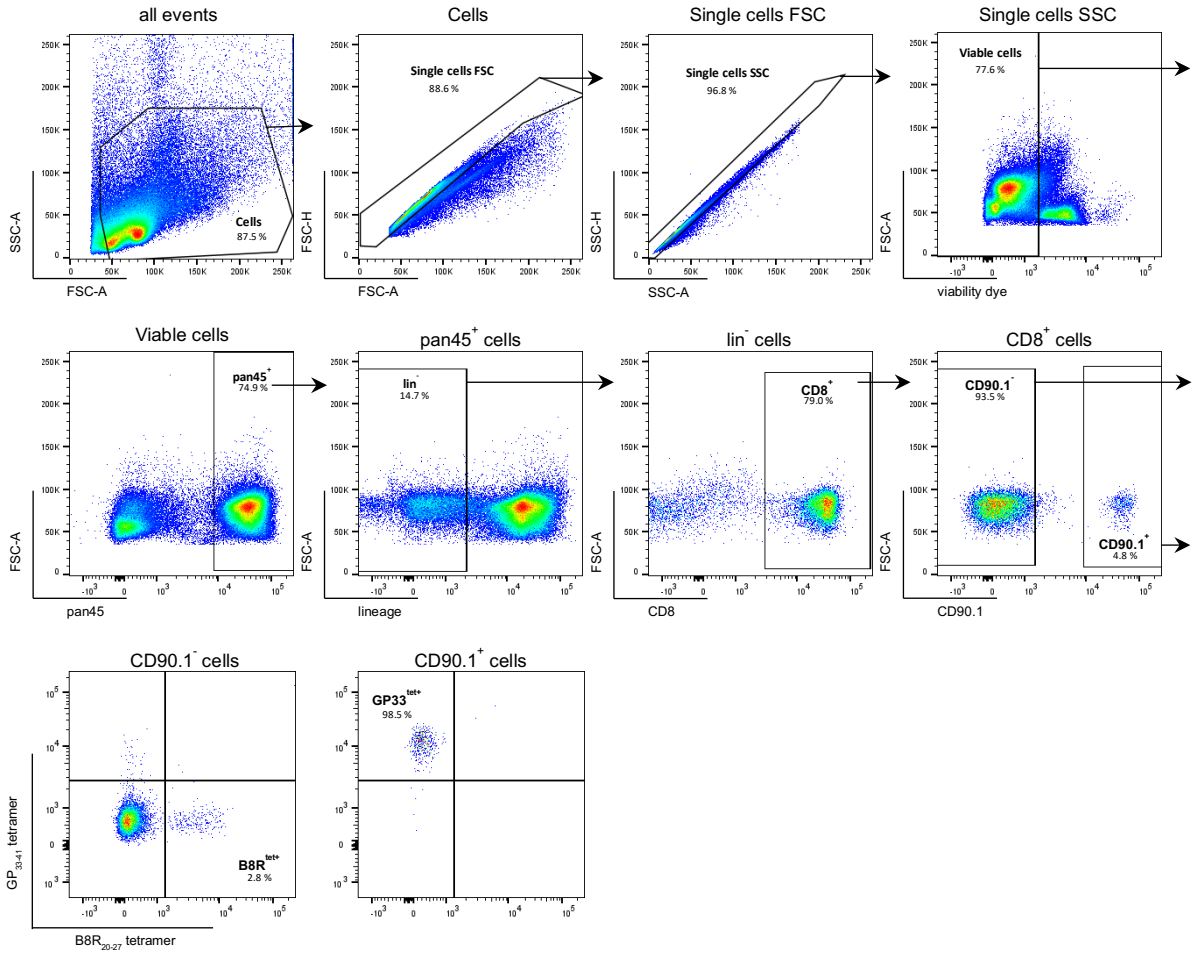

Figure 7. Gating Strategy for the Identification of antiviral CD8^{+} T cells in murine Tissues

Mixed bone marrow chimeric mice were adoptively transferred with GP$_{33\text{-}41}$-specific TCR327 CD8^{+} T cells (CD90.1^{+}) before being infected with recombinant Vaccinia Virus (rVACV-G2) at the ear pinnae. The antiviral cytotoxic T cell response was monitored in the blood, skin, draining lymph node and spleen of infected mice at various days post infection (dpi) using flow cytometry. Representative gating on antiviral CD8^{+} T cells from spleen recovered at 30dpi is shown. In order to identify antiviral CD8^{+} T cells, first cell debris, doublets, dead cells (eF780^{+}) and stromal cells (pan45^{-}) were excluded. From the remaining leukocytes (pan45^{+}), lineage negative cells, i.e. MHC-II^{+} cells (MHC-II^{+}), CD4 T cells (CD4^{+}) and NK cells (NK1.1^{+}), were excluded. From the remaining cells, CD8^{+} cells were gated. The CD8^{+} T cell population was then subdivided into formerly transferred TCR327 CD8^{+} T cells (CD90.1^{+}) and endogenous (CD90.1^{-}) cells, the antigen specificity of these cells was then analysed by tetramer staining. Among the CD90.1^{+} and endogenous (CD90.1^{-}) cells were GP$_{33\text{-}41}$ specific (CD90.1^{+} GP33^{tet+}) and B8R$_{20\text{-}27}$ specific (CD90.1^{-} B8R^{tet+}) cells identified respectively.

In both mBMC types the GP$_{33\text{-}41}$ specific cells among CD8^{+} T cells in the blood increase from 8 dpi to 12 dpi. At 12 dpi the maximum is reached and until then the proportion of GP$_{33\text{-}41}$ specific cells among CD8^{+} T cells declined in the blood. Interestingly, although the frequency of adoptively transferred GP$_{33\text{-}41}$ specific cells among CD8^{+} T cells in the blood of CCR2$^{-/-}$+H-2D$^{b\text{-}/\text{-}}$ mBMC is at 8 dpi equal to that of Wt+H-2D$^{b\text{-}/\text{-}}$ mBMC, it shows a significant reduction from 12 to 30 dpi when CCR2 dependent cells are unable to present the antigen (Figure 8a). The endogenous H-2K^{b} restricted B8R$_{20\text{-}27}$ tetramer positive

CD8⁺ T cell response served as an internal control and showed no significant difference between both experimental groups (Figure 8c). Moreover, the persistence of antigen-specific CD8⁺ T cells as tissue resident memory in the skin at 30 dpi is remarkedly reduced if CCR2 dependent cells lack the ability to present the respective antigen (Figure 8b,d).

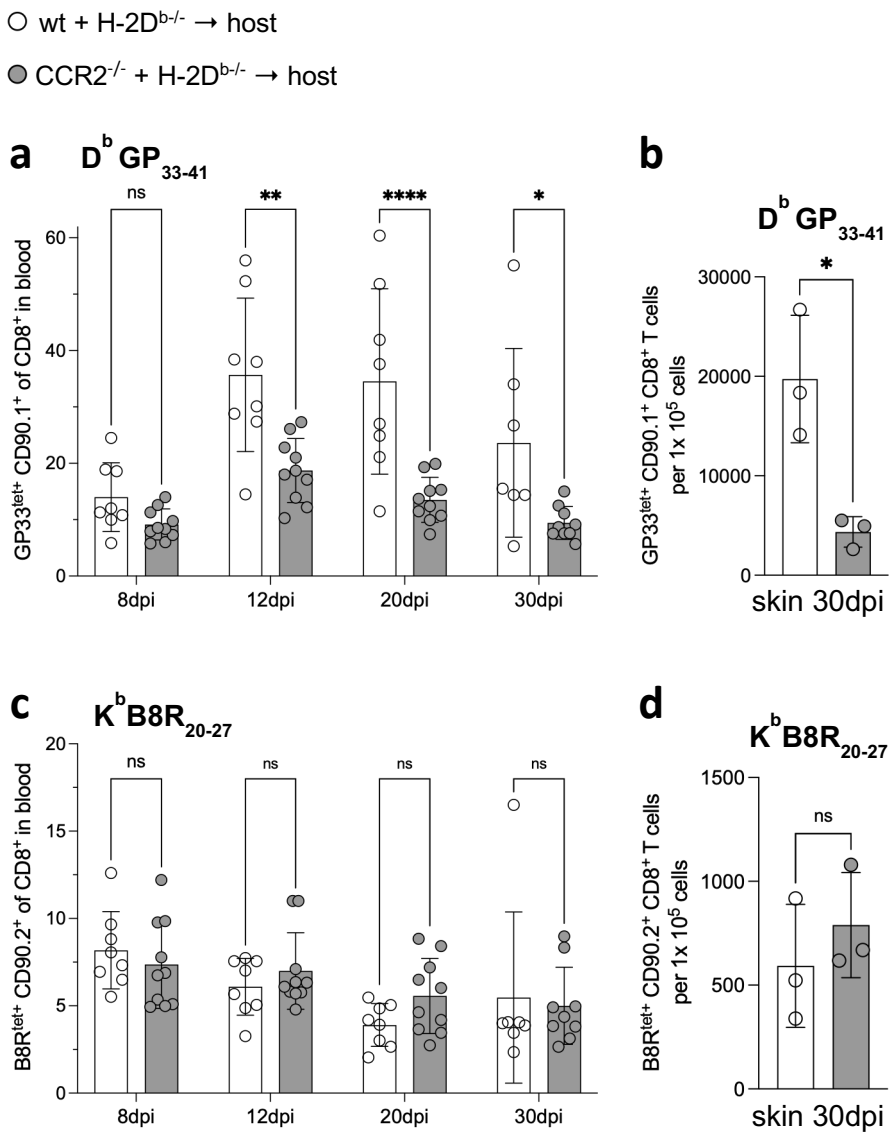

Figure 8. Antigen Presentation by CCR2 dependent mononuclear Phagocytes is required for a sustained antiviral CTL Response and the Differentiation of resident memory CD8⁺ T cells in the Skin

*Wt + H-2D$^{b\,-/-}$ and CCR2$^{-/-}$ + H-2D$^{b\,-/-}$ mixed bone marrow chimeric mice were adoptively transferred with GP$_{33-41}$-specific TCR327 CD8⁺ T cells (CD90.1⁺) before being infected with recombinant Vaccinia Virus (rVACV-G2) at the ear pinnae. The antiviral cytotoxic T cell response was monitored in the blood and the skin of infected mice at the indicated days post infection (dpi) using flow cytometry. After gating on no debris, singlets, viable (efl780⁻) and pan45⁺ cells, lineage negative (CD90.2⁻, NK1.1⁻, CD19⁻) cells were removed and based on their CD8 expression CD8⁺ T cells were identified. The CD8⁺ T cell population was subdivided into CD90.1⁺ and endogenous (CD90.1⁻) cells, the antigen-specificity of those cells was then analysed by tetramer staining. **(a,c)** Cumulative data of the CTL response at the indicated days post infection (dpi) against the D^b restricted GP$_{33-41}$ (CD90.1⁺ GP$_{33-41}$ tetramer⁺) and the K^b restricted B8R$_{20-27}$ epitope (CD90.1⁻ B8R$_{20-27}$ tetramer⁺) in the blood of n=8-10 mice per group from two independent experiments. Vertical bars represent mean ± SD. Statistical significance was determined using two way ANOVA with ****p ≤ 0.0001; **p ≤ 0.01; *p ≤0.1; ns (not significant). **(b,d)** At 30 dpi the number of GP$_{33-41}$ specific (GP$_{33-41}$ tetramer⁺) and endogenous B8R$_{20-27}$ specific (B8R$_{20-27}$ tetramer⁺) CD8⁺ tissue resident memory (T$_{RM}$) per 100.000 viable cells of the skin tissue were determined using flow cytometry. One of two independent experiments is shown with n = 3 mice. Vertical bars represent mean ± SD. Statistical significance was determined using unpaired t-test with *p ≤0.1; ns (not significant).*

To gain a deeper understanding about the function of CCR2 dependent cells for CTL responses to cutaneous virus infection, the potential provision of co-stimulation besides antigen presentation by CCR2 dependent cells was investigated next. For this purpose, mBMC were generated by the reconstitution of irradiated mice with a mixture of $CCR2^{-/-}$ with either $CD40^{-/-}$, $CD70^{-/-}$, $CD80^{-/-}xCD86^{-/-}$ or $CD70^{-/-}xCD80^{-/-}xCD86^{-/-}$. Respectively, as a control, irradiated mice were injected with a mixture of wt bone marrow and one of the four co-stimulatory molecule knockout bone marrows. After complete reconstitution mBMC were adoptively transferred with naïve $327TCR^{+}$ $CD90.1^{+}$ $CD8^{+}$ T cells and one day later mBMC were infected with rVACV-G2. At 30 dpi the relative number of GP_{33-41} specific $CD8^{+}$ T cells was determined in the skin (Figure 9a), skin-draining lymph node (Figure 9b) and spleen (Figure 9c) of mBMC. The antiviral skin-resident memory CD8+ T cells in the skin were significantly impaired in $CCR2^{-/-}$+$CD40^{-/-}$, $CCR2^{-/-}$+$CD80^{-/-}xCD86^{-/-}$ and $CCR2^{-/-}$+$CD70^{-/-}xCD80^{-/-}xCD86^{-/-}$ mBMC, as well in $CCR2^{-/-}$+$CD70^{-/-}$ a tendency of an impaired memory differentiation was observed. In contrast, the relative number of antiviral memory $CD8^{+}$ T cells in the skin-draining lymph node was significantly reduced exclusively, if CCR2-dependent cells lack CD40 expression. Furthermore, the formation of antiviral memory $CD8^{+}$ T cells in spleen was reduced when CCR2-dependent cells were deficient in CD40 or CD70.

To acquire information about, where CCR2-dependent cells interact with $CD8^{+}$ T cells to control the antiviral adaptive immune response, we generated mBMC utilizing CCR7 deficient mice. CCR7 is a chemokine receptor expressed e.g. on activated DC. Through the detection of chemokines these cells are guided to lymphoid organs such as the draining lymph nodes. To study the role of CCR7 on CCR2 dependent cells on the CTL response during viral skin infection, by utilizing mBMC a situation was generated where CCR2 dependent cells are disabled of the migration to lymphoid organs. Irradiated mice were reconstituted with a mixture of $CCR2^{-/-}$ with $CCR7^{-/-}$ bone marrow, or as a control with a mixture of wt and $CCR7^{ko}$ bone marrow. After complete reconstitution mBMC were adoptively transferred with naïve $327TCR^{+}$ $CD90.1^{+}$ $CD8^{+}$ T cells and one day later mBMC were infected with rVACV-G2. At 30 dpi the relative number of GP_{33-41} specific $CD8^{+}$ T cells was determined in skin (Figure 9a), skin-draining lymph node (Figure 9b) and spleen (Figure 9c) of mBMC. We observed no differences in the $CD8^{+}$ memory T cell formation in any tissue analyzed between both types of mBMC, indicating that CCR2-dependent cells do not migrate via gradients of CCR7 ligands, such as CCL19 or CCL21 chemokines, to lymphoid organs for the interaction with antiviral $CD8^{+}$ T cells.

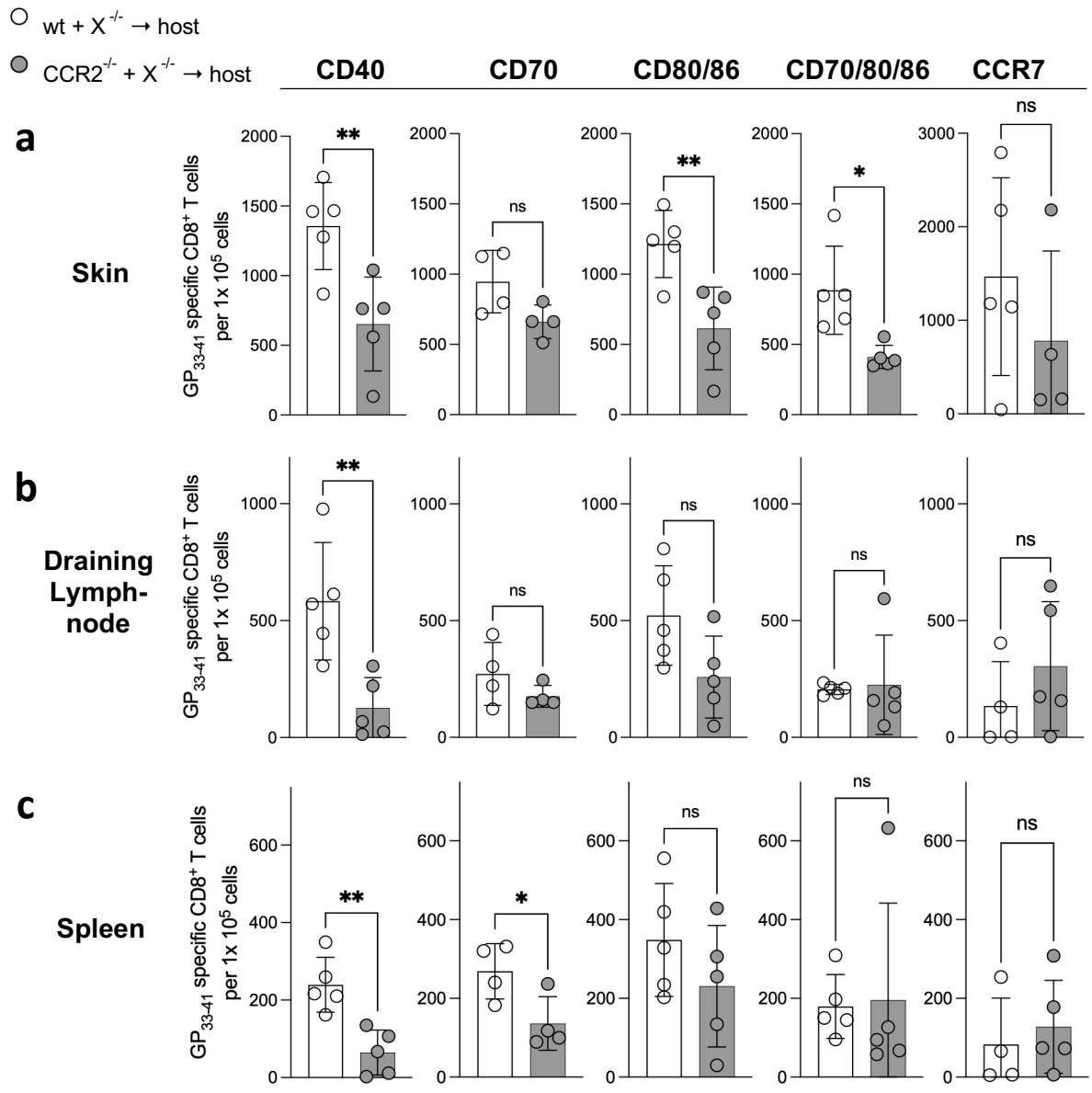

Figure 9. Co-stimulation by CCR2 dependent mononuclear Phagocytes drives the Differentiation into anti-viral Memory CD8⁺ T cells predominantly in Skin Tissue

*Wt + X $^{-/-}$ and CCR2$^{-/-}$ + X$^{-/-}$ mixed bone marrow chimeric mice were adoptively transferred with TCR327 (GP_{33-41}-specific) CD90.1⁺ CD8⁺ T cells before being infected with recombinant Vaccinia Virus (rVACV-G2) at the ear pinnae, thereby X means one of the five knockouts (CD40$^{-/-}$, CD70$^{-/-}$, CD80$^{-/-}$xCD86$^{-/-}$, CD70$^{-/-}$xCD80$^{-/-}$xCD86$^{-/-}$, CCR7$^{-/-}$) as shown in the uppermost row. At 30 dpi the antiviral CD8⁺ memory T cells in the skin (**a**), draining lymph node (**b**) and spleen (**c**) were identified using flow cytometry. After gating on no debris, singlets, viable (efl780-) and pan45⁺cells, lineage negative (CD90.2^{-}, NK1.1^{-}, CD19^{-}) cells were removed and based on their CD8 expression CD8⁺ T cells were identified. The CD8⁺ T cell population was subdivided into CD90.1⁺ and endogenous (CD90.2^{-}) cells, the antigen specificity of those cells was then analyzed by tetramer staining. The numbers of GP_{33-41} specific (GP_{33-41} tetramer⁺) and endogenous $B8R_{20-27}$ specific ($B8R_{20-27}$ tetramer⁺) CD8⁺ T cells among 100.000 viable cells of the respective organ is shown for each investigated knockout (columns). One experiment is shown with n = 4-5. Vertical bars represent mean ± SD. Statistical significance was determined using unpaired t-test with ∗∗p ≤ 0.01; ∗p ≤0.1; ns (not significant).*

In summary, we identified cDC1, cDC2 and a cDC-like population, as a cDC associated subset of the infl. cDC2/moC population, relying on FLT3 signaling for their residency in the infected skin. In addition, monocytes, macrophages and partially the infl. cDC2/moC population were found to require CCR2 for

tissue residency in the infected skin. However, we were unable to distinguish the respective CCR2 and FLT3 subset among the infl. cDC2/moC population identified based on the lineage specific marker expression analysed.

Taken together, in response to a cutaneous pox virus infection antigen presentation and co-stimulation on CCR2 dependent phagocytes seem to play an essential role for sustained CTL responses and subsequently $CD8^+$ T cell memory differentiation, in particular in skin tissue. In this context, CD40 expression on CCR2 dependent cells may also be important for the formation of circulating memory $CD8^+$ T cells in the skin-draining lymph nodes and spleen. The concrete location where CCR2-dependent cells interact with antiviral $CD8^+$ T cells was not determined, but we found, that CTL controlling CCR2-dependent cells might not migrate into the skin draining lymph nodes through CCR7 signaling. We did not address, whether these cells at all migrate to secondary lymphoid organs during viral skin infection.

5.2. Cellular Requirements for the Recall of CD8$^+$ Memory T cells in the murine Skin

5.2.1 Research Objective

Tissue-resident memory T cells (T_{RM}) mediate efficient protection of epithelial tissue against reinfection. In case of reinfection, CD8$^+$ T_{RM} reencounter cognate antigen bound to the respective MHC I on the surface of an antigen-presenting cell (APC), upon recognition of pathogen derived protein fragments through the TCR, T_{RM} an then rapidly initiate the secondary immune response. However, the concrete cellular requirements for activating memory T cells resident in non-lymphoid tissues remains unknown. Theoretically, any infected cell can function as an APC because most cells, including structural cells, express MHC-I molecules. It has been postulated that circulating memory CD8$^+$ T cells require DC for recall, whereas tissue-resident memory CD8$^+$ T cells may not (Roberts and Woodland, 2004; Zammit et al., 2005; Low et al., 2020). However, in the skin, the largest epithelium of the body, the cell types acting as APC and thus are capable of initiating T_{RM} recall have yet to be identified. At epithelia, memory T cell activation may be carried out at least partially by non-DC (Crowe et al., 2003), such as infected structural cells. This study aims to identify the respective contribution of cell types required for the presentation of skin associated antigen to recall local CD8$^+$ memory T cells in the skin, meaning: 'Could any cell induce local recall in the skin?'.

Virally infected cells expressing a foreign antigen are phenocopied through the utilization of a conditional transgenic mouse model. This mouse model targets epidermal structural cells, which are primarily infected by skin-tropic viruses, and allows to monitor antigen-specific local memory recall *in vivo*. In healthy skin, keratinocytes are the most abundant cells of the epidermis. Because of their location close to the environment, keratinocytes might be the site of initial virus infection and the first cell type in which virus replication occurs. During viral reinfection, thus intracellular foreign antigen bound to MHC on infected keratinocytes becomes visible for TCR recognition by local memory CD8$^+$ T lymphocytes crawling through the epidermis. To study the cellular requirements for the recall of T_{RM}, we developed a conditional mouse model for the induction of MHC-I restricted antigen expression in keratinocytes. Similar mouse models for constitutive expression of class I restricted expression of antigen in K14$^+$ cells were developed previously (Kurts et al., 1996; McGargill et al., 2002), our model differs as antigen expression is conditional. This allows to control expression timely and also restrict antigen expression to a locally confined area of the tissue. Furthermore, the initiation of the cognate stimulus can be set without additional triggers such as tissue injury and innate alarm signals through the control of the antigen expression by the topical application of a chemical.

5.2.2 Identifying the Target Cells of Cre in K14Cre Mice's Skin

Epithelial tissues get frequently infected. Local memory cells reside epithelia at this the frontline of defense and mediate protection against reinfections(Wakim et al., 2008; Mackay et al., 2012; Zaid et al., 2014). Most skin-tropic viruses target keratinocytes. Infected Keratinocytes may directly reactivate epidermal $CD8^+$ T_{RM}, which then respond with massive IFN gamma (IFNγ) secretion leading to an antiviral state within the tissue (Ariotti et al., 2014). To induce antigen expression in keratinocytes, we generated a transgenic mouse strain targeting keratinocytes through the Cre/loxP system. In the murine skin, the Cytokeratin14 (K14) expression is restricted to mitotically active keratinocytes of the epidermis close to the basal lamina (Guo et al., 2019). The basal layer of the interfollicular epidermis and the outer root sheath of the hair follicle is composed of Cytokeratin14 (K14)-rich filaments in keratinocytes (Vasioukhin et al., 1999) to target those cells conditionally; we used transgenic $K14CreER_T$ mice. In $K14CreER_T$ mice, the Cre recombinase can be induced by treatment with tamoxifen, specifically in $K14^+$ cells. To assess precisely in which cutaneous cell population Cre recombines floxed sequences, we crossed $K14CreER_T$ mice to Cre-indicator mice (R26R-EYFP). In those mice, Cre activity induces EYFP expression. The double transgenic offspring (K14EYFP mice, Figure 10a) were treated topically with the active metabolite of tamoxifen (4-hydroxy-tamoxifen, 4OHT) on the skin of the ears. Since the topically applied active metabolite of tamoxifen was chosen for the induction of K14Cre recombinase expression, only K14 expressing cells resident at the treated area of skin are targeted. The advantage of topical 4OHT, compared to treatment with tamoxifen systemically, is that the induction of side effects by targeting $K14^+$ cells, which are not resident in the topically treated area of skin, e.g. $K14^+$ cells of the esophagus (Vassar et al., 1989; Byrne et al., 1994; Wang et al., 1997) or cells of another area of skin, is precluded.

After 4OHT treatment of the ears' skin of naïve K14EYFP mice, the induced Cre activity reflected by EYFP expression was determined by flow cytometry of single cell suspension from epidermal and dermal tissue (Figure 10b). EYFP expression was not detected in dermal or $CD45^+$ cells of the treated skin but exclusively found in $CD45^-$ epidermal cells (gating of $CD45^+$ or $CD45^-$ cells was done according to the gating strategy for cell suspensions of the whole ear shown in Figure 10c). On average, 149 ± 72 $CD45^-$ epidermal cells per ear expressed YFP after 4OHT treatment of K14EYFP mice (Figure 10b). To characterize the target cell population of the $K14CreER_T$ in-more detail, we applied a staining panel for keratinocytes to skin samples of 4OHT-treated K14EYFP mice, allowing us to spatially resolve the location of keratinocytes relative to the hair follicle of the skin (Nagao et al., 2012; Tamoutounour et al., 2019). For gating on keratinocyte subsets, first, cell debris was removed from the cells. Then dead cells, doublets, T cells ($CD90.2^+$), NK cells ($NK1.1^+$), B cells ($CD19^+$) and the remaining hematopoietic cells ($CD45^+$) were removed. The expression of the Sca-1 marker identified keratinocyte subsets of the

interfollicular and infundibulum epidermis. EpCAM is expressed throughout the epidermis in adult mice, with higher expression in the infundibulum and isthmus. CD34 was only expressed in the bulge area, where hair follicles and melanocyte stem cells are located. Integrin α6 (CD49f) was uniquely expressed in the basal layers. Based on these expression patterns, keratinocyte subsets were identified from the epidermis: interfollicular (IE), infundibulum (IF), isthmus (IM), basal bulge (BB), and suprabasal bulge (SB) (Figure 10c, Figure 10d).

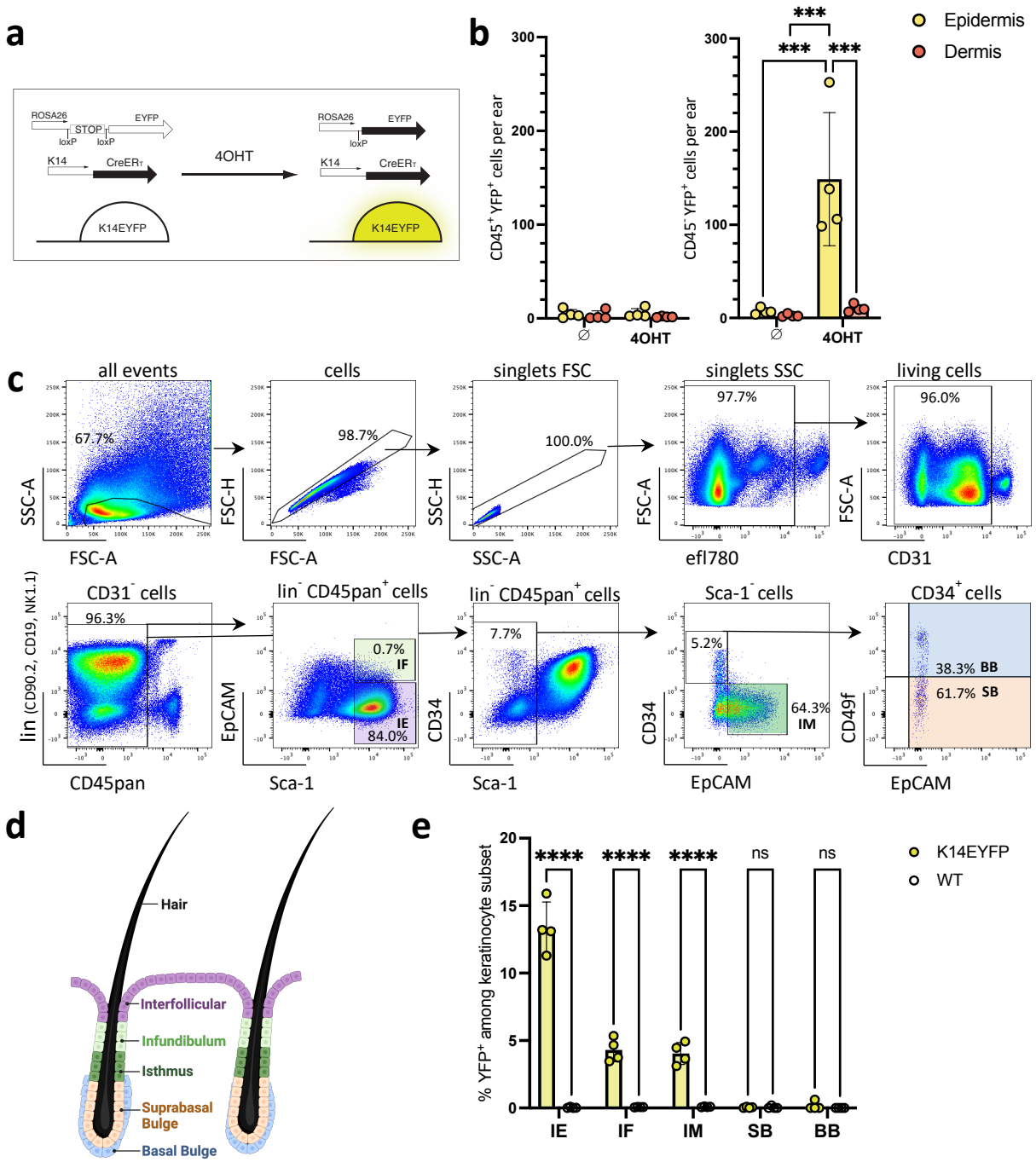

Figure 10. Characterization of K14-Cre Activity in cutaneous Cells using K14EYFP Indicator Mice
*The transgenic mouse model K14EYFP (K14CreER$_T$xR26R-EYFP) allows the 4OHT inducible expression of EYFP in Cytokeratin14 (K14) expressing target cells (**a**). The ear skin of naïve K14EYFP mice was treated topically with 20 µg of the active metabolite of Tamoxifen, 4-hydroxy-tamoxifen (4OHT). 48 h after treatment, the EYFP expression of hematopoietic cells (CD45$^+$) as well as non-hematopoietic cells (CD45$^-$) in the epidermis (yellow) and dermis (red) from the treated ear skin was assessed by flow cytometry. Data from one experiment is shown (**b**). The five keratinocyte subsets interfollicular (IE), infundibulum (IF), Isthmus (IM), Suprabasal Bulge (SB) and Basal Bulge (BB) were identified based on surface marker expression of EpCAM, Sca-1, CD34 and CD49f in the murine epidermis by flow cytometry (**c**). Schematic illustration of keratinocyte subsets in the murine epidermis (**d**). Frequency of EYFP expressing cells among the five keratinocyte subsets. The ear skin of K14EYFP (yellow-green) or WT (white) mice was treated topically daily with 20 µg of 4OHT for three days. 24 h after treatment, the frequency of EYFP-expressing cells among each keratinocyte subset in the treated epidermis was assessed by flow cytometry. Cumulated data from two experiments is shown with n=4 per group (**e**). Vertical bars represent mean ± SD. Statistical significance was determined with two-way ANOVA (B) or unpaired t-test (E); with $*p \leq 0.1$; $**p \leq 0.01$ $***p \leq 0.001$; $****p \leq 0.0001$; ns: not significant.*

The frequency of EYFP$^+$ cells was determined from each keratinocyte subset in epidermal cells of K14EYFP mice after topical 4OHT treatment of the ear skin (Figure 10e). EYFP$^+$ keratinocytes were found in three of five areas: 0 % EYFP$^+$ keratinocytes were observed in the two layers of the bulge, i.e. suprabasal and basal bulge, of the ear skin. In contrast, in the intrafollicular (on average 13.4% EYFP$^+$ of total), infundibulum (on average 4.3 % EYFP$^+$ of total), and isthmus (4.0 % EYFP$^+$ of total), a fraction of K14Cre recombined cells was found. As expected, the K14Cre-targeted primarily keratinocytes of the ear skin are the regions between hair follicles, namely in the interfollicular epidermis.

5.2.3 The double transgenic K14DIETER Mouse Model allows conditional Expression of the GP$_{33-41}$ Model Peptide in Keratinocytes

The following section describes the mouse model K14DIETER, which was developed to study the cellular requirement for tissue-resident memory (T_{RM}) recall. After an infection is cleared, T_{RM} persist at the previous site of infection and can be recalled by cognate antigen stimulation. However, the concrete cellular requirements remain unknown. To identify the cells which are involved in local memory recall in the skin, we generated a novel mouse model to mimic antigen reencounter by the tamoxifen-inducible expression of antigen in the most common structural cell of the skin's uppermost layer, namely the keratinocytes the epidermis. To this end, we crossed K14CreER$_T$ mice to ST33 mice. The offspring (K14CreER$_T$xST33 or K14DIETER) allows 4OHT inducible model peptide expression in keratinocytes, analogous to the DIETER mouse model (CD11cCreER$_T$xST33) targeting CD11c$^+$ cells (Probst et al., 2003) instead of keratinocytes. Double-transgenic mice allow the induction of model antigen expression specifically in the respective target cell. After treating those mice with tamoxifen, Cre recombinase is activated in K14 or CD11c positive cells, the DNA sequence of the STOP cassette is cut out, and the three model antigens are expressed and presented on MHC-I (Figure 11a).

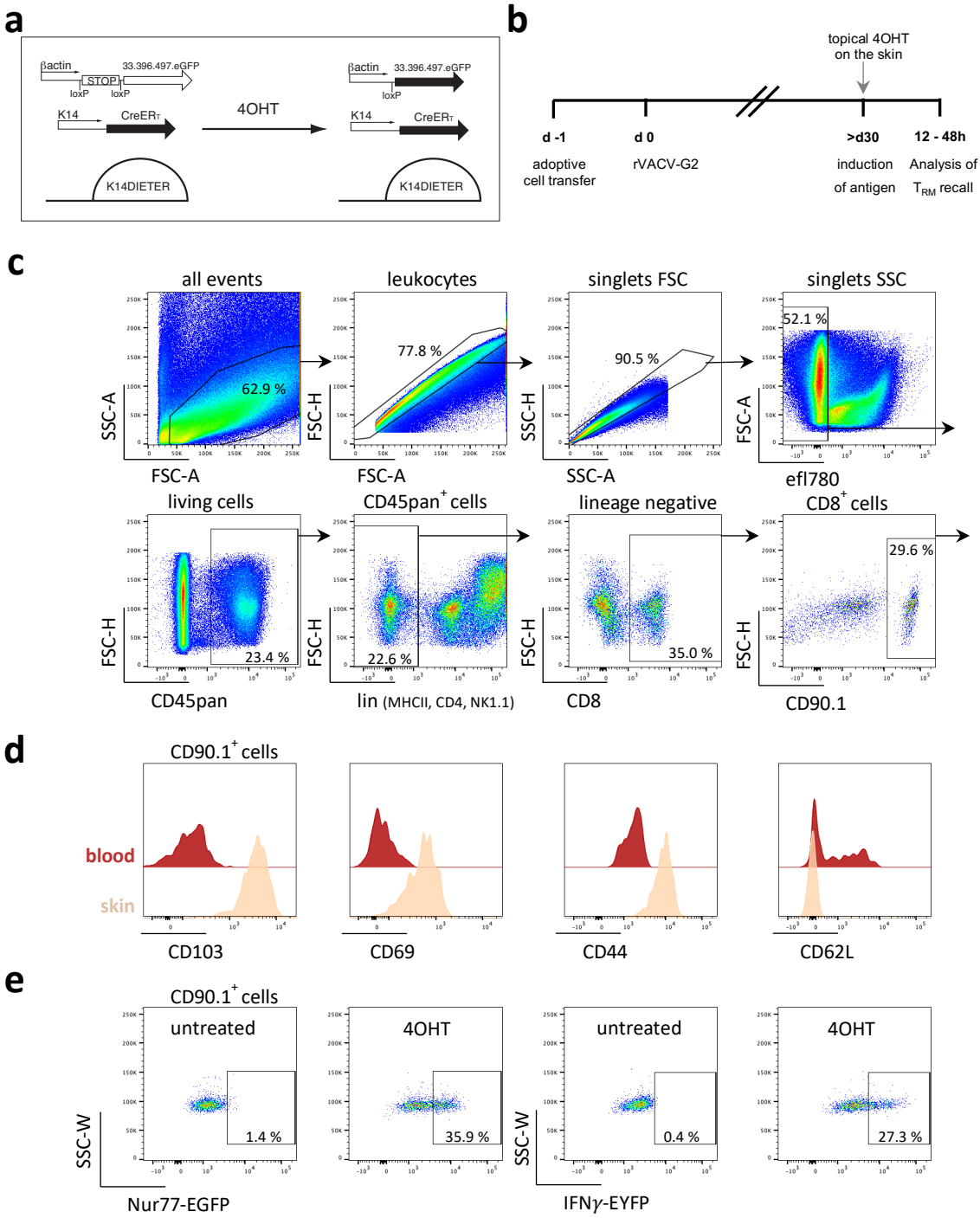

Figure 11. Identification of T_{RM} in the murine Skin by Flow Cytometry

*(**a**) The transgenic mouse model K14DIETER (K14CreER$_T$xST33) allows the 4OHT inducible expression of a model antigen containing the LCMV GP$_{33-41}$ epitope in Cytokeratin 14 expressing keratinocytes of a defined area of skin. (**b**) Procedure for skin resident memory reactivation experiments. K14DIETER mice were adoptively transferred with Nur77-EGFP- or IFNγ-EYFP-TCR327 CD8$^+$ T cells (CD90.1$^+$). One day later, both ears were infected at the ear pinnae with recombinant Vaccinia virus expressing the LCMV GP$_{33-41}$ epitope (rVACV-G2). At 30 dpi, antigen expression was induced by topical 4OHT treatment on the ear skin. (**c**) Representative flow cytometry gating for the identification of CD8$^+$CD90.1$^+$TCR327$^+$ T cells in ear skin and blood of K14DIETER mice is shown. At 30 dpi, ear tissue was enzymatically digested, and the resulting cell suspension was analyzed by flow cytometry after antibody-based staining. To identify skin resident memory T cells in the skin, first cell debris, doublets, dead cells (eF780$^+$) and stromal cells (CD45$^-$) were excluded. From the remaining leukocytes (CD45pan$^+$), lineage-negative cells, i.e. MHCII$^+$ cells (MHCII$^+$), CD4$^+$ T cells (CD4$^+$) and NK cells (NK1.1$^+$), were excluded. From the remaining cells, CD8$^+$ cells were gated, followed by gating for CD90.1$^+$, identifying TCR327 CD8$^+$ T cells. (**d**) After identifying CD90.1$^+$ TCR327 CD8$^+$ T cells in the skin, their phenotype was further compared to the same cell population from blood based on the expression of CD103, CD69, CD62L and CD44. (**e**) Nur77-EGFP or IFNγ-EYFP expression of CD90.1$^+$ T cells was determined in 4-hydroxytamoxifen (4OHT) treated versus untreated skin.*

In general, using these models, the antigen expression can be either systemically or locally induced. For local memory recall, antigen expression was induced in target cells localized in a specific area of skin by the topical application of the active metabolite of tamoxifen, 4-hydroxytamoxifen (4OHT) (Hain et al., 2019). With this method, we aimed to induce a local memory response in skin triggered by keratinocyte-derived antigen to study the participation of various cutaneous cell types in this process. To enable the study of the T_{RM} responses using this transgenic model, we first generated K14DIETER mice harboring antigen-specific T_{RM} in healthy skin (Figure 11b). For this purpose, naïve GP_{33-41} specific TCR transgenic $CD8^+$ T cells from $327TCR^+$ $CD90.1^+$ reporter mice, which allow either the direct read out of Nur77 expression (Nur77-EGFP) or of IFNγ production (IFNγ-EYFP) in antigen specific T cells by flow cytometry, were adoptively transferred in K14DIETER mice. We infected them one day later with recombinant Vaccinia Virus expressing the GP_{33-41} antigen (rVACV-G2) to generate a local virus infection in the skin leading to the priming of the transferred $CD8^+$ T cells and generation of tissue-resident memory T cells. After resolved infection (> 30 dpi), the ears were enzymatically digested, and tissue-resident memory T cells were identified in the cell suspension based on the gating strategy shown in Figure 11c. First, cell debris was removed by gating on SSC low to intermediate events. Then from the remaining events, doublets, dead cells, non-hematopoietic cells ($CD45^-$), antigen-presenting cells ($MHC\text{-}II^+$), CD4 T cells ($CD4^+$) and NK cells ($NK1.1^+$) were removed. Among the remaining population, the $CD8^+$ T cells were identified based on their CD8 expression and among them, the GP_{33-41}-specific $CD8^+$ T cells ($327TCR^+$) were subsequently identified based on the congenic marker expression of CD90.1. On average, about $4x10^3$ antigen-specific $CD8^+$ bona fide T_{RM} had been generated per ear (Figure 12d), indicating the successful introduction of antigen-specific local memory to the skin tissue as required to investigate the mechanism for memory recall *in vivo*. We found that those cells homogenously expressing the T_{RM}-specific markers $CD103^+$ $CD69^+$, as expected. Furthermore, they expressed CD44 and did not express the surface marker CD62L in contrast to circulating $CD90.1^+$ $CD8^+$ T cells from the blood (Figure 11d).

5.2.4 Local Antigen Expression in Keratinocytes leads to a rapid functional Recall of $CD8^+$ T_{RM} in the Epidermis

In response to TCR stimulation, T_{RM} secrete IFNγ inducing a tissue-wide antiviral state (Ariotti et al., 2014). For *in situ* memory re-challenge, 4OHT was topically applied on the ear skin of virus-experienced K14DIETER mice. The IFNγ response of T_{RM} in the skin was quantified by IFNγ-EYFP reporter expression in 327TCR x IFNγ-EYFP $CD8^+$ T cells using flow cytometry (Figure 11e; for the gating strategy of $T_{RM,}$ see Figure 11c and 11d). Compared to the untreated ear samples of K14DIETER mice, in the 4OHT-treated ears, the frequency of IFNγ-$EYFP^+$ cells amongst isolated antigen-specific $CD8^+$ T cells was significantly

elevated within 48 h after treatment (Figure 12a). After topical 4OHT treatment, the tissue-resident memory cells in skin respond antigen-specifically with the expression of IFNγ, which is reflected by the IFNγ-EYFP signal, starting 24 h post treatment and peaking at 96 h post treatment (Figure 12a). Starting 72 h post treatment, antigen-specific T cells in the draining lymph node also respond with IFNγ expression (Figure 12b). When comparing the induced immune response in the layers of the murine skin, it becomes evident that most IFNγ^+ cells are located within the epidermis. In contrast, only a few T_{RM} in the dermis started to respond 48 h after recall, which is a delay of 24 h compared to the epidermal compartment (Figure 12c,d).

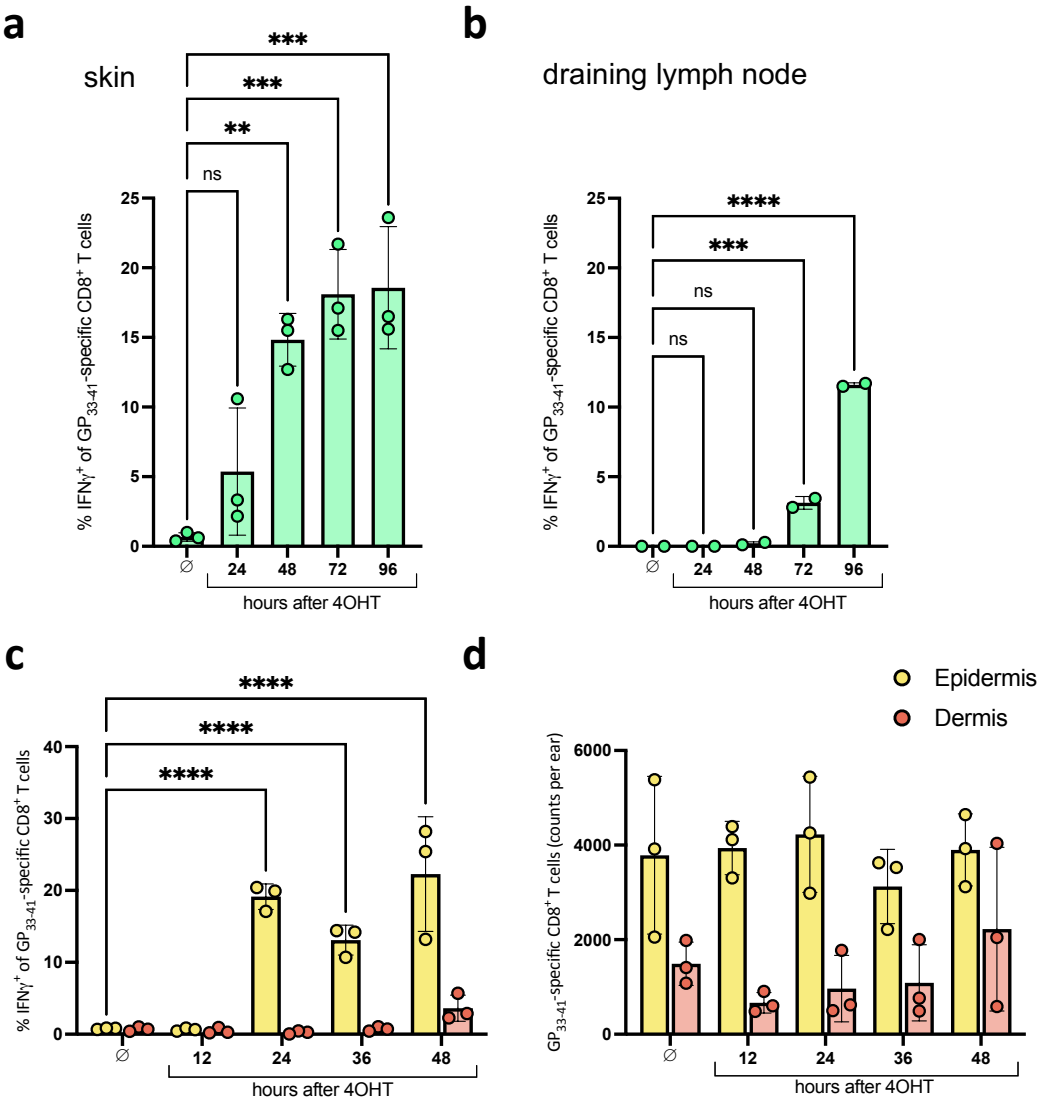

Figure 12. Tissue-resident Memory T cells respond with IFNγ Production after 4OHT Treatment of virus-experienced K14DIETER Mice

*K14DIETER mice were adoptively transferred with IFNγ-EYFPxTCR327 CD8$^+$ T cells (CD90.1$^+$) and infected on the ears with rVACV-G2. At 30 dpi, 4OHT was topically applied to the skin of the ears. (**a,b**) YFP expression in CD8$^+$CD90.1$^+$ IFNγ-EYFP-TCR327 T cells was assessed in the ear skin (**a**) and draining lymph node (**b**) at the indicated time (0-96 h) after 4OHT treatment. (**c,d**) The ear skin was separated into the epidermal and dermal layer, and YFP expression in CD8$^+$CD90.1$^+$ IFNγ-EYFP-TCR327 T cells (**c**) as well as absolute number of CD8$^+$CD90.1$^+$ IFNγ-EYFP TCR327 T cells (**d**) in the epidermis (yellow) and dermis (red) was determined. Vertical bars represent mean ± SD with n=2-3. Statistical significance was determined with one (**a,b**) or two-way (**c,d**) Anova; with *p ≤ 0.1; **p ≤ 0.01***p ≤ 0.001; ****p ≤ 0.0001; ns: not significant (not depicted for two-way ANOVA).*

Despite a massive IFNγ response of antigen-specific T cells, especially in the epidermis, we did not observe a significant increase in the number of antigen-specific $CD8^+$ T cells per ear, neither in the epidermis nor in the dermis (Figure 12d). In summary, our results suggest that, antigen-specific skin resident $CD8^+$ memory T cells respond with IFNγ production, when local expression of the virus derived GP_{33-41} peptide in keratinocytes is induced.

5.2.5 Induced T_{RM} Recall in K14DIETER Mice depends on the Dosage of 4OHT and leads to an increasing Tissue Thickness of the Skin due to the Infiltration of Cells over Time

For topical 4OHT treatment of virus-experienced ear skin of K14DIETER mice, doses ranging from 0 to 20 µg per ear were administered. The amount of 4OHT correlates positively with the frequency of IFNγ^+ T_{RM} in the treated skin of K14DIETER mice (Figure 13a). Besides the direct measurement of IFNγ^+ in T_{RM}, additional tissue-wide signs of an ongoing immune response were detected. A thickening of ear tissue starting from 24 h post 4OHT treatment was observed (Figure 13b). Within 0 to 96 h after 4OHT treatment, the maximum of tissue thickening is measured 96 h after the treatment. This ear thickening is one of the cardinal signs of inflammation besides swelling (tumor). Also, heat (calor), pain (dolor) and redness (rubor) became evident (data not shown). All of these are signs of the immune system responding to a stimulus. Tissue thickening can be due to the infiltration of cells into the skin. To analyze which and how many cells infiltrate the two layers of the murine ear skin, we stained isolated cells from the 4OHT treated tissue for surface markers, focusing on phagocytes. After the antigen-specific recall, most cells in the skin tissue consisted of $CD11b^+$Ly-$6C^+$ monocytes (Figure 13c) and $CD11b^+$Ly-$6G^+$ neutrophilic granulocytes (Figure 13d). In general, higher amounts of infiltrated cells were found in the dermal layer compared to the epidermal layer of the recalled skin tissue. Interestingly, at 48 h after 4OHT, an emerging MHC-II-expressing non-hematopoietic cells subset (MHC-II^+ $CD45^-$) was found in the epidermis (Figure 13e).

In conclusion, these results indicate that structural cells expressing cognate antigens can initiate local memory recall in the epidermis. This leads rapidly to IFNγ production by antigen-specific T_{RM} in the epidermis, the recruitment of monocytes and granulocytes and subsequently results in establishment of an anti-viral state in the skin tissue, suggesting that any infected cell can provoke a rapid antiviral response in the skin.

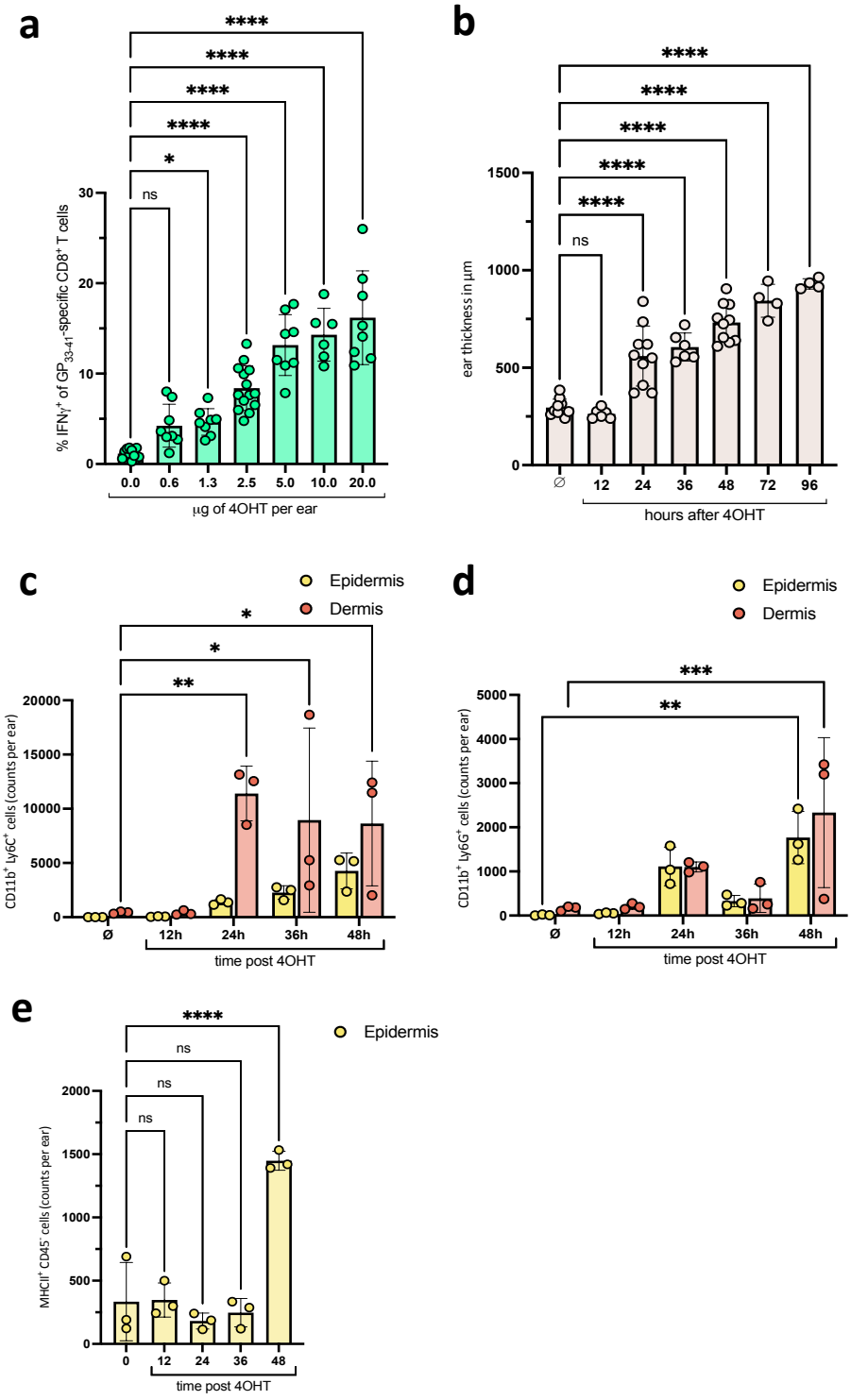

Figure 13. Keratinocyte-derived Antigen Expression triggers an T_{RM} Memory-induced Immune Response in the Skin
*K14DIETER mice were adoptively transferred with IFNγ-EYFPxTCR327 CD8⁺ T cells (CD90.1⁺) and infected on the ears with rVACV-G2. At 30 dpi, 4OHT was topically applied to the skin of the ears. (**a**) The amount of 4OHT for recall induction was varied from 0.6 to 20.0 µg per ear. 48 h after treatment, the EYFP expression in CD8⁺CD90.1⁺ IFNγ-EYFPxTCR327 T cells was assessed in the ear skin. (**b**) The ear thickness was determined at the indicated time after 4OHT treatment. (**c,d**) The influx of cells in the epidermis (yellow) and dermis (red) of ear skin was analyzed from 12-48 h after 4OHT treatment. Absolute numbers of CD11b⁺ Ly-6C⁺ (**c**), CD11b⁺ Ly6G⁺ (**d**), and MHCII⁺CD45⁻ (**e**) cells were determined using flow cytometry. Vertical bars represent mean ± SD with n=3-16. Statistical significance was determined with one (**a,b,e**) or two-way (**c,d**) Anova; with *p ≤ 0.1; **p ≤ 0.01***p ≤ 0.001; ****p ≤ 0.0001; ns: not significant (not depicted for two-way ANOVA).*

5.2.6 The Impact of circulating CD8$^+$ T cells from the Lymph Node on T_{RM} Recall in Skin

To investigate the impact of circulating CD8$^+$ memory T cells on the early phase of memory recall in skin, the drug Fingolimod was utilized. Fingolimod binds to the sphingosine-1-phosphate receptor 1 (S1PR1) on lymphocytes. Therefore, chemotaxis, along a sphingosine-phosphate 1 (S1P) gradient out of the lymph node into the bloodstream, is inhibited. T cells in mice treated with Fingolimod remain trapped in the lymph nodes. Thus, the influx of lymphocytes in peripheral tissues e.g. due to inflammation is reduced. To assess the impact of circulating CD8$^+$ T cells from the lymph node on T_{RM} recall in the skin, changes in the recall response with or without Fingolimod treatment were analyzed. For this purpose, K14DIETER mice were adoptively transferred with TCR327 x IFNγ-EYFP cells (CD90.1$^+$) and infected on the ears with rVACV-G2. At 30 dpi, when skin resident memory was established, antigen expression in keratinocytes was induced by topical 4OHT treatment on one ear of each mouse. Then the mice received orally either Fingolimod or an equivalent amount of diluent daily for four days. Four days after antigen induction, ear thickness was measured and the re-activation of T_{RM} in the epidermis and dermis was assessed by quantifying the IFNγ-EYFP reporter expression using flow cytometry (Figure 14). Fingolimod treatment resulted in a reduced tissue thickening of the 4OHT-treated ear skin compared to without Fingolimod. Reduced thickening might be associated with less lymphocytes migrating from the lymph node to the ear (Figure 14a).

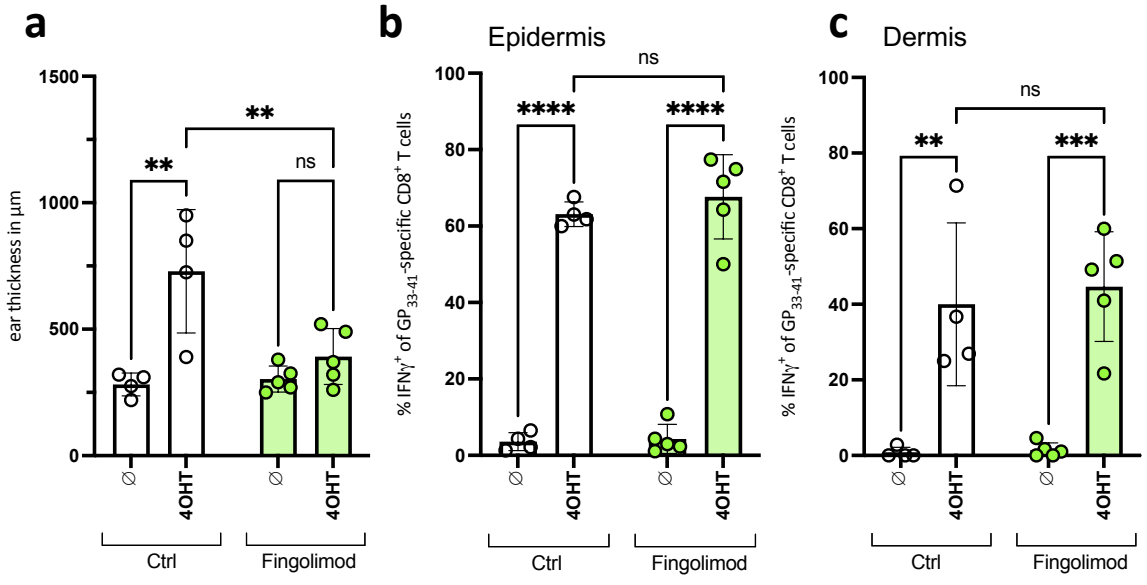

Figure 14.Trapping circulating T cells in the Lymph Nodes by Fingolimod Treatment does not affect T_{RM} Recall in the Skin

*K14DIETER mice were adoptively transferred with IFNγ-EYFP-TCR327 CD8$^+$ T cells (CD90.1$^+$) and infected on the ears with rVACV-G2. Thirty days after infection, 4OHT was topically applied to the skin of one ear (4OHT) and one ear was left untreated (∅). Additionally, the mice received daily either 10µg of Fingolimod (FYT720, green) or an equivalent amount of diluent (Ctrl, white) orally for four days. Four days after 4OHT treatment, the ear thickness (**a**) and the frequency of IFNγ-EYFP$^+$ among CD8$^+$ CD90.1$^+$ IFNγ-EYFP TCR327 T cells in the epidermis (**b**) and dermis (**c**) were determined in Fingolimod-treated (Fingolimod) vs control (Ctrl) mice. Vertical bars represent mean ± SD with n=4-5. Statistical significance was determined with two-way ANOVA, with $*p \leq 0.1$; $**p \leq 0.01$ $***p \leq 0.001$; $****p \leq 0.0001$; ns: not significant.*

Further analysis showed that the frequency of IFNγ-EYFP$^+$ T_{RM} remained unchanged with versus without Fingolimod treatment during recall, both in the epidermis and dermis of the skin, at these early time points analysed (Figure 14b,c). This suggests that Fingolimod, which specifically affects the migration of circulating lymphocytes, has no impact on the induction of T_{RM} recall, meaning local memory recall is independent of circulating cells from lymph nodes. This finding serves as a proof of concept, as we aim to specifically examine the recall of tissue-resident CD8$^+$ memory cells and not of their circulating counterparts. However, after Fingolimod treatment, fewer cells from the lymph node might have migrated into the skin, resulting in less tissue thickening. The identity of the trapped cells within the draining lymph nodes was not assessed in this experiment.

5.2.7 The isolated CD8$^+$ T_{RM} Population from Skin does not contain intravascular CD8$^+$ T cells

Cell influx following local memory recall from the lymph node through the blood into the skin was investigated in the previous experiment. Next, we assessed whether intravascular CD8$^+$ T cells are part of the T_{RM} isolated from the skin and thus are contaminating the isolated T_{RM} population of the skin. For this purpose, K14DIETER mice were adoptively transferred with TCR327 x IFNγ-EYFP cells (CD90.1$^+$) and infected on the ears with rVACV-G2. Thirty days after infection, when skin resident memory was established, antigen expression in keratinocytes was induced by topical 4OHT treatment on one ear of each mouse. Three days after 4OHT treatment and 5 min before euthanasia, a fluorescently labelled anti-CD8β was administered i.v. to stain CD8 T cells in the vasculature and to be able to discriminate between those in blood circulation and those resident in the tissue. Blood, epidermis and dermis were collected, processed for flow cytometry and stained *ex vivo* with anti-CD8α and for tissue-resident memory-specific markers. As expected, CD8 T cells from blood did not express CD69 or CD103, whereas *ex vivo* stained CD8α^+ T cells in the dermis and epidermis express the skin residency markers CD69 and CD103 (Figure 15a). Nevertheless, not all CD8 T cells from the skin homogeneously express those markers, indicating the presence of other, probably circulating, CD8 T cells in those tissues. The intravascular staining revealed that exclusively in blood intravascular stained (CD8α^+) among the *ex vivo* stained (CD8β^+) CD8 T cells were identified. In contrast, in the epidermis and dermis no intravascular CD8 T cells (CD8β^+) were found (Figure 15b), indicating that the *bona fide* T_{RM} population from the epidermis or dermis does not contain CD8$^+$ T cells *in transit* from inside the tissues vasculature.

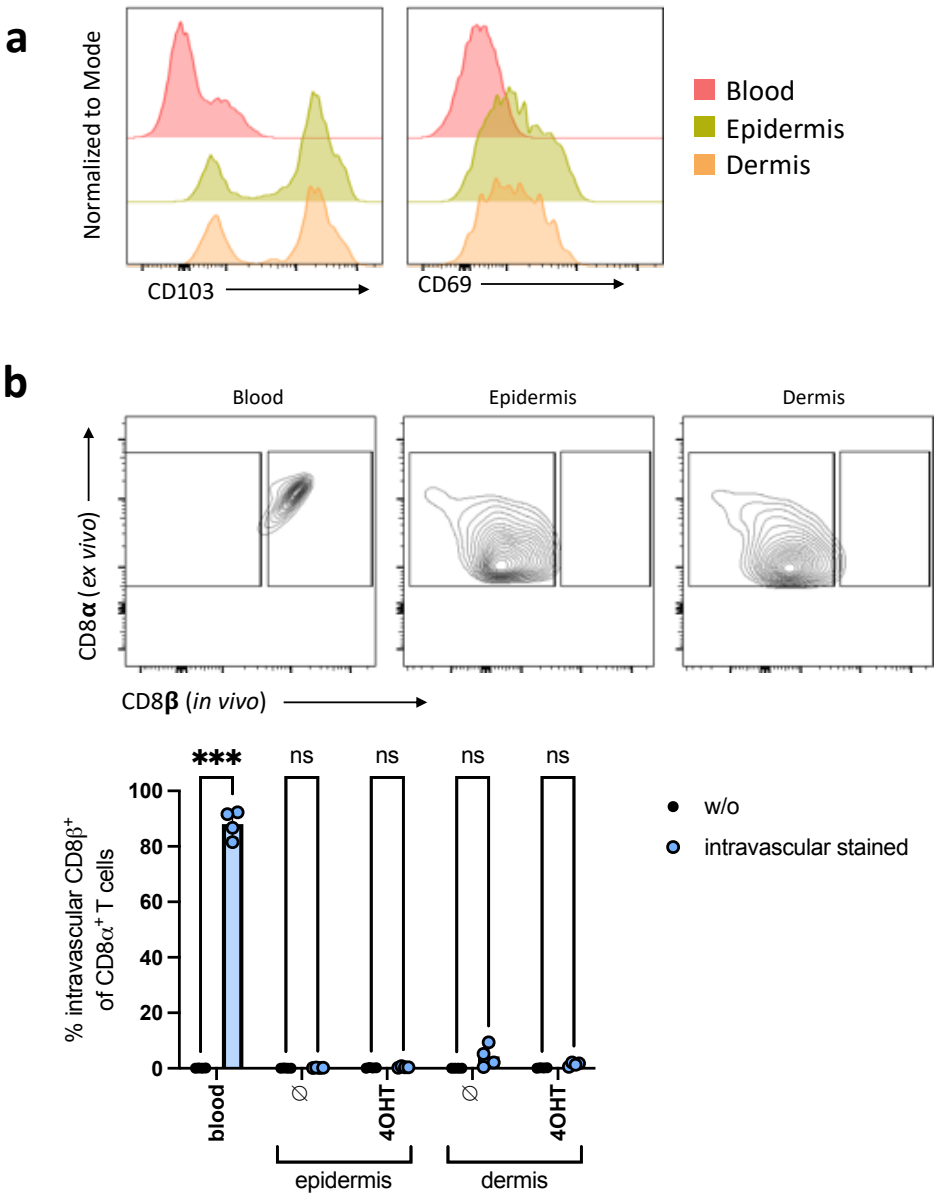

Figure 15. Intravascular Staining to distinguish Memory T cells in Circulation from tissue-resident Memory T cells within the Skin Tissue

*Virus-experienced K14DIETER mice were topically treated with 4OHT at the ear pinnae. Three days after 4OHT treatment, they were injected i.v. with 0.8 µg fluorescently labelled anti-CD8β antibody five minutes before euthanasia. Blood, epidermis and dermis were collected, processed for flow cytometry and stained with anti-CD8α antibody ex vivo. Data shown is gated on total ex vivo stained CD8α+ T cells. (**a**) Expression of CD103 and CD69 in CD8α+ T cells in blood, epidermis and dermis. (**b**) Unstained (black symbols) or intravascular stained CD8β (blue symbols) on ex vivo stained CD8α+ T cells in blood, dermis and epidermis. Frequency of intravascularly stained CD8β+ among ex vivo stained CD8α+ T cells in the blood, in 4OHT treated (4OHT) or untreated (ø) skin tissue (dermis and epidermis is shown separately). Vertical bars represent mean ± SD with n=4. Statistical significance was determined with the student's t-test; with ∗p ≤ 0.1; ∗∗p ≤ 0.01∗∗∗p ≤ 0.001; ∗∗∗∗p ≤ 0.0001; ns: not significant.*

5.2.8 Is Keratinocyte-derived Antigen Stimulation for T_{RM} Recall dependent on other Cells?

The previous data indicates that if an antigen selectively targets keratinocytes in a defined skin area, this results in a rapid antigen-specific reactivation of epidermal $CD8^+$ T_{RM}. Keratinocytes can either directly or indirectly conduct antigen presentation during this process. Besides the possibility of direct activation of T_{RM} by infected epithelial cells, keratinocyte derived antigen might require cross-presentation by a professional APC. In the murine skin, $Langerin^+$ cells have been identified to cross-present MHC-I restricted antigen (Stoitzner et al., 2006; Henri et al., 2010; Iborra et al., 2012).

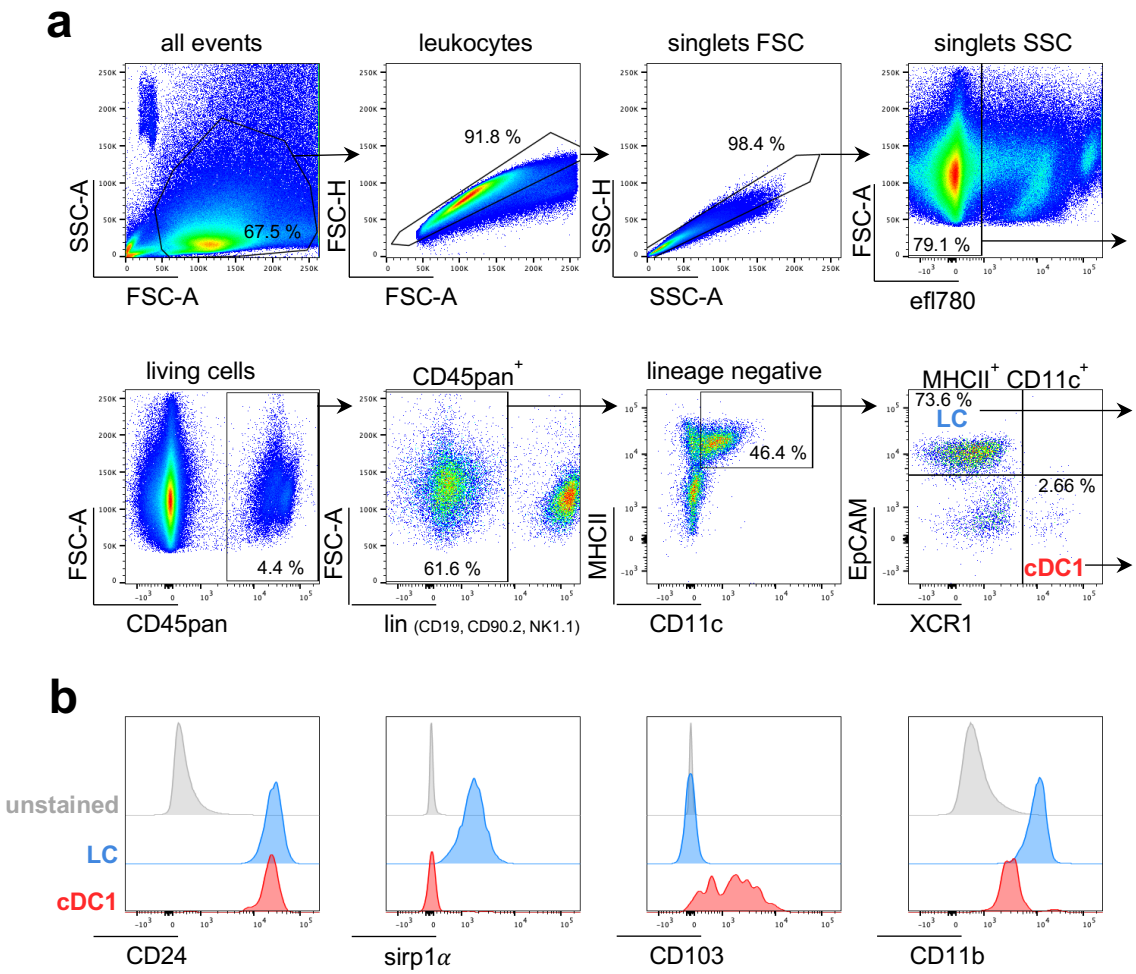

Figure 16. Identification of Langerin$^+$ DC Subsets in the healthy Skin of Mice
*Flow cytometric analysis of single-cell suspensions generated by enzymatic digestion of the ear skin of wild-type mice. (**a**) To identify subsets of Langerin$^+$ ($CD24^+$) DC in the skin, first cell debris, doublets, dead cells ($eF780^+$) and stromal cells ($CD45^-$) are excluded. Then from the remaining leukocyte population ($CD45pan^+$), lineage-negative cells, i.e. B cells ($CD19^+$), T cells ($CD90.2^+$) and NK cells ($NK1.1^+$), were excluded. Then DC ($MHC\text{-}II^+$ $CD11c^+$) were identified based on MHC-II and CD11c expression. Within the DC population, LC ($EpCAM^+$) were distinguished from cDC1 ($XCR1^+$). (**b**) Surface marker expression specific for the cDC1 population (depicted in red; $CD24^+$, $sirp1\alpha^-$, $CD103^+$, $CD11b^-$) and the LC population (shown in blue; $CD24^+$, $sirp1\alpha^+$, $CD103^-$, $CD11b^+$) was verified and compared to the unstained sample (depicted in grey).*

To investigate the contribution of Langerin$^+$ cells in the recall of T_{RM}, we crossed K14DIETER mice to Langerin-DTR mice, that express a human diphtheria toxin receptor from the endogenous Langerin

locus, thus upon Diphtheria-toxin (DT) injection both Langerin$^+$ subsets are depleted (Bennett et al., 2005). In healthy skin, Langerin$^+$ cells include two DC subsets: cDC1 and LC, which can be discriminated by the expression of distinct surface markers such as EpCAM, XCR1, sirp1α and CD11b (Figure 16a,b). cDC1 are a specialized DC subset for cross-presentation in the primary responses (Stoitzner et al., 2006; Henri et al., 2010; Iborra et al., 2012). LC, which are near T_{RM} in the epidermis, were found to be able to cross-present antigen from various sources in humans (Seneschal et al., 2012; Romano et al., 2012; Banchereau et al., 2012).

5.2.9 Are Langerin$^+$ cells required for Memory Recall via keratinocyte-derived Antigen?

The role of Langerin$^+$ cells in the recall of skin resident CD8$^+$ memory T cells was investigated through the depletion of Langerin$^+$ cells (schematically depicted in Figure 17a) during the recall process. To deplete Langerin$^+$ cells during memory recall, K14DIETER mice were crossed to Langerin-diphtheria toxin receptor knock-in (Langerin-DTR) mice (Bennett et al., 2005). Triple transgenic mice (Langerin-DTR$^+$, K14CreER$_T^+$ and ST33$^+$) are referred to as Langerin-DTR x K14DIETER in the following. Langerin-DTR x K14DIETER mice or K14DIETER mice were adoptively transferred with IFNγ-EYFP$^+$ or Nur77-EGFP$^+$ 327TCR$^+$ CD8$^+$ T cells, and one day later they were infected with rVACV G2 on both ears. At 30 dpi, mice were injected twice (d-3, d-1) with diphtheria toxin intravenously. In Langerin-DTR x K14DIETER mice, the efficient depletion of both Langerin$^+$ cell types, LC and cDC1, was verified in Langerin-DTR$^+$ mice by flow cytometric analysis of MHC-II$^+$ CD11c$^+$ cells in the skin one day after the last DT treatment (Figure 17b,c). In general, LC are distinguishable from Langerin$^+$ dermal DC (cDC1), based on their expression of EpCAM (Gaiser et al., 2012), whereas the lineage marker for cDC1 is XCR1 (Bachem et al., 2012) (see gating Figure 16). The recall response of skin resident T_{RM} was analyzed comparing Diphtheria-toxin-treated Langerin-DTR x K14DIETER (Langerin$^+$ cell-depleted) mice versus K14DIETER control mice (Ctrl). 48 h after 4OHT-induced antigen expression in keratinocytes, the ear thickness (Figure 17d), the TCR stimulation by Nur77-EGFP expression (Figure 17e) and the IFNγ production, as indicated by IFNγ-EYFP expression, (Figure 17f) of memory TCR327$^+$ cells was assessed flow cytometrically.

In the presence of Langerin$^+$ cells (Ctrl), ears treated with 4OHT were significantly thicker than the samples from mice depleted of Langerin$^+$ cells (Figure 17d). In line with these findings, EGFP in Nur77-EGFPx327TCR and EYFP-expression in the IFNγ-EYFPx327TCR CD8$^+$ T memory cells was exclusively induced when Langerin$^+$ cells were present in K14DIETER mice (Figure 17e,f). In conclusion, after induced antigen expression in keratinocytes in Langerin$^+$-cell-depleted mice, a lack of cell infiltration

in the skin, TCR stimulation and IFNγ production in antigen-specific T_{RM} was observed, which indicates that Langerin$^+$ cells are crucial for antigen specific recall of T_{RM} in the skin.

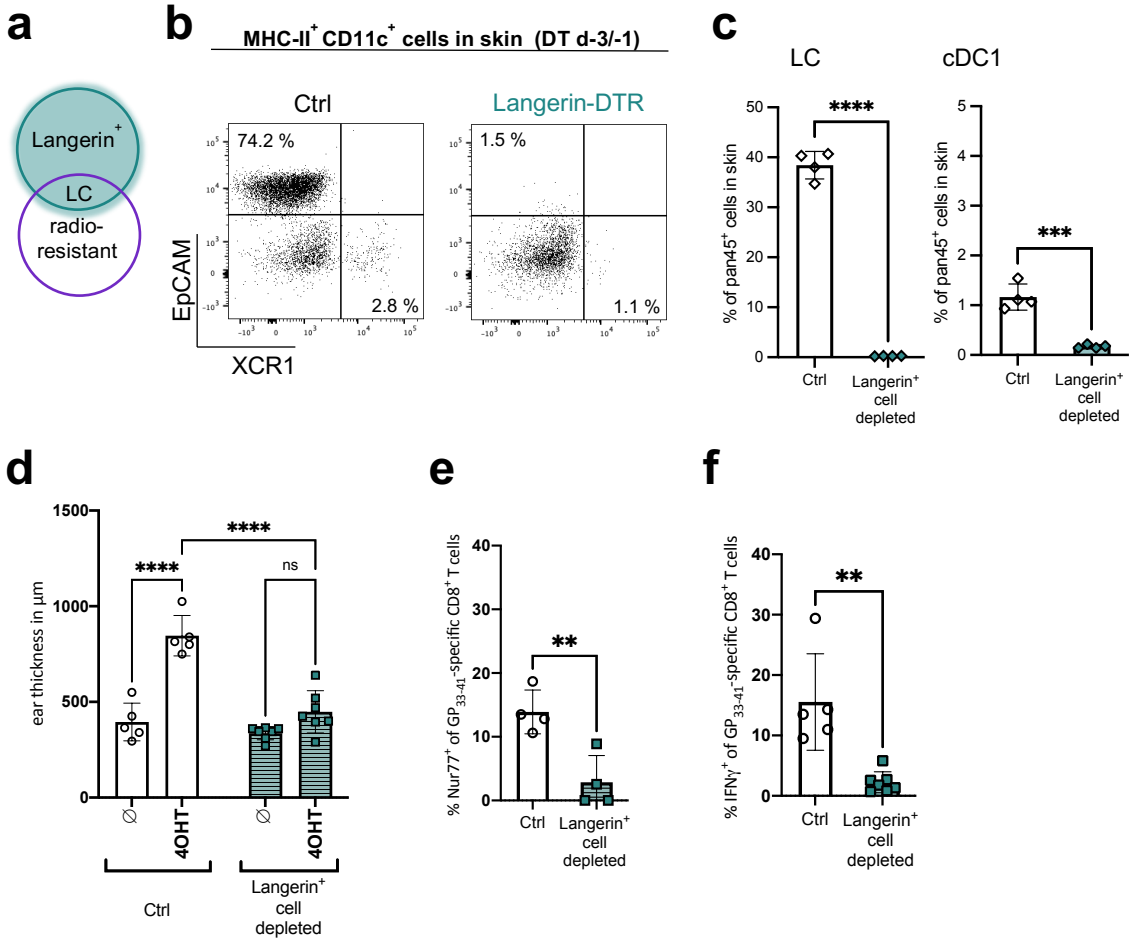

Figure 17. The Depletion of Langerin$^+$ cells ablates Reactivation and Cytokine Production of T_{RM} in the Skin
*K14DIETERxLangerin-DTR or K14DIETER mice were adoptively transferred with IFNγ-EYFP- or Nur77-EGFP TCR327 CD8$^+$ T cells (CD90.1$^+$) and infected on the ears with rVACV-G2. At 30 dpi, mice were injected with DT, depleting Langerin$^+$ cells in Langerin-DTR$^+$ K14DIETER but not in K14DIETER (Ctrl). (**a**) Schematic representation of Langerin-DTR mouse model affecting Langerin$^+$ cells, including LC. (**b**) Representative flow cytometry plot of the EpCAM and XCR1 expression on CD11c$^+$MHC-II$^+$ cells from the ear 4 days after DT treatment in wild-type (Ctrl) and Langerin-DTR mice. (**c**) Frequency of LC and cDC1 among pan45$^+$ cells in the skin of K14DIETER (Ctrl; white squares) and K14DIETERxLangerin-DTR (Langerin$^+$ cell depleted; petrol squares) mice with n=5, showing absence of Langerin$^+$ DC in Langerin-DTR mice. (**d**) Ear swelling, (**e,f**) frequency of Nur77 (**e**) and IFNγ (**f**) expressing cells among reporter-TCR327 CD8$^+$ T cells (CD90.1$^+$) in 4OHT treated skin tissue of K14DIETER (Ctrl; white circles) and K14DIETERxLangerin-DTR (Langerin$^+$ cell depleted; petrol squares) mice 48 hours after induction of antigen expression in keratinocytes. One representative experiment of two with n=4-8 per group is shown. Vertical bars represent mean ± SD. Statistical significance was determined with two-way ANOVA (ear thickness) or unpaired t-test (flow cytometry) with $*p \leq 0.1$; $**p \leq 0.01$ $***p \leq 0.001$; $****p \leq 0.0001$; ns (not significant).*

5.2.10 Are cDC1 dispensable for T_{RM} Recall in the Skin?

Our results indicate that Langerin$^+$ cells are required for T_{RM} reactivation by keratinocyte-derived antigens. To delineate whether the required cell type among the Langerin+ cells in skin is the dermal Langerin$^+$ XCR$^+$ cDC1 or the epidermal Langerin$^+$ EpCAM$^+$ LC, we made use of the differential radio-

sensitivity of these two cell-types. Bone marrow cells from Langerin-DTR mice were transplanted into lethally irradiated K14DIETER mice. After reconstitution, bone marrow chimera were adoptively transferred with IFNγ-EYFPxTCR327 CD8$^+$ T cells (CD90.1$^+$) and ears were infected with rVACV-G2. At 30 dpi, mice were injected twice (d-3, d-1) with PBS (Ctrl) or DT. DT depletes donor-derived DTR expressing Langerin$^+$ cells, whereas the skins radioresistant compartment including LC remain (cDC1 depleted; schematically depicted in Figure 18a). One day after the last injection, depletion of cDC1 in DT treated Langerin-DTR→ K14DIETER chimeras was verified by flow cytometric analysis of MHC-II$^+$ CD11c$^+$ cells in the skin (Figure 18b,c). The ear skin of cDC1 depleted and control mice were treated with 4OHT, two days later the recall response was analyzed. Similar ear swelling was observed in the experimental group depleted of cDC1 compared to the control group (Figure 18d). Interestingly, after 4OHT treatment the frequency of IFNγ expressing TCR327 CD8$^+$ T cells (CD90.1$^+$) was elevated in cDC1 depleted mice compared to the control group (Figure 18e). In conclusion, depletion of radiosensitive, Langerin$^+$ cells in the skin (i.e. cDC1), did not impair functional reactivation of T_{RM} in the skin in response to antigen expression in keratinocytes.

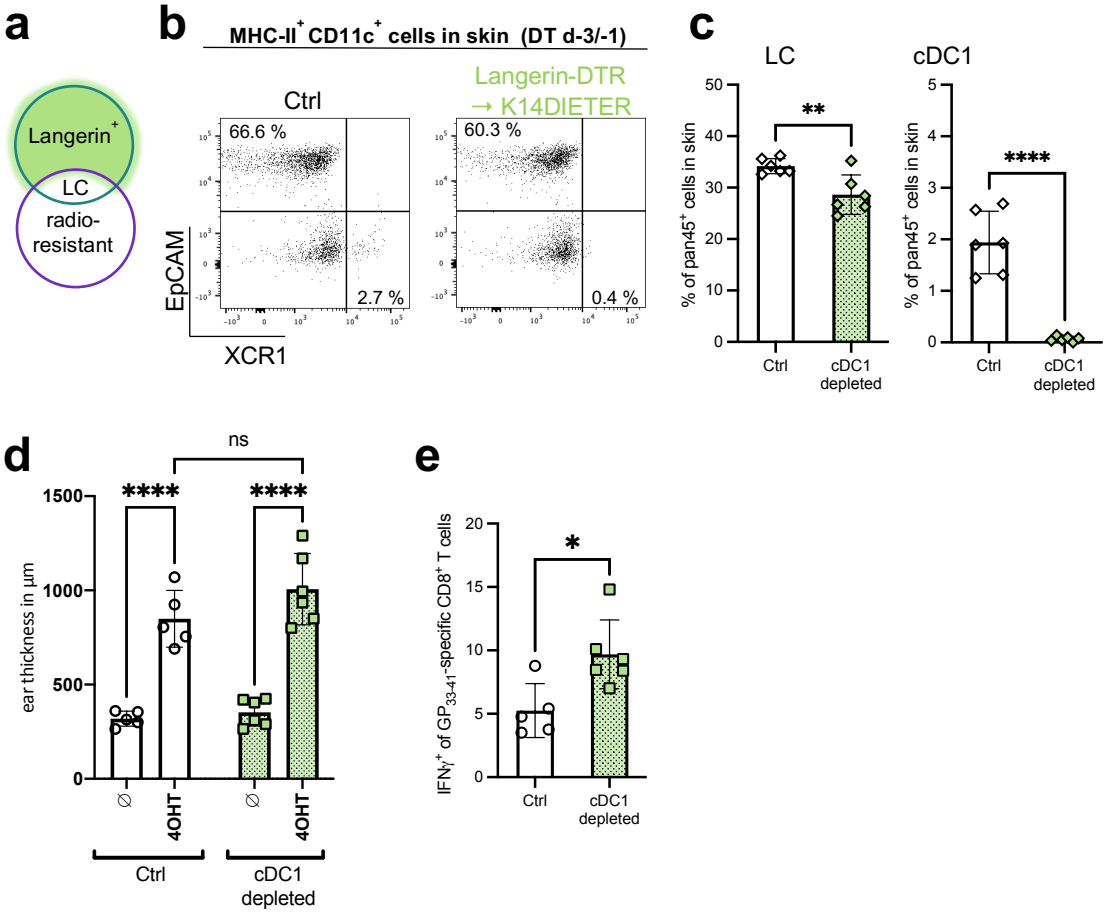

Figure 18. The Langerin$^+$ Cell Subset cDC1 is dispensable for T_{RM} Recall in Skin

*Bone marrow chimeric mice were generated by injecting lethally irradiated K14DIETER mice with bone marrow cells from Langerin-DTR mice. 6-8 weeks after irradiation, mice were adoptively transferred with IFNγ-EYFPxTCR327 CD8$^+$ T cells (CD90.1$^+$) and infected on the ears with rVACV-G2. At 30 dpi, mice were injected twice with either PBS (Ctrl) or DT, depleting DTR-expressing cells in DT treated chimera (cDC1 depleted). (**a**) Schematic representation of bone marrow chimeric model affecting the radiosensitive Langerin$^+$ subset of cDC1 specifically, whereas the skin's radioresistant compartment, including*

*LC, remains. (**b**) Representative flow cytometry plot of the EpCAM and XCR1 expression on CD11c$^+$MHC-II$^+$ cells from the ear 4 days after PBS (Ctrl) or DT (cDC1 depleted) injection in Langerin-DTR → K14DIETER bone marrow chimera. (**c**) Frequency of LC and cDC1 among pan45$^+$ cells in the skin of PBS (Ctrl; white squares) or DT (cDC1 depleted; green squares) treated Langerin-DTR → K14DIETER bone marrow chimera with n=6, showing absence of cDC1after DT treatment. (**d,e**) One day after the first DT or PBS injection, the skin of one ear of Langerin-DTR → K14DIETER bone marrow chimera was treated with 4OHT. (**d**) Ear swelling and (**e**) frequency of expressing cells among TCR327$^+$CD8$^+$ T cells (CD90.1$^+$) in 4OHT treated skin tissue of PBS (Ctrl; white circles) or DT injected Langerin-DTR → K14DIETER bone marrow chimera (cDC1 depleted; green squares) was determined 48 hours after induction of antigen expression in keratinocytes. One experiment with n=5-6 per group is shown. Vertical bars represent mean ± SD. Statistical significance was determined with two-way ANOVA (ear thickness) or unpaired t-test (flow cytometry) with ∗p ≤ 0.1; ∗∗p ≤ 0.01 ∗∗∗p ≤ 0.001; ∗∗∗∗p ≤ 0.0001; ns (not significant).*

5.2.11 Radioresistant Cells present Antigen for CD8$^+$ T_{RM} Recall in the Skin

In the previous experiments, Langerin$^+$ cells were identified as crucial for local memory recall in the skin, whereas the cDC1 subset was irrelevant for the cutaneous memory response following cognate antigen expression in keratinocytes. This finding suggests that other Langerin$^+$ DC subsets than cDC1 cross-present keratinocyte-derived antigens. To test this hypothesis in keratinocyte-mediated T_{RM} recall, an alternative approach was used instead of using the Langerin-DTR/DT system. The alternative approach includes the utilization of bone marrow chimeric mice to modulate the antigen presentation of radioresistant versus radiosensitive cells to identify the required antigen-presenting cell types for local memory recall in the skin. H-2D$^{b-/-}$ mice lacking a functional gene for the MHC class I haplotype H-2D^b, which is required for H-2Db restricted GP_{33-41} peptide presentation towards 327TCR T cells. To disable direct antigen presentation on radioresistant cells in K14DIETER mice, we crossed the K14DIETER strain to H-2D$^{b-/-}$ mice. H-2D$^{b-/-}$ x K14DIETER mice (CD45.2) were lethally irradiated and then reconstituted with wild-type bone marrow (CD45.1). In those WT -> H-2D$^{b-/-}$ x K14DIETER chimeras the H-2D^b knockout is restricted to radioresistant cells, which allows to investigate the role of antigen presentation on radioresistant cells, as depicted schematically in Figure 19a.

As a control, K14DIETER mice were transplanted with the bone marrow of wild-type mice. After reconstitution the host's hematopoietic compartment is replaced by cells from the donor, only radioresistant cells remain from the host. We analyzed MHC-II$^+$ CD11c$^+$ cells in the skin by flow cytometry. In bone marrow chimeric mice, radiosensitive cells like hematopoietic cells (e.g., XCR1$^+$ cDC1) were derived from the wild-type donor compartment (Figure 19b, upper panel), as described before (Henri et al., 2010), and fully functional concerning the repertoire of MHC class I haplotypes, such as H-2D^b (Figure 19b, lower panel). In contrast, the ontogenetically distinct subset of LC (the epidermal Langerin$^+$ DC subset) remains of host origin because they are radioresistant (Merad et al., 2002) (Figure 19b, upper panel), in addition to the radioresistant keratinocytes (CD45$^-$ cells). Therefore, in our H-2D^b bone marrow chimeric model, keratinocytes and LC remain host-derived and cannot

present the GP_{33-41} peptide due to the lack of H-2D^b in these host-derived cells (Figure 19b, lower panel). The Chimeras were utilized to investigate cellular contributions to antigen presentation for T_{RM} recall: In WT -> K14DIETER chimeras (Ctrl), the GP_{33-41} antigen can be presented by radioresistant cells, whereas WT -> H-2D$^{b-/-}$ x K14DIETER chimeras (host H-2D$^{b-/-}$) hold a knockout for the MHC class I haplotype H-2D^b in radioresistant cells. Both chimeras were adoptively transferred with IFNγ-EYFP$^+$ or Nur77-EGFP$^+$ 327TCR$^+$ CD8$^+$ T cells and were infected with rVACV-G2 on both ears one day later. 30 days after the infection, the ear skin of chimeras was treated with 4OHT or left untreated.

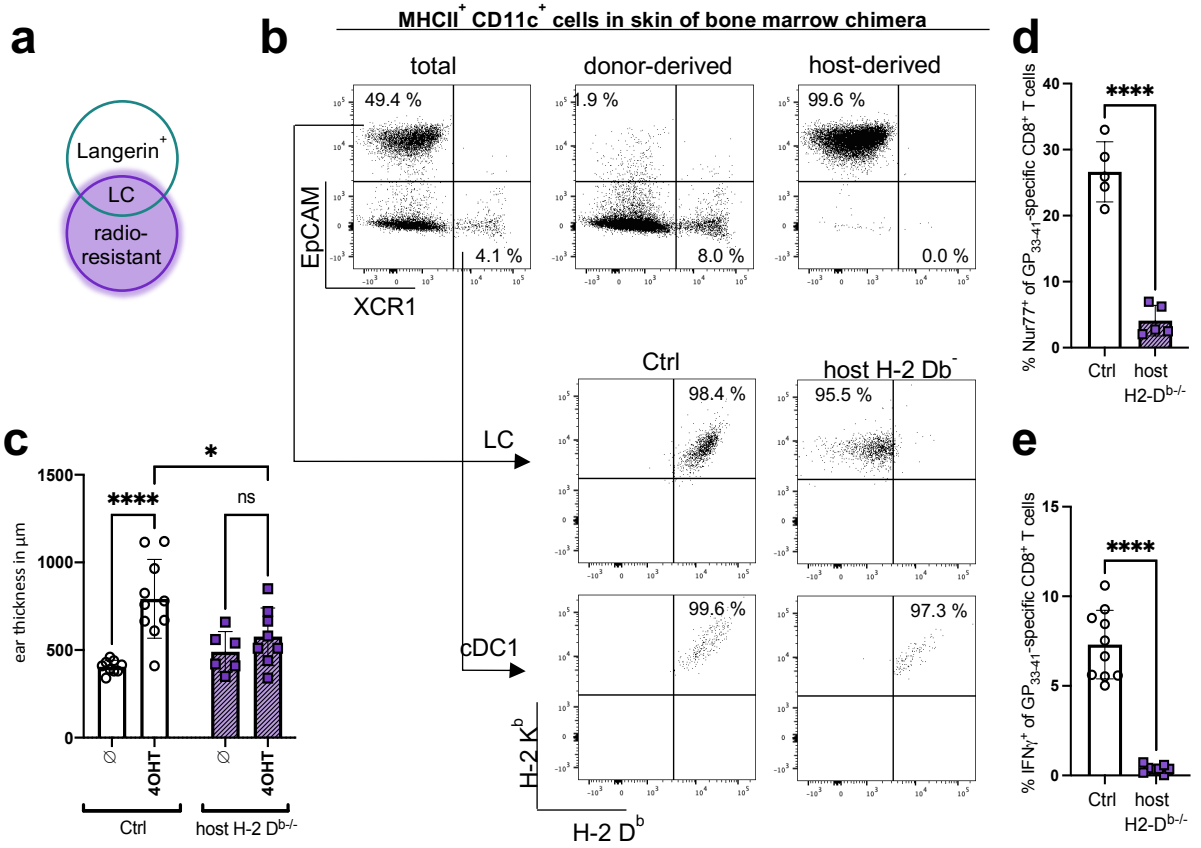

Figure 19. Antigen Presentation by radioresistant Cells is required for Reactivation of T_{RM} in the Skin

*Bone marrow chimeric mice were generated by transplanting lethally irradiated K14DIETER x H-2D$^{b-/-}$ (CD45.2) or K14DIETER (CD45.2) mice with WT (CD45.1) bone marrow. (**a**) The distinctive cellular compartment of the two groups of bone marrow chimera is depicted schematically: After reconstitution in K14DIETER x H-2D$^{b-/-}$ chimera all radioresistant cells (purple) cannot present H-2D^b restricted antigen in contrast to the K14DIETER chimera. (**b upper panel**) DC subsets in ear skin cell suspensions from chimeras were analysed by flow cytometry. MHCII$^+$ CD11c$^+$ cells in the skin were gated based on expression of CD45.1. Representative dot plots show EpCAM$^+$ XCR1$^-$ LC and EpCAM$^-$ XCR1$^+$ cDC1 amongst either total, CD45.1$^+$ (donor derived) or CD45.1$^-$ (host derived) cells. Numbers indicate frequency of cells in the respective quadrant, showing that LC are host derived whereas cDC1 are not. (**b lower panel**). The Expression of MHC class I haplotypes H-2D^b and H-2K^b in EpCAM$^+$ XCR1$^-$ LC and EpCAM$^-$ XCR1$^+$ cDC1 from the ear skin of K14DIETER (Ctrl) and K14DIETER x H-2D$^{b-/-}$ (host H-2D$^{b-/-}$) chimera was investigated by flow cytometry. In the host H-2D$^{b-/-}$ group LC are H-2D$^{b-/-}$, but they express H-2K^b. In contrast the cDC1 express both strain specific haplotypes. (**c,d,e**) Chimeras were adoptively transferred with Nur77-EGFP- or IFNγ-EYFP-TCR327 CD8$^+$ T cells (CD90.1$^+$) and infected on the ears with rVACV-G2. Thirty days after infection antigen expression in keratinocytes of one ear was induced by topical 4OHT treatment (4OHT) or left untreated (Ø). 48h later, ear swelling (**c**), Nur77 expression (**d**) and IFNγ expression (**e**) of reporter-TCR327 CD8$^+$ T (CD90.1$^+$) was recorded. One representative experiment of two with n=5-10 per group is shown. Vertical bars represent mean ± SD. Statistical significance was determined with two-way Anova (ear thickness) or unpaired t-test (flow cytometry) with $*p \leq 0.1$; $**p \leq 0.01$ $***p \leq 0.001$; $****p \leq 0.0001$; ns (not significant).*

Two days after induction of antigen expression in keratinocytes, thickening of the skin tissue was observed in the K14DIETER chimera (Ctrl). In contrast, this ear thickening was absent in the WT -> H-2D$^{b-/-}$ x K14DIETER chimera (host H-2D$^{b-/-}$), indicating fewer cells infiltrating the skin (Figure 19c). To evaluate the recall response after 4OHT treatment the TCR stimulation by Nur77-EGFP expression (Figure 19d) and the IFNγ production by IFNγ-EYFP (Figure 19e) in skin resident memory CD8$^+$ TCR327$^+$ cells were assessed by flow cytometry. Upon induction of antigen expression in keratinocytes, H-2D^b restricted GP$_{33-41}$ specific T$_{RM}$ (327TCR$^+$) responded with Nur77 and IFNγ expression in K14DIETER chimeric mice, but not in K14DIETERx H-2D$^{b-/-}$ chimeras where radioresistant cells lack the MHC class-I molecule required for GP$_{33-41}$ antigen presentation. This result indicates the necessity of antigen presentation on a radioresistant cell population for T$_{RM}$ reactivation in the skin.

Consequently, this experiment reveals that either direct induction of T$_{RM}$ recall by antigen presentation on keratinocytes or cross-presentation of keratinocyte derived antigen by LC may be possible. However, the previous depletion of Langerin$^+$ cells in memory recall suggested that cDC1 or LC cross-present KC-derived antigen for T$_{RM}$ recall. Together with the current finding, that a radioresistant subset is required for antigen-presentation for T$_{RM}$ recall, one crucial cell type for T$_{RM}$ recall in skin became apparent, which expresses Langerin and is radioresistant. Both characteristics are only met by Langerhans cells in the skin. Overall, the results indicate that LC are the critical antigen-presenting cell type for T$_{RM}$ recall in skin, and likely cross-present keratinocyte-derived peptides.

5.2.12 LC cross-present keratinocyte derived Antigen *ex vivo* and mediate TCR-Stimulation of naive antigen specific T cells, whereas Keratinocytes are less potent in activating T cells

To directly address the hypothesis that LC cross-present antigen derived from keratinocytes, LC were isolated and tested in an ex vivo assay for T cell activation. To compare the efficiency of antigen presentation by cross-presenting LC versus direct presentation by keratinocytes using the K14DIETER model, keratinocytes and LC were isolated from 4OHT treated skin of naïve K14DIETER mice. LC, identified as CD45$^+$ MHC-II$^+$ EpCAM$^+$ epidermal cells (Figure 20a), were isolated from 4OHT treated K14DIETER mice and co-cultured in different ratios with purified GP$_{33-41}$ specific CD8$^+$ T cells from naïve Nur77-EGFP x 327TCR mice. After 16 h of Co-culture TCR stimulation of 327TCR CD8$^+$T cells was detected based on Nur77-EGFP expression starting from a DC to T cell ratio of 1 to 1 (Figure 20b). The ability of LC to stimulate antigen specifically the TCR of naïve CD8$^+$ T cells indicates that LC can cross-present keratinocyte-derived antigen to CD8$^+$ T cells. However, isolated keratinocytes can also mediate TCR ligation, reflected by the Nur77-EGFP$^+$ 327TCR$^+$ T cells after in vitro activation, but higher numbers

of keratinocytes compared to LC are necessary. In conclusion, these data show that LC are able to cross-present keratinocyte derived antigen, leading to TCR ligation of antigen-specific T cells *in vitro*.

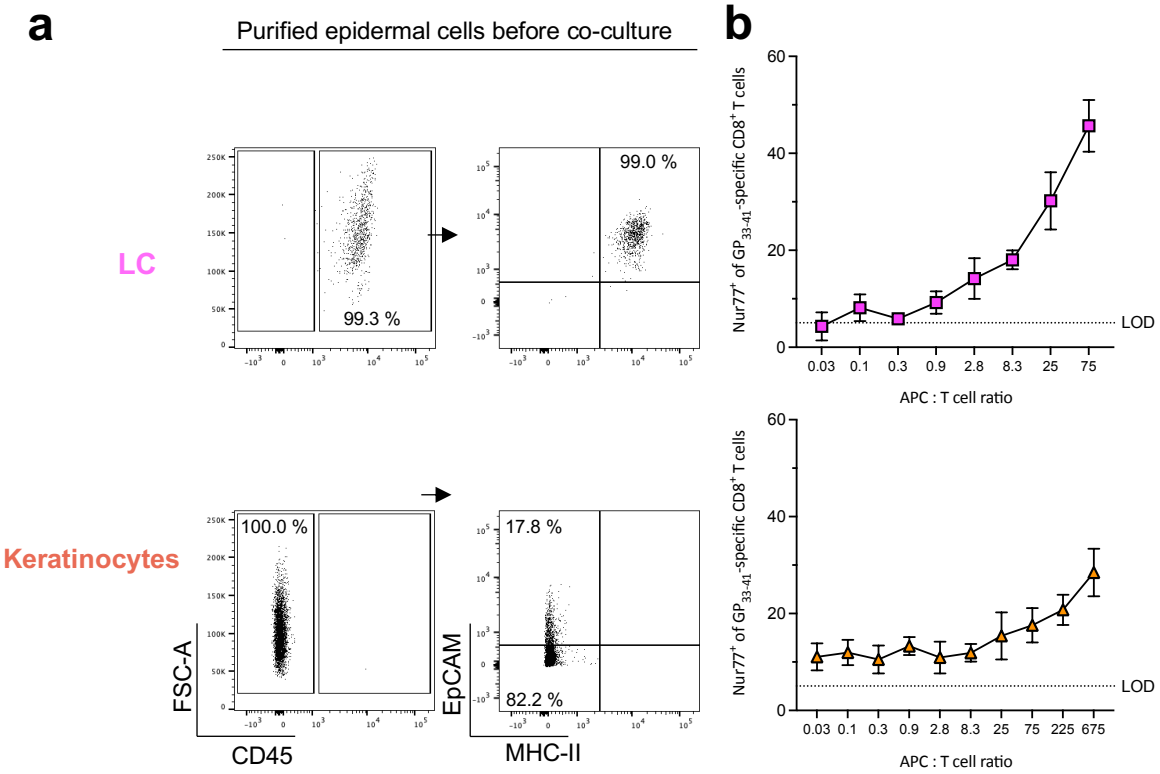

Figure 20. Langerhans Cells cross-present keratinocyte-derived Antigen more efficient than endogenous Antigen presenting Keratinocytes

*Naïve K14DIETER mice were topically treated with 4OHT on the ears daily for three days to induce antigen expression in keratinocytes. One day after the last treatment epidermal explants were prepared from the mouse ear skin with dispase. For the purification of LC: the emigrated LC were collected after 48 h of explant culture, enriched by density gradient centrifugation and then sorted for living CD45⁺ EpCAM⁺ MHC-II⁺ cells. For the isolation of keratinocytes single cell suspensions from the epidermal explants were obtained with collagenase and sorted for living CD45⁻ cells. (**a**) The purified LC fraction (upper panel) expressed homogenously the markers CD45, EpCAM and MHC-II and the purified keratinocyte fraction (lower panel) was CD45⁻ MHC-II⁻ EpCAM$^{negativ\ to\ high}$ as depicted in the dot plots. (**b**) Purified cells (Langerhans cells in pink symbols; keratinocytes in orange symbols) were cultured for 16 hours in serial dilutions with naïve Nur77-EGFPx327TCR CD8⁺ T cells followed by flow cytometric quantification of EGFP expression on CD90.1⁺ CD8⁺ T cells. Detection limit (LOD) was determined using unstimulated CD90.1⁺ Nur77-EGFPx327TCR CD8⁺ T cells. One experiments with n=3-5 is shown. Vertical bars represent mean ± SD.*

5.2.13 LC are required for T_{RM} Recall in Skin

To determine whether exclusively LC fulfil the function of cross-presenting keratinocyte-derived antigen, we depleted only LC during recall and quantified the T_{RM} response to its cognate antigen upon induced antigen expression in keratinocytes. Upon DT treatment of Langerin-DTR mice, Langerin⁺ cells are depleted and repopulate the skin in distinct kinetics. Within a certain timeframe cDC1 have

repopulated the skin, whereas LC are still absent, allowing to study the function of LC *in vivo* (Kissenpfennig et al., 2005; Nagao et al., 2009), schematically depicted in (Figure 21a).

K14DIETER and K14DIETER x Langerin-DTR mice were adoptively transferred with a 1:1 mixture of $CD8^+$ T cells from TCR327 x IFNγ-EYFP ($CD90.1^{+/+}$) and TCR327 x Nur77-EGFP ($CD90.1^{+/}$) and infected on the ears with rVACV-G2. Thirty days later, when skin resident memory was established, LC were depleted in Langerin-DTR x K14DIETER mice through DT injection (d-14/d-12). By DT treatment at 12 and 10 days before 4OHT-induced antigen expression in Keratinocytes of the ears, LC were depleted, but dermal cDC1 had largely recovered. in Langerin-DTR^+ mice (Figure 21b,c). Subsequently, antigen expression by keratinocytes was induced by topical application of 4OHT on the skin of mice or left untreated. 48 h after 4OHT treatment the ears were analyzed regarding their tissue thickness (Figure 21d), Nur77 expression (Figure 21e) and IFNγ production of $327TCR^+$ $CD8^+$ T cells (Figure 21f). In the presence of LC, ears treated with 4OHT were significantly thicker than those depleted of LC. In line with this finding, IFNγ production of skin resident $CD8^+$ memory T cells was induced exclusively when LC were present. These results suggest that LC are required for the functional memory recall in response to keratinocyte expressed antigen. In summary, we found that LC are crucial for the cross-presentation of keratinocyte derived antigens in T_{RM} recall, whereas cDC1 are dispensable in this process. Strikingly, keratinocyte-derived antigen expression in the absence of Langerhans cells results *in vivo* in a lack of TCR ligation, as shown by the absence of $Nur77^+$ $327TCR^+$ T_{RM} in the skin. Suggesting that *in vivo* keratinocytes alone cannot induce TCR ligation, whereas they can do so *in vitro*.

Interestingly, under inflammatory conditions keratinocytes acquire an activated phenotype, e.g. the upregulation of MHC molecules on the cell surface (Fan et al., 2003). In other transgenic mouse models expressing antigen in keratinocytes, keratinocytes were found to act as a primary APC under such inflammatory conditions (Kim et al., 2009). Thus, we question if activated keratinocytes stimulating T cells antigen-specifically is also true for the reactivation of T_{RM} or if cross-presenting LC are nevertheless crucial, independently of the inflammatory status of the tissue, e.g. presence of pathogen-associated molecular pattern (PAMPs). To study whether cross-presentation by LC is independent of the presence of PAMPs, induction of antigen expression in keratinocytes of virus-experienced K14DIETER mice was supplemented with stimulation of PRR at the same area of the skin. This trigger was set by topical application of Imiquimod (IMQ), which is the active ingredient of the nano-emulsion IMI-Sol. After topical application of IMI-Sol on the skin, innate immune activation cascades are triggered by activating the TLR7/MyD88 pathway (Lopez et al., 2017).

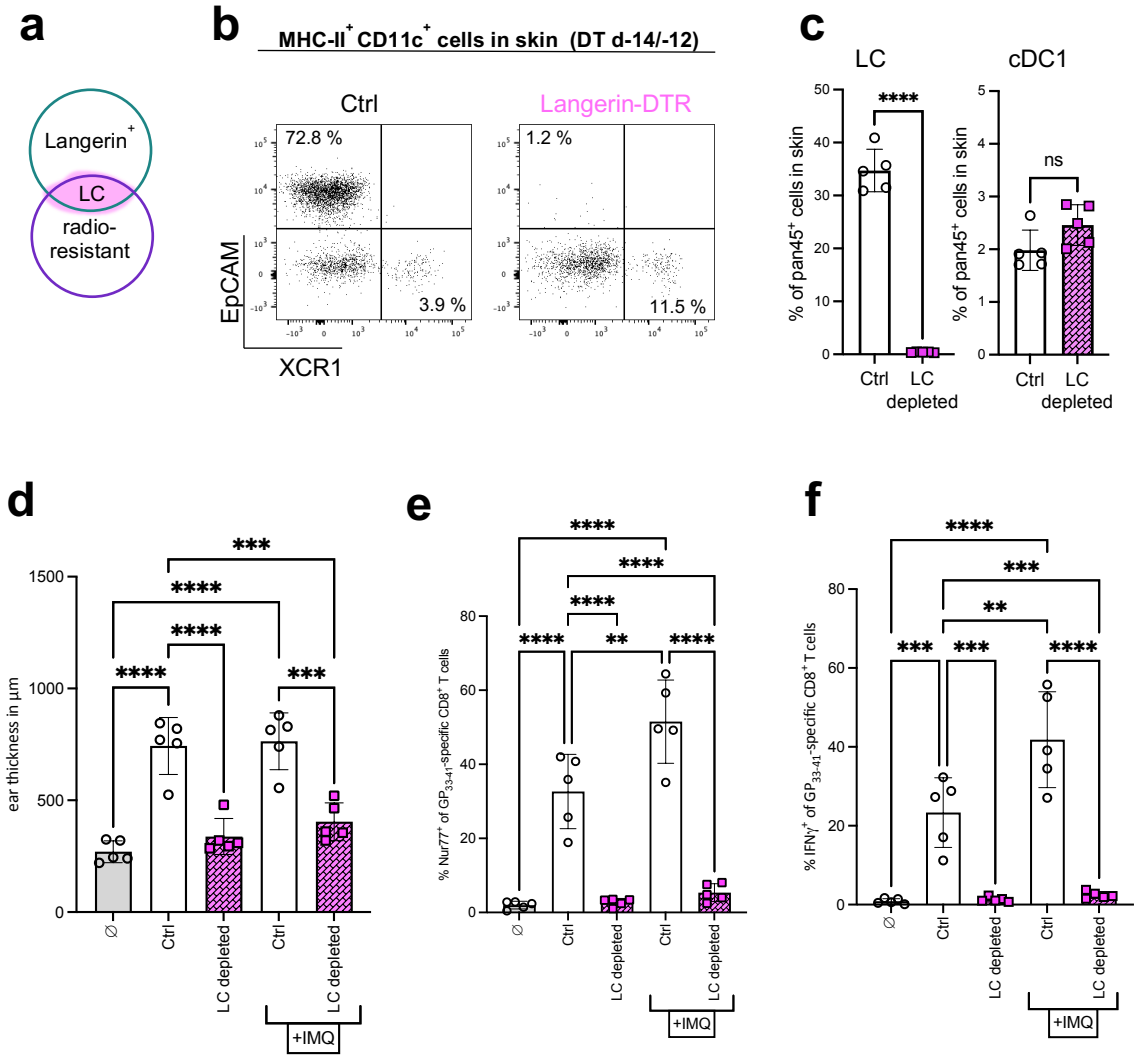

Figure 21. LC are crucial for local Memory Recall in the Skin, independent of innate Immune Activation

*The role of the radioresistant Langerin⁺ cell, i.e. Langerhans cell (LC), in the recall of T_{RM} was investigated (depicted schematically in (**a**). For this purpose, K14DIETERxLangerin-DTR or K14DIETER mice were adoptively transferred with Nur77GFP or IFNγ-EYFP xTCR327 CD8⁺ T cells (CD90.1⁺) and infected on the ears with rVACV-G2. At 30 dpi, mice were injected with DT, depleting Langerin⁺ cells in Langerin-DTRxK14DIETER (Langerin-DTR) but not in K14DIETER (Ctrl). A representative flow cytometry plot of CD11c⁺MHC-II⁺ cells from the ear 14 days after DT treatment shows the absence of EpCAM⁺ LC but repopulation of the skin by XCR1⁺ cDC1 with n=5 (**b,c**). (**d,e,f**) 14 days after DT treatment, ear skin was treated with 4OHT, or 4OHT and Imiquimod (IMQ) or left untreated (Ø). Ear swelling (**d**), Nur77 (**e**) and IFNγ (**f**) expression of reporter-TCR327 CD8⁺ T cells (CD90.1⁺) of ears 24 h after induction of antigen expression in keratinocytes is shown. One representative experiment with n=5 per group is shown. Vertical bars represent mean ± SD. Statistical significance was determined with two-way ANOVA (ear thickness) or unpaired t-test (flow cytometry) with *p ≤ 0.1; **p ≤ 0.01 ***p ≤ 0.001; ****p ≤ 0.0001; ns (not significant).*

To investigate whether the role of LC in the recall of T_{RM} is still crucial even with innate immune activation by IMQ, K14DIETER and K14DIETER x Langerin-DTR mice were adoptively transferred with a 1:1 mixture of CD8⁺ T cells from TCR327 x IFNγ-EYFP (CD90.1$^{+/+}$) and TCR327 x Nur77-EGFP (CD90.1$^{+/-}$) and infected on the ears with rVACV-G2. Thirty days later, when skin resident memory was established, LC were depleted in Langerin-DTR x K14DIETER mice through DT injection (d-14/d-12). Subsequently,

14 days after the first DT treatment, antigen expression by keratinocytes was induced by topical application of 4OHT on the skin of both ears. Two hours after the 4OHT treatment, IMQ was topically applied to one ear's skin. After one day, ear swelling (Figure 21d) and an elevated expression of the Nur77 (Figure 21e), as well as high levels of IFNγ reporter (Figure 21f) were observed in 4OHT treated versus untreated ears in un-depleted mice, reflecting the reactivation of T_{RM}. Furthermore, IMQ treatment enhanced the Nur77 and IFNγ response significantly compared to the 4OHT treatment alone (Figure 21e,f). These signs of recall response were abrogated in LC-depleted mice despite the additional TLR7 stimulus by IMQ. This result clearly indicate that LC are required to reactivate skin T_{RM} in response to an antigen expressed in keratinocytes, even after PRR stimulation.

5.2.14 LC are crucial for T_{RM} mediated Protection against a Virus Re-challenge

Next, we questioned whether protective T_{RM} recall also depends on LC during viral re-challenge of the skin. To investigate the efficiency of T_{RM}-mediated protection with and without LC the viral burden was quantified following vaccinia virus re-challenge. To focus solely on the protective effect of antigen-specific memory CD8 T cells, we generated T_{RM} against GP_{33-41} by transcutaneous vaccination (TCI) in wildtype (WT) and Langerin-DTR mice. For TCI the combinatory approach of Anthralin and Imiquimod was used to induce antigen-specific $CD8^+$ T cells in circulation and within the tissue, i.e. T_{RM} (Sohl et al., 2022). Circulating GP_{33-41}-specific $CD8^+$ T cells were identified in the blood by tetramer staining 28 days after immunization. On average, in wildtype (WT) mice, 1.85 % (SD = 1.03 %) and Langerin-DTR^+ mice, 1.64 % (SD=0.78 %) of $CD8^+$ T cells were GP_{33-41}-tetramer positive. No significant difference was observed in the frequency of tetramer-stained GP_{33-41} specific $CD8^+$ T cells in blood between WT and Langerin-DTR^+ mice (Figure 22a). These immunized mice were then challenged thirty days after immunization (d0) at one ear with a GP_{33-41} expressing recombinant vaccinia virus (rVACV-G2) and at the other ear with another sub-strain (rVACV-OVA), expressing an irrelevant antigen (schematically depicted in Figure 22b). Simultaneous to the virus challenge, LC were depleted by injecting DT (d-14/d-12) in Langerin-DTR^+ mice. Four days after the skin infection (d4), the virus titer was determined in the skin tissue of each ear. In GP_{33-41} immunized WT mice, a lower virus load was observed in the ears, re-challenged with the virus expressing GP_{33-41} peptide (rVACV-G2) compared to the tissue infected with the unknown virus strain (rVACV-OVA) (Figure 22b), indicating that local antigen-specific memory has developed and mediates protection against the virus expressing the GP_{33-41} peptide. Protection was lost when LC were depleted, i.e. DT-treated Langerin-DTR mice, suggesting that LC depletion diminishes the secondary response against a pathogen expressing a known antigen (Figure 22b). In brief, this experiment demonstrates that LC are crucial for the antigen-specific $CD8^+$ T_{RM} recall and its protective effect against viral reinfection of the skin.

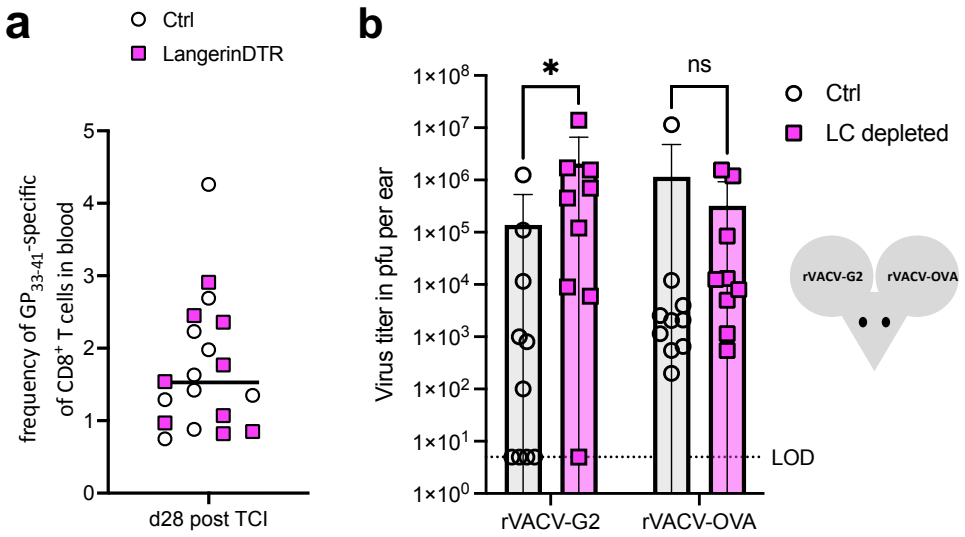

Figure 22. Langerhans Cells mediate antigen-specific Protection against Virus Re-challenge in the Skin

*Wild-type and Langerin-DTR mice were transcutaneously immunised against the GP_{33-41} peptide. 28 days later, the frequency of GP_{33-41} specific $CD8^+$ T cells was determined in the blood by tetramer staining, vertical bar represent mean (**a**). 30 days after infection, the mice were injected with DT to deplete $Langerin^+$ cells in Langerin-DTR^+ (LC depleted) but not in wild-type mice (Ctrl). 14 days after treatment $EpCAM^+$ LC are depleted, whereas $XCR1^+$ cDC1 repopulated the skin. The mice were infected on one ear with rVACV-G2 and at the other with rVACV-OVA. Virus titer was determined four days after infection in the skin tissue, vertical bars represent mean ± SD (**b**). Statistical significance was determined with a non-parametric t-test with $*p \leq 0.1$; $**p \leq 0.01$ $***$, $p \leq 0.001$; $****p \leq 0.0001$; ns (not significant). One experiment with n=9-10 per group is shown.*

6 DISCUSSION

6.1 The Control of cytotoxic T cell Responses in the Skin by CCR2 dependent Phagocytes

6.1.1 Changes in the Phagocyte Network in Skin following Infection

MNP (mononuclear phagocytes) form a dispersed network of migratory and sessile cells with phagocytic activity, which rapidly respond to infection. Within tissues, MNP can persist locally or can be induced upon inflammation, when recruited cells enter the inflamed site from circulation and differentiate into MNP subsets. In skin, blood-born myeloid cells such as CCR2 dependent monocytes differentiate into macrophages or DC-like cells, whereas FLT3 dependent pre-cDC turn into cDC within inflamed tissues (Tamoutounour et al., 2013). While macrophages stay localized, DC can migrate from the skin to dLN upon inflammation to initiate adaptive immune responses (Nakano et al., 2009). Upon vaccinia virus infection of the skin, we characterized early changes within the composition of the cutaneous MNP network based on surface marker expression of single cells using flow cytometry. Our results highlight the role of monocytes and neutrophils as the earliest responder cells upon infection (Fischer et al., 2011; Burn et al., 2021; Ng et al., 2023), reflected by their immediate infiltration in the infected skin. Reactive oxygen and nitrogen species are produced by such inflammatory monocytes and neutrophils to induce damage of cells, DNA and protein in and around viral foci (Roos et al., 2003; Babior, 2004; Halliwell, 2006; Hickman et al., 2013). We observed between 1 to 5 days post-infection that inflammatory monocytes comprise the majority of MNP in the infected skin. Besides their innate effector mechanisms, these inflammatory monocytes, which depend on CCR2, can differentiate on site, migrate into secondary lymphoid organs and might contribute to adaptive immune responses (Domínguez and Ardavín, 2010; Ginhoux and Jung, 2014; Jakubzick et al., 2017).

We characterized the ontogeny of infiltrated MNP in vaccinia virus infected skin using mixed bone marrow chimeric mice. In the early phase of skin infection, we identified three subsets as CCR2 dependent cells: inflammatory monocytes, dermal macrophages and a third subset. Interestingly, this third subset was dependent on CCR2 as well as FLT3, while we were able to assign other clusters either clearly to CCR2 or FLT3 dependency. The proximity of clusters in the UMAP represent similarities among the clusters, implying that the FLT3/CCR2 dependent subsets of cells express a similar set of surface markers like cDC, but also markers of monocytic cells. The hybrid phenotype of the third subset

of CCR2 dependent cells identified in the infected skin is reminiscent of a newly identified cell type, named inflammatory cDC2 (infl. cDC2) (Bosteels et al., 2020a; b). While CCR2 was thought to be required for the infiltration of monocytes into inflamed tissues, also blood-born FLT3-dependent pre-cDC, the precursor of CD11b$^+$ cDC2, require CCR2 for tissue residency in mucosal tissues (Scott et al., 2015; Schlitzer et al., 2015; Nakano et al., 2017) and skin (Tamoutounour et al., 2013). In 2020 the Lambrecht lab discovered among the CD11b$^+$ cDC2 population the infl. cDC2 with migratory properties to the dLN. This subset originates from FLT3 dependent pre-cDC, which are recruited through CCR2 signaling into inflamed tissues, e.g. during viral infection of the lung or infections of the skin by extracellular pathogens (Bosteels et al., 2020a; b). Upon inflammation infl. cDC2 then acquire the expression of the activating Fc receptors CD64 and Mar-1, enabling the internalization of immune complexes to boost MHC-II restricted CD4$^+$ T cell responses, furthermore infl. cDC2 acquire IRF8 expression which allows them to cross-prime CD8$^+$ T cells (Bosteels et al., 2020b). In contrast, CCR2 dependent cells derived from inflammatory monocyte poorly migrate to lung or skin dLN to prime T cell responses (Mesnil et al., 2012; Nakano et al., 2013; Tamoutounour et al., 2013). Although we and others found that inflammatory monocytes make up the biggest part of infiltrating cells in skin during the early phase infection (Hickman et al., 2013). Still the function of such CCR2 dependent cells, be it monocyte derived cells or the infl. cDC2, in the control of the CD8$^+$ T cell response to vaccinia virus infection of the skin, remains unknown. Here we investigated CCR2-dependent cells in their function as APC, as well as surface molecules relevant for the interaction with T cells. The mechanisms through which CCR2-dependent cells can regulate adaptive antiviral responses and the potential site of interaction are elaborated in the following.

6.1.2 Priming and clonal Expansion of CTL

In an acute infection an array of signals is necessary to control the antiviral T cell response in a time dependent manner as well as according to the threat, that is essential to fight infection, restore homeostasis and establish immunological memory.

In general, the antiviral CD8$^+$ T cell response to acute infection is divided into three distinct phases – priming and expansion, contraction and differentiation and subsequently memory. In each phase the response needs to be regulated by the tissue environment as well as by APC, which directly interact with antigen-specifically activated T cells through the presentation of cognate antigen on MHC complexes on their surface (Zammit et al., 2005; Zammit and Lefrançois, 2006; McGill et al., 2008; Ballesteros-Tato et al., 2010) as well as accessory signals, which are co-stimulatory ligand–receptor

interactions (Locksley et al., 2001; Sharpe and Freeman, 2002), as well as soluble ligands such as cytokines (Mescher et al., 2007). The sum of activating and inhibiting signals, through the strength of TCR stimulation and accessory interactions together, dictates the fate of the responding $CD8^+$ T cell and can result in a variety of outcomes for the responding cell: ranging from full activation, to aborted activation, anergy or apoptosis (Mescher et al., 2007; Ream et al., 2010). While TCR stimulation and accessory signals have been studied extensively during naive T cell activation (Locksley et al., 2001; Sharpe and Freeman, 2002), stimuli provided by APC may likewise shape the fate of T cells later in the response when T cells have been primed to effector T cells already. Our results indicate that CCR2 dependent cells play a crucial role for a sustained CTL response. Antigen-presentation, co-stimulation through the receptors CD70, CD80 and CD86 and T cell help through CD40 on CCR2 dependent cells might be important for the interaction with T cells during the time following the acute phase of vaccinia virus infection of the skin.

Upon local intradermal infection of the skin vaccinia virus particles disseminate directly to the dLN and induce initial priming (Lin et al., 2013), other virus infections such as herpes simplex virus infection require migratory DC for the transfer of antigen to the dLN in order to prime naïve T cells (Bedoui et al., 2009). It is known, that for the antiviral $CD8^+$ T cell response to Vaccinia virus infection multiple interactions with both direct and cross-presenting APC in the dLN is required (Norbury et al., 2002; Wiesel et al., 2010; Eickhoff et al., 2015). To what extent migratory DC are required for the antiviral response remains unknown.

Previous reports demonstrated that in general $CD4^+$ help for optimal priming of $CD8^+$ T cells is required, which occurs through the interaction of CD4 and CD8 T cells with a single DC co-presenting MHC-I and MHC-II antigens (Cassell and Forman, 1988; Bennett et al., 1997, 1998), to which T cells are attracted by a chemokine gradients (Castellino et al., 2006). In contrast, upon intradermal vaccinia virus infection it turned out that the initial activation of CD8 and CD4 T cell is segregated by distinct DC in different locations: $CD4^+$ T cells were activated antigen-specifically whilst remaining in the paracortex area of the dLN, whereas the initial activation of virus-specific naive $CD8^+$ T cells occurs through infected cDC in the supcapsular sinus (Hickman et al., 2011; Eickhoff et al., 2015), that brief interaction initiates initial expansion and differentiation (Kaech and Ahmed, 2001; van Stipdonk et al., 2001). Then at a later timepoint (38 hours after infection) antiviral $CD4^+$ and $CD8^+$ T cells cluster around $XCR1^+$ DC, which co-present MHC-I and MHC-II restricted viral antigen, in the peripheral paracortex and cross-prime CTL with the help of $CD4^+$ T cells (Eickhoff et al., 2015; Bennett et al., 1997). Interestingly, the cluster-forming subset of cDC1 was identified as the LN-resident $CD8a^+$ $XCR1^+$ cDC1, since blockage of DC migration or the lack of migratory $CD103^+$ DC in Batf3 knock out mice (Edelson et al., 2010) did not

affected the formation of T cell clusters (Eickhoff et al., 2015). The subset of LN-resident cDC1 cross-presents antigen and transmits $CD4^+$ T cell help to $CD8^+$ T cells via CD40/CD40L interaction, which is required for the optimal stimulation of activated CTL, allowing them to differentiation towards a memory precursor phenotype and to gain the ability to produce IL-2 in memory responses (Feau et al., 2011). The absence of cDC1 resulted in a helpless phenotype of activated $CD8^+$ T cells, induced a shift towards short lived effector cells (SLEC) ($KLRG1^{high}$ $IL\text{-}7R^{low}$) as well as a loss of memory effector precursor cells (MPEC) ($KLRG1^{low}$ $IL\text{-}7R^{high}$) and a reduced IL-2 production of activated $CD8^+$ T cells (Eickhoff et al., 2015). When activated antiviral $CD8^+$ T cells differentiate into effector cytotoxic T lymphocytes (CTL) they undergo simultaneously clonal expansion and acquire the ability to produce IFNγ, TNFα, granzymes and perforin, that allow them to efficiently eliminate infectious particles and infected cells (Kaech and Ahmed, 2001). Subsequently infectious particles are gradually eliminated through the common effort of targeted killing by CTL and innate defense mechanism (Hickman et al., 2013), thereafter it becomes essential to reestablish homeostasis and induce memory formation. Our data demonstrates that sustained proliferation and survival, but not priming, of antiviral $CD8^+$ T cells requires CCR2-dependent cells. Our study sheds light on the stimuli provided by CCR2 dependent cells to antiviral $CD8^+$ T cells in the events following initial priming. In vaccinia virus infected mice lacking antigen-presentation on CCR2 dependent cells the initial priming of antigen-specific CTL response, meaning until 8 dpi, remains unaltered, but the sustained systemic response of antiviral CTL in blood at later timepoints and tissue resident memory generation following skin infection was aberrant. Suggesting that antigen presentation by CCR2 dependent cells is required during the contraction phase of the CTL response and/or memory formation.

6.1.3 Contraction of the CTL Response – Apoptosis or Survival

When virus is cleared and inflammation abates, the response contracts at day 8 after infection (Fischer et al., 2011). The large population of effector T cells differentiates terminally into two subsets with distinct features (Joshi et al., 2007; Obar and Lefranois, 2010). Upon pathogen clearance SLEC become gratuitous and undergo apoptosis, whereas only 5-10 % are MPEC which develop further into memory subsets and can then persist through pro-survival signals (Harty and Badovinac, 2008; Kaech and Cui, 2012). During the phase of limited antigen at the contraction phase of an acute response, most likely effector differentiation occurs (Arens and Schoenberger, 2010). Once the antigen is successfully eliminated, the number of lymphocytes needs to be downregulated to keep them manageable (Boise and Thompson, 1996). Thus, excess cells are removed by apoptosis leading to the contraction of the immune response. Besides antigen-presentation, we identified additional accessory stimuli provided

by CD40, CD70 and CD80/86 on CCR2 dependent cells that control the sustained CTL response. The engagement of CD80 (B7.1) or CD86 (B7.2) on APC and its ligand CD28, that is expressed on T cells, transmits a signal that synergizes with the TCR signal (Lenschow et al., 1996; Lanzavecchia, 1998; Sharpe and Freeman, 2002). Together, the TCR and CD28 signaling in the T cell, promotes survival and proliferation, where TCR- signaling strength correlates with proliferation. Once entered the cell cycle IL-2 promotes survival, during the clonal expansion the activated T cell is sensitive to the induction of cell death, the withdrawal of IL-2 or Fas cross-linking can induce apoptosis (Boise et al., 1995; Van Parijs et al., 1996). Interestingly, the sensitivity of lymphocytes to apoptosis is controlled through the CD28-stimulation by the interacting APC, which augments the production of autocrine IL-2 and induces the expression of the anti-apoptotic proteins Bcl-xL and c-FLIPshort for the protection against activation induced cell death (ACID) (Sperling et al., 1996; Boise and Thompson, 1996; Kirchhoff et al., 2000; Dolfi et al., 2011). Our results indicate that CCR2 dependent cells provide TCR and CD28 signaling to activated T cells during viral skin infection and likely promote through that mechanisms their survival and sustained proliferation. Without the CCR2-dependent cell triggered stimulation of CD27 and simultaneously CD28 on T cells during vaccinia virus infection of the skin, the formation of antiviral central memory $CD8^+$ T cells was largely unaltered, whereas the T_{RM} formation in skin was impaired. We obtained similar results when CCR2 dependent cells lack only CD80/86, but express CD70, indicating that CCR2 dependent cells localized in the skin might induce the required CD28 signaling in $CD8^+$ effector T cells for the establishment of T_{RM} within the skin. Interestingly, we did not observe an impairment on T_{RM} formation in skin, if CCR2 dependent cells lack solely CD70 during viral skin infection, instead the memory subset in spleen was decreased, suggesting that the CD27/CD70 pathway mediated by CCR2 dependent cells might be more important for the development of systemic memory. APC, licensed by $CD4^+$ T cells, promote via CD70/CD27 ligation the secondary expansion and the avoidance of TRAIL-mediated activation-induced cell death (AICD) in $CD8^+$ T cells in case of re-encountering cognate antigen (Feau et al., 2012). The lack of the CD70 ligand, namely CD27, on T cells, attenuates the effector and memory CTL response, but not in all models of viral infection (Feau et al., 2012), our results suggest, that CD70 expression on CCR2-dependent cells might not be important for the formation of local memory T cells specific for skin-tropic virus in skin.

6.1.4 Programming of Memory $CD8^+$ T cells

Programming of memory $CD8^+$ T cells requires the encounter with antigen-presenting DC inducing IL-2 receptor signaling (Williams et al., 2006; Feau et al., 2011), CD40/CD40L co-stimulation (Arens and Schoenberger, 2010) and the activation of the CD70/CD27 pathways (Feau et al., 2012). The fate of

effector CD8$^+$ T cells in becoming either MPEC or SLEC largely depends on a complex differentiation program regulated through the transcription factors Tbet, Runx and Eomesodermin (Cruz-Guilloty et al., 2009). High inflammation or a lack of T cells help promote T-bet expression in effector cells. In turn a sustained high expression of T-bet prevents the transition of effector to the MPEC fate, which then become memory CD8$^+$ T cells (Hamilton et al., 2006; Joshi et al., 2007; Intlekofer et al., 2007). Assuming that helpless CTL differentiate terminally into SLEC with a sustained high Tbet expression, these SLEC can undergo activation-induced cell death (AICD) by Tumour-necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-signaling upon secondary antigen-encounter during contraction (Janssen et al., 2005). Collectively, this suggests, that licensed CCR2 dependent cells by T cell help promote via CD40 the differentiation of interacting effector T cells to become MPEC. This concept would explain our results, where we observed an impaired general formation of memory CD8$^+$ T cells after viral infection in mice lacking CD40 expression in CCR2-dependent cells. Strikingly, this aligns with previous results, which demonstrated, that even when the primary CTL response to acute infection is the same with and without CD4 deficiency, the memory response to reinfection mediated by the memory CD8$^+$ T cell pool is impaired without CD4 T cell help (Sun and Bevan, 2003; Shedlock and Shen, 2003). Importantly, for CTL activation help by an activated CD4$^+$ T cell is mediated through the CD40L/CD40 axes (Schoenberger et al., 1998; Ridge et al., 1998; Bennett et al., 1998), through CD40 ligation APC can be licensed and supersedes direct communication between CD4$^+$ and CD8$^+$ T cells to provide help to CTL (Lanzavecchia, 1998). Moreover, upon CD40 stimulation several activation and maturation mechanisms are induced in APC that regulate the CTL response (Bedoui et al., 2016; Borst et al., 2018). These include: increased antigen-presenting capacity, the upregulation of the expression of the adhesion molecule ICAM-1, the costimulatory ligands CD80, CD86 and CD70 and inflammatory cytokines TNFα, IL-12 or IL-15 (Kennedy et al., 1994; Stout et al., 1996; Cella et al., 1996; Grewal and Flavell, 1996; Koch et al., 1996). The interacting CD8 T cell of a licensed APC then receives stimuli through CD28, CD27 and cytokine receptors, which lead to optimal effector differentiation, proliferation, the upregulation of IFNγ production and the survival of memory CD8$^+$ T cells (Cella et al., 1996; Novy et al., 2007; Ahrends et al., 2017; Borst et al., 2018). Collectively, this leads us to the hypothesis, that CCR2 dependent cells promotes the differentiation and survival of antiviral memory CD8$^+$ T cell mediated through the transmission of co-stimulation and T cell help after skin infection.

6.1.5 Persistence of Memory CD8$^+$ T cell Subsets

Memory CD8$^+$ T cells require pro-survival signals for their persistence (>30 dpi) to establish long-term protection against reinfection, such pro-survival signals include autocrine IL-2 production and

homeostatic cytokines such as interleukin-7 (IL-7) and IL-15 (Schluns et al., 2002; Becker et al., 2002), all of these are required for homeostatic proliferation (Tan et al., 2002; Wherry et al., 2003b). Licensed APC, which are licensed through CD40-signaling by $CD4^+$ T cell help, secrete IL-12 (Obar et al., 2010; Wiesel et al., 2010) as well as IL-15 (Wu et al., 2002; Oh et al., 2008). Both deliver pro-survival signals to interleukin perceiving $CD8^+$ T cells. While IL-15 signaling in CTL can contribute to CD8 memory persistence (Wu et al., 2002; Oh et al., 2008), IL-12 promotes the secondary expansion (Feau et al., 2011; Wolkers et al., 2012). IL-12 receptor stimulation enables autocrine IL-2 secretion in $CD8^+$ T cells, that results in the suppression of the apoptotic pathway TRAIL, the induction of CD25 expression allowing the optimized responsiveness to IL-2 and thus boosts expansion (Obar et al., 2010; Wiesel et al., 2010).

IL-15 act later during the response, and interestingly, the dependence on IL-15 of memory T cells for their survival differs among subsets (Schenkel et al., 2016), while T_{CM} express high levels of CD122 and depend on IL-15 for their long-term survival, T_{EM} do not (Castro et al., 2011; Ahrends et al., 2019). However, we found, that if CCR2-dependent cells were deficient in CD40 expression, after vaccinia virus infection the numbers of persisting antiviral memory $CD8^+$ T cell populations were markedly reduced in all analyzed compartments, which were: the skin, the dLN and the spleen. This suggests that certain memory subsets, namely T_{CM} residing in secondary lymphoid organs and T_{RM} at the previous site of infection, are affected equally. While the formation of T_{CM} might directly depend on IL-15 production by CCR2 dependent cells, the CD40 molecule on CCR2 dependent cells controls the formation of T_{RM} potentially through the induction of the key transcription factor T-bet in $CD8^+$ T cells. Helpless $CD8^+$ T cells, which e.g. had not been in contact with a licensed $CD40^+$ APC, express high levels of T-bet (Intlekofer et al., 2007). A high expression of T-bet leaves $CD8^+$ T cells unresponsive to transforming growth factor-b (TGF-b), and thus prevents the induction of CD103 integrin expression, that is essential for the maintenance of T_{RM} in the tissue (Laidlaw et al., 2014).

Analogous CD40 on CCR2 dependent cells might regulate the formation of T_{RM} in skin by suppressing T-bet in memory precursors after skin-tropic virus infection. It is well established, that during the priming and expansion phase of the $CD8^+$ T cell response the cross-priming $XCR1^+$ cDC1 was identified to be licensed through CD40 ligation and transmits T cell help to $CD8^+$ T cells for optimal and sustained responses (Hor et al., 2015; Eickhoff et al., 2015). However, we found during the contraction phase and later in the antiviral response, that CD40 ligation is required on another APC, a CCR2 dependent subset, for the control of the sustained antiviral CTL response, in particular the formation of the antiviral $CD8^+$ memory T cells.

6.1.6 Cell Migration between skin-draining Lymph Node and Skin

We found that direct interaction of T cells with antigen presenting and co-stimulating CCR2 dependent APC is required for a sustained antiviral response, this interaction can take place at multiple sites, e.g. the skin or the dLN. While the interaction site for CTL priming is clearly assigned to dLN; it remains controversial where interactions of antigen-encountered effector T cells and antigen-bearing APC take place. Since we found that upon vaccinia virus infection CCR2 dependent cells serve as the crucial APC in controlling the antiviral CTL response by antigen-presentation and the provision of accessory stimuli such as CD40 to CTL, during the contraction and memory differentiation phase, we discuss in the following where this interaction of antigen-bearing CCR2-dependent APC and $CD8^+$ T cells might take place. CCR7 expression allows DC in peripheral tissues to move along CCL19 and CCL21 gradients through the afferent lymph into the paracortex of the dLN (Cyster, 1999), where priming of CTL takes place during acute infection. Since we found that CCR2 dependent cells do not depend on CCR7 for their control of the CTL response to viral skin infection, CCR2 dependent cells probably do not migrate from the skin to the dLN through CCR7 signaling. In vaccinia infection the replication competent virus directly inhibits CCR7 signaling, which reduces the CCR7-dependent migration of DC from the infected skin to dLN (Aggio et al., 2021). Whilst skin DC such as infl. cDC2 were found to depend on CCR7 for their migration from the site of infection to the dLN, monocytes can migrate through alternative pathways such as through Ccr5 or CCR2 to the dLN. However, in general CCR2-dependent cells are known for their rapid response to inflammation, those cells get rapidly released from the bone marrow into the blood and get recruited to sites of infection and also draining lymph nodes. All such movements depend on CCR2 (Orozco et al., 2021). A highly mobile subset of inflammatory monocytes in the skin was found to be infected and able to generate infectious virus progeny, in particular from 3-5 days post infection. In line with Hickmann et al, our results show that most infiltrating cells in skin during the early phase infection are inflammatory monocytes, which are attracted by high levels of the monocyte chemoattractant CCL2 (reviewed here (Deshmane et al., 2009). Notably, CCL2 derived from inflamed skin is transported through the lymph to the luminal surface of high endothelial venules (HEV), leading to homing of CCR2 dependent cells such as blood monocytes into the dLN (Weber et al., 1996; Palframan et al., 2001), indicating that CCR2 dependent cells might interact with CTL within the paracortex, where HEV typically traverse the lymph node and blood-born cells transmigrate (Girard et al., 2012). Collectively, either infl. cDC2 are not important in the dLN and other CCR2 dependent cells might be of relevance in the dLN using CCR7 independent ways of migration, or infl. cDC2 use CCR7 independent ways of migration, or in general CTL control takes place outside of secondary lymphoid organs, for example in the skin.

CTL differentiate in the dLN, when they gain cytotoxicity, they simultaneously change homing patterns and migrate to peripheral tissues at about 4dpi (Fischer et al., 2011; Hickman et al., 2013), where the antigen was first captured. Once in the infected skin, these CTL kill infected cells, but every cell presenting viral antigen on an MHC complex are a target for CTL mediated killing, such as viral antigen presenting CCR2-dependent cells. Since our results indicate that these CCR2-dependent cells control the expansion of specific CTL, the feedback loop of APC killing by CTL would limit the response. Interestingly, APC killing was found to limit the response indeed and leads that way to the immunodominance of early expressed viral epitopes (Probst et al., 2003). In particular CCR2 dependent cells might countervail CTL mediated killing because they are produced and mobilzid in very high numbers upon inflammation. This might be the reason why they can promote the sustained CTL response, that our results show in the context of skin infection. Similarly in the context of lung infection, interactions of APC and T cells were described within the infected tissue, APC present viral antigen and provide co-stimulation to CD8 T cells to enhance the response in the lung (McGill et al., 2008; Ballesteros-Tato et al., 2010; Hufford et al., 2010). Additionally, CCR2 dependent cells might also inhibit CTL mediated killing directly by expressing granzyme B inhibitors as described in the lymph node, where DC maturation through CD40 (T cell help) or PRR stimulation was found to prevents CTL mediated killing by the upregulation of the granzyme B inhibitor SPI-6 (Medema et al., 2001; Mueller et al., 2006).

6.1.7 Cytotoxic Effector Function in Skin

At 4 days after vaccinia virus infection of the skin fully activated antiviral $CD8^+$ T cells traffic in an CD62L-dependent manner through the blood stream into the peripheral site of infection (Mohammed et al., 2016; Hickman et al., 2008). Specific $CD8^+$ T cell appear from 5-8 dpi within the infected tissue to eliminate infected monocytes outside viral foci, whereas Ly-$6G^+$ cells control the center of foci, both together achieve the elimination of virus at 15-20 dpi (Fischer et al., 2011; Hickman et al., 2013). The recruitment of effector $CD8^+$ T cells to clear infection is antigen-independent: After immunization against HSV the migration of T_{RM} progenitor cells depends strictly on inflammation and is irrespective of the inflammations cause, be it local HSV infection, which includes reencounter of cognate antigen, or the application of the antigen-independent stimulus by the skin irritant DNFB (Mackay et al., 2012). Fully activated effector $CD8^+$ T cells with the ability to produce IFNγ, TNFα, granzymes and perforin, eliminate infectious particles and infected cells (Kaech and Ahmed, 2001) in skin and after withdrawal of survival signals CTL undergo ACID upon antigen encounter. The key cytokine IFNγ of cytotoxic T cells then programs effector T cell contraction and represses IL-7R expression (Badovinac et al., 2000, 2004),

thus shaping the milieu for memory $CD8^+$ T cell formation. At the end of an acute infection the inflammatory milieu in the formerly infected tissue abates, at that time the inflammation dependent infiltration of CTL into the tissue decreases and subsequently the absence of inflammation favors the formation of MPEC (Joshi et al., 2007), which can then form memory $CD8^+$ T cells *in situ*.

6.1.8 The Formation of Skin resident Memory $CD8^+$ T Cells

Our results show that antigen-presentation and accessory stimuli by CCR2 dependent cells shape the formation of T_{RM} in skin, which requires antigen encounter *in situ* (Khan et al., 2016; Muschaweckh et al., 2016). We observed a massive reduction in the frequency of antiviral T_{RM} in skin, when CCR2 dependent cells lack the capacity to present cognate antigen during skin infection, suggesting that the availability of antigen through CCR2 dependent APC drives the positioning of antiviral T_{RM} at the site of infection. The CCR2 dependent APC, that we identified as crucial APC for the formation of T_{RM}, might be resident in the skin and need to have acquired viral antigen either by being directly infected (direct presentation) or by the engulfment of viral material (cross-presentation) for their function. Either way, the CCR2-dependent APC conveying antigen encounter to $CD8^+$ T cells in the infected skin tissue, is required late during the acute infection, when antigen is low and MPEC have been guided to the former infection site. Moreover, programming of $CD8^+$ memory T cells is facilitated in the context of low inflammation, possibly because inflammatory signals provoke CD8 T cell differentiation toward terminal effector differentiation (Joshi et al., 2007; Pham et al., 2009; Pipkin et al., 2010).

In summary, our data implies that in viral skin infection CCR2 dependent cells shape the local repertoire of persisting memory $CD8^+$ T cells, including T_{RM} in skin, the systemic response and central memory within secondary lymphoid organs. CCR2 dependent cells provide antigen-presentation as well as the required receptors for co-stimulation and the transmission of T cell help in this process. Which concrete subset of CCR2 dependent cells bears viral antigen and controls the response *in situ* in an antigen-specific manner requires further research. In the early phase of vaccinia virus infection, we identified three CCR2 dependent subsets in the infected skin: inflammatory monocytes, dermal macrophages and a population of monocyte derived cells and/or inflammatory cDC2. Further research is necessary to determine, whether pre-cDC derived infl. cDC2 (Bosteels et al., 2020b; a) or monocyte derived cells can function as the APC, which we identified as required for a sustained response in the context of viral infection of the skin. We think, in a sustained antiviral response, co-stimulation and T cell help transmission by CCR2 dependent cells suppresses the activation induced cell death (ACID),

provides pro-survival signals to memory precursors and establish memory $CD8^+$ T cells in an antigen-specific manner.

6.2 Cellular Requirements for the Recall of CD8$^+$ Memory T cells in the murine Skin

After infection with skin tropic virus, such as herpes simplex virus (HSV), vaccinia virus (VACV), molluscum contagiosum virus (MCV), and human papilloma virus (HPV), the protein production machinery of the infected keratinocytes is hijacked by the virus for replication, keratinocytes are the predominant cell type expressing viral antigen (Lei et al., 2020). CD8$^+$ T_{RM} are lodged in the epidermis surrounded by keratinocytes, thus infected keratinocytes might be the first site where antigen becomes visible to memory T cells. After reinfection with the same virus, the recall of T_{RM} is crucial to fight reinfection *in situ*. We generated transgenic mice to experimentally phenocopy this reoccurrence of viral antigen in keratinocytes. This minimalistic model, in comparison to other constitutive expression models, is locally restricted and controlled by topical application, thus being unbiased from any other mechanical or innate cell activating triggers apart from cognate stimulation. At the same time, the *in vivo* model allows the investigation under physiological conditions and to trace antigen-specific memory responses following local T_{RM} reactivation. Using this model, we show that for the recall of antigen-specific CD8$^+$ T_{RM} in skin cognate stimulation is sufficient and that LC are required to cross-present keratinocyte derived antigen and mediate that way protection against reinfection.

6.2.1 Keratinocytes as direct-presenting APC

The discussion around, which APC can activate T cells responding to keratinocyte derived antigen, is controversial. Most studies searching for the identity of the required APC presenting keratinocyte-derived antigen focus on the ability to prime T cells by using adoptive transfer of naïve T cells into mice expressing antigen in keratinocytes. In an *in vivo* study, mice expressing constitutively Ovalbumin (OVA) antigen in keratinocytes were intradermally injected with T cells, specific for the MHC-I restricted OVA (OT-I). They develop a skin phenotype similar to graft versus host disease (GvHD), suggesting keratinocytes can prime directly naïve T cells. In contrast, the same group found that mice which were i.v. injected with antigen-specific OTI T cells do not develop the disease (Shibaki et al., 2004; Kim et al., 2009). The data on human cells from *in vitro* studies is similarly controversial: co-cultured keratinocytes co-stimulate naïve human T cells (Orlik et al., 2020), but in another study, antigen presentation by keratinocytes induce tolerance in T cells (Bal et al., 1990). When integrating the previous studies into *in vivo* studies on priming of naïve T cells, it became apparent that studies based on the hypothesis of naïve T cells encountering keratinocytes *in vivo* require to be very critically reviewed because under physiological conditions, naïve T cells, which circulate in blood and secondary lymphoid organs, will not encounter an APC resident in skin, such as the keratinocyte (von Andrian and

Mackay, 2000). This reinforces the well-established concept that naive cognate T cells need to be activated by interactions with the skin-antigen loaded APC in the lymph node. Our *in vitro* data show when viral peptide expression was induced in keratinocytes, purified keratinocytes present antigen and can activate antigen-specific naïve T cells *in vitro*. In contrast, *in vivo* we found that keratinocytes cannot induce TCR signaling in T_{RM}. Instead, T_{RM} recall in skin requires LC to cross-present keratinocyte-derived antigen.

6.2.2 Why can cross-presenting LC induce the Recall of T_{RM}, but not Keratinocytes?

Interestingly, we found when keratinocytes express antigen, they themselves, but also cross-presenting adjacent LC, can induce TCR stimulation in cognate T cells *in vitro*. Still, lower numbers of LC were found to be required for the activation of naïve T cells, indicating that the reasons for the enhanced T cell activation capacity of LC potentially lies in cell intrinsic specializations of LC supporting the interaction with T_{RM}. This feature might allow LC to activate T_{RM} *in vivo*. So far, we did not delineate, the specialization of LC allowing the optimized interaction with T cells, which keratinocytes lack. Beneficial specializations could include: a high number of antigens on MHC complexes on the cells surface, the expression of adhesion molecules, costimulatory molecules, innate cytokines or attracting chemokines. Moreover, $CD8^+$ T_{RM} cells are highly mobile (Ariotti et al., 2012) and crawling $CD8^+$ T_{RM} cells frequently interact with LC cells after resolved HSV skin infection (Zaid et al., 2014). Similarly, after resolved vaccinia virus, we observed close contact between the cell bodies and dendrites of LC with most T_{RM} cells in the epidermis (Kamenjarin et al., 2023). However, in the study of Zaid et al, the depletion of LC cells reduced the crawling velocity of T_{RM} within the epidermis (Zaid et al., 2014), suggesting a motility-governing function of LC, which could be mediated through a CCL20 chemokine gradient recruiting T cells via CCR6 to LC (Dieu-Nosjean et al., 2000; Schutyser et al., 2003). Moreover the expression of adhesion molecules such as ICAM-1 or VCAM-1 could support the formation of an immunological synapse (Sims and Dustin, 2002), or co-stimulatory molecules could enhance the efficiency of T cell activation as demonstrated on human LC expressing CD70 (Polak et al., 2012). All of such may play a role in T_{RM} recall, providing a superior function of LC compared to keratinocytes in antigen presentation to specific memory T cells in the skin.

6.2.3 Cross-presentation as a Mechanism to overcome viral Immune Escape

Another aspect, why cross presenting LC are superior in T cell activation in comparison to keratinocytes, is that the complete dependence on direct-presentation of antigen by infected cells is

a dangerous venture due to the direct effect of viral immune escape mechanism on infected cells. Classically, endogenous peptides are presented on MHC I molecules of the same cell which produced those peptides. When keratinocytes are infected, they produce viral antigen and present the endogenous antigen directly on MHC-I complexes on their surface. Apart from that way of presentation, the same keratinocyte-derived antigen can become engulfed by phagocytic cells and subsequently exogenous antigen can access the MHC-I–restricted pathway to induce antigen-specific $CD8^+$ T cell responses; this process is called 'cross-presentation' and was first described already about 50 years ago (Bevan, 1976). Cross-presentation is a process restricted to specialized dendritic cells (DC), which are proficient in coordinating immune responses. Thus, as part of a viral immune evasion strategy some viruses do not directly infect DC. Skin-tropic viruses apply immune escape mechanisms encoded in their DNA or RNA to affect infected cells that way, that the induction of anti-viral immune responses is hindered. The cross-presentation pathway is crucial for countervailing such mechanisms by processing extracellular antigen into the MHC-I pathway and make the antigen visible to T cells on MHC class I molecules on the surface of cross-presenting DC. This detour of exogenous antigen into the endogenous antigen pathway by cross-presenting cells has significant implications not only in safeguarding against primary viral infections.

In primary immune responses, both $Langerin^+$ pAPC of the skin, namely LC and cDC1, can migrate to the draining lymph nodes, where antigen-loaded APC encounter naïve T cells and priming takes place. If an APC cross-presents extracellular antigen to prime naïve cytotoxic T cells, this particular process is named cross-priming. Which of the migratory $Langerin^+$ DC subsets in skin carries out cross-priming was investigated in several studies. Early *in vivo* studies investigating $Langerin^+$ cross-priming cells relied on murine Langerin-DTR mice or antigen delivery to DC with antibody-antigen conjugates that cannot distinguish between $Langerin^+$ dDC and LC (Idoyaga et al., 2008; Stoitzner et al., 2006). Cross-presentation of keratinocyte derived OVA inducing OT-I $CD8^+$ T cell proliferation was found to be conducted by both migratory LC and dermal DC (Azukizawa et al., 2003; Waithman et al., 2007). Notably, those studies used the Langerin-DTR/DT system while being unaware of the existence of cDC1, which express Langerin besides LC, implying that the cross-priming capability might have been falsely contributed to LC. Evidence arose that LC cross-present antigen in skin but loose the ability to cross-present after emigration to the lymph node (Stoitzner et al., 2006). In contrast, $Langerin^+$ dDC, isolated from lymph nodes of HSV infected mice efficiently cross-present viral peptides at the site where priming of naïve T cells occurs (Bedoui et al., 2009). The question whether LC are capable of cross-priming *in vivo* (Romani et al., 2012), finally found an answer in an *in vivo* system depleted of $Langerin^+$ cells, where advantage of the distinct repopulation kinetics of $Langerin^+$ dDC and LC was taken to assess the specific role of LC (Bursch et al., 2007; Poulin et al., 2007; Wang et al., 2008). This

approach allowed to identify the Langerin$^+$ CD103$^+$ dDC (cDC1) as the migratory cell responsible for cross-priming and the exclusion of a role of LC in this context (Henri et al., 2010). These findings align with studies documenting the critical role of cDC1 as well as the dispensability of LC in the initiation of CD8$^+$ T cell antiviral immunity after skin infection by cytopathic viruses such as HSV-1 (Allan et al., 2003) and vaccinia (Seneschal et al., 2014). Interestingly, the migration of LC to the draining LN takes about 4 days, whereas cDC1 start migration immediately after skin irritation and peaks two days later (Kissenpfennig et al., 2005; Shklovskaya et al., 2008; Tomura et al., 2014), this delay of LC migration is probably due to multiple steps for the emigration of LC from the epidermis to the dLN, such as detachment from neighboring keratinocytes and crossing the epidermal basement membrane by proteolysis (Gunn et al., 1999; Kabashima et al., 2007). The delayed arrival of LC might be one reason why other DC are more relevant for cross-priming in the draining lymph node.

However, in a setting of restricted antigen availability Langerhans cells were found to be required for the initiation of immune responses, such as in contact hypersensitivity (CHS), which is a model of allergic contact dermatitis. High concentration of hapten increases dissemination into the dermis, then the response was dependent on cutaneous cDC1 (Nagao et al., 2009; Noordegraaf et al., 2010; Clausen and Stoitzner, 2015), indicating that LC are in particular required in situations, when antigen is restricted to the epidermis. Furthermore, the form of antigen dictates the ability to activate T cells by distinct DC subsets. For example, soluble antigen was found to be more efficiently cross-presented by Langerin$^+$ dermal DC, whereas LC favor the cross-presentation of encapsulated antigen (Zaric et al., 2013, 2015). Upon intradermal immunization with antigen conjugated nanoparticles, LC are required for a sufficient number of IFNγ^+ antigen specific CTL as well as in secondary immune responses (Zaric et al., 2013), such as in the induction of antiviral and anti-tumor memory responses (Zaric et al., 2015). These results imply, that LC might be crucial for tissue immunity by interacting with T cells *in situ*. This view is supported by our data and the findings of others in skin showing clearly that *ex vivo* LC from epidermal explants of mice, expressing constitutively antigen in keratinocytes, can cross-present antigen *in vitro* (Stoitzner et al., 2006; Bursch et al., 2009). Moreover, LC expressing an alloantigen are required to license infiltrating epidermal CD8$^+$ T cells to produce effector molecules such as IFNγ, indicating an important role of LC in interacting with T cells within the epidermis (Bennett et al., 2011). Similarly, the depletion of host LC before transplantation of donor alloreactive T cells were found to prevent skin GvHD (Merad et al., 2004), and using three independent GvHD models, the pathogenicity of T cells in the skin was found to be triggered by interactions with LC *in situ* (Santos e Sousa et al., 2018). Collectively, LC were found to induce T cells responses within the epidermis in skin conditions concentrated to the epidermis. We finally unraveled a novel setting in which cross-presentation on LC is required: the distinct role of LC in cross-presenting keratinocyte derived antigen in the epidermis as

the making or breaking point for the local memory recall in skin, which was demonstrated in our *in vivo* and *in vitro* experiments. Neither the keratinocytes in the epidermis nor Langerin$^+$ dermal DC can replace the engagement of LC for early local memory recall in the epidermis. Our results also demonstrate the requirement for LC in recalling T_{RM} during the replicative phase of viral particles in the skin tissue, which implies that the protective function of T_{RM} in the skin requires the initial engagement of LC, which is most crucial in the defense re-occurring skin-tropic virus infection.

6.2.4 Cross-presenting Tissue macrophages in other Tissues

Ontogenetically related cells to LC, such as tissue macrophages (e.g. liver resident Kupffer cells, microglia in the brain) (Ginhoux et al., 2010; Hoeffel et al., 2012; Epelman et al., 2014), potentially fulfil the task of cross-presentation for early T_{RM} recall in other tissues. Non-circulation T_{RM} have been identified across barrier tissues, like the skin (Gebhardt et al., 2009; Wakim et al., 2008), intestine (Masopust et al., 2010), lung (Wakim et al., 2013), female reproductive tract (Mackay et al., 2012; Schenkel et al., 2014b) and liver (Fernandez-Ruiz et al., 2019) and share a common transcriptional program (Mackay et al., 2015, 2016; Milner et al., 2017). Depending on their tissue of residency, T_{RM} were found to be considerably heterogeneous in phenotype and function. This heterogeneity results in distinct responsiveness to certain stimuli and affects the longevity and plasticity of T_{RM} (Yang and Kallies, 2021). In skin, T_{RM} might represent the stereotype of the long-lived, stable, bona fide CD103$^+$ CD69$^+$ T_{RM} phenotype, in contrast to the more variable pool of T_{RM} resident in other tissues. The number of T_{RM} resident in skin is stable for at least 60 days post-infection, in contrast, T_{RM} within the liver form less stable reservoirs because they are not fully committed to their homing tissue compared to skin resident T_{RM} (Christo et al., 2021). Intestinal T_{RM} were found to have either limited recall potential (CD103$^+$) or to be central memory-like T cells located within the intestine (CD103$^-$) (Fung et al., 2022). Notably, CD103$^+$ T_{RM} of the lung are short-lived and are quickly supplemented from circulating effector memory T cells after antigen re-exposure (Van Braeckel-Budimir et al., 2018). This is likely due to the high vascularization of the lung, which provides easy access for circulating effector memory T cells into the epithelial tissues: In turn the discrimination between resident memory T cells and circulating T cells might be a challenging task within the lung tissue even early after recall.

In 2020 the Keach lab used a viral re-infection model to study the effect of encountering antigen on different antigen-presenting cells for T_{RM} recall within the lung and found that CD169$^+$ tissue macrophages are required for the recall of lung resident memory T cells (Low et al., 2020), furthermore, alveolar macrophages were found to be the source of persistent antigen after

immunization and are thus required for enhanced longevity of specific $CD8^+$ lung T_{RM} (Lobby et al., 2022). Surprisingly, the function of tissue macrophages for the recall of T_{RM} within the lung was found to be redundant: Various lung-resident cell populations, including non-hematopoietic and hematopoietic cells, have been found to reactivate $CD8^+$ T_{RM} (Low et al., 2020). Interestingly, the type of cell delivering the cognate stimulus governs the functional output, reflected in the transcriptional differences of recalled lung resident T cells (Low et al., 2020). How tissue-specific factors such as the engaging antigen-presenting cells shape T_{RM} cell responses is largely unknown, but how this affects the T_{RM} fate is a key question in the current research on T_{RM}. We have conclusively demonstrated the critical and protective function of LC-mediated cross-presentation for the recall of $CD8^+$ T cells in both conditions, when using our very controlled inducible recall system or in case of a reinfection, which includes tissue injury and exposure to PAMPs. This suggests, that the dependence of the recall of T_{RM} in skin on LC is unaltered even when a pathogen triggers innate immune activation, which is the case during reinfection.

6.2.5 LC in Human

Human LC are homologous cells to murine LC: In both species, LC express common gene signatures, such as the expression of MHC class I related genes at a high-level (Carpentier et al., 2016) and expression of the surface molecule Langerin (CD207) (Merad et al., 2008). Furthermore, human LC were found to cross-present antigen (Klechevsky et al., 2008; Seneschal et al., 2012; Polak et al., 2012), indicating that LC might full fill the same cross-presenting role for skin resident $CD8^+$ memory recall in human, as we observed in mice. In mice, we found LC cross-presenting keratinocyte-derived peptide for the functional and protective local recall of T_{RM} in skin against local viral re-infection. The early reactivation of skin-resident T_{RM} induces the tissue-wide expression of antiviral genes through IFNγ signaling and prompts the recruitment and activation of other immune cells for an efficient secondary immune response.

6.2.6 Human pathogenic Viruses

Analogous to our experimental system, which allows the inducible expression of a viral antigen in keratinocytes, papilloma virus infection of the skin might have similar implications, while inducing the expression of several instead of one viral peptide within the host-cell. Papillomavirus are non-lytic viruses infecting mitotically active basal keratinocytes. Viral gene expression is minimal (circa 50-100 copies per cell) till the infected cell enters the differentiated layer of the epidermis. Viral replication

occurs in nondividing keratinocytes, the virus reactivates the cellular DNA synthesis machinery of those cells, this subsequently leads to abnormal proliferation and differentiation, resulting in the formation of warts becoming visible at the skin surface (Doorbar, 2005; Pyeon et al., 2009). Genes encoded by DNA viruses, such as the early genes E5 and E7 of HPV or mechanism of poxviruses, directly interfere with antigen processing and MHC expression of infected cells and thus have the capacity to perturbate antigen recognition (Bal et al., 1990; Maudsley, 1991; Woodworth and Simpson, 1993; McFadden and Kane, 1994; Ehrlich, 1997), providing a selective advantage to the virus. Immune evasion strategies of viruses act on the infected cell, thus uninfected cells cross-presenting viral antigen is a mechanism to overcome escape mechanisms caused by viral components within infected cells such as blockage of cytosolic PRR recognition, Type I interferons and antigen presentation. As an example, blockage of sensing the virus through cytosolic pattern recognition receptors (PRR) is employed by large DNA viruses such as papilloma viruses, poxviruses and herpesviruses (García-Sastre, 2017; Parekh et al., 2019; Zhao et al., 2021; Hong and Laimins, 2017). The endosomal PRR TLR7 can be stimulated experimentally using the TLR7-agonist Imiquimod (IMQ). Interestingly, in clinical trials topical IMQ, was efficient for the treatment of HPV-induced warts (Edwards et al., 1998). TLR7 Stimulation by topical imiquimod on the skin leads to an activated phenotype of LC associated with an enlargement of the cell and the formation of more dendrites (Suzuki et al., 2000). Furthermore, TLR stimulation enhances cross-presentation of antigen to $CD8^+$ T cells (Oh et al., 2011; Nair-Gupta et al., 2014). In line with this view is our observation of an elevated IFNγ response of recalled T_{RM} upon topical IMQ treatment, likely due to an TLR7 mediated enhanced cross-presentation by LC. Then the early IFNγ production by T_{RM}, activated by a cross-presenting uninfected LC, can efficiently overcome viral immune escape strategies.

6.2.7 Human Skin Diseases

While T_{RM} recall is pivotal for anti-viral defense, memory activation can also cause the relapse of skin diseases, when pathogenic T_{RM} reside in the skin. This is particularly evident in patchy re-occurring skin diseases, where inflamed lesions tend to resurface in specific areas of the skin, even after initially successful therapy. This recurring pattern has been observed in various skin conditions such as fixed drug eruption (FDE), vitiligo and psoriasis (Nicolaidou et al., 2007; Masson Regnault et al., 2017; Carbone, 2020). The consistent reappearance of disease-related lesions in formerly affected areas of skin implies that the recall of skin resident T_{RM} in these sites can trigger flare-ups. Indeed, studies in mice and the analysis of biopsies of patients indicate that pathogenic $CD8^+$ T_{RM} in resting lesions within the epidermis drives perseverative relapses in the context of the FDE (Mizukawa et al., 2008), vitiligo

and psoriasis (Boyman et al., 2004; Cheuk et al., 2014; Boniface et al., 2018). The intervening therapy for such diseases focuses on suppressing immune responses to reduce inflammation, e.g. by biologicals (such as anti-TNFα or anti-IL-23) or narrow-band UVB (NB-UVB) radiation. UVB radiation of the skin resulting in immune suppression was found to be mediated through LC: UVB leads to a reduction of LC density (Taguchi et al., 2013), the remaining LC induce skin resident T_{reg} suppressing CHS responses (Schwarz et al., 2010) and $CD8^+$ T cells were found to express less IFNγ (Sigmundsdottir et al., 2005), suggesting that such phenomena could be linked through the lack of antigenic stimulation of memory $CD8^+$ T cells upstream. Prevention of the recall of pathogenic T_{RM} located in skin lesions and flare-ups might be inhibited by UVB radiation due to the lack of LC and thus align with our findings about antigen cross-presenting LC for T_{RM} recall. Collectively, our findings reveal a novel target to treat local relapse of skin diseases driven by pathogenic skin resident memory T cells: the cross-presenting LC.

7 APPENDICES

7.1 Abbreviations

%	percent or frequency
°C	Degrees Celsius
+/+	Homozygotous knockin
+/-	Heterozygotous knockout/knockin
-/-	Homozygotous knockout
4OHT	4-hydroxy-tamoxifen
7-AAD	7-actinomycin D
APC	Antigenpresenting cell or Allophycocyanin
BM	Bone marrow
BMC	Bone marrow chimera
BUV	Brilliant Ultra Violet
BV	Brilliant Violet
CCL	C-C chemokine ligand
CCR	C-C chemokine receptor
CD	Cluster of differentiation
cDC	Conventional Dendritic cell
CHS	Contact hypersensitivity
CTL	Cytotoxic T Lymphocyte
Ctrl	Control
Cy	Cyanin
DC	Dendritic cell
dLN	Draining lymph node
DNA	Desoxyribonucleic acid
DNAse	Desoxyribonuclease
dpi	Days post infection
DT	Diphtheriatoxin
DTR	Diphtheriatoxin receptor
EDTA	Ethylendiamintetraacet
EGFP	Enhanced Green flourescent Protein
EYFP	Enhanced Yellow flourescent Protein

FACS	Fluorescence activated cell sorter or sorting
FCS	Fetal calf serum
FDE	Fixed drug eruption
FITC	Flourescein isothiocyanat
FlowSOM	Flow Self-Organizing Maps
FLT3	Fms related receptor tyrosin kinase 3
FSC	Forward scatter
fwd	Forward
G2	Glycoprotein 2
GvHD	Graft versus host disease
h	Hours
HSC	Hematopoietic stem cell
HPV	Human papilloma virus
HSV	Herpes simplex virus
IFNγ	Interferon gamma
IMQ	Imiquimod
Infl.	inflammatory
ILC	Innate lymphoid cell
i.p.	Intraperitoneal
i.v.	intravenous
K14	Cytokeratin14
KO	knockout
LC	Langerhans cell
µg	Microgramm
mBMC	mixed bone marrow chimera
min	Minutes
mL	Milliliter
moC	monocyte derived Cells
MNP	Mononuclear Phagocytes
µL	Microliter
NET	Neutrophil extracellular traps
NK cell	Natural killer cell
nm	nano meter
OVA	Ovalbumin
P	professional

PAMP	Pathogen Associated Molecular Pattern
PB	Pacific Blue flourochrome
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pDC	Plasmacytoid Dendritic Cell
PE	Phycoerythrin
PerCP	Peridinin chlorophyll-A protein
Pop	Population
PRR	Pattern Recognition Receptor
PSI	Pounds per square inch
r	recombinant
rcf	Relative centrifugal force
rev	Reverse
s	Seconds
S1P	Sphingosine-phosphate-1
S1PR1	Sphingosine-phosphate-1 receptor 1
SD	Standard deviation
SSC	Sideward scatter
TCI	Transcutaneous Immunisation
T_{CM}	Central Memory T cell
TCR	T cell receptor
T_{EM}	Effector Memory T cell
tet	Tetramer
TLR	Toll-like receptor
TNFα	Tumor Necrosis Factor alpha
T_{RM}	Tissue resident Memory T cell
U	Units
UMAP	Uniform Manifold Approximation and Projection
UV	Ultraviolett
V	Volt
VACV	Vaccinia Virus
w/w	weight per weight
WT	wildtype

7.2 Bibliography

Abbas Abul K., A.H. Lichtman;, and S. Pillai. 2014. Cellular and Molecular Immunology. 8th edition. 544 pp.

Aggio, J.B., V. Krmeská, B.J. Ferguson, P.F. Wowk, and A.G. Rothfuchs. 2021. Vaccinia Virus Infection Inhibits Skin Dendritic Cell Migration to the Draining Lymph Node. *The Journal of Immunology*. 206:776–784. doi:10.4049/jimmunol.2000928.

Ahrends, T., J. Busselaar, T.M. Severson, N. Bąbała, E. de Vries, A. Bovens, L. Wessels, F. van Leeuwen, and J. Borst. 2019. CD4+ T cell help creates memory CD8+ T cells with innate and help-independent recall capacities. *Nat Commun*. 10:5531. doi:10.1038/s41467-019-13438-1.

Ahrends, T., A. Spanjaard, B. Pilzecker, N. Bąbała, A. Bovens, Y. Xiao, H. Jacobs, and J. Borst. 2017. CD4+ T Cell Help Confers a Cytotoxic T Cell Effector Program Including Coinhibitory Receptor Downregulation and Increased Tissue Invasiveness. *Immunity*. 47:848-861.e5. doi:10.1016/j.immuni.2017.10.009.

Albanesi, C., C. Scarponi, S. Sebastiani, A. Cavani, M. Federici, S. Sozzani, and G. Girolomoni. 2001. A cytokine-to-chemokine axis between T lymphocytes and keratinocytes can favor Th1 cell accumulation in chronic inflammatory skin diseases. *Journal of leukocyte biology*. 70:617–623.

Aldridge, J.R., C.E. Moseley, D.A. Boltz, N.J. Negovetich, C. Reynolds, J. Franks, S.A. Brown, P.C. Doherty, R.G. Webster, and P.G. Thomas. 2009. TNF/iNOS-producing dendritic cells are the necessary evil of lethal influenza virus infection. *Proceedings of the National Academy of Sciences of the United States of America*. 106:5306–5311. doi:10.1073/pnas.0900655106.

Allan, R.S., C.M. Smith, G.T. Belz, A.L. van Lint, L.M. Wakim, W.R. Heath, and F.R. Carbone. 2003. Epidermal viral immunity induced by CD8alpha+ dendritic cells but not by Langerhans cells. *Science*. 301:1925–1928. doi:10.1126/science.1087576.

Alvarez, D., E.H. Vollmann, and U.H. von Andrian. 2008. Mechanisms and Consequences of Dendritic Cell Migration. *Immunity*. 29:325–342. doi:10.1016/j.immuni.2008.08.006.

von Andrian, U.H., and C.R. Mackay. 2000. T-Cell Function and Migration — Two Sides of the Same Coin. *N Engl J Med*. 343:1020–1034. doi:10.1056/NEJM200010053431407.

Arens, R., and S.P. Schoenberger. 2010. Plasticity in programming of effector and memory CD8 $^{+}$ T-cell formation: Plasticity in CD8 $^{+}$ T-cell formation. *Immunological Reviews*. 235:190–205. doi:10.1111/j.0105-2896.2010.00899.x.

Ariotti, S., J.B. Beltman, G. Chodaczek, M.E. Hoekstra, A.E. van Beek, R. Gomez-Eerland, L. Ritsma, J. van Rheenen, A.F.M. Maree, T. Zal, R.J. de Boer, J.B.A.G. Haanen, and T.N. Schumacher. 2012. Tissue-resident memory CD8+ T cells continuously patrol skin epithelia to quickly recognize local antigen. *Proceedings of the National Academy of Sciences*. 109:19739–19744. doi:10.1073/pnas.1208927109.

Ariotti, S., M.A. Hogenbirk, F.E. Dijkgraaf, L.L. Visser, M.E. Hoekstra, J.Y. Song, H. Jacobs, J.B. Haanen, and T.N. Schumacher. 2014. Skin-resident memory CD8+T cells trigger a state of tissue-wide pathogen alert. *Science*. 346:101–105. doi:10.1126/science.1254803.

Arosa, F.A., A.J. Esgalhado, C.A. Padrão, and E.M. Cardoso. 2017. Divide, Conquer, and Sense: CD8+CD28− T Cells in Perspective. *Frontiers in Immunology*. 7.

Auffray, C., D. Fogg, M. Garfa, G. Elain, O. Join-Lambert, S. Kayal, S. Sarnacki, A. Cumano, G. Lauvau, and F. Geissmann. 2007. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science*. 317:666–670. doi:10.1126/science.1142883.

Auffray, C., M.H. Sieweke, and F. Geissmann. 2009. Blood Monocytes: Development, Heterogeneity, and Relationship with Dendritic Cells. *Annual Review of Immunology*. 27:669–692. doi:10.1146/annurev.immunol.021908.132557.

Azukizawa, H., H. Kosaka, S. Sano, W.R. Heath, I. Takahashi, X.-H. Gao, Y. Sumikawa, M. Okabe, K. Yoshikawa, and S. Itami. 2003. Induction of T-cell-mediated skin disease specific for antigen transgenically expressed in keratinocytes. *European Journal of Immunology*. 33:1879–1888. doi:10.1002/eji.200323630.

Babior, B.M. 2004. NADPH oxidase. *Current Opinion in Immunology*. 16:42–47. doi:10.1016/j.coi.2003.12.001.

Bachem, A., E. Hartung, S. Güttler, A. Mora, X. Zhou, A. Hegemann, M. Plantinga, E. Mazzini, P. Stoitzner, S. Gurka, V. Henn, H. Mages, and R. Kroczek. 2012. Expression of XCR1 Characterizes the Batf3-Dependent Lineage of Dendritic Cells Capable of Antigen Cross-Presentation. *Frontiers in Immunology*. 3.

Bachmann, M.F., A. Gallimore, S. Linkert, V. Cerundolo, A. Lanzavecchia, M. Kopf, and A. Viola. 1999. Developmental regulation of Lck targeting to the CD8 coreceptor controls signaling in naive and memory T cells. *J Exp Med*. 189:1521–1530. doi:10.1084/jem.189.10.1521.

Badovinac, V.P., B.B. Porter, and J.T. Harty. 2004. CD8+ T cell contraction is controlled by early inflammation. *Nat Immunol*. 5:809–817. doi:10.1038/ni1098.

Badovinac, V.P., A.R. Tvinnereim, and J.T. Harty. 2000. Regulation of Antigen-Specific CD8+ T Cell Homeostasis by Perforin and Interferon-γ. *Science*. 290:1354–1357. doi:10.1126/science.290.5495.1354.

Bal, V., A. McIndoe, G. Denton, D. Hudson, G. Lombardi, J. Lamb, and R. Lechler. 1990. Antigen presentation by keratinocytes induces tolerance in human T cells. *Eur J Immunol*. 20:1893–1897. doi:10.1002/eji.1830200904.

Ballesteros-Tato, A., B. León, F.E. Lund, and T.D. Randall. 2010. Temporal changes in dendritic cell subsets, cross-priming and costimulation via CD70 control CD8+ T cell responses to influenza. *Nat Immunol*. 11:216–224. doi:10.1038/ni.1838.

Banchereau, J., L.A. Thompson-Snipes, S. Zurawski, J.P. Blanck, Y. Cao, S. Clayton, J.P. Gorvel, G. Zurawski, and E. Klechevsky. 2012. The differential production of cytokines by human Langerhans cells and dermal CD14+ DCs controls CTL priming. *Blood*. 119:5742. doi:10.1182/BLOOD-2011-08-371245.

Becker, T.C., E.J. Wherry, D. Boone, K. Murali-Krishna, R. Antia, A. Ma, and R. Ahmed. 2002. Interleukin 15 is required for proliferative renewal of virus-specific memory CD8 T cells. *J Exp Med*. 195:1541–1548. doi:10.1084/jem.20020369.

Bedoui, S., W.R. Heath, and S.N. Mueller. 2016. CD4(+) T-cell help amplifies innate signals for primary CD8(+) T-cell immunity. *Immunol Rev*. 272:52–64. doi:10.1111/imr.12426.

Bedoui, S., P.G. Whitney, J. Waithman, L. Eidsmo, L. Wakim, I. Caminschi, R.S. Allan, M. Wojtasiak, K. Shortman, F.R. Carbone, A.G. Brooks, and W.R. Heath. 2009. Cross-presentation of viral and self antigens by skin-derived CD103+ dendritic cells. *Nature Immunology*. 10:488–495. doi:10.1038/ni.1724.

Belz, G.T., N.S. Wilson, C.M. Smith, A.M. Mount, F.R. Carbone, and W.R. Heath. 2006. Bone marrow-derived cells expand memory CD8+ T cells in response to viral infections of the lung and skin. *Eur J Immunol*. 36:327–335. doi:10.1002/eji.200535432.

Bennett, C.L., F. Fallah-Arani, T. Conlan, C. Trouillet, H. Goold, L. Chorro, B. Flutter, T.K. Means, F. Geissmann, and R. Chakraverty. 2011. Langerhans cells regulate cutaneous injury by licensing CD8 effector cells recruited to the skin. *Blood*. 117:7063–7069. doi:10.1182/blood-2011-01-329185.

Bennett, C.L., E. van Rijn, S. Jung, K. Inaba, R.M. Steinman, M.L. Kapsenberg, and B.E. Clausen. 2005. Inducible ablation of mouse Langerhans cells diminishes but fails to abrogate contact hypersensitivity. *J Cell Biol*. 169:569–576. doi:10.1083/jcb.200501071.

Bennett, S.R., F.R. Carbone, F. Karamalis, R.A. Flavell, J.F. Miller, and W.R. Heath. 1998. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature*. 393:478–480. doi:10.1038/30996.

Bennett, S.R., F.R. Carbone, F. Karamalis, J.F. Miller, and W.R. Heath. 1997. Induction of a CD8+ cytotoxic T lymphocyte response by cross-priming requires cognate CD4+ T cell help. *J Exp Med*. 186:65–70. doi:10.1084/jem.186.1.65.

Bertram, E.M., W. Dawicki, B. Sedgmen, J.L. Bramson, D.H. Lynch, and T.H. Watts. 2004. A switch in costimulation from CD28 to 4-1BB during primary versus secondary CD8 T cell response to influenza in vivo. *J Immunol*. 172:981–988. doi:10.4049/jimmunol.172.2.981.

Bevan, M.J. 1976. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. *J Exp Med*. 143:1283–1288. doi:10.1084/jem.143.5.1283.

Boise, L.H., A.J. Minn, P.J. Noel, C.H. June, M.A. Accavitti, T. Lindsten, and C.B. Thompson. 1995. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-XL. *Immunity*. 3:87–98. doi:10.1016/1074-7613(95)90161-2.

Boise, L.H., and C.B. Thompson. 1996. Hierarchical Control of Lymphocyte Survival. *Science*. 274:67–68. doi:10.1126/science.274.5284.67.

Boniface, K., C. Jacquemin, A.-S. Darrigade, B. Dessarthe, C. Martins, N. Boukhedouni, C. Vernisse, A. Grasseau, D. Thiolat, J. Rambert, F. Lucchese, A. Bertolotti, K. Ezzedine, A. Taieb, and J. Seneschal. 2018. Vitiligo Skin Is Imprinted with Resident Memory CD8 T Cells Expressing CXCR3. *Journal of Investigative Dermatology*. 138:355–364. doi:10.1016/j.jid.2017.08.038.

Bonilla, F.A., and H.C. Oettgen. 2010. Adaptive immunity. *Journal of Allergy and Clinical Immunology*. 125. doi:10.1016/j.jaci.2009.09.017.

Boring, L., J. Gosling, S.W. Chensue, S.L. Kunkel, R.V. Farese, H.E. Broxmeyer, and I.F. Charo. 1997. Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C-C chemokine receptor 2 knockout mice. *J Clin Invest*. 100:2552–2561. doi:10.1172/JCI119798.

Borowski, A.B., A.C. Boesteanu, Y.M. Mueller, C. Carafides, D.J. Topham, J.D. Altman, S.R. Jennings, and P.D. Katsikis. 2007. Memory CD8+ T Cells Require CD28 Costimulation. *The Journal of Immunology*. 179:6494–6503. doi:10.4049/JIMMUNOL.179.10.6494.

Borriello, F., M.P. Sethna, S.D. Boyd, A.N. Schweitzer, E.A. Tivol, D. Jacoby, T.B. Strom, E.M. Simpson, G.J. Freeman, and A.H. Sharpe. 1997. B7-1 and B7-2 have overlapping, critical roles in immunoglobulin class switching and germinal center formation. *Immunity*. 6:303–313. doi:10.1016/s1074-7613(00)80333-7.

Borst, J., T. Ahrends, N. Bąbała, C.J.M. Melief, and W. Kastenmüller. 2018. CD4+ T cell help in cancer immunology and immunotherapy. *Nat Rev Immunol*. 18:635–647. doi:10.1038/s41577-018-0044-0.

Bosteels, C., K. Fierens, S. De Prijck, J. Van Moorleghem, M. Vanheerswynghels, C. De Wolf, A. Chalon, C. Collignon, H. Hammad, A.M. Didierlaurent, and B.N. Lambrecht. 2020a. CCR2- and Flt3-Dependent Inflammatory Conventional Type 2 Dendritic Cells Are Necessary for the Induction of Adaptive Immunity by the Human Vaccine Adjuvant System AS01. *Front Immunol*. 11:606805. doi:10.3389/fimmu.2020.606805.

Bosteels, C., K. Neyt, M. Vanheerswynghels, M.J. van Helden, D. Sichien, N. Debeuf, S. De Prijck, V. Bosteels, N. Vandamme, L. Martens, Y. Saeys, E. Louagie, M. Lesage, D.L. Williams, S.-C. Tang, J.U. Mayer, F. Ronchese, C.L. Scott, H. Hammad, M. Guilliams, and B.N. Lambrecht. 2020b. Inflammatory Type 2 cDCs Acquire Features of cDC1s and Macrophages to Orchestrate Immunity to Respiratory Virus Infection. *Immunity*. 52:1039-1056.e9. doi:10.1016/j.immuni.2020.04.005.

Bousso, P., and E. Robey. 2003. Dynamics of CD8+ T cell priming by dendritic cells in intact lymph nodes. *Nat Immunol*. 4:579–585. doi:10.1038/ni928.

Boyman, O., H.P. Hefti, C. Conrad, B.J. Nickoloff, M. Suter, and F.O. Nestle. 2004. Spontaneous Development of Psoriasis in a New Animal Model Shows an Essential Role for Resident T Cells and Tumor Necrosis Factor-α. *Journal of Experimental Medicine*. 199:731–736. doi:10.1084/jem.20031482.

Brunham, R.C., and K.M. Coombs. 1998. In celebration of the 200th anniversary of Edward Jenner's Inquiry into the causes and effects of the variolae vaccinae. *The Canadian journal of infectious diseases = Journal canadien des maladies infectieuses*. 9:310–3.

Burn, G.L., A. Foti, G. Marsman, D.F. Patel, and A. Zychlinsky. 2021. The Neutrophil. *Immunity*. 54:1377–1391. doi:10.1016/j.immuni.2021.06.006.

Burnet, F.M. 1976. A modification of {J}erne's theory of antibody production using the concept of clonal selection. *The Australian Journal of Science*. 20:67–69. doi:10.3322/canjclin.26.2.119.

Bursch, L.S., B.E. Rich, and K.A. Hogquist. 2009. Langerhans Cells Are Not Required for the CD8 T Cell Response to Epidermal Self-Antigens 1. *The Journal of Immunology*. 182:4657–4664. doi:10.4049/jimmunol.0803656.

Bursch, L.S., L. Wang, B. Igyarto, A. Kissenpfennig, B. Malissen, D.H. Kaplan, and K.A. Hogquist. 2007. Identification of a novel population of Langerin+ dendritic cells. *J Exp Med*. 204:3147–3156. doi:10.1084/jem.20071966.

Byrne, C., M. Tainsky, and E. Fuchs. 1994. Programming gene expression in developing epidermis. *Development*. 120:2369–2383. doi:10.1242/dev.120.9.2369.

Carbone, F.R. 2020. Immovable memories: the journey to permanent residency. *Nat Immunol*. 21:698–699. doi:10.1038/s41590-020-0651-3.

Carbone, F.R., L.K. Mackay, W.R. Heath, and T. Gebhardt. 2013. Distinct resident and recirculating memory T cell subsets in non-lymphoid tissues. *Current Opinion in Immunology*. 25:329–333. doi:10.1016/j.coi.2013.05.007.

Carlin, L.M., E.G. Stamatiades, C. Auffray, R.N. Hanna, L. Glover, G. Vizcay-Barrena, C.C. Hedrick, H.T. Cook, S. Diebold, and F. Geissmann. 2013. Nr4a1-dependent Ly6C(low) monocytes monitor endothelial cells and orchestrate their disposal. *Cell*. 153:362–375. doi:10.1016/j.cell.2013.03.010.

Carpenter, A.C., and R. Bosselut. 2010. Decision checkpoints in the thymus. *Nature immunology*. 11:666–673. doi:10.1038/ni.1887.

Carpentier, S., T.P. Vu Manh, R. Chelbi, S. Henri, B. Malissen, M. Haniffa, F. Ginhoux, and M. Dalod. 2016. Comparative genomics analysis of mononuclear phagocyte subsets confirms homology between lymphoid tissue-resident and dermal XCR1(+) DCs in mouse and human and distinguishes them from Langerhans cells. *Journal of immunological methods*. 432:35–49. doi:10.1016/J.JIM.2016.02.023.

Cassell, D., and J. Forman. 1988. Linked recognition of helper and cytotoxic antigenic determinants for the generation of cytotoxic T lymphocytes. *Ann N Y Acad Sci*. 532:51–60. doi:10.1111/j.1749-6632.1988.tb36325.x.

Castellino, F., A.Y. Huang, G. Altan-Bonnet, S. Stoll, C. Scheinecker, and R.N. Germain. 2006. Chemokines enhance immunity by guiding naive CD8+ T cells to sites of CD4+ T cell-dendritic cell interaction. *Nature*. 440:890–895. doi:10.1038/nature04651.

Castiglioni, P., D.S. Hall, E.L. Jacovetty, E. Ingulli, and M. Zanetti. 2008. Protection against Influenza A Virus by Memory CD8 T Cells Requires Reactivation by Bone Marrow-Derived Dendritic Cells1. *The Journal of Immunology*. 180:4956–4964. doi:10.4049/jimmunol.180.7.4956.

Castro, I., A. Yu, M.J. Dee, and T.R. Malek. 2011. The basis of distinctive IL-2- and IL-15-dependent signaling: weak CD122-dependent signaling favors CD8+ T central-memory cell survival but not T effector-memory cell development. *J Immunol*. 187:5170–5182. doi:10.4049/jimmunol.1003961.

Cella, M., D. Scheidegger, K. Palmer-Lehmann, P. Lane, A. Lanzavecchia, and G. Alber. 1996. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J Exp Med*. 184:747–752. doi:10.1084/jem.184.2.747.

Chambers, E.S., and M. Vukmanovic-Stejic. 2020. Skin barrier immunity and ageing. *Immunology*. 160:116–125. doi:10.1111/IMM.13152.

Cheuk, S., M. Wikén, L. Blomqvist, S. Nylén, T. Talme, M. Ståhle, and L. Eidsmo. 2014. Epidermal Th22 and Tc17 cells form a localized disease memory in clinically healed psoriasis. *J Immunol*. 192:3111–3120. doi:10.4049/jimmunol.1302313.

Chorro, L., A. Sarde, M. Li, K.J. Woollard, P. Chambon, B. Malissen, A. Kissenpfennig, J.-B. Barbaroux, R. Groves, and F. Geissmann. 2009. Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and

inflammation-associated expansion of the epidermal LC network. *The Journal of experimental medicine*. 206:3089–100. doi:10.1084/jem.20091586.

Christo, S.N., M. Evrard, S.L. Park, L.C. Gandolfo, T.N. Burn, R. Fonseca, D.M. Newman, Y.O. Alexandre, N. Collins, N.M. Zamudio, F. Souza-Fonseca-Guimaraes, D.G. Pellicci, D. Chisanga, W. Shi, L. Bartholin, G.T. Belz, N.D. Huntington, A. Lucas, M. Lucas, S.N. Mueller, W.R. Heath, F. Ginhoux, T.P. Speed, F.R. Carbone, A. Kallies, and L.K. Mackay. 2021. Discrete tissue microenvironments instruct diversity in resident memory T cell function and plasticity. *Nature Immunology 2021 22:9*. 22:1140–1151. doi:10.1038/s41590-021-01004-1.

Clausen, B.E., and P. Stoitzner. 2015. Functional specialization of skin dendritic cell subsets in regulating T cell responses. *Frontiers in Immunology*. doi:10.3389/fimmu.2015.00534.

Cohen, J.A., J. Wu, and D.H. Kaplan. 2020. Neuronal Regulation of Cutaneous Immunity. *The Journal of Immunology*. 204:264–270. doi:10.4049/jimmunol.1901109.

Crowe, S.R., S.J. Turner, S.C. Miller, A.D. Roberts, R.A. Rappolo, P.C. Doherty, K.H. Ely, and D.L. Woodland. 2003. Differential Antigen Presentation Regulates the Changing Patterns of CD8 + T Cell Immunodominance in Primary and Secondary Influenza Virus Infections. *The Journal of Experimental Medicine*. 198:399–410. doi:10.1084/jem.20022151.

Cruz-Guilloty, F., M.E. Pipkin, I.M. Djuretic, D. Levanon, J. Lotem, M.G. Lichtenheld, Y. Groner, and A. Rao. 2009. Runx3 and T-box proteins cooperate to establish the transcriptional program of effector CTLs. *Journal of Experimental Medicine*. 206:51–59. doi:10.1084/jem.20081242.

Cui, W., and S.M. Kaech. 2010. Generation of effector CD8+ T cells and their conversion to memory T cells. *Immunological Reviews*. 236:151–166. doi:10.1111/j.1600-065X.2010.00926.x.

Cyster, J.G. 1999. Chemokines and Cell Migration in Secondary Lymphoid Organs. *Science*. 286:2098–2102. doi:10.1126/science.286.5447.2098.

Deshmane, S.L., S. Kremlev, S. Amini, and B.E. Sawaya. 2009. Monocyte Chemoattractant Protein-1 (MCP-1): An Overview. *J Interferon Cytokine Res*. 29:313–326. doi:10.1089/jir.2008.0027.

Di Meglio, P., G.K. Perera, and F.O. Nestle. 2011. The Multitasking Organ: Recent Insights into Skin Immune Function. *Immunity*. 35:857–869. doi:10.1016/j.immuni.2011.12.003.

Dieu-Nosjean, M.-C., C. Massacrier, B. Homey, B. Vanbervliet, J.-J. Pin, A. Vicari, S. Lebecque, C. Dezutter-Dambuyant, D. Schmitt, A. Zlotnik, and C. Caux. 2000. Macrophage Inflammatory Protein 3α Is Expressed at Inflamed Epithelial Surfaces and Is the Most Potent Chemokine Known in Attracting Langerhans Cell Precursors. *Journal of Experimental Medicine*. 192:705–718. doi:10.1084/jem.192.5.705.

Dolfi, D.V., P.A. Duttagupta, A.C. Boesteanu, Y.M. Mueller, C.H. Oliai, A.B. Borowski, and P.D. Katsikis. 2011. Dendritic Cells and CD28 Costimulation Are Required To Sustain Virus-Specific CD8+ T Cell Responses during the Effector Phase In Vivo. *The Journal of Immunology*. 186:4599–4608. doi:10.4049/jimmunol.1001972.

Domínguez, P.M., and C. Ardavín. 2010. Differentiation and function of mouse monocyte-derived dendritic cells in steady state and inflammation. *Immunological Reviews*. 234:90–104.

Doorbar, J. 2005. The papillomavirus life cycle. *Journal of Clinical Virology*. 32:7–15. doi:10.1016/J.JCV.2004.12.006.

Edelson, B.T., W. Kc, R. Juang, M. Kohyama, L.A. Benoit, P.A. Klekotka, C. Moon, J.C. Albring, W. Ise, D.G. Michael, D. Bhattacharya, T.S. Stappenbeck, M.J. Holtzman, S.-S.J. Sung, T.L. Murphy, K. Hildner, and K.M. Murphy. 2010. Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. *J Exp Med*. 207:823–836. doi:10.1084/jem.20091627.

Edwards, L., A. Ferenczy, L. Eron, D. Baker, M.L. Owens, T.L. Fox, A.J. Hougham, and K.A. Schmitt. 1998. Self-administered topical 5% imiquimod cream for external anogenital warts. HPV Study Group. Human PapillomaVirus. *Arch Dermatol*. 134:25–30. doi:10.1001/archderm.134.1.25.

Ehrlich, R. 1997. Modulation of antigen processing and presentation by persistent virus infections and in tumors. *Hum Immunol*. 54:104–116. doi:10.1016/s0198-8859(97)00083-9.

Eickhoff, S., A. Brewitz, M.Y. Gerner, F. Klauschen, K. Komander, H. Hemmi, N. Garbi, T. Kaisho, R.N. Germain, and W. Kastenmüller. 2015. Robust Anti-viral Immunity Requires Multiple Distinct T Cell-Dendritic Cell Interactions. *Cell*. 162:1322–1337. doi:10.1016/j.cell.2015.08.004.

Epelman, S., K.J. Lavine, and G.J. Randolph. 2014. Origin and Functions of Tissue Macrophages. *Immunity*. 41:21–35. doi:10.1016/j.immuni.2014.06.013.

Fan, L., B.W. Busser, T.Q. Lifsted, D. Lo, and T.M. Laufer. 2003. Antigen presentation by keratinocytes directs autoimmune skin disease. *Proceedings of the National Academy of Sciences*. 100:3386–3391. doi:10.1073/pnas.0437899100.

Feau, S., R. Arens, S. Togher, and S.P. Schoenberger. 2011. Autocrine IL-2 is required for secondary population expansion of CD8(+) memory T cells. *Nat Immunol*. 12:908–913. doi:10.1038/ni.2079.

Feau, S., Z. Garcia, R. Arens, H. Yagita, J. Borst, and S.P. Schoenberger. 2012. The CD4+ T-cell help signal is transmitted from APC to CD8+ T-cells via CD27–CD70 interactions. *Nat Commun*. 3:948. doi:10.1038/ncomms1948.

Fernandez-Ruiz, D., W.Y. Ng, L.E. Holz, J.Z. Ma, A. Zaid, Y.C. Wong, L.S. Lau, V. Mollard, A. Cozijnsen, N. Collins, J. Li, G.M. Davey, Y. Kato, S. Devi, R. Skandari, M. Pauley, J.H. Manton, D.I. Godfrey, A. Braun, S.S. Tay, P.S. Tan, D.G. Bowen, F. Koch-Nolte, B. Rissiek, F.R. Carbone, B.S. Crabb, M. Lahoud, I.A. Cockburn, S.N. Mueller, P. Bertolino, G.I. McFadden, I. Caminschi, and W.R. Heath. 2019. Liver-Resident Memory CD8+ T Cells Form a Front-Line Defense against Malaria Liver-Stage Infection. *Immunity*. 51:780. doi:10.1016/j.immuni.2019.09.019.

Fischer, M.A., M.L. Davies, I.E. Reider, E.L. Heipertz, M.R. Epler, J.J. Sei, M.A. Ingersoll, N.V. Rooijen, G.J. Randolph, and C.C. Norbury. 2011. CD11b+, Ly6G+ Cells Produce Type I Interferon and Exhibit Tissue Protective Properties Following Peripheral Virus Infection. *PLOS Pathogens*. 7:e1002374. doi:10.1371/journal.ppat.1002374.

Früh, K., and Y. Yang. 1999. Antigen presentation by MHC class I and its regulation by interferon ?? *Current Opinion in Immunology*. 11:76–81. doi:10.1016/S0952-7915(99)80014-4.

Fung, H.Y., M. Teryek, A.D. Lemenze, and T. Bergsbaken. 2022. CD103 fate mapping reveals that intestinal CD103- tissue-resident memory T cells are the primary responders to secondary infection. *Sci Immunol*. 7:eabl9925. doi:10.1126/sciimmunol.abl9925.

van Furth, R. 1981. 27 - IDENTIFICATION OF MONONUCLEAR PHAGOCYTES: OVERVIEW AND DEFINITIONS. *In* Methods for Studying Mononuclear Phagocytes. D.O. Adams, P.J. Edelson, and H.S. Koren, editors. Academic Press. 243–251.

van Furth, R., and Z.A. Cohn. 1968. The origin and kinetics of mononuclear phagocytes. *J Exp Med*. 128:415–435. doi:10.1084/jem.128.3.415.

van Furth, R., Z.A. Cohn, J.G. Hirsch, J.H. Humphrey, W.G. Spector, and H.L. Langevoort. 1972. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. *Bull World Health Organ*. 46:845–852.

Fuse, S., W. Zhang, and E.J. Usherwood. 2008. Control of memory CD8+ T cell differentiation by CD80/CD86-CD28 costimulation and restoration by IL-2 during the recall response. *J Immunol*. 180:1148–1157. doi:10.4049/jimmunol.180.2.1148.

Gaiser, M.R., T. Lämmermann, X. Feng, B.Z. Igyarto, D.H. Kaplan, L. Tessarollo, R.N. Germain, and M.C. Udey. 2012. Cancer-associated epithelial cell adhesion molecule (EpCAM; CD326) enables epidermal Langerhans cell motility and migration in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 109. doi:10.1073/pnas.1117674109.

García-Sastre, A. 2017. Ten Strategies of Interferon Evasion by Viruses. *Cell Host & Microbe*. 22:176–184.

Van Gassen, S., B. Callebaut, M.J. Van Helden, B.N. Lambrecht, P. Demeester, T. Dhaene, and Y. Saeys. 2015. FlowSOM: Using self-organizing maps for visualization and interpretation of cytometry data. *Cytometry Part A*. 87:636–645. doi:10.1002/CYTO.A.22625.

Gebhardt, T., and L.K. Mackay. 2012. Local immunity by tissue-resident CD8+ memory T cells. *Frontiers in Immunology*. 3. doi:10.3389/fimmu.2012.00340.

Gebhardt, T., L.M. Wakim, L. Eidsmo, P.C. Reading, W.R. Heath, and F.R. Carbone. 2009. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nature Immunology*. 10:524–530. doi:10.1038/ni.1718.

Gebhardt, T., P.G. Whitney, A. Zaid, L.K. Mackay, A.G. Brooks, W.R. Heath, F.R. Carbone, and S.N. Mueller. 2011. Different patterns of peripheral migration by memory CD4+ and CD8+ T cells. *Nature*. 477:216–219. doi:10.1038/nature10339.

Geissmann, F., S. Jung, and D.R. Littman. 2003. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity*. 19:71–82. doi:10.1016/s1074-7613(03)00174-2.

Ginhoux, F., M. Greter, M. Leboeuf, S. Nandi, P. See, S. Gokhan, M.F. Mehler, S.J. Conway, L.G. Ng, E.R. Stanley, I.M. Samokhvalov, and M. Merad. 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science*. 330:841–845. doi:10.1126/science.1194637.

Ginhoux, F., and M. Guilliams. 2016. Tissue-Resident Macrophage Ontogeny and Homeostasis. *Immunity*. 44:439–449. doi:10.1016/j.immuni.2016.02.024.

Ginhoux, F., and S. Jung. 2014. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nature reviews. Immunology*. 14:392–404. doi:10.1038/nri3671.

Girard, J.-P., C. Moussion, and R. Förster. 2012. HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. *Nat Rev Immunol*. 12:762–773. doi:10.1038/nri3298.

Gordon, S. 2002. Pattern recognition receptors: doubling up for the innate immune response. *Cell*. 111:927–930. doi:S0092867402012011.

Gordon, S. 2016. Phagocytosis: The Legacy of Metchnikoff. *Cell*. 166:1065–1068. doi:10.1016/j.cell.2016.08.017.

Grawunder, U., R.B. West, and M.R. Lieber. 1998. Antigen receptor gene rearrangement. *Current opinion in immunology*. 10:172–80.

Grewal, I.S., and R.A. Flavell. 1996. The role of CD40 ligand in costimulation and T-cell activation. *Immunol Rev*. 153:85–106. doi:10.1111/j.1600-065x.1996.tb00921.x.

Grewal, I.S., and R.A. Flavell. 1997. The Cd40 ligand. *Immunol Res*. 16:59–70. doi:10.1007/BF02786323.

Guilliams, M., C.-A. Dutertre, C.L. Scott, N. McGovern, D. Sichien, S. Chakarov, S. Van Gassen, J. Chen, M. Poidinger, S. De Prijck, S.J. Tavernier, I. Low, S.E. Irac, C.N. Mattar, H.R. Sumatoh, G.H.L. Low, T.J.K. Chung, D.K.H. Chan, K.K. Tan, T.L.K. Hon, E. Fossum, B. Bogen, M. Choolani, J.K.Y. Chan, A. Larbi, H. Luche, S. Henri, Y. Saeys, E.W. Newell, B.N. Lambrecht, B. Malissen, and F. Ginhoux. 2016. Unsupervised High-Dimensional Analysis Aligns Dendritic Cells across Tissues and Species. *Immunity*. 45:669–684. doi:10.1016/j.immuni.2016.08.015.

Guilliams, M., F. Ginhoux, C. Jakubzick, S.H. Naik, N. Onai, B.U. Schraml, E. Segura, R. Tussiwand, and S. Yona. 2014. Dendritic cells, monocytes and macrophages: A unified nomenclature based on ontogeny. *Nature Reviews Immunology*. 14:571–578. doi:10.1038/nri3712.

Gunn, M.D., S. Kyuwa, C. Tam, T. Kakiuchi, A. Matsuzawa, L.T. Williams, and H. Nakano. 1999. Mice Lacking Expression of Secondary Lymphoid Organ Chemokine Have Defects in Lymphocyte Homing and Dendritic Cell Localization. *Journal of Experimental Medicine*. 189:451–460. doi:10.1084/jem.189.3.451.

Guo, Y., K.A. Leacock, C. Redmond, V. Jaskula-Ranga, and P.A. Coulombe. 2019. Keratin 14-dependent disulfides regulate epidermal homeostasis and barrier function via 14-3-3σ and YAP1. 824219. doi:10.1101/824219.

Hain, T., F. Melchior, N. Kamenjarin, S. Muth, H. Weslati, B.E. Clausen, K. Mahnke, C. Silva-Vilches, K. Schütze, J. Sohl, M.P. Radsak, G. Bündgen, T. Bopp, S. Danckwardt, H. Schild, and H.C. Probst. 2019. Dermal CD207-Negative Migratory Dendritic Cells Are Fully Competent to Prime Protective, Skin Homing Cytotoxic T-Lymphocyte Responses. *Journal of Investigative Dermatology*. 139:422–429. doi:10.1016/j.jid.2018.08.022.

Halliwell, B. 2006. Phagocyte-derived reactive species: salvation or suicide? *Trends in Biochemical Sciences*. 31:509–515. doi:10.1016/j.tibs.2006.07.005.

Hamilton, S.E., M.C. Wolkers, S.P. Schoenberger, and S.C. Jameson. 2006. The generation of protective memory-like CD8+ T cells during homeostatic proliferation requires CD4+ T cells. *Nat Immunol*. 7:475–481. doi:10.1038/ni1326.

Hammarlund, E., M.W. Lewis, S.G. Hansen, L.I. Strelow, J.A. Nelson, G.J. Sexton, J.M. Hanifin, and M.K. Slifka. 2003. Duration of antiviral immunity after smallpox vaccination. *Nat Med*. 9:1131–1137. doi:10.1038/nm917.

Hany, M., S. Oehen, M. Schulz, H. Hengartner, M. Mackett, D.H.L. Bishop, H. Overton, and R.M. Zinkernagel. 1989. Anti-viral protection and prevention of lymphocytic choriomeningitis or of the local footpad swelling reaction in mice by immunization with vaccinia-recombinant virus expressing LCMV-WE nucleoprotein or glycoprotein. *European journal of immunology*. 19:417–424. doi:10.1002/EJI.1830190302.

Harty, J.T., and V.P. Badovinac. 2008. Shaping and reshaping CD8+ T-cell memory. *Nature Reviews Immunology*. 8:107–119. doi:10.1038/nri2251.

Hashimoto, D., A. Chow, C. Noizat, P. Teo, M.B. Beasley, M. Leboeuf, C.D. Becker, P. See, J. Price, D. Lucas, M. Greter, A. Mortha, S.W. Boyer, E.C. Forsberg, M. Tanaka, N. van Rooijen, A. García-Sastre, E.R. Stanley, F. Ginhoux, P.S. Frenette, and M. Merad. 2013. Tissue-Resident Macrophages Self-Maintain Locally throughout Adult Life with Minimal Contribution from Circulating Monocytes. *Immunity*. 38:792–804. doi:10.1016/j.immuni.2013.04.004.

Heath, W.R., and F.R. Carbone. 2013. The skin-resident and migratory immune system in steady state and memory: Innate lymphocytes, dendritic cells and T cells. *Nature Immunology*. 14:978–985. doi:10.1038/ni.2680.

Henri, S., L.F. Poulin, S. Tamoutounour, L. Ardouin, M. Guilliams, B. de Bovis, E. Devilard, C. Viret, H. Azukizawa, A. Kissenpfennig, and B. Malissen. 2010. CD207+ CD103+ dermal dendritic cells cross-present keratinocyte-derived antigens irrespective of the presence of Langerhans cells. *The Journal of experimental medicine*. 207:189–206. doi:10.1084/jem.20091964.

Hershberg, R.M., P.E. Framson, D.H. Cho, L.Y. Lee, S. Kovats, J. Beitz, J.S. Blum, and G.T. Nepom. 1997. Intestinal epithelial cells use two distinct pathways for HLA class II antigen processing. *J Clin Invest*. 100:204–215. doi:10.1172/JCI119514.

Hickman, H.D., L. Li, G.V. Reynoso, E.J. Rubin, C.N. Skon, J.W. Mays, J. Gibbs, O. Schwartz, J.R. Bennink, and J.W. Yewdell. 2011. Chemokines control naive CD8+ T cell selection of optimal lymph node antigen presenting cells. *J Exp Med*. 208:2511–2524. doi:10.1084/jem.20102545.

Hickman, H.D., G.V. Reynoso, B.F. Ngudiankama, E.J. Rubin, J.G. Magadán, S.S. Cush, J. Gibbs, B. Molon, V. Bronte, J.R. Bennink, and J.W. Yewdell. 2013. Anatomically Restricted Synergistic Antiviral Activities of Innate and Adaptive Immune Cells in the Skin. *Cell Host & Microbe*. 13:155–168. doi:10.1016/j.chom.2013.01.004.

Hickman, H.D., K. Takeda, C.N. Skon, F.R. Murray, S.E. Hensley, J. Loomis, G.N. Barber, J.R. Bennink, and J.W. Yewdell. 2008. Direct priming of antiviral CD8+ T cells in the peripheral interfollicular region of lymph nodes. *Nat Immunol*. 9:155–165. doi:10.1038/ni1557.

Hintzen, G., L. Ohl, M.-L. del Rio, J.-I. Rodriguez-Barbosa, O. Pabst, J.R. Kocks, J. Krege, S. Hardtke, and R. Förster. 2006. Induction of Tolerance to Innocuous Inhaled Antigen Relies on a CCR7-Dependent Dendritic Cell-Mediated Antigen Transport to the Bronchial Lymph Node1. *The Journal of Immunology*. 177:7346–7354. doi:10.4049/jimmunol.177.10.7346.

Hoebe, K., E. Janssen, and B. Beutler. 2004. The interface between innate and adaptive immunity. *Nature Immunology*. 5:971–974. doi:10.1038/ni1004-971.

Hoeffel, G., J. Chen, Y. Lavin, D. Low, F.F. Almeida, P. See, A.E. Beaudin, J. Lum, I. Low, E.C. Forsberg, M. Poidinger, F. Zolezzi, A. Larbi, L.G. Ng, J.K.Y. Chan, M. Greter, B. Becher, I.M. Samokhvalov, M. Merad, and F. Ginhoux. 2015. C-Myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity*. 42:665–678. doi:10.1016/j.immuni.2015.03.011.

Hoeffel, G., Y. Wang, M. Greter, P. See, P. Teo, B. Malleret, M. Leboeuf, D. Low, G. Oller, F. Almeida, S.H.Y. Choy, M. Grisotto, L. Renia, S.J. Conway, E.R. Stanley, J.K.Y. Chan, L.G. Ng, I.M. Samokhvalov, M. Merad, and F. Ginhoux. 2012. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac–derived macrophages. *The Journal of Experimental Medicine*. 209:1167–1181. doi:10.1084/jem.20120340.

Hong, S., and L.A. Laimins. 2017. Manipulation of the innate immune response by human papillomaviruses. *Virus research*. 231:34. doi:10.1016/J.VIRUSRES.2016.11.004.

Höpken, U.E., J. Droese, J.-P. Li, J. Joergensen, D. Breitfeld, H.-G. Zerwes, and M. Lipp. 2004. The chemokine receptor CCR7 controls lymph node-dependent cytotoxic T cell priming in alloimmune responses. *Eur J Immunol*. 34:461–470. doi:10.1002/eji.200324690.

Hor, J.L., P.G. Whitney, A. Zaid, A.G. Brooks, W.R. Heath, and S.N. Mueller. 2015. Spatiotemporally Distinct Interactions with Dendritic Cell Subsets Facilitates CD4+ and CD8+ T Cell Activation to Localized Viral Infection. *Immunity*. 43:554–565. doi:10.1016/j.immuni.2015.07.020.

Hufford, M.M., T.S. Kim, J. Sun, and T.J. Braciale. 2010. Antiviral CD8+ T cell effector activities in situ are regulated by target cell type. *Journal of Experimental Medicine*. 208:167–180. doi:10.1084/jem.20101850.

Iborra, S., H.M. Izquierdo, M. Martínez-López, N. Blanco-Menéndez, C. Reis E Sousa, and D. Sancho. 2012. The DC receptor DNGR-1 mediates cross-priming of CTLs during vaccinia virus infection in mice. *The Journal of clinical investigation*. 122:1628–1643. doi:10.1172/JCI60660.

Idoyaga, J., C. Cheong, K. Suda, N. Suda, J.Y. Kim, H. Lee, C.G. Park, and R.M. Steinman. 2008. Cutting Edge: Langerin/CD207 Receptor on Dendritic Cells Mediates Efficient Antigen Presentation on MHC I and II Products In Vivo1. *The Journal of Immunology*. 180:3647–3650. doi:10.4049/jimmunol.180.6.3647.

Inaba, K., M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu, and R.M. Steinman. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J.Exp.Med.* 176:1693–1702. doi:10.1084/jem.176.6.1693.

Intlekofer, A.M., N. Takemoto, C. Kao, A. Banerjee, F. Schambach, J.K. Northrop, H. Shen, E.J. Wherry, and S.L. Reiner. 2007. Requirement for T-bet in the aberrant differentiation of unhelped memory CD8+ T cells. *Journal of Experimental Medicine*. 204:2015–2021. doi:10.1084/jem.20070841.

Iwasaki, A., and R. Medzhitov. 2010. Regulation of adaptive immunity by the innate immune system. *Science (New York, N.Y.)*. 327:291–295. doi:10.1126/science.1183021.

Jakubzick, C.V., G.J. Randolph, and P.M. Henson. 2017. Monocyte differentiation and antigen-presenting functions. *Nat Rev Immunol*. 17:349–362. doi:10.1038/nri.2017.28.

Janssen, E.M., N.M. Droin, E.E. Lemmens, M.J. Pinkoski, S.J. Bensinger, B.D. Ehst, T.S. Griffith, D.R. Green, and S.P. Schoenberger. 2005. CD4+ T-cell help controls CD8+ T-cell memory via TRAIL-mediated activation-induced cell death. *Nature*. 434:88–93. doi:10.1038/nature03337.

Jenkins, S.J., and D.A. Hume. 2014. Homeostasis in the mononuclear phagocyte system. *Trends in Immunology*. 35:358–367. doi:10.1016/j.it.2014.06.006.

Jeske, D.J., J. Jarvis, C. Milstein, and J.D. Capra. 1984. Junctional diversity is essential to antibody activity. *Journal of immunology (Baltimore, Md. : 1950)*. 133:1090–2.

Jiang, X., R.A. Clark, L. Liu, A.J. Wagers, R.C. Fuhlbrigge, and T.S. Kupper. 2012. Skin infection generates non-migratory memory CD8+ TRM cells providing global skin immunity. *Nature*. 483:227–231. doi:10.1038/nature10851.

Jonuleit, H., U. Kühn, G. Müller, K. Steinbrink, L. Paragnik, E. Schmitt, J. Knop, and A.H. Enk. 1997. Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. *European Journal of Immunology*. 27:3135–3142. doi:10.1002/eji.1830271209.

Joshi, N.S., W. Cui, A. Chandele, H.K. Lee, D.R. Urso, J. Hagman, L. Gapin, and S.M. Kaech. 2007. Inflammation Directs Memory Precursor and Short-Lived Effector CD8+ T Cell Fates via the Graded Expression of T-bet Transcription Factor. *Immunity*. 27:281–295. doi:10.1016/j.immuni.2007.07.010.

Kabashima, K., N. Shiraishi, K. Sugita, T. Mori, A. Onoue, M. Kobayashi, J. Sakabe, R. Yoshiki, H. Tamamura, N. Fujii, K. Inaba, and Y. Tokura. 2007. CXCL12-CXCR4 Engagement Is Required for Migration of Cutaneous Dendritic Cells. *The American Journal of Pathology*. 171:1249–1257. doi:10.2353/ajpath.2007.070225.

Kaech, S.M., and R. Ahmed. 2001. Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naïve cells. *Nat Immunol*. 2:415–422. doi:10.1038/87720.

Kaech, S.M., and W. Cui. 2012. Transcriptional control of effector and memory CD8+ T cell differentiation. *Nature Reviews Immunology*. 12:749–761. doi:10.1038/nri3307.

Kamenjarin, N., K. Hodapp, F. Melchior, G. Harms, A.-K. Hartmann, J. Bartneck, S. Muth, V.K. Raker, C. Becker, A. Brand, B.E. Clausen, M.P. Radsak, H. Schild, and H.C. Probst. 2023. Cross-presenting Langerhans cells are required for the early reactivation of resident CD8+ memory T cells in the epidermis. *Proceedings of the National Academy of Sciences*. 120:e2219932120. doi:10.1073/pnas.2219932120.

Kawabe, T., T. Naka, K. Yoshida, T. Tanaka, H. Fujiwara, S. Suematsu, N. Yoshida, T. Kishimoto, and H. Kikutani. 1994. The immune responses in CD40-deficient mice: impaired immunoglobulin class switching and germinal center formation. *Immunity*. 1:167–178. doi:10.1016/1074-7613(94)90095-7.

Kennedy, M.K., K.M. Mohler, K.D. Shanebeck, P.R. Baum, K.S. Picha, C.A. Otten-Evans, C.A. Janeway Jr., and K.H. Grabstein. 1994. Induction of B cell costimulatory function by recombinant murine CD40 ligand. *European Journal of Immunology*. 24:116–123. doi:10.1002/eji.1830240118.

Khan, T.N., J.L. Mooster, A.M. Kilgore, J.F. Osborn, and J.C. Nolz. 2016. Local antigen in nonlymphoid tissue promotes resident memory CD8+ T cell formation during viral infection. *The Journal of experimental medicine*. 213:951–66. doi:10.1084/jem.20151855.

Kim, B.S., F. Miyagawa, Y.-H. Cho, C.L. Bennett, B.E. Clausen, and S.I. Katz. 2009. Keratinocytes Function as Accessory Cells for Presentation of Endogenous Antigen Expressed in the Epidermis. *Journal of Investigative Dermatology*. 129:2805–2817. doi:10.1038/jid.2009.176.

Kimbrell, D. a, and B. Beutler. 2001. The evolution and genetics of innate immunity. *Nature reviews. Genetics*. 2:256–267. doi:10.1038/35066006.

Kirchhoff, S., W.W. Müller, M. Li-Weber, and P.H. Krammer. 2000. Up-regulation of c-FLIPshort and reduction of activation-induced cell death in CD28-costimulated human T cells. *Eur J Immunol*. 30:2765–2774. doi:10.1002/1521-4141(200010)30:10<2765::AID-IMMU2765>3.0.CO;2-W.

Kissenpfennig, A., S. Henri, B. Dubois, C. Laplace-Builhé, P. Perrin, N. Romani, C.H. Tripp, P. Douillard, L. Leserman, D. Kaiserlian, S. Saeland, J. Davoust, and B. Malissen. 2005. Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. *Immunity*. 22:643–654. doi:10.1016/J.IMMUNI.2005.04.004.

Kitano, Y., and N. Okada. 1983. Separation of the epidermal sheet by dispase. *British Journal of Dermatology*. 108:555–560. doi:10.1111/J.1365-2133.1983.TB01056.X.

Klechevsky, E., R. Morita, M. Liu, Y. Cao, S. Coquery, L.A. Thompson-Snipes, F. Briere, D. Chaussabel, G. Zurawski, A.K. Palucka, Y. Reiter, J. Banchereau, and H. Ueno. 2008. Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. *Immunity*. 29:497–510. doi:10.1016/J.IMMUNI.2008.07.013.

Kobayashi, T., R.R. Ricardo-Gonzalez, and K. Moro. 2020. Skin-Resident Innate Lymphoid Cells – Cutaneous Innate Guardians and Regulators. *Trends Immunol*. 41:100–112. doi:10.1016/j.it.2019.12.004.

Koch, F., U. Stanzl, P. Jennewein, K. Janke, C. Heufler, E. Kämpgen, N. Romani, and G. Schuler. 1996. High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. *Journal of Experimental Medicine*. 184:741–746. doi:10.1084/jem.184.2.741.

Kondo, M. 2010. Lymphoid and myeloid lineage commitment in multipotent hematopoietic progenitors. *Immunological Reviews*. 238:37–46. doi:10.1111/j.1600-065X.2010.00963.x.

van Kooten, C., and J. Banchereau. 2000. CD40-CD40 ligand. *Journal of Leukocyte Biology*. 67:2–17. doi:10.1002/jlb.67.1.2.

Kurts, C., W.R. Heath, F.R. Carbone, J. Allison, J.F. Miller, and H. Kosaka. 1996. Constitutive class I-restricted exogenous presentation of self antigens in vivo. *Journal of Experimental Medicine*. 184:923–930. doi:10.1084/jem.184.3.923.

Laidlaw, B.J., N. Zhang, H.D. Marshall, M.M. Staron, T. Guan, Y. Hu, L.S. Cauley, J. Craft, and S.M. Kaech. 2014. CD4+ T cell help guides formation of CD103+ lung-resident memory CD8+ T cells during influenza viral infection. *Immunity*. 41:633–645. doi:10.1016/j.immuni.2014.09.007.

Lanzavecchia, A. 1998. Licence to kill. *Nature*. 393:413–414. doi:10.1038/30845.

Lei, V., A.J. Petty, A.R. Atwater, S.A. Wolfe, and A.S. MacLeod. 2020. Skin Viral Infections: Host Antiviral Innate Immunity and Viral Immune Evasion. *Front Immunol*. 11:593901. doi:10.3389/fimmu.2020.593901.

Lenschow, D.J., T.L. Walunas, and J.A. Bluestone. 1996. CD28/B7 system of T cell costimulation. *Annu Rev Immunol*. 14:233–258. doi:10.1146/annurev.immunol.14.1.233.

Lenz, L.L., E.A. Butz, and M.J. Bevan. 2000. Requirements for bone marrow-derived antigen-presenting cells in priming cytotoxic T cell responses to intracellular pathogens. *J Exp Med*. 192:1135–1142. doi:10.1084/jem.192.8.1135.

León, B., and C. Ardavín. 2008. Monocyte-derived dendritic cells in innate and adaptive immunity. *Immunology and cell biology*. 86:320–4. doi:10.1038/icb.2008.14.

Lin, L.C.W., I.E.A. Flesch, and D.C. Tscharke. 2013. Immunodomination during Peripheral Vaccinia Virus Infection. *PLoS Pathog*. 9:e1003329. doi:10.1371/journal.ppat.1003329.

Liu, L., R.C. Fuhlbrigge, K. Karibian, T. Tian, and T.S. Kupper. 2006. Dynamic programming of CD8+ T cell trafficking after live viral immunization. *Immunity*. 25:511–520. doi:10.1016/j.immuni.2006.06.019.

Liu, L., Q. Zhong, T. Tian, K. Dubin, S.K. Athale, and T.S. Kupper. 2010. Epidermal injury and infection during poxvirus immunization is crucial for the generation of highly protective T cell-mediated immunity. *Nat Med*. 16:224–227. doi:10.1038/nm.2078.

Lobby, J.L., I. Uddbäck, C.D. Scharer, T. Mi, J.M. Boss, A.R. Thomsen, J.P. Christensen, and J.E. Kohlmeier. 2022. Persistent Antigen Harbored by Alveolar Macrophages Enhances the Maintenance of Lung-Resident Memory CD8+ T Cells. *J Immunol*. 209:1778–1787. doi:10.4049/jimmunol.2200082.

Locksley, R.M., N. Killeen, and M.J. Lenardo. 2001. The TNF and TNF Receptor Superfamilies: Integrating Mammalian Biology. *Cell*. 104:487–501. doi:10.1016/S0092-8674(01)00237-9.

Lopez, P.A., M. Denny, A.-K. Hartmann, A. Alflen, H.C. Probst, E. von Stebut, S. Tenzer, H. Schild, M. Stassen, P. Langguth, and M.P. Radsak. 2017. Transcutaneous immunization with a novel imiquimod nanoemulsion induces superior T cell responses and virus protection. *Journal of Dermatological Science*. 87:252–259. doi:10.1016/j.jdermsci.2017.06.012.

Low, J.S., Y. Farsakoglu, M.C. Amezcua Vesely, E. Sefik, J.B. Kelly, C.C.D. Harman, R. Jackson, J.A. Shyer, X. Jiang, L.S. Cauley, R.A. Flavell, and S.M. Kaech. 2020. Tissue-resident memory T cell reactivation by diverse antigen-presenting cells imparts distinct functional responses. *The Journal of experimental medicine*. 217. doi:10.1084/JEM.20192291.

Mackarehtschian, K., J.D. Hardin, K.A. Moore, S. Boast, S.P. Goff, and I.R. Lemischka. 1995. Targeted disruption of the flk2/flt3 gene leads to deficiencies in primitive hematopoietic progenitors. *Immunity*. 3:147–161. doi:10.1016/1074-7613(95)90167-1.

Mackay, L.K., M. Minnich, N.A.M. Kragten, Y. Liao, B. Nota, C. Seillet, A. Zaid, K. Man, S. Preston, D. Freestone, A. Braun, E. Wynne-Jones, F.M. Behr, R. Stark, D.G. Pellicci, D.I. Godfrey, G.T. Belz, M. Pellegrini, T. Gebhardt, M. Busslinger, W. Shi, F.R. Carbone, R.A.W. Van Lier, A. Kallies, and K.P.J.M. Van Gisbergen. 2016. Hobit and Blimp1 instruct a universal

transcriptional program of tissue residency in lymphocytes. *Science (New York, N.Y.)*. 352:459–463. doi:10.1126/SCIENCE.AAD2035.

Mackay, L.K., A.T. Stock, J.Z. Ma, C.M. Jones, S.J. Kent, S.N. Mueller, W.R. Heath, F.R. Carbone, and T. Gebhardt. 2012. Long-lived epithelial immunity by tissue-resident memory T (TRM) cells in the absence of persisting local antigen presentation. *Proc Natl Acad Sci U S A*. 109:7037–7042. doi:10.1073/pnas.1202288109.

Mackay, L.K., E. Wynne-Jones, D. Freestone, D.G. Pellicci, L.A. Mielke, D.M. Newman, A. Braun, F. Masson, A. Kallies, G.T. Belz, and F.R. Carbone. 2015. T-box Transcription Factors Combine with the Cytokines TGF-β and IL-15 to Control Tissue-Resident Memory T Cell Fate. *Immunity*. 43:1101–1111. doi:10.1016/j.immuni.2015.11.008.

Malissen, B., S. Tamoutounour, and S. Henri. 2014. The origins and functions of dendritic cells and macrophages in the skin. *Nature Publishing Group*. 14. doi:10.1038/nri3683.

Masopust, D., D. Choo, V. Vezys, E.J. Wherry, J. Duraiswamy, R. Akondy, J. Wang, K.A. Casey, D.L. Barber, K.S. Kawamura, K.A. Fraser, R.J. Webby, V. Brinkmann, E.C. Butcher, K.A. Newell, and R. Ahmed. 2010. Dynamic T cell migration program provides resident memory within intestinal epithelium. *J Exp Med*. 207:553–564. doi:10.1084/jem.20090858.

Masopust, D., V. Vezys, A.L. Marzo, L. Lefrançois, D. Kagi, B. Ledermann, K. Burki, R.M. Zinkernagel, H. Hengartner, J.T. Harty, A.R. Tvinnereim, D.W. White, R. Dobber, A. Hertogh-Huijbregts, J. Rozing, K. Bottomly, L. Nagelkerken, C. Zimmermann, K. Brduscha-Riem, C. Blaser, R.M. Zinkernagel, H. Pircher, E.A. Butz, M.J. Bevan, D.H. Busch, I.M. Pilip, S. Vijh, E.G. Pamer, S.K. Kim, K.S. Schluns, L. Lefrançois, L. Bruno, J. Kirberg, H. von Boehmer, D.H. Busch, I. Pilip, E.G. Pamer, E.C. Butcher, L.J. Picker, R.W. Dutton, L.M. Bradley, S.L. Swain, U.H. von Andrian, C.R. Mackay, F. Sallusto, D. Lenig, R. Forster, M. Lipp, A. Lanzavecchia, G.M.V. Bleek, S.G. Nathenson, J.D. Altman, B.K. Cho, C. Wang, S. Sugawa, H.N. Eisen, J. Chen, L. Puddington, M.J. Bevan, J.K. Rose, L. Lefrançois, L.K. Selin, R.M. Welsh, D. Masopust, J. Jiang, H. Shen, L. Lefrançois, M.K. Slifka, J.K. Whitmire, R. Ahmed, G.T. Belz, J.D. Altman, and P.C. Doherty. 2001. Preferential localization of effector memory cells in nonlymphoid tissue. *Science (New York, N.Y.)*. 291:2413–7. doi:10.1126/science.1058867.

Masson Regnault, M., M.-P. Konstantinou, A. Khemis, Y. Poulin, M. Bourcier, F. Amelot, C. Bulaï Livideanu, and C. Paul. 2017. Early relapse of psoriasis after stopping brodalumab: a retrospective cohort study in 77 patients. *Journal of the European Academy of Dermatology and Venereology*. 31:1491–1496. doi:10.1111/jdv.14387.

Maudsley, D.J. 1991. Role of oncogenes in the regulation of MHC antigen expression. *Biochem Soc Trans*. 19:291–296. doi:10.1042/bst0190291.

McFadden, G., and K. Kane. 1994. How Dna Viruses Perturb Functional Mhc Expression To Alter Immune Recognition. *In* Advances in Cancer Research. G.F. Vande Woude and G. Klein, editors. Academic Press. 117–209.

McGargill, M.A., D. Mayerova, H.E. Stefanski, B. Koehn, E.A. Parke, S.C. Jameson, A. Panoskaltsis-Mortari, and K.A. Hogquist. 2002. A Spontaneous CD8 T Cell-Dependent Autoimmune Disease to an Antigen Expressed Under the Human Keratin 14 Promoter1. *The Journal of Immunology*. 169:2141–2147. doi:10.4049/jimmunol.169.4.2141.

McGill, J., N. Van Rooijen, and K.L. Legge. 2008. Protective influenza-specific CD8 T cell responses require interactions with dendritic cells in the lungs. *Journal of Experimental Medicine*. 205:1635–1646. doi:10.1084/jem.20080314.

McInnes, L., J. Healy, N. Saul, and L. Großberger. 2018. UMAP: Uniform Manifold Approximation and Projection. *Journal of Open Source Software*. 3:861. doi:10.21105/JOSS.00861.

Medema, J.P., D.H. Schuurhuis, D. Rea, J. van Tongeren, J. de Jong, S.A. Bres, S. Laban, R.E. Toes, M. Toebes, T.N. Schumacher, B.A. Bladergroen, F. Ossendorp, J.A. Kummer, C.J. Melief, and R. Offringa. 2001. Expression of the serpin serine protease inhibitor 6 protects dendritic cells from cytotoxic T lymphocyte-induced apoptosis: differential modulation by T helper type 1 and type 2 cells. *J Exp Med*. 194:657–667. doi:10.1084/jem.194.5.657.

Medzhitov, R., and C. Janeway Jr. 2000. Innate immune recognition: mechanisms and pathways. *Immunol Rev*. 173:89–97. doi:10.1034/j.1600-065X.2000.917309.x.

Medzhitov, R., and C. Janeway. 2000. Innate immunity. *The New England Journal of Medicine*. 343:338–344. doi:10.1016/j.coi.2003.11.017.

Merad, M., F. Ginhoux, and M. Collin. 2008. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Nature reviews. Immunology*. 8:935–947. doi:10.1038/NRI2455.

Merad, M., P. Hoffmann, E. Ranheim, S. Slaymaker, M.G. Manz, S.A. Lira, I. Charo, D.N. Cook, I.L. Weissman, S. Strober, and E.G. Engleman. 2004. Depletion of host Langerhans cells before transplantation of donor alloreactive T cells prevents skin graft-versus-host disease. *Nat Med*. 10:510–517. doi:10.1038/nm1038.

Merad, M., M.G. Manz, H. Karsunky, A. Wagers, W. Peters, I. Charo, I.L. Weissman, J.G. Cyster, and E.G. Engleman. 2002. Langerhans cells renew in the skin throughout life under steady-state conditions. *Nature immunology*. 3:1135–1141. doi:10.1038/NI852.

Merad, M., P. Sathe, J. Helft, J. Miller, and A. Mortha. 2013. The Dendritic Cell Lineage: Ontogeny and Function of Dendritic Cells and Their Subsets in the Steady State and the Inflamed Setting. *Annual Review of Immunology*. 31:563–604. doi:10.1146/annurev-immunol-020711-074950.

Mescher, M.F., P. Agarwal, K.A. Casey, C.D. Hammerbeck, Z. Xiao, and J.M. Curtsinger. 2007. Molecular basis for checkpoints in the CD8 T cell response: Tolerance versus activation. *Seminars in Immunology*. 19:153–161. doi:10.1016/j.smim.2007.02.007.

Mesnil, C., C.M. Sabatel, T. Marichal, M. Toussaint, D. Cataldo, P.-V. Drion, P. Lekeux, F. Bureau, and C.J. Desmet. 2012. Resident CD11b(+)Ly6C(-) lung dendritic cells are responsible for allergic airway sensitization to house dust mite in mice. *PLoS One*. 7:e53242. doi:10.1371/journal.pone.0053242.

Metlay, J.P., M.D. Witmer-Pack, R. Agger, M.T. Crowley, D. Lawless, and R.M. Steinman. 1990. The distinct leukocyte integrins of mouse spleen dendritic cells as identified with new hamster monoclonal antibodies. *J Exp Med*. 171:1753–1771. doi:10.1084/jem.171.5.1753.

Milner, J.J., C. Toma, B. Yu, K. Zhang, K. Omilusik, A.T. Phan, D. Wang, A.J. Getzler, T. Nguyen, S. Crotty, W. Wang, M.E. Pipkin, and A.W. Goldrath. 2017. Runx3 programs CD8+ T cell residency in non-lymphoid tissues and tumours. *Nature*. 552:253–257. doi:10.1038/nature24993.

Mizukawa, Y., Y. Yamazaki, and T. Shiohara. 2008. In vivo dynamics of intraepidermal CD8+ T cells and CD4+ T cells during the evolution of fixed drug eruption. *British Journal of Dermatology*. 158:1230–1238. doi:10.1111/j.1365-2133.2008.08516.x.

Modrow, S., D. Falke, U. Truyen, and H. Schätzl. 2013. Molecular virology. 9783642207. 1–1016 pp.

Mohammed, R.N., H.A. Watson, M. Vigar, J. Ohme, A. Thomson, I.R. Humphreys, and A. Ager. 2016. L-selectin Is Essential for Delivery of Activated CD8+ T Cells to Virus-Infected Organs for Protective Immunity. *Cell Rep*. 14:760–771. doi:10.1016/j.celrep.2015.12.090.

Moran, A.E., K.L. Holzapfel, Y. Xing, N.R. Cunningham, J.S. Maltzman, J. Punt, and K.A. Hogquist. 2011. T cell receptor signal strength in Treg and iNKT cell development demonstrated by a novel fluorescent reporter mouse. *J Exp Med*. 208:1279–1289. doi:jem.20110308 [pii] 10.1084/jem.20110308.

Morrison, S.J., and D.T. Scadden. 2014. The bone marrow niche for haematopoietic stem cells. *Nature*. 505:327–34. doi:10.1038/nature12984.

Mueller, S.N., C.M. Jones, A.T. Stock, M. Suter, W.R. Heath, and F.R. Carbone. 2006. CD4+ T Cells Can Protect APC from CTL-Mediated Elimination1. *The Journal of Immunology*. 176:7379–7384. doi:10.4049/jimmunol.176.12.7379.

Munitic, I., M. Kuka, A. Allam, J.P. Scoville, and J.D. Ashwell. 2013. CD70 deficiency impairs effector CD8 T cell generation and viral clearance but is dispensable for the recall response to lymphocytic choriomeningitis virus. *J Immunol*. 190:1169–1179. doi:10.4049/jimmunol.1202353.

Murphy, K.M., P. Travers, and M. Walport. 2009. Janeway Immunologie. 7th edition. 1119 pp.

Muschaweckh, A., V.R. Buchholz, A. Fellenzer, C. Hessel, P.-A. König, S. Tao, R. Tao, M. Heikenwälder, D.H. Busch, T. Korn, W. Kastenmüller, I. Drexler, and G. Gasteiger. 2016. Antigen-dependent competition shapes the local repertoire of

tissue-resident memory CD8 + T cells. *The Journal of Experimental Medicine*. jem.20160888. doi:10.1084/jem.20160888.

Nagao, K., F. Ginhoux, W.W. Leitner, S.I. Motegi, C.L. Bennett, B.E. Clausen, M. Merad, and M.C. Udey. 2009. Murine epidermal Langerhans cells and langerin-expressing dermal dendritic cells are unrelated and exhibit distinct functions. *Proceedings of the National Academy of Sciences of the United States of America*. 106:3312–3317. doi:10.1073/PNAS.0807126106.

Nagao, K., T. Kobayashi, K. Moro, M. Ohyama, T. Adachi, D.Y. Kitashima, S. Ueha, K. Horiuchi, H. Tanizaki, K. Kabashima, A. Kubo, Y. Cho, B.E. Clausen, K. Matsushima, M. Suematsu, G.C. Furtado, S.A. Lira, J.M. Farber, M.C. Udey, and M. Amagai. 2012. Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin. *Nat Immunol*. 13:744–752. doi:10.1038/ni.2353.

Nair-Gupta, P., A. Baccarini, N. Tung, F. Seyffer, O. Florey, Y. Huang, M. Banerjee, M. Overholtzer, P.A. Roche, R. Tampé, B.D. Brown, D. Amsen, S.W. Whiteheart, and J.M. Blander. 2014. TLR signals induce phagosomal MHC-I delivery from the endosomal recycling compartment to allow cross-presentation. *Cell*. 158:506–521. doi:10.1016/j.cell.2014.04.054.

Nakano, H., J.E. Burgents, K. Nakano, G.S. Whitehead, C. Cheong, C.D. Bortner, and D.N. Cook. 2013. Migratory properties of pulmonary dendritic cells are determined by their developmental lineage. *Mucosal Immunol*. 6:678–691. doi:10.1038/mi.2012.106.

Nakano, H., K.L. Lin, M. Yanagita, C. Charbonneau, D.N. Cook, T. Kakiuchi, and M.D. Gunn. 2009. Blood-derived inflammatory dendritic cells in lymph nodes stimulate acute T helper type 1 immune responses. *Nature immunology*. 10:394–402. doi:10.1038/ni.1707.

Nakano, H., M.R. Lyons-Cohen, G.S. Whitehead, K. Nakano, and D.N. Cook. 2017. Distinct functions of CXCR4, CCR2, and CX3CR1 direct dendritic cell precursors from the bone marrow to the lung. *Journal of Leukocyte Biology*. 101:1143–1153. doi:10.1189/jlb.1A0616-285R.

Ng, L.G., Z. Liu, I. Kwok, and F. Ginhoux. 2023. Origin and Heterogeneity of Tissue Myeloid Cells: A Focus on GMP-Derived Monocytes and Neutrophils. *Annu. Rev. Immunol.* 41:375–404. doi:10.1146/annurev-immunol-081022-113627.

Nicolaidou, E., C. Antoniou, A.J. Stratigos, C. Stefanaki, and A.D. Katsambas. 2007. Efficacy, predictors of response, and long-term follow-up in patients with vitiligo treated with narrowband UVB phototherapy. *Journal of the American Academy of Dermatology*. 56:274–278. doi:10.1016/j.jaad.2006.09.004.

Nielsen, M.M., D.A. Witherden, and W.L. Havran. 2017. γδ T cells in homeostasis and host defence of epithelial barrier tissues. *Nature reviews. Immunology*. 17:733–745. doi:10.1038/NRI.2017.101.

Noordegraaf, M., V. Flacher, P. Stoitzner, and B.E. Clausen. 2010. Functional redundancy of Langerhans cells and Langerin+ dermal dendritic cells in contact hypersensitivity. *J Invest Dermatol.* 130:2752–2759. doi:10.1038/jid.2010.223.

Norbury, C.C., D. Malide, J.S. Gibbs, J.R. Bennink, and J.W. Yewdell. 2002. Visualizing priming of virus-specific CD8+ T cells by infected dendritic cells in vivo. *Nat Immunol*. 3:265–271. doi:10.1038/ni762.

Novy, P., M. Quigley, X. Huang, and Y. Yang. 2007. CD4 T Cells Are Required for CD8 T Cell Survival during Both Primary and Memory Recall Responses1. *The Journal of Immunology*. 179:8243–8251. doi:10.4049/jimmunol.179.12.8243.

Nussenzweig, M.C., R.M. Steinman, J.C. Unkeless, M.D. Witmer, B. Gutchinov, and Z.A. Cohn. 1981. Studies of the cell surface of mouse dendritic cells and other leukocytes. *J Exp Med*. 154:168–187. doi:10.1084/jem.154.1.168.

Obar, J.J., and L. Lefranois. 2010. Memory CD8+ T cell differentiation. *Annals of the New York Academy of Sciences*. 1183:251–266. doi:10.1111/j.1749-6632.2009.05126.x.

Obar, J.J., M.J. Molloy, E.R. Jellison, T.A. Stoklasek, W. Zhang, E.J. Usherwood, and L. Lefrançois. 2010. CD4+ T cell regulation of CD25 expression controls development of short-lived effector CD8+ T cells in primary and secondary responses. *Proc Natl Acad Sci U S A*. 107:193–198. doi:10.1073/pnas.0909945107.

Ochsenbein, A.F., D.D. Pinschewer, S. Sierro, E. Horvath, H. Hengartner, and R.M. Zinkernagel. 2000. Protective long-term antibody memory by antigen-driven and T help-dependent differentiation of long-lived memory B cells to short-lived plasma cells independent of secondary lymphoid organs. *Proceedings of the National Academy of Sciences of the United States of America*. 97:13263–13268. doi:10.1073/pnas.230417497.

Oh, J.Z., J.S. Kurche, M.A. Burchill, and R.M. Kedl. 2011. TLR7 enables cross-presentation by multiple dendritic cell subsets through a type I IFN-dependent pathway. *Blood*. 118:3028–3038. doi:10.1182/blood-2011-04-348839.

Oh, S., L.P. Perera, M. Terabe, L. Ni, T.A. Waldmann, and J.A. Berzofsky. 2008. IL-15 as a mediator of CD4+ help for CD8+ T cell longevity and avoidance of TRAIL-mediated apoptosis. *Proc Natl Acad Sci U S A*. 105:5201–5206. doi:10.1073/pnas.0801003105.

Orlik, C., D. Deibel, J. Küblbeck, E. Balta, S. Ganskih, J. Habicht, B. Niesler, J. Schröder-Braunstein, K. Schäkel, G. Wabnitz, and Y. Samstag. 2020. Keratinocytes costimulate naive human T cells via CD2: a potential target to prevent the development of proinflammatory Th1 cells in the skin. *Cell Mol Immunol*. 17:380–394. doi:10.1038/s41423-019-0261-x.

Orozco, S.L., S.P. Canny, and J.A. Hamerman. 2021. Signals governing monocyte differentiation during inflammation. *Current Opinion in Immunology*. 73:16–24. doi:10.1016/j.coi.2021.07.007.

Palframan, R.T., S. Jung, G. Cheng, W. Weninger, Y. Luo, M. Dorf, D.R. Littman, B.J. Rollins, H. Zweerink, and A. Rot. 2001. Inflammatory Chemokine Transport and Presentation in HEV: A Remote Control Mechanism for Monocyte Recruitment to Lymph Nodes in Inflamed Tissues.

Parekh, N.J., T.E. Krouse, I.E. Reider, R.P. Hobbs, B.M. Ward, and C.C. Norbury. 2019. Type I interferon-dependent CCL4 is induced by a cGAS/STING pathway that bypasses viral inhibition and protects infected tissue, independent of viral burden. *PLOS Pathogens*. 15:e1007778. doi:10.1371/JOURNAL.PPAT.1007778.

Parker, S., D.A. Schultz, H. Meyer, and R.M. Buller. 2014. Smallpox and Monkeypox Viruses☆. *In* Reference Module in Biomedical Sciences. Elsevier.

Pasparakis, M., I. Haase, and F.O. Nestle. 2014. Mechanisms regulating skin immunity and inflammation. *Nature Reviews Immunology*. 14:289–301. doi:10.1038/nri3646.

Pham, N.-L.L., V.P. Badovinac, and J.T. Harty. 2009. A Default Pathway of Memory CD8 T Cell Differentiation after Dendritic Cell Immunization Is Deflected by Encounter with Inflammatory Cytokines during Antigen-Driven Proliferation1. *The Journal of Immunology*. 183:2337–2348. doi:10.4049/jimmunol.0901203.

Pipkin, M.E., J.A. Sacks, F. Cruz-Guilloty, M.G. Lichtenheld, M.J. Bevan, and A. Rao. 2010. Interleukin-2 and Inflammation Induce Distinct Transcriptional Programs that Promote the Differentiation of Effector Cytolytic T Cells. *Immunity*. 32:79–90. doi:10.1016/j.immuni.2009.11.012.

Pircher, H., K. Bürki, R. Lang, H. Hengartner, and R.M. Zinkernagel. 1989. Tolerance induction in double specific T-cell receptor transgenic mice varies with antigen. *Nature*. 342:559–561. doi:10.1038/342559a0.

Polak, M.E., L. Newell, V.Y. Taraban, C. Pickard, E. Healy, P.S. Friedmann, A. Al-Shamkhani, and M.R. Ardern-Jones. 2012. CD70–CD27 Interaction Augments CD8+ T-Cell Activation by Human Epidermal Langerhans Cells. *Journal of Investigative Dermatology*. 132:1636–1644. doi:10.1038/jid.2012.26.

Poltorak, M.P., and B.U. Schraml. 2015. Fate Mapping of Dendritic Cells. *Front Immunol*. 6:199. doi:10.3389/fimmu.2015.00199.

Poulin, L.F., S. Henri, B. de Bovis, E. Devilard, A. Kissenpfennig, and B. Malissen. 2007. The dermis contains langerin+ dendritic cells that develop and function independently of epidermal Langerhans cells. *Journal of Experimental Medicine*. 204:3119–3131. doi:10.1084/jem.20071724.

Price, J.G., J. Idoyaga, H. Salmon, B. Hogstad, C.L. Bigarella, S. Ghaffari, M. Leboeuf, and M. Merad. 2015. The cell cycle inhibitor Cdkn1a regulates Langerhans cell radiation resistance and promotes T regulatory cell generation upon exposure to ionizing irradiation. *Nat Immunol*. 16:1060–1068. doi:10.1038/ni.3270.

Probst, H.C., J. Lagnel, G. Kollias, and M. van den Broek. 2003. Inducible transgenic mice reveal resting dendritic cells as potent inducers of CD8+ T cell tolerance. *Immunity*. 18:713–720. doi:10.1016/s1074-7613(03)00120-1.

Probst, H.C., P. Stoitzner, L. Amon, R.A. Backer, A. Brand, J. Chen, B.E. Clausen, S. Dieckmann, D. Dudziak, L. Heger, K. Hodapp, F. Hornsteiner, A. Hovav, L. Jacobi, X. Ji, N. Kamenjarin, K. Lahl, I. Lahmar, J. Lakus, C.H.K. Lehmann, D. Ortner, M. Picard, M.P. Roberti, L. Rossnagel, Y. Saba, C. Schalla, A. Schlitzer, B.U. Schraml, K. Schütze, A. Seichter, K. Seré, A. Seretis, S. Sopper, H. Strandt, M.M. Sykora, H. Theobald, C.H. Tripp, and L. Zitvogel. 2022. Guidelines for DC preparation and flow cytometry analysis of mouse nonlymphoid tissues. *Eur J Immunol*. eji.202249819. doi:10.1002/eji.202249819.

Pyeon, D., S.M. Pearce, S.M. Lank, P. Ahlquist, and P.F. Lambert. 2009. Establishment of human papillomavirus infection requires cell cycle progression. *PLoS Pathog*. 5:e1000318. doi:10.1371/journal.ppat.1000318.

Ream, R.M., J. Sun, and T.J. Braciale. 2010. Stimulation of Naive CD8+ T Cells by a Variant Viral Epitope Induces Activation and Enhanced Apoptosis. *The Journal of Immunology*. 184:2401–2409. doi:10.4049/jimmunol.0902448.

Ridge, J.P., F. Di Rosa, and P. Matzinger. 1998. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature*. 393:474–478. doi:10.1038/30989.

Roberts, A.D., and D.L. Woodland. 2004. Cutting edge: effector memory CD8+ T cells play a prominent role in recall responses to secondary viral infection in the lung. *Journal of immunology (Baltimore, Md. : 1950)*. 172:6533–7. doi:10.4049/JIMMUNOL.172.11.6533.

Romani, N., P.M. Brunner, and G. Stingl. 2012. Changing Views of the Role of Langerhans Cells. *J Invest Dermatol*. 132:872–881. doi:10.1038/jid.2011.437.

Romano, E., J.W. Cotari, R.B. Da Silva, B.C. Betts, D.J. Chung, F. Avogadri, M.J. Fink, E.T. St Angelo, B. Mehrara, G. Heller, C. Münz, G. Altan-Bonnet, and J.W. Young. 2012. Human Langerhans cells use an IL-15R-α/IL-15/pSTAT5-dependent mechanism to break T-cell tolerance against the self-differentiation tumor antigen WT1. *Blood*. 119:5182–5190. doi:10.1182/BLOOD-2011-09-382200.

Roos, D., R. van Bruggen, and C. Meischl. 2003. Oxidative killing of microbes by neutrophils. *Microbes and Infection*. 5:1307–1315. doi:10.1016/j.micinf.2003.09.009.

Rosato, P.C., L.K. Beura, and D. Masopust. 2017. Tissue resident memory T cells and viral immunity. *Current Opinion in Virology*. 22:44–50. doi:10.1016/j.coviro.2016.11.011.

Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *Journal of Experimental Medicine*. 179:1109–1118. doi:10.1084/jem.179.4.1109.

Sallusto, F., D. Lenig, R. Förster, M. Lipp, and A. Lanzavecchia. 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 401:708–12. doi:10.1038/44385.

Scarborough, J., F. Mueller, R. Arban, C. Dorner-Ciossek, U. Weber-Stadlbauer, H. Rosenbrock, U. Meyer, and J. Richetto. 2020. Preclinical validation of the micropipette-guided drug administration (MDA) method in the maternal immune activation model of neurodevelopmental disorders. *Brain, Behavior, and Immunity*. 88:461–470. doi:10.1016/j.bbi.2020.04.015.

Schenkel, J.M., K. a Fraser, L.K. Beura, K.E. Pauken, D. Masopust, V. Vezys, and D. Masopust. 2014a. Resident memory CD8 T cells trigger protective innate and adaptive immune responses. *Science (New York, N.Y.)*. 346:98–101. doi:10.1126/science.1254536.

Schenkel, J.M., K.A. Fraser, L.K. Beura, K.E. Pauken, V. Vezys, and D. Masopust. 2014b. Resident memory CD8 T cells trigger protective innate and adaptive immune responses. *Science (New York, NY)*. 346:98–101.

Schenkel, J.M., K.A. Fraser, K.A. Casey, L.K. Beura, K.E. Pauken, V. Vezys, and D. Masopust. 2016. IL-15-Independent Maintenance of Tissue-Resident and Boosted Effector Memory CD8 T Cells. *J Immunol*. 196:3920–3926. doi:10.4049/jimmunol.1502337.

Schenkel, J.M., and D. Masopust. 2014. Tissue-Resident Memory T Cells. *Immunity*. 41:886–897. doi:10.1016/j.immuni.2014.12.007.

Schlitzer, A., V. Sivakamasundari, J. Chen, H.R.B. Sumatoh, J. Schreuder, J. Lum, B. Malleret, S. Zhang, A. Larbi, F. Zolezzi, L. Renia, M. Poidinger, S. Naik, E.W. Newell, P. Robson, and F. Ginhoux. 2015. Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. *Nat Immunol*. 16:718–728. doi:10.1038/ni.3200.

Schluns, K.S., K. Williams, A. Ma, X.X. Zheng, and L. Lefrançois. 2002. Cutting edge: requirement for IL-15 in the generation of primary and memory antigen-specific CD8 T cells. *J Immunol*. 168:4827–4831. doi:10.4049/jimmunol.168.10.4827.

Schoenberger, S.P., R.E. Toes, E.I. van der Voort, R. Offringa, and C.J. Melief. 1998. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature*. 393:480–483. doi:10.1038/31002.

Schutyser, E., S. Struyf, and J. Van Damme. 2003. The CC chemokine CCL20 and its receptor CCR6. *Cytokine & Growth Factor Reviews*. 14:409–426. doi:10.1016/S1359-6101(03)00049-2.

Schwarz, A., M. Noordegraaf, A. Maeda, K. Torii, B.E. Clausen, and T. Schwarz. 2010. Langerhans Cells Are Required for UVR-Induced Immunosuppression. *Journal of Investigative Dermatology*. 130:1419–1427. doi:10.1038/jid.2009.429.

Scott, C.L., C.C. Bain, P.B. Wright, D. Sichien, K. Kotarsky, E.K. Persson, K. Luda, M. Guilliams, B.N. Lambrecht, W.W. Agace, S.W. Milling, and A.M. Mowat. 2015. CCR2+CD103− intestinal dendritic cells develop from DC-committed precursors and induce interleukin-17 production by T cells. *Mucosal Immunology*. 8:327–339. doi:10.1038/mi.2014.70.

Seneschal, J., R.A. Clark, A. Gehad, C.M. Baecher-Allan, and T.S. Kupper. 2012. Human epidermal Langerhans cells maintain immune homeostasis in skin by activating skin resident regulatory T cells. *Immunity*. 36:873–884. doi:10.1016/J.IMMUNI.2012.03.018.

Seneschal, J., X. Jiang, and T.S. Kupper. 2014. Langerin+ dermal DC, but not Langerhans cells, are required for effective CD8-mediated immune responses after skin scarification with vaccinia virus. *J Invest Dermatol*. 134:686–694. doi:10.1038/jid.2013.418.

Serbina, N.V., T. Jia, T.M. Hohl, and E.G. Pamer. 2008. Monocyte-mediated defense against microbial pathogens. *Annual review of immunology*. 26:421–52. doi:10.1146/annurev.immunol.26.021607.090326.

Sharpe, A.H., and G.J. Freeman. 2002. The B7–CD28 superfamily. *Nat Rev Immunol*. 2:116–126. doi:10.1038/nri727.

Shedlock, D.J., and H. Shen. 2003. Requirement for CD4 T cell help in generating functional CD8 T cell memory. *Science*. 300:337–339. doi:10.1126/science.1082305.

Shibaki, A., A. Sato, J.C. Vogel, F. Miyagawa, and S.I. Katz. 2004. Induction of GVHD-like skin disease by passively transferred CD8(+) T-cell receptor transgenic T cells into keratin 14-ovalbumin transgenic mice. *J Invest Dermatol*. 123:109–115. doi:10.1111/j.0022-202X.2004.22701.x.

Shin, H., Y. Kumamoto, S. Gopinath, and A. Iwasaki. 2016. CD301b+ dendritic cells stimulate tissue-resident memory CD8+ T cells to protect against genital HSV-2. *Nat Commun*. 7:13346. doi:10.1038/ncomms13346.

Shklovskaya, E., B. Roediger, and B. Fazekas de St Groth. 2008. Epidermal and dermal dendritic cells display differential activation and migratory behavior while sharing the ability to stimulate CD4+ T cell proliferation in vivo. *J Immunol*. 181:418–430. doi:10.4049/jimmunol.181.1.418.

Sigal, L.J., S. Crotty, R. Andino, and K.L. Rock. 1999. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. *Nature*. 398:77–80. doi:10.1038/18038.

Sigal, L.J., and K.L. Rock. 2000. Bone marrow-derived antigen-presenting cells are required for the generation of cytotoxic T lymphocyte responses to viruses and use transporter associated with antigen presentation (TAP)-dependent and -independent pathways of antigen presentation. *J Exp Med*. 192:1143–1150. doi:10.1084/jem.192.8.1143.

Sigmundsdottir, H., A. Johnston, J.E. Gudjonsson, and H. Valdimarsson. 2005. Narrowband–UVB irradiation decreases the production of pro-inflammatory cytokines by stimulated T cells. *Arch Dermatol Res*. 297:39–42. doi:10.1007/s00403-005-0565-9.

Sims, T.N., and M.L. Dustin. 2002. The immunological synapse: integrins take the stage. *Immunological Reviews*. 186:100–117. doi:10.1034/j.1600-065X.2002.18610.x.

Sohl, J., A.-K. Hartmann, J. Hahlbrock, J. Bartneck, M. Stassen, M. Klein, M. Bros, S. Grabbe, F. Marini, K. Woods, B. Guezguez, M. Mack, H. Schild, S. Muth, F. Melchior, H.C. Probst, P. Langguth, and M.P. Radsak. 2022. Dithranol as novel co-adjuvant for non-invasive dermal vaccination. *npj Vaccines*. 7:1–13. doi:10.1038/s41541-022-00530-9.

Sperling, A.I., J.A. Auger, B.D. Ehst, I.C. Rulifson, C.B. Thompson, and J.A. Bluestone. 1996. CD28/B7 interactions deliver a unique signal to naive T cells that regulates cell survival but not early proliferation. *J Immunol*. 157:3909–3917.

Sporri, R., and S. Reis e. 2005. Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4+ T cell populations lacking helper function. *Nat.Immunol.* 6:163–170. doi:10.1038/ni1162.

Sprent, J., D. Lo, E.-K. Gao, and Y. Ron. 1988. T Cell Selection in the Thymus. *Immunological Reviews*. 101:173–190. doi:10.1111/j.1600-065X.1988.tb00737.x.

Srinivas, S., T. Watanabe, C.S. Lin, C.M. William, Y. Tanabe, T.M. Jessell, and F. Costantini. 2001. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol*. 1:4. doi:10.1186/1471-213x-1-4.

Starr, T.K., S.C. Jameson, and K.A. Hogquist. 2003. Positive and negative selection of T cells. *Annual review of immunology*. 21:139–76. doi:10.1146/annurev.immunol.21.120601.141107.

Steinert, E.M., J.M. Schenkel, K.A. Fraser, L.K. Beura, L.S. Manlove, B.Z. Igyártó, P.J. Southern, and D. Masopust. 2015. Quantifying memory CD8 T cells reveals regionalization of immunosurveillance. *Cell*. doi:10.1016/j.cell.2015.03.031.

Steinman, R.M., and Z. a Cohn. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *The Journal of experimental medicine*. 137:1142–1162. doi:10.1084/jem.137.5.1142.

Steinman, R.M., G. Kaplan, M.D. Witmer, and Z.A. Cohn. 1979. Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance in vitro. *J Exp Med*. 149:1–16. doi:10.1084/jem.149.1.1.

Stewart, A.J., and P.M. Devlin. 2006. The history of the smallpox vaccine. *Journal of Infection*. 52:329–334. doi:10.1016/j.jinf.2005.07.021.

van Stipdonk, M.J.B., E.E. Lemmens, and S.P. Schoenberger. 2001. Naïve CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. *Nat Immunol*. 2:423–429. doi:10.1038/87730.

Stoitzner, P., C.H. Tripp, A. Eberhart, K.M. Price, J.Y. Jung, L. Bursch, F. Ronchese, and N. Romani. 2006. Langerhans cells cross-present antigen derived from skin. *Proceedings of the National Academy of Sciences of the United States of America*. 103:7783–7788. doi:10.1073/PNAS.0509307103.

Stout, R.D., J. Suttles, J. Xu, I.S. Grewal, and R.A. Flavell. 1996. Impaired T cell-mediated macrophage activation in CD40 ligand-deficient mice. *J Immunol*. 156:8–11.

Sun, J.C., and M.J. Bevan. 2003. Defective CD8 T cell memory following acute infection without CD4 T cell help. *Science*. 300:339–342. doi:10.1126/science.1083317.

Suzuki, H., B. Wang, G.M. Shivji, P. Toto, P. Amerio, M.A. Tomai, R.L. Miller, and D.N. Sauder. 2000. Imiquimod, a topical immune response modifier, induces migration of Langerhans cells. *J Invest Dermatol*. 114:135–141. doi:10.1046/j.1523-1747.2000.00833.x.

Swirski, F.K., M. Nahrendorf, M. Etzrodt, M. Wildgruber, V. Cortez-Retamozo, P. Panizzi, J.-L. Figueiredo, R.H. Kohler, A. Chudnovskiy, P. Waterman, E. Aikawa, T.R. Mempel, P. Libby, R. Weissleder, and M.J. Pittet. 2009. Identification of

Splenic Reservoir Monocytes and Their Deployment to Inflammatory Sites. *Science*. 325:612–616. doi:10.1126/science.1175202.

Taguchi, K., A. Fukunaga, K. Ogura, and C. Nishigori. 2013. The role of epidermal Langerhans cells in NB-UVB-induced immunosuppression. *Kobe J Med Sci*. 59:E1-9.

Takahama, Y. 2006. Journey through the thymus: stromal guides for T-cell development and selection. *Nature reviews. Immunology*. 6:127–35. doi:10.1038/nri1781.

Tamoutounour, S., M. Guilliams, F. MontananaSanchis, H. Liu, D. Terhorst, C. Malosse, E. Pollet, L. Ardouin, H. Luche, C. Sanchez, M. Dalod, B. Malissen, and S. Henri. 2013. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity*. 39:925–938. doi:10.1016/j.immuni.2013.10.004.

Tamoutounour, S., S.-J. Han, J. Deckers, M.G. Constantinides, C. Hurabielle, O.J. Harrison, N. Bouladoux, J.L. Linehan, V.M. Link, I. Vujkovic-Cvijin, P.J. Perez-Chaparro, S.P. Rosshart, B. Rehermann, V. Lazarevic, and Y. Belkaid. 2019. Keratinocyte-intrinsic MHCII expression controls microbiota-induced Th1 cell responses. *Proceedings of the National Academy of Sciences*. 116:23643–23652. doi:10.1073/pnas.1912432116.

Tan, J.T., B. Ernst, W.C. Kieper, E. LeRoy, J. Sprent, and C.D. Surh. 2002. Interleukin (IL)-15 and IL-7 Jointly Regulate Homeostatic Proliferation of Memory Phenotype CD8+ Cells but Are Not Required for Memory Phenotype CD4+ Cells. *Journal of Experimental Medicine*. 195:1523–1532. doi:10.1084/jem.20020066.

Thomas, G., R. Tacke, C.C. Hedrick, and R.N. Hanna. 2015. Nonclassical Patrolling Monocyte Function in the Vasculature. *Arterioscler Thromb Vasc Biol*. 35:1306–1316. doi:10.1161/ATVBAHA.114.304650.

Tomura, M., A. Hata, S. Matsuoka, F.H.W. Shand, Y. Nakanishi, R. Ikebuchi, S. Ueha, H. Tsutsui, K. Inaba, K. Matsushima, A. Miyawaki, K. Kabashima, T. Watanabe, and O. Kanagawa. 2014. Tracking and quantification of dendritic cell migration and antigen trafficking between the skin and lymph nodes. *Sci Rep*. 4:6030. doi:10.1038/srep06030.

Topham, D.J., and E.C. Reilly. 2018. Tissue-Resident Memory CD8+ T Cells: From Phenotype to Function. *Frontiers in Immunology*. 9.

Tripp, C.H., B. Haid, V. Flacher, M. Sixt, H. Peter, J. Farkas, R. Gschwentner, L. Sorokin, N. Romani, and P. Stoitzner. 2008. The lymph vessel network in mouse skin visualised with antibodies against the hyaluronan receptor LYVE-1. *Immunobiology*. 213:715–728. doi:10.1016/j.imbio.2008.07.025.

Tscharke, D.C., and G.L. Smith. 1999. A model for vaccinia virus pathogenesis and immunity based on intradermal injection of mouse ear pinnae. *Journal of General Virology*. 80:2751–2755. doi:10.1099/0022-1317-80-10-2751.

Tsou, C.-L., W. Peters, Y. Si, S. Slaymaker, A.M. Aslanian, S.P. Weisberg, M. Mack, and I.F. Charo. 2007. Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J Clin Invest*. 117:902–909. doi:10.1172/JCI29919.

Uematsu, S., and S. Akira. 2008. Pathogen recognition by innate immunity. *Skin Research*. 7:1–11. doi:10.1007/s10156-008-0596-1.

Van Braeckel-Budimir, N., S.M. Varga, V.P. Badovinac, and J.T. Harty. 2018. Repeated Antigen Exposure Extends the Durability of Influenza-Specific Lung-Resident Memory CD8+ T Cells and Heterosubtypic Immunity. *Cell Rep*. 24:3374-3382.e3. doi:10.1016/j.celrep.2018.08.073.

Van Parijs, L., A. Ibraghimov, and A.K. Abbas. 1996. The roles of costimulation and Fas in T cell apoptosis and peripheral tolerance. *Immunity*. 4:321–328. doi:10.1016/s1074-7613(00)80440-9.

Varol, C., A. Mildner, and S. Jung. 2015. Macrophages: Development and Tissue Specialization. *Annual Review of Immunology*. 33:643–675. doi:10.1146/annurev-immunol-032414-112220.

Vasioukhin, V., L. Degenstein, B. Wise, and E. Fuchs. 1999. The magical touch: genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. *Proc Natl Acad Sci U S A*. 96:8551–8556.

Vassar, R., M. Rosenberg, S. Ross, A. Tyner, and E. Fuchs. 1989. Tissue-specific and differentiation-specific expression of a human K14 keratin gene in transgenic mice. *Proceedings of the National Academy of Sciences*. 86:1563–1567. doi:10.1073/pnas.86.5.1563.

Vermaelen, K.Y., I. Carro-Muino, B.N. Lambrecht, and R.A. Pauwels. 2000. Specific Migratory Dendritic Cells Rapidly Transport Antigen from the Airways to the Thoracic Lymph Nodes. *Journal of Experimental Medicine*. 193:51–60. doi:10.1084/jem.193.1.51.

Vonderheide, R.H. 2018. The Immune Revolution: A Case for Priming, Not Checkpoint. *Cancer Cell*. 33:563–569. doi:10.1016/j.ccell.2018.03.008.

Vugmeyster, Y., R. Glas, B. Pérarnau, F.A. Lemonnier, H. Eisen, and H. Ploegh. 1998. Major histocompatibility complex (MHC) class I KbDb -/- deficient mice possess functional CD8+ T cells and natural killer cells. *Proceedings of the National Academy of Sciences of the United States of America*. 95:12492–12497. doi:10.1073/PNAS.95.21.12492.

Waithman, J., R.S. Allan, H. Kosaka, H. Azukizawa, K. Shortman, M.B. Lutz, W.R. Heath, F.R. Carbone, and G.T. Belz. 2007. Skin-Derived Dendritic Cells Can Mediate Deletional Tolerance of Class I-Restricted Self-Reactive T Cells. *The Journal of Immunology*. 179:4535–4541. doi:10.4049/jimmunol.179.7.4535.

Wakim, L.M., N. Gupta, J.D. Mintern, and J.A. Villadangos. 2013. Enhanced survival of lung tissue-resident memory CD8+ T cells during infection with influenza virus due to selective expression of IFITM3. *Nature Immunology*. 14:238–245. doi:10.1038/ni.2525.

Wakim, L.M., J. Waithman, N. van Rooijen, W.R. Heath, and F.R. Carbone. 2008. Dendritic cell-induced memory T cell activation in nonlymphoid tissues. *Science (New York, N.Y.)*. 319:198–202. doi:10.1126/science.1151869.

Wang, L., L.S. Bursch, A. Kissenpfennig, B. Malissen, S.C. Jameson, and K.A. Hogquist. 2008. Langerin expressing cells promote skin immune responses under defined conditions. *J Immunol*. 180:4722–4727. doi:10.4049/jimmunol.180.7.4722.

Wang, X., S. Zinkel, K. Polonsky, and E. Fuchs. 1997. Transgenic studies with a keratin promoter-driven growth hormone transgene: Prospects for gene therapy. *Proceedings of the National Academy of Sciences*. 94:219–226. doi:10.1073/pnas.94.1.219.

Watt, F.M. 2014. Mammalian skin cell biology: at the interface between laboratory and clinic. *Science (New York, N.Y.)*. 346:937–940. doi:10.1126/SCIENCE.1253734.

Weber, C., R. Alon, B. Moser, and T.A. Springer. 1996. Sequential regulation of alpha 4 beta 1 and alpha 5 beta 1 integrin avidity by CC chemokines in monocytes: implications for transendothelial chemotaxis. *J Cell Biol*. 134:1063–1073. doi:10.1083/jcb.134.4.1063.

Weinlich, G., M. Heine, H. Stossel, M. Zanella, P. Stoitzner, U. Ortner, J. Smolle, F. Koch, N.T. Sepp, G. Schuler, and N. Romani. 1998. Entry Into Afferent Lymphatics and Maturation In Situ of Migrating Murine Cutaneous Dendritic Cells. *Journal of Investigative Dermatology*. 110:441–448. doi:10.1046/J.1523-1747.1998.00161.X.

Wherry, E.J., J.N. Blattman, K. Murali-Krishna, R. van der Most, and R. Ahmed. 2003a. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *Journal of virology*. 77:4911–27. doi:10.1128/jvi.77.8.4911-4927.2003.

Wherry, E.J., V. Teichgräber, T.C. Becker, D. Masopust, S.M. Kaech, R. Antia, U.H. von Andrian, and R. Ahmed. 2003b. Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat Immunol*. 4:225–234. doi:10.1038/ni889.

Wiesel, M., N. Joller, A.-K. Ehlert, J. Crouse, R. Spörri, M.F. Bachmann, and A. Oxenius. 2010. Th cells act via two synergistic pathways to promote antiviral CD8+ T cell responses. *J Immunol*. 185:5188–5197. doi:10.4049/jimmunol.1001990.

Williams, M.A., A.J. Tyznik, and M.J. Bevan. 2006. Interleukin-2 signals during priming are required for secondary expansion of CD8+memory T cells. *Nature*. 441:890–893. doi:10.1038/nature04790.

Wilson, A., and A. Trumpp. 2006. Bone-marrow haematopoietic-stem-cell niches. *Nature reviews. Immunology*. 6:93–106. doi:10.1038/nri1779.

Wolkers, M.C., C. Gerlach, R. Arens, E.M. Janssen, P. Fitzgerald, T.N. Schumacher, J.P. Medema, D.R. Green, and S.P. Schoenberger. 2012. Nab2 regulates secondary CD8+ T-cell responses through control of TRAIL expression. *Blood*. 119:798–804. doi:10.1182/blood-2011-08-373910.

Woodworth, C.D., and S. Simpson. 1993. Comparative lymphokine secretion by cultured normal human cervical keratinocytes, papillomavirus-immortalized, and carcinoma cell lines. *Am J Pathol*. 142:1544–1555.

Wu, T.-S., J.-M. Lee, Y.-G. Lai, J.-C. Hsu, C.-Y. Tsai, Y.-H. Lee, and N.-S. Liao. 2002. Reduced expression of Bcl-2 in CD8+ T cells deficient in the IL-15 receptor alpha-chain. *J Immunol*. 168:705–712. doi:10.4049/jimmunol.168.2.705.

Yang, K., and A. Kallies. 2021. Tissue-specific differentiation of CD8+ resident memory T cells. *Trends Immunol*. 42:876–890. doi:10.1016/j.it.2021.08.002.

Yona, S., K.W. Kim, Y. Wolf, A. Mildner, D. Varol, M. Breker, D. Strauss-Ayali, S. Viukov, M. Guilliams, A. Misharin, D.A. Hume, H. Perlman, B. Malissen, E. Zelzer, and S. Jung. 2013. Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages under Homeostasis. *Immunity*. 38:79–91. doi:10.1016/J.IMMUNI.2012.12.001.

Zaid, A., L.K. Mackay, A. Rahimpour, A. Braun, M. Veldhoen, F.R. Carbone, J.H. Manton, W.R. Heath, and S.N. Mueller. 2014. Persistence of skin-resident memory T cells within an epidermal niche. *Proceedings of the National Academy of Sciences*. doi:10.1073/pnas.1322292111.

Zammit, D.J., L.S. Cauley, Q.-M. Pham, and L. Lefrançois. 2005. Dendritic Cells Maximize the Memory CD8 T Cell Response to Infection. *Immunity*. 22:561–570. doi:10.1016/j.immuni.2005.03.005.

Zammit, D.J., and L. Lefrançois. 2006. Dendritic cell–T cell interactions in the generation and maintenance of CD8 T cell memory. *Microbes and Infection*. 8:1108–1115. doi:10.1016/J.MICINF.2005.12.002.

Zaric, M., O. Lyubomska, C. Poux, M.L. Hanna, M.T. McCrudden, B. Malissen, R.J. Ingram, U.F. Power, C.J. Scott, R.F. Donnelly, and A. Kissenpfennig. 2015. Dissolving microneedle delivery of nanoparticle-encapsulated antigen elicits efficient cross-priming and Th1 immune responses by murine Langerhans cells. *J Invest Dermatol*. 135:425–434. doi:10.1038/jid.2014.415.

Zaric, M., O. Lyubomska, O. Touzelet, C. Poux, S. Al-Zahrani, F. Fay, L. Wallace, D. Terhorst, B. Malissen, S. Henri, U.F. Power, C.J. Scott, R.F. Donnelly, and A. Kissenpfennig. 2013. Skin dendritic cell targeting via microneedle arrays laden with antigen-encapsulated poly-D,L-lactide-co-glycolide nanoparticles induces efficient antitumor and antiviral immune responses. *ACS Nano*. 7:2042–2055. doi:10.1021/nn304235j.

Zhao, J., C. Qin, Y. Liu, Y. Rao, and P. Feng. 2021. Herpes Simplex Virus and Pattern Recognition Receptors: An Arms Race. *Frontiers in Immunology*. 11:3680. doi:10.3389/FIMMU.2020.613799/BIBTEX.

Zhou, F. 2009. Molecular mechanisms of IFN-gamma to up-regulate MHC class I antigen processing and presentation. *International reviews of immunology*. 28:239–260. doi:10.1080/08830180902978120.

Zinkernagel, R.M., and P.C. Doherty. 1997. The discovery of MHC restriction. *Immunology Today*. 18:14–17. doi:10.1016/S0167-5699(97)80008-4.

7.3 List of Figures

7.4 List of Tables

7.5 Curriculum Vitae

7.6 Authorships

Parts of this thesis are already published:

- **Nadine Kamenjarin**, Katrin Hodapp, Felix Melchior, Gregory S Harms, Ann-Kathrin Hartmann, Joschka Bartneck, Sabine Muth, Verena K. Raker, Becker Christian, Anna Brand, Björn E. Clausen, Markus P. Radsak, Hansjörg Schild, Hans Christian Probst
  'Cross-presenting Langerhans cells are required for the early reactivation of resident CD8$^+$ memory T cells in the epidermis.'
  *Proceedings of the National Academy of Sciences (PNAS) 120:e2219932120 (**2023**)*

- Probst Hans Christian and Patrizia Stoitzner, Lukas Amon, Ronald A Backer, Anna Brand, Jianzhou Chen, Björn E Clausen, Sophie Dieckmann, Diana Dudziak, Lukas Heger, Katrin Hodapp, Florian Hornsteiner, Avi-Hai Hovav, Lukas Jacobi, Xingqi Ji, **Nadine Kamenjarin**, Katharina Lahl, Imran Lahmar, Jelena Lakus, Christina H K Lehmann, Daniela Ortner, Marion Picard, Maria Paula Roberti, Lukas Rossnagel, Yasmin Saba, Carmen Schalla, Andreas Schlitzer, Barbara U Schraml, Kristian Schütze, Anna Seichter, Kristin Seré, Athanasios Seretis, Sieghart Sopper, Helen Strandt, Martina M Sykora, Hannah Theobald, Christoph H Tripp, Laurence Zitvogel
  'Guidelines for DC preparation and flow cytometry analysis of mouse nonlymphoid tissues.'
  *European Journal of Immunology (EJI) eji.202249819 (**2022**)*

Other publications released as a PhD student:

- Tanja Klaus, Alicia S. Wilson, Elisabeth Vicari, Eva Hadaschik, Matthias Klein, Sara Salome Clara Helbich, **Nadine Kamenjarin**, Katrin Hodapp, Jenny Schunke, Maximilian Haist, Florian Butsch, Hans Christian Probst, Alexander H. Enk, Karsten Mahnke, Ari Waisman, Monika Bednarczyk, Matthias Bros, Tobias Bopp and Stephan Grabbe
  'Impaired Treg-DC interactions contribute to autoimmunity in leukocyte adhesion deficiency type 1.'
  JCI Insight 7, (**2022**).

- Cristina Ferreira, Leandro Barros, Marta Baptista, Birte Blankenhaus, André Barros, Patrícia Figueiredo-Campos, Špela Konjar, Alexandra Lainé, **Nadine Kamenjarin**, Ana Stojanovic, Adelheid Cerwenka, Hans Christian Probst, Julien C. Marie and Marc Veldhoen
  'Type 1 Treg cells promote the generation of CD8$^+$ tissue-resident memory T cells.'
  Nature Immunology 21, 766–776, (**2020**)

- Tobias Hain and Felix Melchior, **Nadine Kamenjarin**, Sabine Muth, Hela Weslati, Björn E. Clausen, Karsten Mahnke, Cinthia Silva-Vilches, Kristian Schütze, Julian Sohl, Markus P. Radsak, Georg Büdgen, Tobias Bopp, Sven Danckwardt, Hansjörg Schild and Hans Christian Probst
  'Dermal CD207-Negative Migratory Dendritic Cells Are Fully Competent to Prime Protective, Skin Homing Cytotoxic T-Lymphocyte Responses.'
  Journal of Investigative Dermatology 139, 422–429 (**2019**)

- Louise Saul, Iris Mair, Alasdair Ivens, Pamela Brown, Kay Samuel, John D. M. Campbell, Daniel Y. Soong, **Nadine Kamenjarin** and Richard J. Mellanby
  '1,25-Dihydroxyvitamin D3 Restrains CD4$^+$ T Cell Priming Ability of CD11c$^+$ Dendritic Cells by Upregulating Expression of CD31.'
  Frontiers in Immunology 10, (**2019**).

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