From Department of Medicine, Solna
Karolinska Institutet, Stockholm, Sweden

# RESIDENT T CELLS IN HUMAN SKIN - FUNCTIONAL HETEROGENEITY AND CLINICAL IMPLICATIONS

**Stanley Sing Hoi Cheuk**
卓星愷


Karolinska Institutet

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# Resident T Cells in Human Skin - Functional Heterogeneity and Clinical Implications
# THESIS FOR DOCTORAL DEGREE (Ph.D.)

By


## **Stanley Sing Hoi Cheuk**
卓星愷


*Principal Supervisor:*
Liv Eidsmo, Docent
Karolinska Institutet
Department of Medicine, Solna
Unit of Dermatology and Venereology

*Co-supervisor(s):*
Mona Ståhle, Professor
Karolinska Institutet
Department of Medicine, Solna
Unit of Dermatology and Venereology

Bence Rethi, Docent
Karolinska Institutet
Department of Medicine, Solna
Unit of Rheumatology

John Andersson, Docent
Karolinska Institutet
Department of Medicine, Solna
Translational Immunology Unit

*Opponent:*
Onur Boyman, Professor
University of Zurich
Department of Immunology

*Examination Board:*
Johan Sandberg, Professor
Karolinska Institutet
Department of Medicine, Huddinge
Center for Infection Medicine

Anna-Lena Spetz, Professor
Stockholms Universitet
Department of Molecular Biosciences
The Wenner-Gren Institute

Mohammad Alimohammadi, Docent
Uppsala Universitet
Department of Medical Sciences
Division of Dermatology and Venereology

# ABSTRACT

The skin forms a critical barrier against the external environment and is therefore frequently challenged by infections and subjected to immune-mediated diseases as well as malignancies. The Tissue-Resident Memory T ($T_{RM}$) cell is a subset of T cells that resides at sites of previous infection in the skin and other epithelial tissues. Upon re-activation, $T_{RM}$ cells provide rapid, robust and localized adaptive immune defence against re-infection. The role of $T_{RM}$ cells in different human diseases is increasingly appreciated. This thesis aims to explore the functional capacity and regulatory mechanisms of resident T cells in human skin and their potential roles in two different immune-mediated skin diseases, vitiligo and psoriasis.

**PAPER I**: Human skin contains heterogeneous populations of T cells. CD49a expression marks a functionally distinct subpopulation of epidermal CD8 $T_{RM}$ cells that are highly poised towards IFN-γ production and cytolytic function, whereas CD49a$^-$ $T_{RM}$ cells preferentially produced IL-17. The cytotoxic potential of CD49a$^+$ $T_{RM}$ cell was specifically unleashed by IL-15 stimulation. In vitiligo, an acquired chronic depigmenting disorder of the skin, CD49a$^+$ $T_{RM}$ cells accumulated in both epidermis and dermis in lesions implicating a pathogenic role of CD49a$^+$ $T_{RM}$ cells.

**PAPER II**: In psoriasis, a common chronic inflammatory skin disease, a large proportion of epidermal T cells, but not dermal T cells, expressed the pathogenic cytokines IL-17 and IL-22 during active disease (**PAPER II**). Upon clinical remission, T cells with pathogenic capacity were retained in the epidermis of resolved lesions. Upon reactivation, CD4 T cells responded with IL-22 production, whereas CD8 T cells with $T_{RM}$ cell phenotypes responded with IL-17. A model of localized disease memory based on $T_{RM}$ cells in resolved psoriasis was proposed.

**PAPER III**: CD8 T cells in active psoriasis lesions expressed granzyme A, but not granzyme B or perforin. *In vitro* experiments showed that granzyme A specifically promotes chemokine expression in IL-17 stimulated keratinocytes. Thus, granzyme A expression in skin-resident CD8 T cells may provide proinflammatory signals in psoriasis.

**PAPER IV**: In cohorts of Caucasian psoriasis patients and healthy controls, genetic association of variants within *IL22* promoter is confined to patients with disease on-set before puberty. The risk haplotype of the *IL22* promoter led to higher transcriptional activity and higher IL-22 production in CD4 T cells from psoriasis patients, underscoring the impact of genetic heterogeneity and their functional consequences in immune-mediated skin diseases.

Through characterization of resident T cells in human skin in healthy and inflammatory conditions, this thesis demonstrates the functional heterogeneity of skin-resident T cells in healthy skin, vitiligo and psoriasis. Further understanding of the formation, homeostatic, regulatory and effector mechanisms of $T_{RM}$ cell may unveil novel therapeutic strategies and improve disease management in a wide range of skin conditions.

# LIST OF SCIENTIFIC PAPERS

I. **IL-15 Promotes Rapid Induction of Cellular Cytotoxicity by a Subset of $CD8^+CD49a^+$ Tissue-Resident T Cells in Human Epidermis**
Stanley Cheuk, Heinrich Schlums, Irène Gallais Sérézal, Samuel Chiang, Elisa Martini, Nicole Marquardt, Anna Gibbs, Andrea Introini, Marianne Forkel, Annelie Tjernlund, Jakob Michaelsson, Lasse Folkersson, Jenny Mjösberg, Marcus Ehrström, Mona Ståhle, Yenan Bryceson, Liv Eidsmo
*Manuscript*

II. **Epidermal Th22 and Tc17 Cells Form a Localized Disease Memory in Clinically Healed Psoriasis**
Stanley Cheuk, Maria Wikén, Lennart Blomqvist, Susanne Nylén, Toomas Talme, Mona Ståhle, Liv Eidsmo
*Journal of Immunology. 2014; 192:3111-3120*
doi: 10.4049/jimmunol.1302313


III. **Granzyme A Potentiates Chemokine Production in IL-17 Stimulated Keratinocytes**
Stanley Cheuk, Elisa Martini, David Chang, Kerstin Bergh, Liv Eidsmo
*Manuscript*

IV. **Genetic Variants of the *IL22* Promoter Associate to Onset of Psoriasis before Puberty and Increased IL-22 Production in T Cells**
Pernilla Nikamo, Stanley Cheuk , Josefin Lysell, Charlotta Enerbäck, Kerstin Bergh, Ning Xu Landén, Liv Eidsmo, Mona Ståhle
*Journal of Investigative Dermatology. 2014, vol. 134, page. 1535-1541*

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ADCC | Antibody-Dependent Cellular Cytotoxicity |
| AHR | Aryl Hydrocarbon Receptor |
| AMPs | Antimicrobial peptides |
| B2M | Beta-2 microglobulin |
| CCL | Chemokine (C-C motif) ligand |
| CD | Cluster of differentiation |
| CDRs | Complementarity Determining Regions |
| CLA | Cutaneous Lymphocyte-Associated Antigen |
| CTLA4 | Cytotoxic T-Lymphocyte-Associated Protein-4 |
| CTLs | Cytotoxic T Lymphocytes |
| CXCL | Chemokine (C-X-C Motif) Ligand |
| DC | Dendritic Cell |
| DETCs | Dendritic Epidermal T cells |
| DTH | Delayed-Type Hypersensitivity |
| ERAP1 | Endoplasmic Reticulum Aminopeptidase 1 |
| FBS | Fetal Bovine Serum |
| FDR | False Discovery Rate |
| GZM | Granzyme |
| HLA | Human Leukocyte Antigen |
| HPV | Human Papillomavirus |
| HSV | Herpes simplex virus |
| IELs | Intraepithelial T Lymphocytes |
| IFN | Interferon |
| IL | Interleukin |
| ILCs | Innate Lymphoid Cells |
| iSALT | Inducible Skin-associated Lymphoid Tissue |
| LCs | Langerhans cells |
| MAIT cell | Mucosal-associated Invariant T cell |
| MALT | Mucosa-associated Lymphoid Tissue |
| MHC | Major Histocompatibility Complex |
| nb-UVB | Narrow-band UVB |
| NFκB | Nuclear Factor kappa-light-chain-enhancer of activated B cells |

| | |
|---|---|
| NKG2D | Natural Killer Group 2D |
| PAMPs | Pathogen-associated molecular patterns |
| PBMC | Peripheral blood mononuclear cells |
| PD-1 | Programmed cell death-1 |
| pDC | Plasmacytoid dendritic cells |
| PMA | Phorbol 12-myristate 13-acetate |
| PRF | Perforin |
| PRRs | Pattern Recognition Receptors |
| S1P1 | Sphingosine 1-Phosophate Receptor-1 |
| SALT | Skin-associated Lymphoid Tissue |
| SNP | Single Nucleotide Polymorphism |
| STAT | Signal Transducer and Activator of Transcription |
| Tc cell | Cytotoxic T cell |
| $T_{CM}$ cell | Central Memory T cell |
| TCR | T cells receptor |
| $T_{EM}$ cell | Effector Memory T cell |
| TGF | Transforming Growth Factor |
| Th cell | Helper T cell |
| TLDA | TaqMan Low density Array |
| TLRs | Toll-like Receptors |
| TNF | Tumor Necrosis Factor |
| Treg | Regulatory T cell |
| $T_{RM}$ cell | Tissue-resident memory T cell |
| TYR | Tyrosinase |
| UV | Ultraviolet |
| VLA-1 | Very Late Antigen-1 |

# 1 INTRODUCTION

The immune system protects multicellular-organisms from infections. In higher vertebrates the immune system is classically divided into two major subsystems: the innate and adaptive immune systems.

**The innate immune system** provides rapid response to pathogens but has limited specificity. Innate immune cells include granulocytes, macrophages, dendritic cells and innate lymphoid cells. Despite limited specificity, each type of innate immune cells has a specialized function: engulfing pathogens or dying cells (neutrophils, macrophages, dendritic cells), secreting inflammatory mediators and enzymes (mast cells, basophils and eosinophils), killing of aberrant cells (natural killer cells) and presenting antigens to helper T cells (macrophages, dendritic cells). In broad terms, most cells in the body possess various degrees of innate immune function. Epithelial cells that form the border of the body are particularly important in providing the first line of innate immune defense, and therefore, could also be considered as part of the innate immune system. **The adaptive immune system** targets pathogens with specificity and is characterised by the formation of immunologic memory. B lymphocytes (B cells) and **T lymphocytes (T cells)** are the primary cell types responsible for establishing adaptive immunity, in which B cells mediate humoral immunity by producing antibodies; whilst T cells mediate cellular immunity through helping other immune cell types or targeted killing of virally infected cells (Kindt *et al.*, 2007; Abbas and Lichtman, 2011; Murphy *et al.*, 2012).

**The skin** is one of the largest interfaces between the body and outer environments. It requires immune defense against various forms of insult and violation. In this regard, the skin is a crucial part of the immune system, sensing and responding to foreign entities. Such immune-surveillance is established by both the innate and adaptive immune cells. T cells in particular can be recruited to the skin during active inflammation. Recently, resident T cells were shown to establish on-site adaptive immunity in the skin after virus infection, providing the first line of adaptive immune defense against re-infection (Gebhardt *et al.*, 2009; Jiang *et al.*, 2012). However, T cells also play key pathogenic roles in many skin diseases. Complex interactions between genetic susceptibility and environmental triggers may result in an over-reaction of immune responses that could cause unwanted inflammation leading to tissue destruction, autoimmunity or inflammatory diseases. In **vitiligo**, auto-reactive T cells may cause disappearance of melanocytes in the skin, resulting in loss of skin pigments. In **psoriasis**, hyper-inflammatory T cells in skin help to sustain chronic inflammation. Although effective treatments are available for psoriasis, local relapse is still a major therapeutic challenge and a "molecular scar" of psoriasis have been proposed (Suárez-Fariñas *et al.*, 2011).

Through characterizing the resident T cells in human skin, this thesis aims to uncover their functional heterogeneity and explore their roles in two chronic immune-mediated skin diseases, psoriasis and vitiligo.

# 2 T CELLS

T cells are lymphocytes that play a central role in establishing and maintaining cellular adaptive immunity in vertebrates. A T cell, which has gone through the maturation process in thymus, expresses a clonotype of functional T cells receptor (TCR) on its surface. In the past few decades, various subsets of T cells have been identified and classified by their functional capacities. The T cell population can be subdivided into two main categories, the "conventional" T cells and the "unconventional" T cells. The former recognize peptide antigens bound onto classical Major Histocompatibility Complex (MHC) molecules with their TCR αβ chain; whereas the latter, such as γδT cells, Natural Killer T (NKT) cells and Mucosal-associated invariant T (MAIT) cells, recognize antigens presented by other molecules, like Cluster of differentiation (CD)1a-d or MHC related protein-1(MR1) molecules (Godfrey *et al.*, 2015).

## 2.1 T Cell – the Overture

One of the classical characteristics of adaptive immunity is the ability to mount specific responses against foreign antigens, while limiting auto-reactivity by distinguishing self from non-self. Conventional T cells achieve this through the self-restricted interaction between their membrane-bound αβ chains of the T cell receptor (TCR) and peptide bound to the self-MHC- molecule expressed on the target cells or antigen presenting cells. The αβ chains of TCR form the functional TCR complex together with the CD3 molecules, which relay the downstream signaling (Smith-Garvin *et al.*, 2009).

### 2.1.1 T Cell Selection - The Making of a Mature but Naïve T cell

The huge diversity of T cell receptor (TCR) within a single individual is generated by V-(D)-J recombination, which is a form of somatic DNA rearrangement that takes place in the thymus (Schatz and Swanson, 2011). This process provides the molecular basis for recognizing numerous antigens. Productive recombination of the TCR gene results from the joining of the V (Variable) and J (Joining) segments in the α chain, and the V, D (Diversity) and J segments in the β chain (Figure 2.1). The most variable parts of each chain of TCR are the three Complementarity Determining Regions (CDRs). The CDR1 and CDR2 are determined by the sequence of the V segment; the CDR3 is the junction between V, (D), and J segments. Random addition or deletion during recombination adds further variability to CDR3, making it the most variable region of the TCR (Siu *et al.*, 1984; Schatz and Swanson, 2011; Attaf *et al.*, 2015).

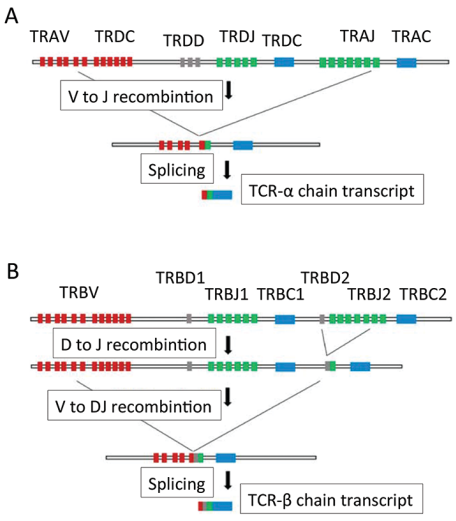


**Figure 2.1. V(D)J rearrangement of T cells , Adaptation of (Attaf *et al.*, 2015)**

It has been estimated that V-(D)-J recombination can theoretically generate ~ $10^{18}$ TCR clonotypes in humans and ~ $10^{15}$ in mice (Davis and Bjorkman, 1988; Attaf *et al.*, 2015). However, the estimated size of T cell repertoire is much smaller (< $10^{8}$) than the theoretical value (Arstila *et al.*, 1999; Robins *et al.*, 2010). This is due to several factors. First, carrying >$10^{18}$ T cells with unique TCRs within a human body would be biologically infeasible. Secondly, a large number of the possible recombined TCRs would not recognize self-MHC and would not be functional. Thirdly, some of the TCRs may recognize self-antigens strongly, potentially causing autoimmunity. According to the classical affinity model of thymic selection for conventional T cells, T cells bearing functional TCR with a relatively low affinity to self-peptides presented on self-MHC molecules will be released to the circulation; whereas T cells with a non-functional or non-self-restrictive TCR and T cells bearing TCR with high affinity to self-peptide:MHC will be eliminated (Klein *et al.*, 2014). However, the assumption of the absolute specificity of TCR implies that a lot of potential foreign peptides cannot be detected (Mason, 1998). It has been postulated and later supported by experimental data that a single TCR may cross-react with numerous of different peptides presented on self-MHC, filling the gap in the unmatched foreign peptide diversity (Mason, 1998; Sewell, 2012; Wooldridge *et al.*, 2012). Together, these features warrant the self-restriction nature of the TCR:MHC interaction and provide the basis for detecting foreign antigens, whilst limiting the chance of autoimmunity.

### 2.1.2 T Cell Activation

After the thymic selection, extrathymic T cells are "mature" but "naïve" (Sprent and Tough, 1994). Naïve T cells circulate through secondary lymphoid organs. In case of an infection, the pathogen-specific naïve T cells can be activated by antigen presenting cells in secondary lymphoid tissues and expand, eventually differentiating into effector and memory T cells (Sprent and Tough, 1994). Dendritic cells (DCs), amongst the professional antigen presenting cells, can migrated to or resided in lymph nodes, providing potent activation to T cells. However, MHC:TCR ligation alone does not result in full activation of naïve T cells. A second stimulus is required in the form of co-stimulation, as exemplified by the co-stimulatory molecule CD28. Downstream signals of CD28 induce expression of interleukin (IL)-2 that promote clonal expansion and survival of T cells (Sharpe and Freeman, 2002). On the other hand, co-inhibitory signals, such as cytotoxic T-lymphocyte-associated protein-4 (CTLA4) and Programmed cell death-1 (PD-1), limit the scale of T cell-mediated immune response (Sharpe and Freeman, 2002). Cytokine stimulation from antigen presenting cells is considered as the third signal required for T cell activation (Curtsinger and Mescher, 2010) that determines the functional fate of the T cells upon activation (see the section below).

## 2.2 Conventional T Cell Subsets

Two major types of conventional αβ T cells are classified by their expression of co-receptor CD4 or CD8. CD4 T cells recognize peptides presented by MHC class II molecules, which are expressed on professional antigen presenting cells. Activated CD4 T cells mediate their effector functions through cytokine production. On the contrary, CD8 T cells recognize

peptides bound on MHC class I molecules expressed on most cell types. And in addition to cytokine production, these cells are specialized in killing of target cells.

### 2.2.1 CD4 T Cell – The Helper

Depending on the cytokine expression after activation, CD4 T cells can be classified into different T helper (Th) subsets. As early as in the 1980s, Mosmann and Coffman showed that there were at least two types of T helper cell clones from mice, distinguished by cytokine expression (Mosmann *et al.*, 1986; Mosmann and Coffman, 1989). These two subsets were termed Th1 that produced interferon (IFN)-γ and IL-2, and Th2 that produced IL-4 (then known as B cell stimulating factor 1, BSF-1) (Mosmann *et al.*, 1986). It was later determined that Th2 produced mainly IL-4, IL-5 and IL-13 which were critical for IgE production and clearance of extracellular parasites by enhancing function of eosinophils (Ansel *et al.*, 2006; Zhu *et al.*, 2010). On the other hand, Th1 produced high level of IFN-γ and were instrumental in inducing macrophages phagocytosis and cellular cytotoxicity, which were essential in clearing intracellular pathogens (Mosmann and Coffman, 1989; Szabo *et al.*, 2003). The importance of this functional dichotomy was illustrated by murine cutaneous Leishmania infection where a resistant mouse strain (C57BL/6) imposes an effective Th1 response towards the parasites, whereas the susceptible strain (BALB/c) responses with Th2 inflammation that fail to constrain the infection, leading to chronic infection (Locksley *et al.*, 1987; Heinzel *et al.*, 1989). In the mid-2000s, a third subset of helper T cells was proposed as Th17, which was characterized by its expression of IL-17 (Harrington *et al.*, 2005; Langrish, 2005; Park *et al.*, 2005). Although being named Th17, these cells produce not only IL-17 but also IL-21, IL-22 or IL-26 (Korn *et al.*, 2009). A wide-range of extracellular pathogens, including Candida and Streptococcus, can trigger Th17 response (Korn *et al.*, 2009; Zielinski *et al.*, 2012). Since its discovery, the Th17 cell has been found to be potent inducers of autoimmune tissue inflammation and implicated in many inflammatory diseases, such as multiple sclerosis, rheumatoid arthritis and psoriasis (Huang *et al.*, 2004; Hirota *et al.*, 2007a; Hirota *et al.*, 2007b; Lowes *et al.*, 2008; Korn *et al.*, 2009). In addition, a population of IL-22 producing T cells without IL-17 production is sometime designated as Th22, and has been shown to play a role in epithelial homeostasis (Duhen *et al.*, 2009; Eyerich *et al.*, 2009).

The development and transcriptional regulations of different Th subsets have been studied extensively in recent years. Soon after the discovery of distinct cytokine production patterns in different Th subsets, it became clear that specific sets of cytokines were crucial in Th subsets differentiation. Each of these Th subsets can also be defined by their expression of lineage defining transcriptional regulators, inducing or suppressing the expression of various sets of genes (Figure 2.2). IL-12 and IFN-γ, in particular, are important in Th1 differentiation with the lineage defining transcriptional factor T-bet; similarly, IL-4 and the expression of GATA3 are crucial for Th2 differentiation (Szabo *et al.*, 2003; Ansel *et al.*, 2006; Zhu *et al.*, 2010). For Th17, RORγT was identified as the lineage defining transcriptional factor, and combinations of Transforming Growth Factor (TGF) β, IL-1β, IL-6, IL-21, and IL-23 induce differentiation of Th17 (Korn *et al.*, 2009).

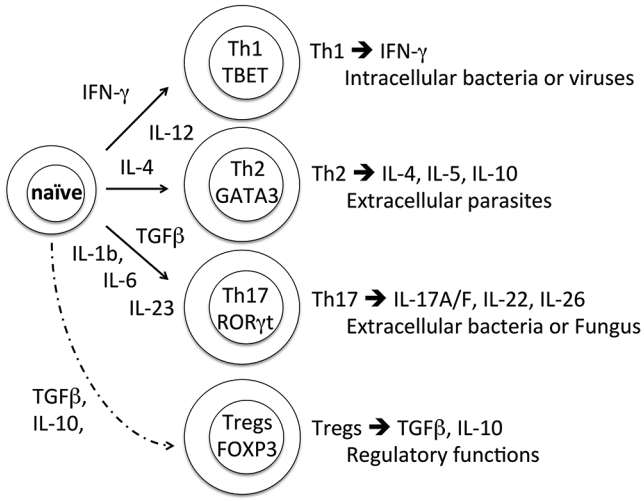


**Figure 2.2 CD4 T helper subsets.**
Adopted and modified
(Di Cesare *et al.*, 2009)

The regulatory T cell (Treg) is another member of the CD4 T cell family. In contrast to other subsets described above, Treg is characterized by its suppressive function in immune responses. In the mid-1990s, Sakaguchi et al. showed that transfer of $CD25^+$ CD4 T cells could attenuate allogenic response towards skin grafts and suggested CD25 as a marker for Tregs (Sakaguchi *et al.*, 1995). Foxp3 was subsequently identified as the master transcriptional regulator for Treg (Hori *et al.*, 2003) following the discovery that *FOXP3* mutation is the underlying cause for IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked) syndrome in humans and the scruffy phenotype in mice (Bennett *et al.*, 2001; Wildin *et al.*, 2001). *FOXP3* deficiency in humans and mice led to the development of multiple inflammatory disorders (Bennett *et al.*, 2001; Wildin *et al.*, 2001). Tregs exert the suppressive function through multiple mechanisms, such as secretion of suppressive cytokines (TGFβ, IL-10), or by inducing tolerogenic antigen presenting cells (Shevach, 2009).

Classification of Th cells by means of effector cytokine production helps to understand how the immune system respond to different pathogens with tailor-made responses. However, their functional distinction is not always fixed (Cosmi *et al.*, 2013; Geginat *et al.*, 2014). Th17 cells seemed to be particularly unstable that could shift to Th1 (Annunziato *et al.*, 2007; Lee *et al.*, 2009), whereas Th1 cells could become IL-10 producing cells and limit inflammatory responses (Cope *et al.*, 2011). These examples demonstrate the functional plasticity of CD4 T cells.

### 2.2.2 CD8 T Cell – The Killer

Activated CD8 T cells are usually called cytotoxic T lymphocytes (CTLs). Distinct from their CD4 counterpart, CD8 T cells have a more specific role in immune defense: killing of infected or aberrant cells. Upon activation, CD8 T cells up-regulate perforin and granzymes, which are proteins stored in cytotoxic granules responsible for cellular cytotoxicity. When encountering a virally infected cell, antigen-specific cytotoxic T cell degranulates, and the cytotoxic granule constituents are released through the immunological synapse (Voskoboinik *et al.*, 2015). Perforin forms holes on target cells, allowing granzymes to enter target cells and

induce apoptosis by activating the caspase pathway or the mitochondrial cell death pathway (Masson and Tschopp, 1985; Young *et al.*, 1986; Talanian *et al.*, 1997; Heibein *et al.*, 2000; Sutton *et al.*, 2000). Among the granzymes, a family of serine proteases stored in cytotoxic granules, granzyme B has a clear cytotoxic role and rapidly induce apoptosis via a caspase-dependent pathway (Voskoboinik *et al.*, 2015). In contrast, the role of granzyme A in mediating target cell apoptosis remains controversial (Lieberman and Fan, 2003; Metkar *et al.*, 2008; Trapani and Bird, 2008; Susanto *et al.*, 2013). Granzyme A-mediated killing of target cells takes longer time, requires much higher concentration, and may act through cleavage of nuclear proteins leading to DNA damages (Lieberman and Fan, 2003; Metkar *et al.*, 2008). Other evidence suggested granzyme A alongside with granzyme M and K could act as proinflammatory proteases (Metkar *et al.*, 2008; Voskoboinik *et al.*, 2015; Wensink *et al.*, 2015). In addition, granulysin, another cytotoxic granule protein, possesses bactericidal activity (Linde *et al.*, 2005). CD8 T cells are also an important source of proinflammatory cytokines, in particular, IFN-γ, IL-2, and Tumor Necrosis Factor (TNF). CD8 T cells producing IL-4, IL-13 and IL-17 have also been identified (Geginat *et al.*, 2003; Yen *et al.*, 2009); and are sometimes classified based on cytokine productions, paralleling the classification of Th subsets, i.e. Tc1, Tc2, and Tc17.

## 2.3 T Cell Memory

Developing antigen-specific immunologic memory is the hallmark of adaptive immunity. Together with recall antibody responses, memory T cells form long-term adaptive immunologic defense against pathogens. Once naïve T cells receive sufficient initial activation by pathogen antigen recognition, co-stimulation, and appropriate cytokine stimulation, they undergo rapid clonal expansion and most become effector cells. After pathogens are eradicated, most of the effector cells die in the contraction phase, but a small population of antigen-specific memory T cells persists for long time in the absence of cognate antigens. Upon re-exposure to the same pathogen, memory T cells mount a more rapid, robust and effective recall response; thereby, providing an augmented immunologic protection (Figure 2.3) (Williams and Bevan, 2007).

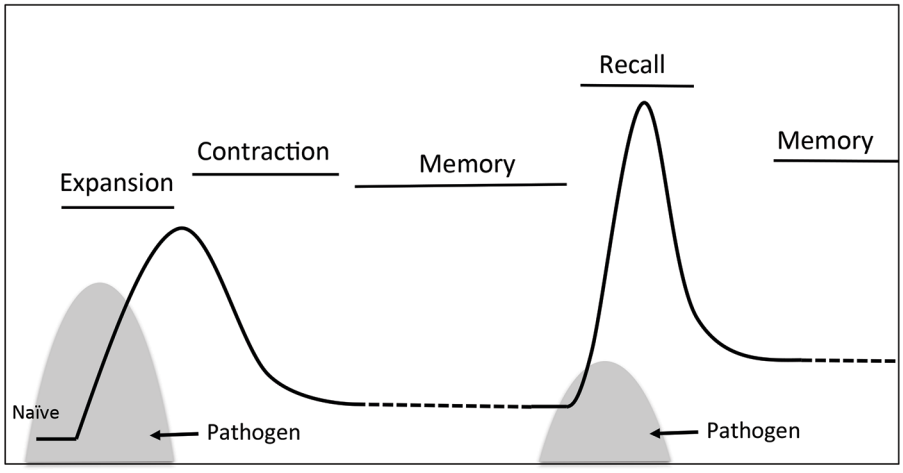


**Figure 2.3. Kinetics of T cells activation and immunologic memory.**
Adapted and modified from (Williams and Bevan, 2007) and (Jameson and Masopust, 2009).

### 2.3.1 Memory T Cell Subsets

The pool of memory T cells includes T cell subsets of different functional capacity that preferentially recirculate or reside in different tissues in the body. In humans, CD45RO and CD45RA display reciprocal expression in the T cell population. $CD45RO^+$ cells were initially ascribed as memory T cells and $CD45RA^+$ cells as naïve cells due to their difference in functional capability (Akbar *et al.*, 1988; Byrne *et al.*, 1988). But it was later found that CD45RA also marked an effector-like population in $CD27^-$ CD8 T cells (Hamann *et al.*, 1997). Sallusto and colleagues further showed that memory cells could be sub-grouped. CCR7 and CD62L expression marked a subset of $CD45RO^+$ memory cells, coined central memory T ($T_{CM}$) cells (Sallusto *et al.*, 1999), which recirculate to secondary lymphoid organs, such as lymph nodes. Another subset lacking CCR7 and CD62L expression was shown to have a higher cytokine production capacity and perforin expression; thus, it was termed the effector memory T ($T_{EM}$) cells (Sallusto *et al.*, 1999). $T_{EM}$ cells have been postulated to patrol through non-lymphoid tissues. Indeed, a majority of T cells in peripheral tissue are phenotypically and functionally similar to $T_{EM}$ cells (Campbell *et al.*, 2001; Masopust, 2001; Reinhardt *et al.*, 2001). Functionally, as compared to $T_{CM}$ cell, $T_{EM}$ cell display less proliferative potential upon antigenic stimulation (Geginat *et al.*, 2003) and express higher level of perforin or granzymes (Takata and Takiguchi, 2006; Romero *et al.*, 2007). An effector-like $T_{EM}$ cell subset expressing CD45RA (sometimes depicted as $T_{EMRA}$) constitutively expressed cytotoxic granule constituents, and had superior cytotoxic capacity; resembling terminally differentiated cells (Sallusto *et al.*, 1999; Geginat *et al.*, 2003). Therefore, apart from preferential homing destinations, the classification of memory phenotype can distinguish the readiness of cytokine production and cytolytic function of T cells under steady state.

### 2.3.2 Tissue Resident Memory T cells

The $T_{CM}$ / $T_{EM}$ model of memory T cell provided a theoretical framework of T cell immunologic memory that combined the functional specialization of T cell with their migration and localization pattern (Sallusto *et al.*, 1999). However, this simplified framework failed to capture the complexity of diverse T cell memory response (Jameson and Masopust, 2009). The majority of T cells in non-lymphoid organs do not express CCR7 or CD62L (Spetz *et al.*, 1996; Campbell *et al.*, 2001; Clark *et al.*, 2006), implicating a $T_{EM}$ phenotype. However, it has been shown that memory T cells egress from nonlymphoid tissue in a CCR7 dependent manner (Bromley *et al.*, 2005; Debes *et al.*, 2005; Bromley *et al.*, 2013). This implies that at least part of the memory T cell population patrolling the peripheral tissues would express CCR7; therefore, violating of the $T_{CM}$ / $T_{EM}$ model. In parabiotic mice model, non-circulating T cell population was found in many non-lymphoid tissues (e.g. intestine, female reproductive tract), whereas T cell population from lung and liver equilibrated with the circulating population (Klonowski *et al.*, 2004; Masopust *et al.*, 2010; Steinert *et al.*, 2015). This illustrates complexity of the diversity of the migratory pattern of the $T_{EM}$ population. One example of such non-recirculating resident T cells population is the

intraepithelial T lymphocytes (IELs) in the intestine that could exert antigen-specific cytolysis upon reactivation (Masopust, 2001).

The studies on skin and nerve-tropic herpes simplex virus (HSV) infection and recall response to the virus in experimental murine model illustrated the pivotal role of resident T cell population in tissue immunology. HSV can infect the skin of both human and mice. In humans, the virus resides within sensory ganglia in a latent state; upon reactivation, skin lesions relapse at previously affected sites (Koelle and Corey, 2008). In contrast, "spontaneous" reactivation does not occur *in vivo* at the periphery in murine HSV infection despite the presence of latent virus in sensory ganglia (Feldman *et al.*, 2002). It was found that, in mouse, HSV-specific memory $CD8^+$ T cells are retained in the sensory ganglia during latency (Khanna *et al.*, 2003), and can expand locally upon antigenic activation (Wakim *et al.*, 2008). Gebhardt and colleagues illustrated that after primary HSV cutaneous infection, HSV-specific CD8 T cells preferentially persist in the previously infected skin epithelia and provide local recall response against HSV reinfection in the skin (Gebhardt *et al.*, 2009). This resident population was then termed the **Tissue-resident memory T ($T_{RM}$) cells**. The protective role of local adaptive immune defence mediated by $T_{RM}$ cells was further confirmed in murine viral infection models of the brain (Wakim *et al.*, 2010), gut mucosa (Masopust *et al.*, 2010; Zhang and Bevan, 2013), lung (Wakim *et al.*, 2013), and female productive tract (Schenkel *et al.*, 2013; Schenkel *et al.*, 2014) as well as skin vaccinia virus infection (Jiang *et al.*, 2012). Apart from viral infection, microbiota and non-infectious inflammation could induce the formation of $T_{RM}$ cells (Mackay *et al.*, 2012; Naik *et al.*, 2012).

A definitive characteristic of $T_{RM}$ cells is their ability to reside in non-lymphoid tissue and, thus, do not recirculate (Gebhardt *et al.*, 2011; Clark *et al.*, 2012; Jiang *et al.*, 2012; Mueller *et al.*, 2013; Steinert *et al.*, 2015). Similar transcriptome profiles of $T_{RM}$ cells from different tissues, gut, lung, brain and skin showed gene expression signature that favours tissue retention (Wakim *et al.*, 2012; Mackay *et al.*, 2013). This ability to persist in tissue is thought to be mediated by the expression of $T_{RM}$ cell markers, CD103, CD69 and CD49a (Gebhardt *et al.*, 2009; Wakim *et al.*, 2010; Zhang and Bevan, 2013). CD103, also known as integrin αE, is part of the heterodimer integrin αEβ7 that binds to E-cadherin (Cepek *et al.*, 1994), which forms adherent junctions widely expressed in epithelial cells (Hartsock and Nelson, 2008) and some dendritic cells (Tang *et al.*, 1993). It was postulated that the CD103 expression is required for $T_{RM}$ during epithelial adhesion and retention (Pauls *et al.*, 2001). CD69, on the other hand, is infamously known as the "early activation marker" for lymphocytes; however, CD69 can also suppress sphingosine 1-phosophate receptor-1 (S1P1) expression (Bankovich *et al.*, 2010) and allow $T_{RM}$ to stay in non-lymphoid tissue (Mackay *et al.*, 2015a). Indeed, down-regulation of S1P1 is required for resident memory $CD8^+$ T cell formation (Skon *et al.*, 2013). CD49a, also known as Very Late Antigen-1 (VLA-1), is the α1 subunit of the α1β1 integrin (Hemler, 1990; Hynes, 2002) that could adhere to collagen IV, a component of the basement membrane between epidermis and dermis. CD49a has been suggested to mediate epidermal-tropic migration and is critical in development of psoriasis in

a spontaneous xenotransplant model (Conrad *et al.*, 2007). Additionally, CD49a marks lung-resident T cells, and was proposed to mediate their tissue retention after active viral infection (Ray *et al.*, 2004; Chapman and Topham, 2010; Piet *et al.*, 2011; Purwar *et al.*, 2011). Importantly, the expression of these $T_{RM}$ markers do not capture all the non-recirculating T cells in the tissue (Steinert *et al.*, 2015). Nonetheless, these markers provide the tools to identify $T_{RM}$ cells in peripheral tissue.

The development of $T_{RM}$ cell depends on TGF-β and IL-15. TGF β up-regulates $T_{RM}$ markers, CD103, CD69 and CD49 (Mackay *et al.*, 2013; Zhang and Bevan, 2013), whereas IL-15 provides survival signals (Adachi *et al.*, 2015; Mackay *et al.*, 2015b). Most functional studies on $T_{RM}$ cell have focused on CD8 $T_{RM}$, but both CD4 and CD8 $T_{RM}$ have been identified and characterized in the lung, intestine, female genital tracts and skin (Gebhardt *et al.*, 2011; Iijima and Iwasaki, 2014; Thome *et al.*, 2014; Turner and Farber, 2014; Glennie *et al.*, 2015; Watanabe *et al.*, 2015). Whether CD4 T cells are re-circulating or lodging in these non-lymphoid tissue is, however, less clear. Even in the absence of cognate antigens, CD8 $T_{RM}$ cells constantly crawl within the epithelial allowing rapid detection of antigen (Ariotti *et al.*, 2012; Zaid *et al.*, 2014). Among the proposed effector mechanisms, IFN-γ induced inflammation has been suggested as the major protective mechanism of the $T_{RM}$-mediated local recall response (Schenkel *et al.*, 2013; Ariotti *et al.*, 2014; Schenkel *et al.*, 2014; Glennie *et al.*, 2015) (Figure 2.4).

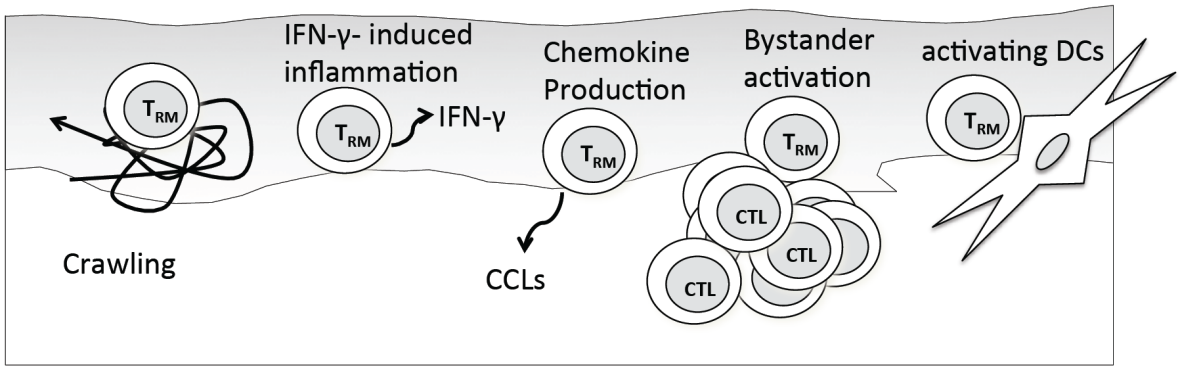


**Figure 2.4. Protective Effector Mechanism of CD8 $T_{RM}$ cells**. Even without the presence of cognate antigens, $T_{RM}$ constantly crawl within the epithelium (Ariotti *et al.*, 2012; Zaid *et al.*, 2014). Upon antigen stimulation, their protective function through multiple mechanisms: (1) IFN- γ induced inflammation; (2) chemokine production; (3) bystander activation of cytotoxic lymphocytes and; (4) induction of DC maturation (Schenkel *et al.*, 2013; Ariotti *et al.*, 2014; Schenkel *et al.*, 2014).

# 3 THE SKIN

The skin serves as the interface between the body and the surrounding environment. It forms a critical barrier against physical and chemical insults, as well as foreign entities such as pathogens and commensal microbes. Structurally, human skin is composed of three basic layers, the epidermis, dermis, and subcutis (Figure. 3.1). The subcutis lies beneath dermis, consisting of connective and adipose tissues. The skin also contains various skin appendages, such as hair, sebaceous glands and sweat glands that exert further physiological functions.

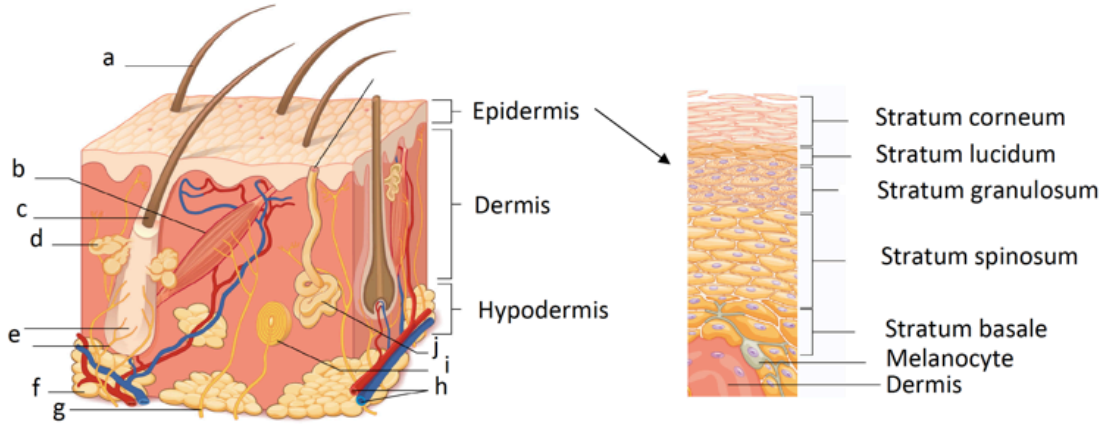


**Figure 3.1. Structure of the human skin** Left. Overall structure of human skin with epidermis and dermis as well as hypodermis. (a. hair shaft, b. arrector pili muscle, c. hair, d. sebaceous gland, e hair root and follicle, f, adipose tissue, g, sensory nerve fibre, h. blood vessels, i. pacinian corpuscle, j. eccrine sweat gland) Right. Architecture of epidermal layers. (Images downloaded for free and modified from OpenStax College, Anatomy & Physiology. OpenStax CNX. http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22. Creative Commons Attribution 4.0 International License.)

The epidermis is composed of multiple layers of tightly packed keratinocytes that form the basis for the physical barrier. Under homeostatic conditions, keratinocytes in the basal layer proliferate, migrate upward, differentiate and, ultimately, form the cornified layer, *stratum corneum*, before being gradually exfoliated (Figure 3.1). Melanocytes, which can produce skin pigment, also situated in the basal layer of the epidermis. A collagen-rich basement membrane anchors the two distinct layers and forms the epidermal-dermal junction. The dermis is connective tissue made of collagen and elastic fibres; thereby, supporting the epidermis and allowing elasticity of the skin. Fibroblasts and nerve fibres lie within the dermis. Blood vessels and lymphatics also drain to the dermis, supplying nutrients and circulation to the skin.

## 3.1 The Cutaneous Immune System

The skin consists of both innate and adaptive immune cell types. In the 1980s, based on evidence of the presence of immunocompetent cells in skin, Streilein proposed the concept of skin-associated lymphoid tissue (SALT), which could protect against persistent infection or neoplasms, (Streilein, 1983). However, in contrast to its mucosal counterpart, i.e. mucosa-associated lymphoid tissue (MALT), the skin at steady state lacks B cells (Bos *et al.*, 1987) and well-defined lymphoid tissue structure (Pitzalis *et al.*, 2014). Aside from keratinocytes,

which provide the first line of innate defence, many immune cell types are present in the skin during healthy or inflammatory states. Among the immune cells, dendritic cells and T cells play a critical role in adaptive immune response; whereas mast cells, neutrophils, innate lymphoid cells (ILCs), macrophages, fibroblasts and endothelial cells secrete inflammatory mediators and take part in inflammation. Recently, Kabashima and colleagues proposed the formation of inducible SALT (iSALT) during inflammation, which involves formation of T cell – dendritic cell clusters and requires initial innate signals from macrophages and keratinocytes (Natsuaki *et al.*, 2014), illustrating the dynamic interaction of immune and stromal cells in skin inflammation.

### 3.1.1 Keratinocytes

The keratinocyte is the most abundant cell type in the human epidermis and forms a stratified epithelium. Such cellular architecture is the basis of the physical barrier. Indeed, an intact physical barrier limits microbial growth and pathogen invasion (Goodarzi *et al.*, 2007). When the physical barrier is compromised, immune mechanisms are required to be in place for pathogen detection and elimination. The innate immune function of keratinocytes, therefore, serves as the first line of defense against intruders.

The innate immune system rapidly acts against a broad spectrum of pathogens. Compared with adaptive immune cells, innate cell types have a limited set of receptors recognizing common structural patterns of pathogens that are called pathogen-associated molecular patterns (PAMPs) (Janeway, 1992). Pattern Recognition Receptors (PRRs) sense the presence of pathogens. Toll-like Receptors (TLRs) are amongst the most studied PPRs. There are ten human TLRs, which are expressed either on the cell surface or the endosomal compartments, sensing different evolutionarily conserved PAMPs (Kumar *et al.*, 2011). Keratinocytes express a specific set of TLRs (TLR 1-5 and 9) (Miller and Modlin, 2007; Nestle *et al.*, 2009a). Upon TLR-activation, keratinocytes produce various cytokines and chemokines. Among them, the IL-8, TNF, and type I interferons, for example, further amplify the inflammatory response (Miller and Modlin, 2007; Kawai and Akira, 2010). These initial proinflammatory mediators derived from keratinocyte would regulate further inflammatory responses, including recruitment and activation of other cell types, like neutrophils, and dendritic cells.

Antimicrobial peptides (AMPs), like defensin, cathelicidin (LL37) and psoriasin (S100A7) are also produced by keratinocytes (Gallo and Hooper, 2012). The bactericidal effects of AMPs are usually mediated through targeting bacterial cytoplasmic membrane (Gallo and Hooper, 2012). In addition to their antimicrobial activities, AMPs can be induced during inflammation and wounding (Frohm *et al.*, 1997) . And it is increasingly recognized that some AMPs have non-bactericidal functions in inflammatory processes; for instance, AMPs binding with nucleic acids could also activate cells through TLRs, showing multiple functions of these small peptides (Lande *et al.*, 2007; Gilliet and Lande, 2008; Ganguly *et al.*, 2009).

### 3.1.2 Dendritic Cells in the Skin

Dendritic cells (DCs) are professional antigen presenting cells that link the innate and adaptive immunity. As professional antigen presenting cells, they express MHC class-II and are capable of activating CD4 T helper cells. Certain subsets of DCs may also present extracellular antigens on MHC class I molecules to CD8 T cells through a process called cross-presentation (Segura and Amigorena, 2014). Through antigen presentation, co-stimulation/co-inhibition and cytokine production, DCs serve crucial stimulatory and regulating functions for T cell immunity (Steinman, 2012).

At steady state, the skin is the home to different subsets of tissue dendritic cells distinct by their anatomical distribution. The Langerhans cell (LC) is the major DCs type in the epidermis, whereas a more diverse group of dermal DCs reside in the dermis, including $CD1c^+$ DCs and $CD141^+$DCs during steady state, as well as plasmacytoid DCs, TIP (TNF- and iNOS-producing) DCs and SLAN DCs during inflammation (Lowes *et al.*, 2005; Zaba *et al.*, 2008; Brunner *et al.*, 2013; McGovern *et al.*, 2014). LCs were once considered as the classical model of tissue resident DCs. The "Langerhans cell paradigm" postulated that LCs capture and process the antigens from the pathogens in the skin, and simultaneously become activated by the inflammatory milieu (Romani *et al.*, 2010). Subsequently, LCs migrate to the lymph node to present foreign antigens to T cells; thereby, initiating the T cell activation. However, this paradigm has been questioned; LCs alone could not initiate cytotoxic T cells response to viral infection in epidermis (Allan *et al.*, 2003). It was later found that dermal DCs, but not LCs, were able to carry antigen to draining lymph node (Allan *et al.*, 2006) and induce T cell proliferation (Fukunaga *et al.*, 2008) or CD8-mediated response (Bedoui *et al.*, 2009). Some experimental contact hypersensitivity models, however, suggested that LCs and dermal DCs might be redundant or compensatory in T cell priming (Noordegraaf *et al.*, 2010; Clausen and Stoitzner, 2015). In light of the polarization of T helper cell subsets, recent studies showed that Langerhans cells are in favour of inducing Th17 (Kashem *et al.*, 2015) or Th22 (Fujita *et al.*, 2009) while dermal DCs promote Th1 differentiation (Igyártó *et al.*, 2011). LCs may also activate skin resident Tregs (Seneschal *et al.*, 2012). Discrepancy in the literature about the function of LCs and dermal DCs illustrates the multifunctional nature of these skin DC subsets and the precise function might be highly dependent on the context of the investigation. Importantly, epidermal $T_{RM}$ cells in skin interact with Langerhans cells and, thus, LCs may be important function in regulating functions of $T_{RM}$ cells (Zaid *et al.*, 2014).

### 3.1.3 T Cells in the Skin

Circulating T cells homing to skin predominately express cutaneous lymphocyte-associated antigen (CLA), which is an inducible modification of P-selectin glycoprotein ligand-1 (PSGL-1) (Fuhlbrigge *et al.*, 1997). However, the majority of CLA expressing T cells reside in the skin, and it has been estimated that there are two times more T cells in the skin than that in the circulation (Clark *et al.*, 2006). In steady state, epidermal T cells are often situated

in the basal layer along the rete ridges; whereas dermal T cells are positioned just beneath the epidermal-dermal junction or surrounding blood vessels in both papillary and reticular dermis (Bos *et al.*, 1987; Foster *et al.*, 1990). Most T cells in human skin are αβ T cells, and both CD4 and CD8 T cells reside in epidermis and dermis (Bos *et al.*, 1987; Foster *et al.*, 1990; Spetz *et al.*, 1996). Interestingly, epidermis from sole of the feet has significantly higher T cell density as compared to buttock, limbs and thorax, indicating regional difference (Foster *et al.*, 1990). Early functional analysis showed that cutaneous T cells could activate autologous keratinocytes to express ICAM-1 (Sugerman and Bigby, 2000). HSV specific CD8 T cells persist in human genital skin and accumulate close to nerve ending during subclinical HSV activation (Zhu *et al.*, 2007; Zhu *et al.*, 2013). During clinical quiescence CD8 T cells at epidermal-dermal junction retain effector-like gene expression profile (Peng *et al.*, 2012). Apart from HSV, skin resident T cells specific to other viruses have also been identified, e.g. Varicella Zoster Virus Skin (VZV) (Vukmanovic-Stejic *et al.*, 2015) and human papillomavirus (HPV) (Viac *et al.*, 1992), implicating a general role in immune surveillance.

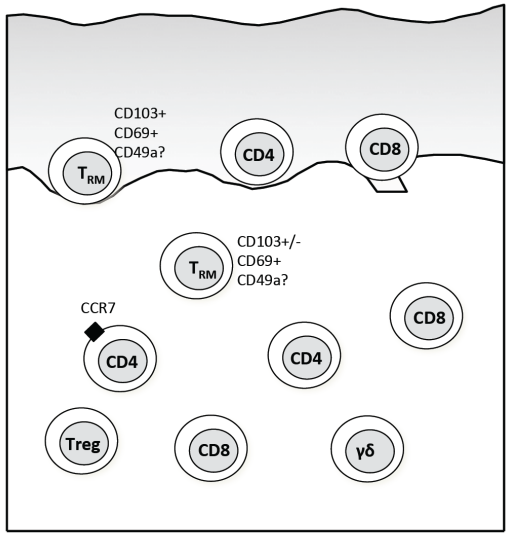


**Figure 3.2. T cell subsets in the human skin**

Most T cells in epidermis and dermis express CD45RO and lack CD62L, indicating a $T_{EM}$ cell phenotype (Spetz *et al.*, 1996; Clark *et al.*, 2006). CD103 is expressed in a significant population of epidermal CD8 T cells (Spetz *et al.*, 1996) and has been proposed to mediate adhesion to epidermis (Pauls *et al.*, 2001). $CD45RO^+$ $Foxp3^+$ Tregs often reside in hair follicles (Sanchez Rodriguez *et al.*, 2014). Apart from CLA, skin homing or resident T cells may express a set of chemokine receptors including CCR4, CCR6, CCR8, and CCR10. (Homey *et al.*, 2002; Hudak *et al.*, 2002; Clark *et al.*, 2006; McCully *et al.*, 2012). Since $T_{RM}$ cells were first identified in murine skin model, $CD69^+$ $CD103^+$ $T_{RM}$ cells have also been identified in human skin and display a higher potential for producing IFNγ, TNF and IL-22 as compared to the $CD103^-$ population (Watanabe *et al.*, 2015). The expression of CD49a in human $T_{RM}$ cells from healthy skin is less studied. However, early histology studies and long-term explant culture did show the presence of $CD49a^+$ in healthy skin (Foster *et al.*, 1990; Purwar *et al.*, 2011). In murine models, $T_{RM}$ cells can be defined by the tissue retention and non-recirculating properties. In human, such functional characterization is difficult, but application of alemtuzumab (anti-CD52) to cutaneous lymphoma patients and skin transplant onto immunodeficient mice demonstrated retention of $T_{RM}$ cells or skin resident $T_{EM}$ cells in human skin (Clark *et al.*, 2012; Watanabe *et al.*, 2015). A re-circulating T cell population lacking CD103 and CD69 expression that expresses CCR7 has also been identified; thus,

human skin is protected by both resident and re-circulating T cell populations (Watanabe *et al.*, 2015).

Animals raised in a germ-free environment had few skin resident T cells (Clark, 2015). In line with that, few resident T cells were found in prenatal and newborn skin (Schuster *et al.*, 2012; Watanabe *et al.*, 2015). The lack of αβ $T_{RM}$ cells prior to infection indicates that $T_{RM}$ cells are likely to develop with time when the skin tissue is challenged immunologically. In mice, formation of $T_{RM}$ cells requires either viral challenge or epithelial inflammation (Mackay *et al.*, 2012). In addition, over $10^{10}$ bacterial cells cover the skin of a healthy human body (Grice *et al.*, 2008) and the colonization of specific commensal bacteria leads to the development of resident T cell subset with specialized immune response (Naik *et al.*, 2012; Naik *et al.*, 2015). A recent study suggested Tregs rapidly migrate into the neonatal skin and form the peripheral tolerance to microbial commensals (Scharschmidt *et al.*, 2015). Therefore, the skin microbiota play a crucial part in the development of the cutaneous immune system but the microbial burden also imposes immunologic pressure on the skin.

Apart from providing adaptive immune surveillance and immune-tolerance to the skin, T cells drive pathology in many types of skin diseases. In lesions of common chronic inflammatory skin disorders such as atopic dermatitis and psoriasis, T cells with distinct cytokine expression profiles were observed. Atopic dermatitis lesions contain distinct Th2 and Th22 subsets while chronic lesions also contain Th1 cells; whereas psoriasis lesions contain Th/Tc1 and Th/Tc17 subsets (Lowes *et al.*, 2008; Nograles *et al.*, 2009; Eyerich *et al.*, 2011; Gittler *et al.*, 2012; Hijnen *et al.*, 2013). The distinct cytokine patterns of the two diseases contribute to the different histological and clinical outcome (Guttman-Yassky *et al.*, 2011). Allergic contact dermatitis is characterised by the delayed type hypersensitive reaction mediated by Th1 and CD8 T cells (Fyhrquist *et al.*, 2014; Gulati *et al.*, 2014). Cytotoxic function of T cells has also been implicated particularly in diseases such as vitiligo and alopecia (Mandelcorn-Monson *et al.*, 2003; Xing *et al.*, 2014). In fixed drug eruption, intraepidermal CD8 T cells respond rapidly to clinical challenge of causative drugs with IFN-γ, perforin and granzyme B expression (Teraki and Shiohara, 2003; Mizukawa *et al.*, 2008). Also, in acute wounds, T cells are able to produce insulin-like growth factor 1, potentially participating in wound healing process (Toulon *et al.*, 2009). These examples highlight the contribution of T cells in a broad spectrum of diseases and inflammation.

### 3.1.4 Cytokines

Cytokines are a group of relatively small proteins that mediate communication between cells. Cytokine signals transduce through specific cytokine receptors expressed on the cell surface. They usually have a short range of effects through autocrine, paracrine or juxtacrine stimulation. Every cell type in the skin is able to produce some cytokines (Nickoloff *et al.*, 2007). Keratinocytes, in particular, can produce many cytokines, among them, IL-1, IL-6, IL-10, IL-18 and TNF (Nestle *et al.*, 2009a). These cytokines serve as potent amplifiers of local inflammatory responses and act on multiple cell types. The IL-2 family cytokines are

particularly important in survival or activation of T cells. In skin, hair follicle-derived IL-7 and **IL-15**, members of the IL-2 family cytokines, mediate homeostasis of memory T cells in the murine skin (Adachi *et al.*, 2015). Aside from being a potent cytokine producer, keratinocytes can respond to various cytokines produced by other cell types; in particular, **IFN-γ**, **IL-17** and **IL-22** from T cells; thereby, forming a T cell - keratinocyte cytokine network in skin inflammation.

**IL-15** is an important cytokine for homeostasis of CD8 T cells and Natural Killer (NK) cells. Its receptor (IL-15R) shares the same β subunit (CD122) and the common γ chain (CD132) with the IL-2 receptor (Liao *et al.*, 2011). Its downstream signaling, which involves Janus kinase (Jak) 1 and 3, and STAT5, promotes survival signals for T cells (Liao *et al.*, 2011). Homeostatic proliferation of memory CD8 T cell is depended on IL-15 (Goldrath, 2002; Judge *et al.*, 2002). In contrast to IL-2, which usually acts in either autocrine or paracrine manner, IL-15 can, additionally, be trans-presented by IL-15Rα to activate T cells (Dubois *et al.*, 2002). Apart from its role in homeostasis of CD8 T cells, IL-15 could also promote optimal memory T cell-mediated response (Richer *et al.*, 2015). In a murine HSV skin infection model, IL-15 is critical in the formation and maintenance of $T_{RM}$ cells in (Mackay *et al.*, 2013; Mackay *et al.*, 2015b). In addition, IL-15 stimulation may promote proliferation of regulatory T cells in human skin (Clark and Kupper, 2007).

**IFN-γ** is the characteristic cytokine produced by Th1, NK cells and CD8 T cells. IFN-γ promotes expression of MHC molecules and boosts cellular antiviral machinery (Samuel, 2001). Of interest, unlike other proinflammatory cytokines, IFN-γ inhibits proliferation of keratinocytes (Hattori *et al.*, 2002). It can induce expression of more than 200 genes related to inflammation in epithelial cell (Sanda *et al.*, 2006) and production of cytokines (e.g. IL-1, IL-6 IL-15) by keratinocytes (Teunissen *et al.*, 1998). In addition, IFN-γ induces production of chemokines (CCL5, CXCL9, CXCL10, and CXCL11) and up-regulation of adhesion molecules (Rashighi *et al.*, 2014). $T_{RM}$ cells generated from viral infection particularly employ IFN-γ to induce localized inflammation and antiviral responses (Chapman *et al.*, 2005; Schenkel *et al.*, 2013; Ariotti *et al.*, 2014; Schenkel *et al.*, 2014).

**IL-17** is the signature cytokine produced by Th17 and Tc17 cells. Neutrophils, mast cells, γδ T cells and innate lymphoid cells can also produce IL-17 in skin during inflammatory conditions such as psoriasis (Res *et al.*, 2010; Laggner *et al.*, 2011; Lin *et al.*, 2011; Teunissen *et al.*, 2014a; Villanova *et al.*, 2014). Essentially, IL-17 is not one single cytokine but a family of cytokines, namely, IL-17A-F. Among them, IL-17A and IL-17F are the most studied. They could either form homodimer or heterodimer, and signal through IL-17 receptor complex composed of IL-17RA and IL-17RC (Gaffen, 2009). IL-17 is a potent inducer of proinflammatory cytokines and chemokines, especially CXCL1, IL-8, and CCL20, as well as antimicrobial peptides (Liang *et al.*, 2006). Skin resident T cell subsets respond to commensals with IL-17 production (Naik *et al.*, 2012). Genetic deficiency within the IL-17 and Th17- related pathway is associated with chronic mucocutaneous candidiasis (Cooper *et*

*al.*, 2015; Ling *et al.*, 2015). In addition, IL-17 is the critical pathogenic cytokine in psoriasis and serves as an effective target for therapy (see **Psoriasis** section).

**IL-22** is a member of the IL-10 cytokine family. The IL-22 receptor is expressed on epithelial cells, but not on immune cells (Wolk *et al.*, 2004). IL-22 is a key cytokine during epithelial homeostasis and inflammation. Initially, it was considered that IL-22 was expressed by Th17 cells, but it was later found that IL-22 could also be expressed by Th1, or, a subset which did not produce IFN-γ or IL-17, i.e. Th22 (Duhen *et al.*, 2009; Eyerich *et al.*, 2009). Recently, mast cells and innate lymphoid cells have been suggested to be efficient IL-22 producers (Cupedo *et al.*, 2008; BPharm *et al.*, 2015)). The gene expression of *IL22* is regulated by Aryl Hydrocarbon receptor (AHR), which can be activated by xenobiotic chemicals (Veldhoen *et al.*, 2008; Ramirez *et al.*, 2010). On keratinocytes, IL-22 induces strong antimicrobial response and promotes hyper-proliferation of keratinocytes (Wolk *et al.*, 2004; Wolk *et al.*, 2006). It can also potentiate inflammatory response mediated by TNF (Eyerich *et al.*, 2009). IL-22 has been proposed as a pathogenic cytokine in psoriasis and has also been implicated in atopic dermatitis (Nograles *et al.*, 2009; Eyerich *et al.*, 2011). However, IL-22 has a protective role in inflammatory bowel disease and gut pathology, indicating its tissue-specific roles (Ouyang, 2010).

### 3.2 Human vs. Mouse

Much of our understanding about immunology and skin inflammation is derived from experimental murine models. While a large proportion of the knowledge is transferable between species, it is important to bear in mind the differences between human and mouse. Mouse skin is covered by fur and has a higher density of hair follicles than human skin. The epidermis of mice contain only 2-3 layers of keratinocytes and is only one-quarter of the thickness of human epidermis; therefore, mice skin also has a higher turnover rate (Gudjonsson *et al.*, 2007). Although LCs are present in both human and mice epidermis, dermal DCs with a different set of phenotypic markers were observed in human and mouse skin (Heath and Carbone, 2009; Haniffa *et al.*, 2015). Attempts have been made to correlate the functionality of different subsets of DCs across species (Ginhoux *et al.*, 2012; McGovern *et al.*, 2014). Sharply contrasting with human skin is the constitution of dendritic epidermal γδ T cells (DETCs) in mouse epidermis. DETCs bear the invariant Vγ1 Vδ6 TCR and form immune synapse-like structures with squamous keratinocyte tight junctions in the steady state, indicating that DECTs may recognize a self-ligand expressed by keratinocyte (Chodaczek *et al.*, 2012). Such interactions are crucial in the development of epidermis as mice without DETCs has aberrant keratinocyte homeostasis and impaired wound healing (Jameson *et al.*, 2002). Previous studies also showed that DETCs might mediate immune surveillance through signaling by NKG2D, which senses stress-induced ligands (Girardi *et al.*, 2001; Hayday and Tigelaar, 2003; Strid *et al.*, 2011). In the murine HSV model, DETCs

and αβ $T_{RM}$ cells shared epidermal niches and the formation of $T_{RM}$ cell is associated with the decrease density of DETCs in the infected region (Zaid *et al.*, 2014). Some of the immunosurveillance strategies of DETCs may be employed by $T_{RM}$ cells in human, but the lack of DETCs signifies the difference between the human and mouse system.

# 4 SKIN DISEASES

The human skin can be affected by a broad spectrum of diseases, ranging from infections to malignancies and immune-mediated diseases. Forming the external barrier, the skin is subjected to immunologic challenges of different pathogens. Exposure to chemical substances, UV radiation, and certain pathogens, such as HPV, increase the risk of developing skin malignancy (Akgül *et al.*, 2006) and lymphomas derived from different T cell subsets also affect the skin (Campbell *et al.*, 2010). Several groups of immune-mediated diseases target the skin: autoimmune diseases like pemphigus; common inflammatory diseases such as psoriasis; and allergic diseases exemplified by allergic dermatitis. In some cases, this classification fails to recognize the complexity of the pathogenic mechanism as evidenced by psoriasis and vitiligo, in which both autoimmune and inflammatory mechanisms have been proposed. Nonetheless, the etiology of these diseases, so-called multifactorial diseases, involves predisposition of multiple genetic factors interacting with environmental triggers that lead to dysregulation of the immune system.

## 4.1 Psoriasis

Psoriasis is a common chronic inflammatory skin disease affecting 2-3 % of the world population (Lowes *et al.*, 2007; Nestle *et al.*, 2009b). Psoriasis can occur early on in childhood, but its onset usually starts in late adolescence and affects males and females equally (Farber and Nall, 1974; Mallbris *et al.*, 2005). The severity and area of affected skin vary among patients and can be assessed by Psoriasis Area and Severity Index (PASI), Physician Global Assessment (PGA) or Body Surface Area (BSA). There is a huge diversity in the phenotypes of psoriasis, ranging from acute widespread small lesions associated with throat infection (guttate psoriasis) to pustular lesions affecting the palms and soles (palmo-plantar psoriasis) or the whole body (generalized pustular psoriasis). Plaque psoriasis is the most common type of psoriasis accounting for 90% of all psoriasis cases (Boehncke and Schön, 2015). Plaque psoriasis is clinically defined by the formation of demarcated erythematous plaques with silvery scales (Nestle *et al.*, 2009b) (Figure 4.1). Although primarily affecting the skin, psoriasis could affect patients systemically with psoriatic arthritis, nail dystrophy, metabolic dysregulation, cardiovascular diseases and other inflammatory conditions as co-morbidities (Nestle *et al.*, 2009b; Davidovici *et al.*, 2010). Nowadays, effective treatments are available, but psoriasis cannot be cured and may relapse preferentially at sites of previous inflammation (Clark, 2011).

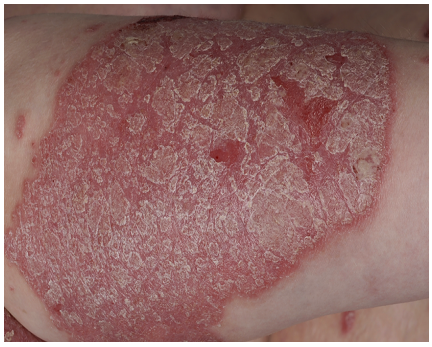

**Figure 4.1 Plaque Psoriasis**
**Courtesy of Dr. Liv Eidsmo**

### 4.1.1 Genetics

The genetic impact of psoriasis is evident in family and twin studies where concordance rate of monozygotic twins and dizygotic twins has been estimated as 35–72% and 12–23% respectively (Farber *et al.*, 1974; Bowcock, 2005). Early tissue typing studies suggested that a high proportion of psoriasis patients carried a specific HLA-C type (McMichael *et al.*, 1978). Indeed, through classical linkage and sequencing analysis, the *HLAC* region has been identified as the most significantly associated locus with psoriasis susceptibility (Asahina *et al.*, 1991; Trembath *et al.*, 1997; Nair *et al.*, 2000), with *HLA-Cw6* (*HLA-C*0602*) being identified as the susceptible risk allele (Nair *et al.*, 2006). Additionally, *HLA-C*0602* has found to be particularly prevalent in patients with early onset of diseases or with the guttate phenotype (Enerbäck *et al.*, 1997; Gudjonsson *et al.*, 2002; Lysell *et al.*, 2013). Human Leukocyte Antigen (HLA)-C is one of the classical MHC class I molecule in human alongside with HLA-A and HLA-B. HLA molecules are highly polymorphic with some of the most variable genetic traits in the human population. As a classical MHC class I molecule, HLA-C could present peptide antigens to CD8 T cells and trigger potent cytotoxic and cytokine responses (Blais *et al.*, 2011). With the technological advance of the genome wide association study (GWAS) on large cohorts of patients, another gene within antigen presentation pathway, *ERAP1*, has been identified as a psoriasis susceptible gene (Consortium *et al.*, 2010; Sun *et al.*, 2010). *ERAP1* encodes for the endoplasmic reticulum aminopeptidase 1, which cleaves peptides for MHC-class I presentation. Interestingly, in Swedish patients, the genetic association of *ERAP1* is only confined to patients with disease onset between 10-20 years of age (Lysell *et al.*, 2013).

Apart from *ERAP1*, many new loci have been identified associated with psoriasis by GWAS within to the IL-23/Th17 pathways (*IL12B*, *IL23A*, *IL23R*), innate immunity, TNF receptor signaling and downstream NFκB pathway (e.g. *TNIP1*, *TNFAIP3*, *CARD14)* (Liu *et al.*, 2008; Nair *et al.*, 2009; Jordan *et al.*, 2012; Tsoi *et al.*, 2012; Harden *et al.*, 2015b). Functionally, Th17 cells from individuals with the protective allele of *IL23R* show IL-23 unresponsiveness, indicating functional consequence of genetic association (Di Meglio *et al.*, 2013); while variants in *CARD14* could increase NFκB signaling and lead to increased production of proinflammatory cytokines or chemokines production in keratinocytes, providing functional links to these genetic associations (Jordan *et al.*, 2012). Despite being primarily a skin condition, surprisingly few genes closely related to skin function are associated with psoriasis. *LCE* genes, encoding for the late cornified envelope (LCE) proteins important for epidermal differentiation, remain the few examples of susceptible genes related to skin function (de Cid *et al.*, 2009; Zhang *et al.*, 2009).

The identification of psoriasis associated genetic variants has increased our understanding of the pathogenesis of psoriasis in which both innate and adaptive immunity are involved. Some genetic variations are more enriched in certain subgroups of patients, highlighting the delicate interaction between genetic and environmental factors that could result in different clinical outcomes.

### 4.1.2 Pathogenesis

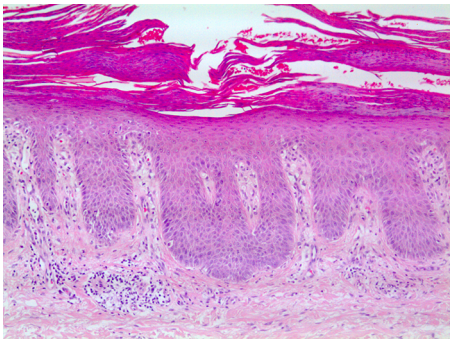

**Figure 4.2 Histology of Plaque Psoriasis. Courtesy of Dr. Mari-Anne Hedblad**

The epidermal pathology of psoriasis is characterized by acanthosis, parakeratosis and elongation of rete ridges (Figure 4.2), which are the results of hyperproliferation of keratinocytes and dysregulation of keratinocyte differentiation. Dysfunctional keratinocytes were once thought to be the sole cause for psoriasis, but it is now appreciated that the massive infiltration of inflammatory cells, including T cells, dendritic cells, neutrophils, mast cells (Lin *et al.*, 2011), innate lymphoid cells (Teunissen *et al.*, 2014a; Villanova *et al.*, 2014) among others, provide proinflammatory stimuli to the keratinocytes. T cells producing high level of proinflammatory cytokines, in particular, play a vital role to activate keratinocytes in psoriasis (Boyman *et al.*, 2007; Nestle *et al.*, 2009a). Over the past two decades, much effort has been put into understanding different aspects of the pathogenesis of psoriasis (Lowes *et al.*, 2007; Nestle *et al.*, 2009b; Boehncke and Schön, 2015) (Figure. 4.3).

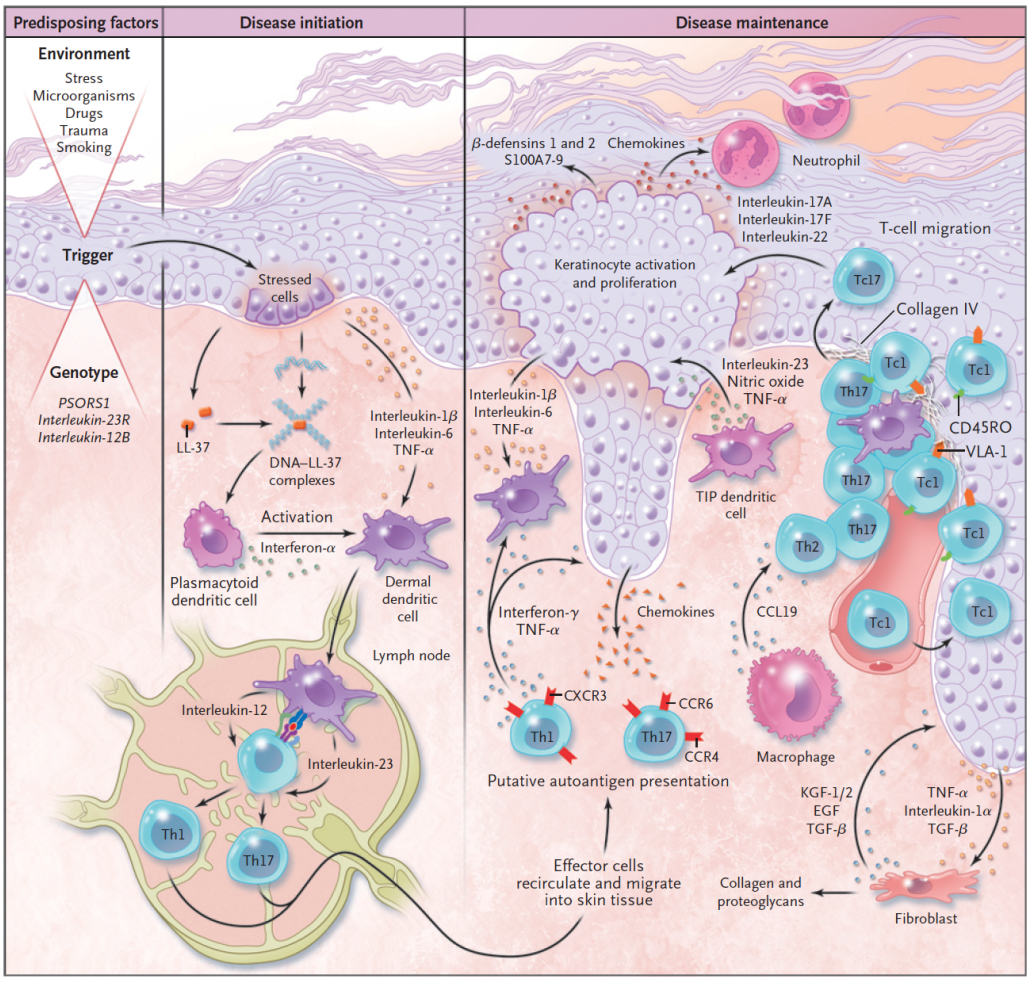


**Figure 4.3. Proposed Immunopathogenesis of Psoriasis. Reproduced with permission from (Nestle *et al.*, 2009b). Copyright Massachusetts Medical Scociety.**

#### *4.1.2.1 Innate Immune Cell Initiates Psoriasis Plaques Formation*

Although the exact triggers for psoriasis are yet to be identified, the initiation of psoriasis inflammation may stem from cellular stress imposed on keratinocytes. These potential triggers include infections, drugs, injuries and psychological stress (Nestle *et al.*, 2009b). Keratinocytes produce proinflammatory mediators including cytokines, chemokines, and antimicrobial peptides in response to cellular stress. The cathelicidin antimicrobial peptide, LL37, in particular, could form complexes with nucleic acids and activate different DCs subsets through TLR's signaling. For instance, LL-37-RNA complexes activate mDC through TLR7/8 (Ganguly *et al.*, 2009), whereas LL-37-DNA complexes activate pDC through TLR9 which promotes IFNα production (Lande *et al.*, 2007). In a xenotransplant mouse model of psoriasis, IFNα production from plasmacytoid dendritic cells (pDC) was crucial for the initiation of psoriasis inflammation (Nestle *et al.*, 2005). The role of IFNα in precipitating psoriasis has also been demonstrated clinically when patients receive IFNα therapy (Funk *et al.*, 1991).

#### *4.1.2.2 Chronic Psoriasis Results from Cytokine Overexpression*

Given the early inflammatory milieu created by both keratinocytes, pDCs, and other local cell types, it has been proposed that activated dermal dendritic cells would migrate to the draining lymph nodes, and subsequently, activate and polarize T cells to Th/Tc1 or Th/Tc17 responses. Activated T cells released from skin draining lymph nodes preferentially enter the skin at inflamed sites due to the increase in chemokines such as CXCL9, CXL10 and CCL20 and secrete pathogenic cytokines IFNγ, TNF, IL-17, and IL-22 (Fierlbeck *et al.*, 1990; Schlaak *et al.*, 1994; Lowes *et al.*, 2008; Nograles *et al.*, 2008; Ortega *et al.*, 2009; Res *et al.*, 2010). The proinflammatory environment also prevents the Tregs mediated immunosuppression (Goodman *et al.*, 2009) and Tregs shifting to become IL-17 producing cells has been reported (Bovenschen *et al.*, 2011). Activated neutrophils, mast cells and innate lymphoid cells accumulated in psoriasis lesions are additional sources of IL-17 and IL-22 cytokines; therefore, a number of immune-cells contribute the pathogenesis to the disease (Fuentes-Duculan *et al.*, 2010; Lin *et al.*, 2011; Teunissen *et al.*, 2014a; Villanova *et al.*, 2014; BPharm *et al.*, 2015). Together, these pathogenic cytokines activate keratinocytes to hyper-proliferate and produce more antimicrobial peptides, proinflammatory cytokines, and chemokines; thereby, expanding the recruitment of immune cells into the lesion and creating a positive feedback vicious cycle (Figure. 4.3).

#### *4.1.2.3 Pathogenic Role of T Cells in Psoriasis*

The pathogenic involvement of T cells in psoriasis has been shown in different settings long before the discovery of IL-23/Th17 as the key pathogenic pathway. Early clinical reports showed that bone marrow transplantation from a donor with psoriasis could lead to onset of the disease in recipient with no history of psoriasis (Gardembas-Pain *et al.*, 1990), indicating that psoriasis can be transferred by blood-borne cells. Infusion of monoclonal antibodies depleting $CD4^+$ T cells has been shown to reduce severity of psoriasis (Prinz *et al.*, 1991; Morel *et al.*, 1992). In animal models of psoriasis, where human skin is transplanted onto the

severe combined immunodeficiency (SCID) mice, maintenance of skin pathology within grafted lesional psoriasis skin is T cell-dependent (Gilhar *et al.*, 1997). Besides, intradermal injection of blood derived activated $CD4^+$ cells to uninvolved skin from psoriasis patients could induce active psoriasis (Nickoloff and Wrone-Smith, 1999). Transplanting uninvolved skin from psoriasis patients onto severely immunocompromised (AGR) mice spontaneously induced psoriasis without blood derived cells; blocking of T cells in this model showed that the spontaneously psoriasis development was resident T cell- dependent (Boyman *et al.*, 2004). Using the same model, the group further demonstrated that the infiltration of $CD49a^+$ epidermal T cells, but not dermal T cells, is associated with the development of psoriatic inflammation (Conrad *et al.*, 2007). Clinically, Alefacept (a soluble LFA-3Ig) showed therapeutic efficacy associated with depletion of T cells, which is the major cell type expressing CD2 in the lesion (Chamian *et al.*, 2007). Overall, overwhelming evidences support the involvement of T cells and their cytokine production in psoriasis pathogenesis. Within the T cell population, although conventional αβ T cells are the primary T cell population present in human psoriasis lesion, IL-17 producing γδT and MAIT cells have also been implicated in human psoriasis (Laggner *et al.*, 2011; Teunissen *et al.*, 2014b), whereas IL-17 producing γδT cells are the primary pathogenic cells type in the imiquimod induced psoriasiform inflammation in the murine model (Cai *et al.*, 2011). Recently, TCR sequencing of psoriasis lesional biopsies has also revealed distinct clonal signatures of γδ TCR (Harden *et al.*, 2015a).

#### *4.1.2.4 In Search for the Autoantigens for Psoriasis*

Oligoclonal expansion of T cells has been found in psoriasis lesions (Chang *et al.*, 1994; Menssen *et al.*, 1995; Lin *et al.*, 2001), and sampling from recurrent psoriasis lesions found retained clonal expansion in relapsing lesions that was absent in uninvolved skin from the same donors (Vollmer *et al.*, 2001). Taken the prominent role of T cells in psoriasis pathogenesis and the genetic associations with the antigen presentation pathway (*HLAC* and *ERAP1*), a very obvious question is what antigen(s) T cells recognize in psoriasis and whether the response to autoantigen drives psoriasis pathogenesis. Many autoantigens have been proposed, some of which (e.g. Ezrin, Maspin) resemble peptides derived from streptococcus, supporting a molecular mimicry hypothesis (Besgen *et al.*, 2010). Recently, LL37, the AMP triggered initial innate activation in psoriasis, was proposed to be an auto-antigen (Lande *et al.*, 2014). Thus, LL37 has putative roles in two distinct pathogenic mechanisms through activating pDCs and as a T cell antigen. Another recent report suggested that melanocyte protein ADAMTSL5 could trigger IL-17 production by CD8 T cells in human psoriasis (Arakawa *et al.*, 2015). Nevertheless, the direct causal role of these autoantigens or autoreactive T cells in psoriasis has not been shown yet.

### 4.1.3 Treatments

Effective therapies that control the symptoms of psoriasis are available today and most of these treatments mediate their therapeutic effects through different forms of immunosuppression. In general, therapies for psoriasis can be classified as topical,

phototherapy and systemic therapy. Treatment is generally decided according to the severity of the disease. Topical vitamin D analogues and glucocorticoids can be applied on affected skin of patients and promote “local” immunosuppression. For patients with moderate to severe psoriasis affecting large areas of the skin, topical treatment is insufficient. Narrow-band UVB (nb-UVB) phototherapy is typically the next choice of therapy for patients where topical therapies are not effective and nb-UVB is often used together with vitamin D analogues. The exact mechanism of the therapeutic effect of nb-UVB is likely to be the mixed effects of induction of apoptosis in skin cells (keratinocytes and immune cells) and immunosuppression by lowering transcription of inflammatory cytokines (Ozawa *et al.*, 1999; Walters *et al.*, 2003). Cyclosporine, targeting Calcineurin, and Methotrexate, limiting cell division by inhibiting folic acid metabolism, are systemic treatments that are used to treat moderate to severe psoriasis patients (Heydendael *et al.*, 2003).

Biologic agents that target molecules involved in the pathogenesis of psoriasis have revolutionized the treatment for patients with severe psoriasis during the last decade. TNF antagonists including Infliximab, Etanacept and Adalimumab, and anti-IL-12/23p40, Ustekinumab, are effective treatments, which highlight key role of TNF and IL-12/23 in psoriasis pathogenesis (Lowes *et al.*, 2007). Anti-IL-23p19, Guselkumab, and Tildrakizumab, show promising results in recent clinical trials (Gordon *et al.*, 2015; Papp *et al.*, 2015), and Secukinumab, an IL-17A monoclonal antibody is already approved for treatment of psoriasis and psoriatic arthritis with Ixekizumab directed against the same target follows closely behind (Langley *et al.*, 2014; Griffiths *et al.*, 2015; Mease *et al.*, 2015). Together these drugs further verify the central role of IL-23/Th17 axis in psoriasis. The development of biological agents has significantly improved the treatment options for moderate to severe psoriasis patients, but considering the logistic and cost of biologic agents, there is still a need for alternatives. Small molecules for oral use are currently being developed. Apremilast, inhibiting PDE4, an enzyme breaking down cyclic adenosine monophosphate (cAMP), is already approved and widely used (Poole and Ballantyne, 2014). Tofacitinib, a JAK1/3 inhibitor, showed promising therapeutic effect in a clinical trail and may provide more options to moderate to severe patients in the future (Bachelez *et al.*, 2015). Nonetheless, all of these immunosuppressive treatments may cause adverse effects like infection and malignancies, due to their immunomodulatory effects.

### 4.1.4 Molecular Scar of Resolved Psoriasis

Although effective treatments are available for psoriasis and results in suppression of inflammation, resolution of epidermal thickness and clinical remission, psoriasis cannot be cured (Clark, 2011). Psoriasis often recurs at site of the previous lesions upon withdrawal of treatment, indicating the existence of “localized disease memory". Krueger and colleagues addressed this question by following patients treated with etanercept and found a set of up-regulated genes related to T cells and inflammatory cytokines remaining in successfully treated lesion as compared to uninvolved skin after three months of anti-TNF therapy (Suárez-Fariñas *et al.*, 2011). This proposed “molecular scar” may form the basis of the

"localized disease memory" underlying the recurrence of psoriasis in previous lesions (Suárez-Fariñas *et al.*, 2011) and $T_{RM}$ cell has been proposed to be involved in forming site specific disease memories (Clark, 2011). The role of $T_{RM}$ cells in "localized disease memory" in resolved psoriasis is examined in this thesis (Figure 4.4).

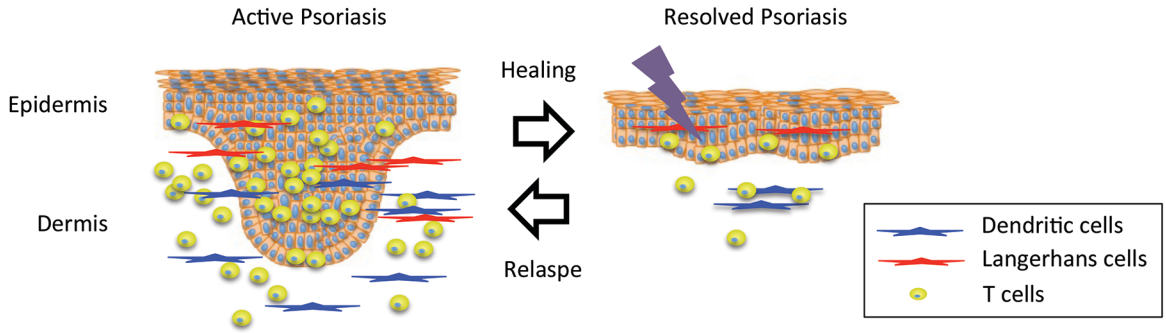


**Figure 4.4. Schema of proposed "localized disease memory" in resolved psoriasis.**
Upon healing by successful treatment, pathogenic memory T cells reside in the epidermis of resolved lesion. Upon re-stimulation, these memory T cells will be activated *in situ*; thereby, giving rise to a local inflammatory response that further recruits immune cells to the lesion and leads to relapse of psoriasis at previous affected areas.

## 4.2 Vitiligo

Vitiligo is an acquired chronic disorder of the skin that affects 0.5-1% of the population with equal incidence in men and women (Ezzedine *et al.*, 2015). Vitiligo is characterized by depigmented macules (Figure 4.5) as a result of local loss of melanocytes in affected patches of the skin. Half of the patients develop the disease before 20 years of age (Majumder *et al.*, 1993) and vitiligo is often associated with other autoimmune diseases: Addison's disease, autoimmune thyroid disease, type I diabetes mellitus, alopecia areata and psoriasis (Rezaei *et al.*, 2007; Sheth *et al.*, 2013).

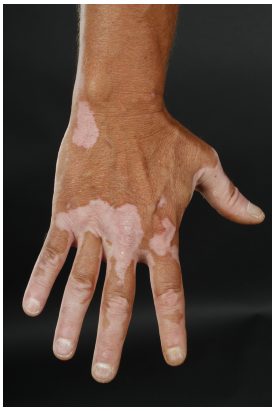

**Figure 4.5. Vitiligo. Courtesy of Dr. Jakob Wikström**

### 4.2.1 Genetics

Vitiligo can run in families indicating a strong genetic predisposition to the disease. A study showed that the concordance of vitiligo in monozygotic twins is 23% while 6-8% of probands' first-degree relatives of patients also have vitiligo (Alkhateeb *et al.*, 2003). The relative risk of vitiligo has been estimated 13 -18 times higher in patients' first-degree relatives as compared to the general population (Alkhateeb *et al.*, 2003). Genetic associations between different *HLA* alleles with vitiligo were not consistent with *HLA-A2*, *DR4*, and *DR7* being reported as susceptible *HLAs* in different ethnic groups (Rezaei *et al.*, 2007; Tarlé *et al.*, 2014). GWAS identified tyrosinase, *TYR*, as the key susceptible gene of vitiligo (Jin Y et al., NEJM, 2010). Tyrosinase is a key enzyme in melanin synthesis and is one of the proposed autoantigen in vitiligo (Song *et al.*, 1994; Ezzedine *et al.*, 2015). The majority of

other genes associated with vitiligo identified from the GWAS were primarily involved in inflammation or immune responses (e.g. *PTPN22*, *IL2RA*, *GZMB*, *FOXP3*, *CD80*, *CCR6*, *NLRP1*, *IFIH1*, *TRIF*, *CASP7*, and *C1QTNF6*.) (Jin *et al.*, 2010; Spritz, 2012). However, with only 23% disease concordance in monozygotic twins indicates other factors also play a role in the pathogenesis.

### 4.2.2 Pathogenesis

The etiology of vitiligo is complex and not fully understood. The pathology of vitiligo is characterized by the loss of melanocytes in the epidermis. The oxidative stress theory of the pathogenesis of vitiligo provided certain insights on the biology of melanocytes, pathogenesis of vitiligo and possible environmental link to the disease. According to the oxidative stress theory, during melanin synthesis, quinones and indoles intermediate products are generated and the accumulation of these intermediates induce reactive oxygen species formation, which could lead to apoptosis of melanocytes (Boissy and Manga, 2004; Kroll *et al.*, 2005) (Figure 4.6 A). However, GWAS identified susceptible genes that predominantly suggested vitiligo as an autoimmune disease (Jin *et al.*, 2010; Spritz, 2012).

Serologic evidence showed that autoantibodies, capable of binding to melanocytes circulate in the serum of vitiligo patients; these autoantibodies were used to identify several vitiligo autoantigens, for example, tyrosinase, TRP-2, SOX-10 and melanin concentrating hormone receptor (MCHR) (Song *et al.*, 1994; Kemp *et al.*, 2007; Waterman *et al.*, 2010). Antibodies from vitiligo patients are capable of binding to melanocytes; mediating antibody-dependent cellular cytotoxicity (ADCC); and inducing HLA-DR, ICAM1, and IL-8 expression by blocking MCHR, facilitating the immune response against melanocytes (Norris *et al.*, 1988; Gilhar *et al.*, 1995; Gottumukkala *et al.*, 2006; Kemp *et al.*, 2007) (Figure 4.6 A).

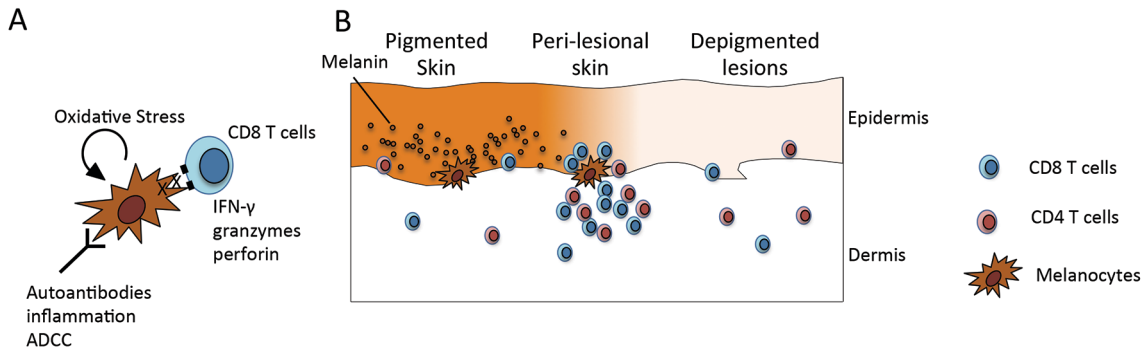


**Figure. 4.6 Schematic drawing of pathogenic mechanisms in vitiligo**. (A) Loss of melanocytes is the hallmark of vitligo pathology. This can be triggered by different mechanisms. Oxidative stress may lead to apoptosis of melanocytes; autoantibodies targeting melanocytes may promote inflammation or kill melanocytes by antibody-dependent cellular cytotoxicity (ADCC); cytotoxic CD8 T cells may kill melanocytes through target killing or induce apoptosis of melanocytes by IFN-γ. (B) Illustration of T cell infiltration in vitiligo lesion.

In parallel with the antibody response, T cell-mediated cellular immune response is also implicated in the pathogenesis of vitiligo. Tyrosinase, Melan-A (MART1), and gp100 have been identified as T cell autoantigens in vitiligo (Palermo *et al.*, 2005; Oyarbide-Valencia *et al.*, 2006) and a high frequency of melanocyte-specific skin homing CD8 T cells can be

found in peripheral blood of vitiligo patients (Ogg *et al.*, 1998). Immune cells including macrophages, CD4, and CD8 T cells, infiltrate the perilesional vitiligo skin but not the non-lesional skin from vitiligo patients, providing evidence for a localized immune response (van den Wijngaard *et al.*, 2000). Higher density of perforin or granzyme B expressing cells populate the perilesional skin in close proximity to disappearing melanocytes, compared to the central part of individual lesions, non-lesional or control skin from non-affected individuals (van den Wijngaard *et al.*, 2000) (Figure 4.6 A.B). Furthermore, T cells isolated from the perilesional skin of vitiligo can target and kill autologous melanocytes (van den Boorn *et al.*, 2009; Wu *et al.*, 2013). Lately, a mouse model of vitiligo identified IFN-γ/CXCL10 pathway as the key pathogenic pathway (Harris *et al.*, 2012; Rashighi *et al.*, 2014). IFN-γ was shown to reduce proliferation and to induce apoptosis in melanocytes (Yang *et al.*, 2015); and the IFN-γ induced downstream chemokine CXCL10 was implicated in recruitment of T cells into vitiligo lesions (Rashighi *et al.*, 2014) (Figure 4.6 A.). Induced recruitment of Tregs in spontaneous vitiligo mouse model showed reduced depigmentation, suggesting a regulatory role of Treg in vitiligo (Eby *et al.*, 2015). In addition, Treg induced anergy of self reactive CD8 T cells targeting vitiligo antigen (Maeda *et al.*, 2014).

### 4.2.3 Treatments

As compared to psoriasis, vitiligo patients have limited treatment options. Melanocytes renew locally in the skin, and the bulge area of the hair follicles act as a reservoir of melanocyte precursors (Cui *et al.*, 1991). Successful vitiligo treatments do not only require suppression of the immune response but also the repopulation of melanocytes to affected skin from these reservoirs. Hairless skin or skin with depigmented hairs cannot repigment by medical treatment and may require surgical grafting of autologous skin or transplant of cultured melanocytes (Shaffrali and Gawkrodger, 2000). Topical immunomodulatory drugs such as calcineurin inhibitors (tacrolimus and pimecrolimus) and corticosteroids are first-line vitiligo treatments. Phototherapy with nb-UVB can trigger proliferation of melanocyte precursors (Goldstein *et al.*, 2015) and is often combined with application of topical agents like steroids and vitamin D analogues (Shaffrali and Gawkrodger, 2000; Taieb *et al.*, 2012). Systemic steroid treatment, cyclosporine or anti-TNF show limited effect in inducing effective repigmentation (Taieb *et al.*, 2012).

# 5 AIMS

This thesis aims to study the functional properties of tissue-resident memory T ($T_{RM}$) cells in healthy human skin and in two immune-mediated skin diseases, psoriasis and vitiligo.

The specific objectives are:

- To dissect the functional heterogeneity of skin resident T cells in healthy skin and vitiligo lesions (**PAPER I**);
- To investigate the potential role of skin resident T cells in the proposed localized disease memory in psoriasis (**PAPER II**);
- To explore proinflammatory properties of granzyme A in psoriasis pathology (**PAPER III**); and
- To study the genetic association of *IL22* promoter in psoriasis by stratification for age at onset and the functional consequences of carrying these genetic variants (**PAPER IV**).

# 6 MATERIALS AND METHODS

Tissue samples (**PAPER I, II, III, IV**)

Healthy skin samples were obtained from reconstructive surgery at the AdVita Clinic at St. Göran Hospitial (Stockholm, Sweden) (**PAPER I-III**) or the reconstructive surgery department at Karolinska University Hospital (Stockholm, Sweden) (**PAPER I**). Four milimeters punch biopsies (one to five per individual) were obtained from the trunk or thighs under local anaesthesia from plaque psoriasis patients recruited from either psoriasis association (Sundyberg, Sweden) or Dermatology Department at Karolinska University Hospital (Stockholm, Sweden) (**PAPER II,III**). Previous psoriasis lesions upon clinical remission were located by photos taken at times of active disease, reliable clinical information, or post-inflammatory hyperpigmentation (**PAPER II**). Patients in the genetic study (**PAPER IV**) were diagnosed with psoriasis after clinical examination by a dermatologist at the department of Dermatology, Karolinska University Hospital, Stockholm, Sweden or at the Swedish Psoriasis Association, Gothenburg, Sweden. Vitiligo patients were recruited from the Vitiligo Patient Association in Sweden and the Dermatology Department at Karolinska University Hospital (Stockholm, Sweden). Lesional biopsies were sampled from within 1 cm to the edge of depigmented lesion border. Non-lesional biopsies were taken at least 10 cm away from depigmented lesions.

All studies were approved by Stockholm Regional Committee of Ethics and performed according to the Declaration of Helsinki Principles. For patients below 18 years of age from **PAPER IV**, parental written consent was obtained. Ethical Permits: **PAPER I**: 2012/50-31/2; amendment: 2015/0041-31,2015/933-32, 2012/1900-31/1, 2013/1800-32; amendment: 2015/1078-32. **PAPER II**: 2005/977-31/3, 2007/20-31/2, 2012/50-31/2. **PAPER III**: 2012/50-31/2. **PAPER IV**: 99-296, 02-241, amendment 02-241 - 2011/1601-32, 03-198

Genetic analysis (**PAPER III**)

The *IL22* promoter in 12 psoriasis patients was sequenced using ABI3730 (Applied Biosystem) according to manufacturer's protocols. Putative binding sites for AhR-ARNT complex were identified by RAVEN (http://www.cisreg.ca/cgi-bin/RAVEN/a) and TFBIND (tfbind.hgc.jp). Single nucleotide polymorphisms (SNPs) (rs12307915, rs2227473, and rs2227483) were typed with TaqMan SNP Genotyping assay (Applied Biosystems). And the INDEL, rs35774195/rs10784699 (AT/-) was typed with customized TaqMan Genotyping assay with one probe bound with the sequence with AT insertion and the other probe without the AT insertion. The SNP rs10784699 was imputed from the genotype of rs2227477.

Cell culture

Cells were cultured at 37°C, 5% CO2. For Peripheral blood mononuclear cells (PBMCs), skin cell suspensions and sorted T cells, cells were cultured in Complete Medium: Roswell Park Memorial Institute medium (RPMI) 1640 supplemented with GlutaMAX™-I, 10%

Fetal Bovine Serum (FBS) (Hyclone) and 100U/mL Penicillin-streptomycin (PEST) (all from Life Technologies). P815 cell were courtesy of Dr. Yenan Brycesson, Center for infectious medicine, Huddinge. HeLa cells were courtesy of Dr. Ning Xu, Center for molecule medicine, Solna.

Keratinocyte culture and stimulation (**PAPER IV**)

Four mm punch biopsies were incubated in 5U/mL dispase (life technologies) overnight at 4°C. Epidermis was manually separated from dermis and incubated in 0.05% Trypsin/ Ethylenediaminetetraacetic acid (EDTA) (Life Technologies) at 37°C for 30 min. 10% FBS in Dulbecco's Modified Eagle Medium (DMEM) was added to the cells to inhibit trypsin digestion. Cells were washed and resuspended in EpiLife medium supplemented with Human Keratinocyte Growth Supplement (HKGS), 100 U/mL penicillin/streptomycin and 1% Fungizone (all from Life Technologies), and plated in dishes pre-treated with collagen (VWR). The keratinocytes were passaged twice and then cryopreserved for further use.

Thawed human keratinocytes were seeded in EpiLife/HKGS/PEST/Fungizone medium in a 24-well plate at a density of 40000 cells/well/mL and left to recover for 24 hrs. A final concentration of 35nM GrzA (Enzo Life Sciences), 10ng/mL IL17A (R&D) or a combination of both was prepared in medium excluding HKGS. Keratinocytes were washed with PBS before adding 0.5 mL of each culture mix in triplicates. After 48 hours of incubation at 37°C the supernatants were collected and immediately put on dry ice and then stored at -80°C.

Preparation of cell suspension from tissue samples (**PAPER I**, **II, III**)

Whole skin biopsies were placed in 5U/mL dispase (Life Technologies) overnight at 4℃ and epidermis was manually separated from dermis. Epidermis was cut into small pieces. In **PAPER I**, **II** (Figure 5,6) and **PAPER III**, both epidermis and dermis were digested with collagenase III (3 mg/ml; Worthington) for 90 min with DNase (5 mg/ml) in complete RPMI 1640 medium. In **PAPER II** (Figure 1-4), epidermis was placed in trypsin (0.025%)/EDTA (0.01%) (Life Technologies) for 15 min at 37°C. Epidermal single-cell suspension was prepared by repeated pipetting. The digested tissue suspensions were then diluted with 5-10 times volume of complete medium, 70-μm filtered, centrifuged and resuspended in complete medium for further experiments. PBMCs were prepared by Ficoll Plaque (GE Healthcare) gradient isolation. For cell sorting for cytotoxicity or functional assays, a relatively large number of T cells was required. Epidermal sheets were placed in complete RPMI medium with or without IL-15 (20ng/mL, R&D System) for 48 hours to allow cell migration out of the tissue. Cells in explant culture would consist of relatively enriched lymphocytes populations.

Flow Cytometry (**PAPER I**, **II, III, IV**)

In **PAPER II** and **IV**, cell suspensions were stained with Live/Dead Yellow Kit (Invitrogen) and, subsequently, the surface staining was performed with a cocktail of fluorochrome-

conjugated Abs at 4°C for 30-40 min. In **PAPER I** and **III**, both Live/Dead Yellow Kit (Invitrogen) staining and surface staining were done in the same step at 4°C for 30-40 min. For phenotypic analysis, cell suspensions were kept in complete medium for 18 hours. For some intracellular cytokine staining, cell suspensions were stimulated with phorbol 12-myristate 13-acetate (PMA) (50 ng/ml) and ionomycin (1 μg /ml) for 5 hours in the presence of brefeldin A (BD Biosciences) in the last 4 hours of stimulation following the manufacturer's protocols. BD Cytofix/Cytoperm Kit (BD Bioscience) was employed for the fixation and permeabilisation for intracellular staining of cytokines and cytotoxic granule constituent proteins according to manufacturer's protocols. Stained cells were acquired by a CyAn ADP analyzer (Beckman Coulter), LSR-II (BD Biosciences) or LSRFortessa flow cytometer (BD Biosciences), and analyzed using Flowjo (Tree Star). In **PAPER II**, the number of cells was quantified by the density of cells in unit surface area of skin and presented as "number of cells/$mm^2$".

Cytokine stimulation on cell suspension (**PAPER I**)

Epidermal cell suspensions were plated at 1-2 million cells/well in a 6-well plate and incubated with IL-15 (20ng/mL), IL-1β (20ng/mL), IL-6 (20ng/mL), IL-23 (20ng/mL), IL-7 (20ng/mL), IL-12 (50ng/mL), Type I IFN (2000U/mL) (all from R&D system) or medium only in the final volume of 4mL for 48 hours. Cells were passed through a 70-μm cell strainer before staining for flow cytometry.

Cell sorting (**PAPER I**, **II**)

For RNA and DNA extraction from specific population of cells, cell suspensions were stained for surface antigens immediately after preparation and sorted at Department of Microbiology, Tumor and Cell Biology in Karolinska Institutet using MoFlo XDP (Beckman Coulter) cell sorter. Stained cells were kept on ice before sorting. For RNA extraction, cells were sorted directly into Qiazol (Qiagen) and stored at -80°C for later RNA extraction. For DNA extraction, cell were sorted directly into cell lysis solution (Qiagen, Puregene Kit) and stored at room temperature before DNA extraction according to manufacturer's protocols. For functional and cytotoxicity assays in **PAPER I**, cells collected from explant culture were stained at 4°C and kept on ice before sorting. The sorting was performed on a BD FACZJAZZ at the Unit of Translational Immunology, Karolinska University Hospital. Cells were sorted into RPMI with 50% FBS at kept at 4°C. Cells were placed in complete medium overnight at 37°C before further experiments.

RNA purification and quantitative RT-PCR (**PAPER I**)

RNA was purified using miRNeasy Mini Kit (Qiagen) and reverse transcribed by High Capacity cDNA Reverse Transcription Kits (Applied Biosystems). The quantitative RT-PCR was performed using TaqMan PreAmp Master Mix Kit (Applied Bioscience) and the TaqMan Gene Expression Master Mix (Applied Bioscience) with custom-made TaqMan Low density array (TLDA) plates pre-coated with TaqMan assays (see below) following the

manufacturers' protocols. Relative expression value was calculated as: $2^{-\Delta CT}$, with beta-2 microglobulin (*B2M*) being the house-keeping gene.

| | | |
|---|---|---|
| AXIN2-Hs00610344_m1 | GAPDH-Hs99999905_m1 | ITGA1-Hs00235006_m1 |
| B2M-Hs00984230_m1 | GATA3-Hs00231122_m1 | ITGAE-Hs01025372_m1 |
| BCL2-Hs00608023_m1 | GZMA-Hs00989184_m1 | KLRG1-Hs00929964_m1 |
| BCL6-Hs00277037_m1 | GZMB-Hs01554355_m1 | KLRK1;KLRC4-KLRK1-Hs00183683_m1 |
| CCR6-Hs01890706_s1 | ID2-Hs00747379_m1 | PDCD1-Hs00169472_m1 |
| CD207-Hs00210451_m1 | ID3-Hs00171409_m1 | PRDM1-Hs00153357_m1 |
| CD3E-Hs01062241_m1 | IFNG-Hs00989291_m1 | PRF1-Hs00169473_m1 |
| CD4-Hs00181217_m1 | IL10-Hs00961622_m1 | RORC-Hs01076122_m1 |
| CD68-Hs00154355_m1 | IL15RA-Hs00542604_m1 | SELL-Hs00174151_m1 |
| CD8A-Hs00233520_m1 | IL17A-Hs00174383_m1 | TBX21-Hs00203436_m1 |
| CRLF2-Hs00845692_m1 | IL2-Hs00174114_m1 | TCF7-Hs00175273_m1 |
| CTLA4-Hs03044418_m1 | IL21R-Hs00222310_m1 | TCF7L1-Hs01064103_m1 |
| EOMES-Hs00172872_m1 | IL22-Hs01574154_m1 | TCF7L2-Hs01009044_m1 |
| FAS-Hs00236330_m1 | IL2RA-Hs00907779_m1 | TGFB1-Hs00998133_m1 |
| FASLG-Hs00181225_m1 | IL2RB-Hs01081697_m1 | TNF-Hs00174128_m1 |
| FOXP3-Hs01085834_m1 | IL7R-Hs00902334_m1 | TNFSF10-Hs00921974_m1 |

**Table: Assays on TaqMan Low density array (TLDA).**

RNA-seq (**PAPER II**)

Extracted RNA was stored at -80°C. Concentration and integrity of RNA was checked with a Bioanalyzer using RNA 6000 Pico Kit (Agilent) before library construction using SMART-Seq v4 Ultra Low Input RNA Kit (ClonTech) and sequenced by Illumina HiSeqTM 2000, at Beijing Genomic Institute, Hong Kong. RNA-seq analysis was performed using RStudio. Differential gene expression analysis was performed using EdgeR package (Robinson *et al.*, 2009) on Bioconductor using rounded expected read count data by RSEM (Li and Dewey, 2011). $CD103^+CD49a^+$ and $CD103^+CD49a^-$ $T_{RM}$ cells from the same donor were paired. Likelihood ratio test was used to calculate p-values and false discovery rate (FDR) method was employed to correct for multiple comparisons. Gene Enrichment analysis was performed by DAVID bioinformatics tool (Huang *et al.*, 2009).

TCR-seq (**PAPER II**)

DNA was extracted by using Puregene Kit (Qiagen). Extracted DNA was stored at -20°C and sent to Adaptive Biotechnogies, Seattle, US, for ImmunoSEQ analysis. The TCR Vβ CDR3 region was sequenced with the ImmunoSEQ platform (Robins *et al.*, 2009; Robins, 2013). Non-productive sequences were omitted from the data analysis.

Functional assay on sorted T cells (**PAPER II**)

Sorted CD8 T cell subpopulations were resuspended and kept in complete RPMI medium overnight at 37°C before use. 5000-10000 cells were plated per well on 96-well V-bottom plates. For time-course experiment, cells were stimulated with IL-15 (20ng/mL) for 1, 2, 4, 8 and 48 hours. Actinomycin-D (Act-D, 5μg/mL) was added prior to IL-15 to inhibit *de novo* synthesis of mRNA in the transcriptional blocking group. For antigenic stimulation, sorted CD8 T cells plated in the same way were cultured with P815 cells in 1:1 ratio with the presence of anti-CD3 (1μg/mL, OCT-3) and brefeldin A (1:1000, BD) for 5 hours at 37°C.

Cytotoxicity assay (**PAPER II**)

Sorted CD8 T cell subpopulations were resuspended and kept in complete RPMI medium overnight at 37°C before use as effector cells in cytotoxicity assay. The cytotoxic assays were performed at the Center of Infectious Medicine, Karolinska University Hospital, Huddinge, as described in (Chiang *et al.*, 2013). Effector cells were incubated with 2000 $^{51}$Cr-labelled P815 cells with 0.5μg/mL of anti-CD3 (clone S4.1) with the effector-to-target cell ratios ranged from 10 to 0.3, and incubated for 4 hours at 37°C. $^{51}$Cr release in supernatant was measured as count per minute (cpm) on a γ-counter (Wizard2, PerkinElmer). Specific lysis was calculated as (mean experimental cpm – mean spontaneous cpm) / (mean maximum cpm – mean spontaneous cpm) x 100.

Confocal microscopy (**PAPER I**)

Cryosections (12μm) from frozen skin biopsies were fixed by acetone and then stained with primary Antibodies at 4°C overnight and then with secondary antibodies conjugated to Alexa Fluor dyes (Invitrogen) for 15 min in room temperature. Nuclei were stained with DAPI (Sigma-Aldrich) for 1 min. Images were acquired with a laser scanning confocal microscopy (LSM700) with Zen 2011 software (Zeiss) and analyzed with ImageJ (http://imagej.nih.gov/ij/).

Luciferase assay (**PAPER III**)

In **PAPER III**, a luciferase assay was employed to determine whether promoter with high-risk or low-risk variants would affect the transcriptional activity of *IL22*. HeLa cells were seeded onto 96-well plates ($10^4$ cells per well) and cultured at 37°C in DMEM supplemented with 10% FBS, 100 U/ml and 1X penicillin/streptomycin (Invitrogen, Stockholm, Sweden) one day before transfection. Luciferase construct with either the high-risk *IL22* promoter (rs2227473 (A), INDEL (AY)) or the low-risk promoter (rs2227473 (G), INDEL (–)) were synthesized and cloned into pGL3 basic vector by GenScript (Piscataway, NJ). HeLa cells were transfected with the firefly luciferase reporter plasmids (100ng per well) together with a renilla luciferase reporter (5ng/well) plasmid as internal control. All transfections were carried out by Fugene HD (Promega, Stockholm, Sweden) following the manufacturer's protocol, in antibiotics free DMEM medium with 10% FBS. Transfected cells were left in

complete DMEM medium for 12 hours before PMA (50 ng/ml, Sigma Aldrich) treatment for 24 hours in six replicates. Luciferase activity was analyzed by using Dual Luciferase Reporter Assay System (Promega) following the manufacturer's instruction. Relative luciferase activity was determined by the ratio of luciferase signal at 24-hours after PMA treatment to the luciferase signal prior stimulation.

Statistical analysis (**PAPER I, II, III, IV**)

Mann-Whitney U test or two-tailed Wilcoxon matched-pairs signed rank test were generally used to determine statistical significant of flow cytometry or qRT-PCR data points from independent or paired samples. Two-sided Student's T test was used to determine statistical significance within single experiment with triplicates or more. P-values were corrected for multiple testing by Holm–Bonferroni method. Detailed statistical analysis for genetic study can be found in **PAPER IV**. Statistic analysis and graphical presentation were computed using PRISM 6 (GraphPad) software.

# 7 RESULTS

## 7.1 Healthy Human Skin Contains a Heterogeneous Population of T Cells.

In **PAPER I**, we studied the composition of skin-resident T cell populations in epidermis and dermis from healthy individuals. Peripheral blood was also obtained from paired donors for comparison. Consistent with previous findings (Foster *et al.*, 1990; Spetz *et al.*, 1996), the majority of T cells in the skin, both epidermal and dermal, were αβ T cells. The proportion of γδ T cells among the whole T cell population in healthy skin was generally lower in the epidermis (2.2% ± 2.1) and dermis (2.2% ± 1.8), than the proportion of γδ T cells in the circulation (4.7% ± 2.7) (Figure R1. A-D). It has been suggested that CD8 T cells primarily home to epidermis while CD4 T cells localized in the dermis of human skin (Nestle *et al.*, 2009a) but inconsistent CD4:CD8 T cells ratio in epidermis has been reported, ranging from absolute dominance of CD8 T cells to a 2:1 , CD4:CD8, ratio (Bos *et al.*, 1987; Foster *et al.*, 1990; Spetz *et al.*, 1996). Our data suggested a slight dominant of CD4 T cells over CD8 T cells in the epidermis and dermis (Figure R1.E). We further characterized the classical memory phenotype of skin-resident T cells in healthy skin according to their CD62L and CD45RA expression, and found that CD8 T cells from skin comprise mostly $T_{EM}$ ($CD45RA^-CD62L^-$) or $T_{EMRA}$ ($CD45RA^+CD62L^-$) phenotypes, while CD4 T cells displayed mainly a $T_{EM}$ phenotype with a smaller proportion of CD4 T cells expressing CD62L, i.e. $T_{CM}$ phenotype ($CD45RA^-CD62L^+$), in epidermal and dermal T cells (Figure R1).

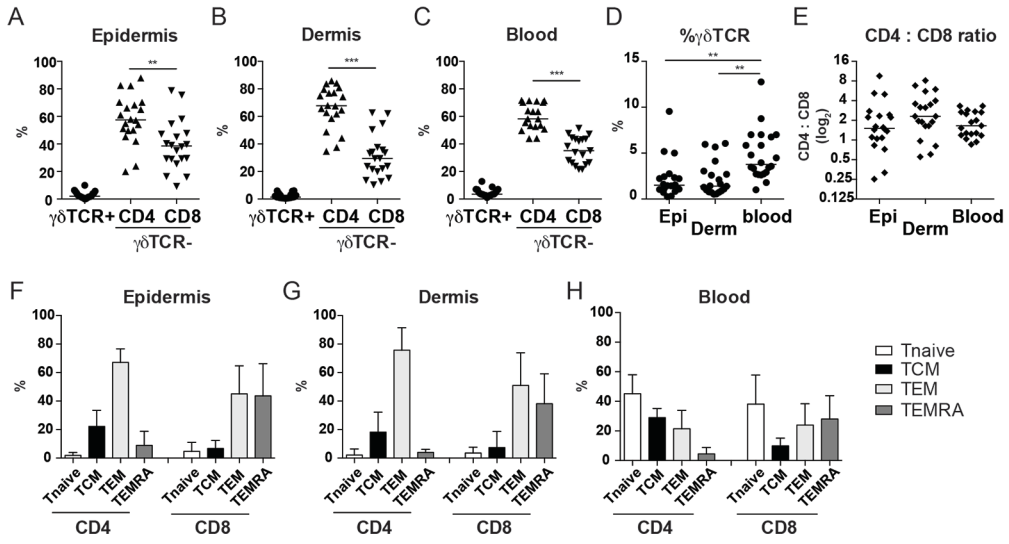


**Figure R1. Characterization of major T cell populations in healthy human skin.**
(A-C) Proportion of γδ T cells, CD4 T cells and CD8 T cells among total $CD3^+$ T cells population within epidermis, dermis and peripheral blood from healthy donors. (D) Proportion of γδ T cells among total $CD3^+$ T cells population within epidermis, dermis and peripheral blood from healthy donors. Wilcoxon test on paried samples. (E) CD4 to CD8 T cell ratio in epidermis, dermis and peripheral blood from healthy donors. Lines indicate median of the data in scattered dots plots (n=20). (F-H) Proportion of naive T cells (Tnaive), central memory $T_{CM}$, $T_{EM}$ and $T_{EMRA}$ T cells among total $CD4^+$ or $CD8^+$ T cells population within epidermis, dermis and peripheral blood from healthy donors. Mean $^+$/- SD (n=14) is depicted in bar charts. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Mouse studies demonstrated that expression of $T_{RM}$ cell surface markers, CD103 and CD69, identified majority, if not all, of the non-recirculating resident T cells in non-lymphoid epithelial tissues (Gebhardt *et al.*, 2009; Steinert *et al.*, 2015). In humans, expression of CD103 and CD69 has previously been used for characterizing $T_{RM}$ cells in the lung, intestine and skin tissues (Sathaliyawala *et al.*, 2013; Thome *et al.*, 2014; Watanabe *et al.*, 2015). In healthy skin, Watanabe *et al.* showed that epidermis contained a large proportion of $CD103^+CD69^+$ $T_{RM}$ cells, whereas dermis consisted a slightly higher proportion of $CD103^-$ $CD69^+$ $T_{RM}$ cells as compared to the $CD103^+CD69^+$ $T_{RM}$ cells (Watanabe *et al.*, 2015). In agreement with Watanabe at al., we found CD103 and CD69 expressing CD4 or CD8 T cells highly enriched in the epidermis and to a lesser extent in the dermis as compared to the circulating T cells (Figure R2). The majority of epidermal CD8 T cells (53.0% ± 17.8) co-expressed CD103 and CD69 while less than 10% of dermal CD8 T cells co-expressed both markers (6.0% ± 3.1) (**PAPER I**, Figure 1). Similar CD103 and CD69 expression patterns were observed in skin-resident CD4 T cells, with a lower proportion (38.8% ± 11.0) in the epidermal population co-expressing CD103 and CD69 as compared to their CD8 counterparts. CD49a is another marker found to be expressed in virus-specific $T_{RM}$ cells in the skin and lung (Gebhardt *et al.*, 2009; Piet *et al.*, 2011). In human skin, Purwar et al. found that only 15% of total T cells expressed CD49a after a 4-week explant culture (Purwar *et al.*, 2011). Our data showed that CD49a expression was restricted in epidermal CD8 T cells, particularly within the $CD8^+CD103^+CD69^+$ $T_{RM}$ cell subset in the healthy human skin (Figure R2 C, D). However, a high variation of CD49a expression was noted among healthy donors ranging from 0 to 70% of CD8 T cells (Figure R2.C, D; **PAPER I,** Figure. 1). Sequencing analysis of the CDR3 region of TCRβ on sorted $T_{RM}$ cell subpopulations from 5 individuals revealed reduced clonal diversity and enrichment of dominant clonotypes in epidermal $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells as compared to $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells, dermal T cells and circulating $T_{EM}$ cells (**PAPER I** Figure 2) implicating a possible previous clonal expansion of the $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells.

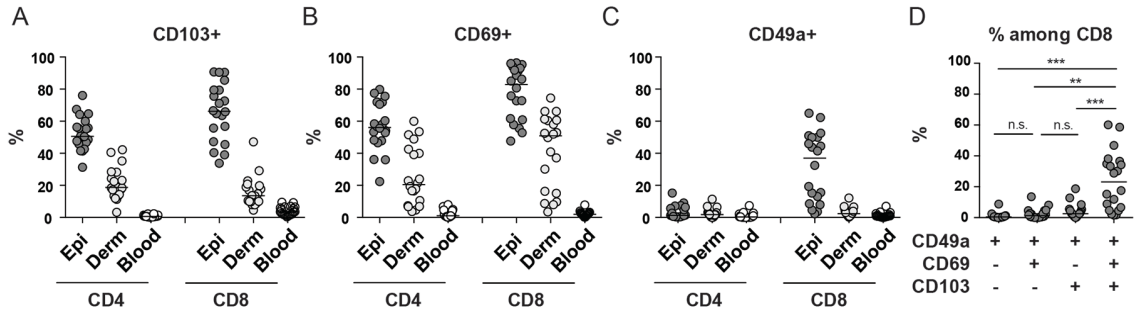


**Figure R2. Characterization of $T_{RM}$ cell surface markers' expression in T cells from healthy skin.**
(A-C) Proportion of $CD103^+$ (A), $CD69^+$(B) and $CD49a^+$(C) among CD4 or CD8 T cells from epidermis, dermis and peripheral blood from healthy donors. (D) Proportion of $CD49a^+$ among CD8 T cells subsets defined by their CD69 and CD103 expression. Wilcoxon test, corrected for mutliple comparison (n=20) *** $p<0.001$, ** $p<0.001$, n.s. depicted not significant.

Taken together, we found that heterogeneous populations of CD4 and CD8 T cells resided in both epidermis and dermis of the healthy human skin. $T_{RM}$ cells expressing both CD103 and CD69 were highly congregated in the epidermis. Interestingly, a minor population of CD4 T cells present in both epidermis and dermis expressed CD62L that may imply a recirculating capacity. In resolved murine cutaneous HSV infection, virus-specific resident CD4 and CD8 T cells are often localized in different regions of the skin. CD8 $T_{RM}$ resided in the epithelia, with confined mobility; whereas CD4 T cells localized in the dermis, rapidly trafficking between skin tissue and circulation (Gebhardt *et al.*, 2011). Identification of migratory T cell subsets in healthy human skin and cutaneous lymphomas provided further support for a recirculating T cell subset within the skin (Watanabe *et al.*, 2015). Our results highlighted the specific expression of CD49a in a subset of $CD8^+CD103^+CD69^+$ $T_{RM}$ cells. By understanding the basic function of different $T_{RM}$ cell subpopulations in the skin, we aimed to further elucidate their clinical implication in skin diseases.

## 7.2 CD49a Marks Functionally Distinct CD8 $T_{RM}$ Cells in Healthy Skin

The high variation in the frequency of $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells in the healthy skin led us to postulate that $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells might be functionally distinct from their $CD49a^-$ counterparts in the healthy epidermis. Indeed, epidermal $CD8^+CD103^+CD49a^+$ and $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells exhibited differential cytokine-producing capacity. In **PAPER I**, IFN-γ and IL-17A production capacity in different subpopulations of skin resident T cells were assessed upon PMA and ionomycin stimulation of the whole epidermal or dermal cell suspension (**PAPER I**, Figure 1). No strong preference was found in IFN-γ and IL-17 production between $CD4^+CD103^+$ and $CD4^+CD103^-$ T cells (**PAPER I**, Figure 1). On the contrary, epidermal $CD8^+$ $T_{RM}$ cell subpopulations displayed distinct cytokine expression patterns: IL-17A was expressed in $CD8^+CD103^+CD49a^-$ T cells in both epidermis and dermis, whereas $CD8^+CD103^+CD49a^+$ epidermal $T_{RM}$ cells preferentially produced IFN-γ (**PAPER I**, Figure 1). Therefore, a clear functional dichotomy of epidermal $CD8^+CD103^+$ $T_{RM}$ cells distinguished by their CD49a expression was unveiled.

PMA and ionomycin stimulation strongly induces Protein kinase C (PKC) activation and $Ca^{2+}$ signaling in T cells (Smith-Garvin *et al.*, 2009). This stimulation regimen is a great tool to study the functional potential of T cells but the activation bypass surface receptor ligation. A more physiological way to activate T cells would be to stimulate with their cognate antigen together with antigen presenting cells. However, such approach requires in-depth knowledge on what the population of interest recognises, which is difficult when using skin material from a heterogeneous population. To mimic TCR:MHC ligation, we employed an antibody re-directed functional assay in which P815 cells presented a stimulatory anti-CD3 Abs to sorted $CD8^+$ $CD103^+$ $T_{RM}$ cells, mimicking an antigenic stimulation. Although the frequency of cytokine expression is lower than PMA and ionomycin stimulation, epidermal CD8 T cells activation through anti-CD3 stimulation showed the same trend of cytokine response as the PMA and ionomycin stimulation (**PAPER I**, Supp. Figure. 1). In addition, pre-treatment with

IL-15 strongly enhanced both the IFN-γ production in $CD8^+CD103^+CD49a^+$ cells and the IL-17A production in $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells (**PAPER I**, Supp. Figure. 1).

To further explore the functional differences between $T_{RM}$ cell subpopulations, $CD8^+CD103^+CD49a^+$ and $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells were sorted for RNA-seq analysis. Transcriptome analysis by RNA-seq confirmed the differential cytokine expression capacity between the two subpopulations and further demonstrated chemokine-producing and cytolysis capacity in $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells (**PAPER I**, Figure 3). In spite of the strong indication of a cytotoxic function in $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells, profiling of the cytotoxic granule constituent protein expression by flow cytometry surprisingly showed unstimulated T cells from freshly isolated epidermal cell suspension were devoid of significant expression of perforin, granzyme A, and granzyme B (**PAPER I**, Sup. Figure 3). We reasoned that $T_{RM}$ cells residing in the healthy skin could be in a quiescent state and may require specific stimulation to acquire cytotoxic granule constituent protein expression. Indeed, IL-15 preferentially up-regulated granzyme A, B and perforin in $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells but not in $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells (**PAPER I**, Figure 4), verifying the cytotoxic potential implicated by the transcriptome analysis. The cytotoxic capacity of IL-15 stimulated $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells was confirmed with an antibody-redirected Cr-release cytotoxicity assay (**PAPER I**, Figure 4). What is intriguing is that $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells failed to elicit cytotoxic function without IL-15 stimulation, emphasizing the role of IL-15 in regulating the cytotoxic function of $T_{RM}$ cells (**PAPER I**, Figure 4).

### 7.3 Cytotoxic $CD49a^+$ $T_{RM}$ Cells Accumulated in Vitiligo Lesions

T cell-mediated cellular cytotoxicity is crucial in controlling viral infection, but could also cause pathology in autoimmunity by targeting self-antigens. In vitiligo, IFNγ-mediated pathology and cytotoxicity against melanocytes have been implicated (Le Poole *et al.*, 2004; Harris *et al.*, 2012; Ezzedine *et al.*, 2015; Yang *et al.*, 2015). In light of the functional division between $CD8^+CD103^+CD49a^+$ and $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells in the healthy skin, we sought to explore the potential implication of $T_{RM}$ cell in the pathogenesis of vitiligo. In **PAPER I**, we found that epidermal and dermal CD8 T cells with $CD8^+CD103^+CD49a^+$ $T_{RM}$ cell phenotype amassed within the depigmented lesions of vitiligo patients as compared to pigmented skin of vitiligo patients or skin from healthy donors (**PAPER I**, Figure 5). Interestingly, T cells with $CD8^+CD103^+CD49a^+$ $T_{RM}$ cell phenotype accumulated in the dermis of depigmented lesions of vitiligo patients, whereas these cells were virtually absent in dermis of healthy donors or non-lesional skin of vitiligo patients (**PAPER I**, Figure 5). Furthermore, a significantly larger proportion of dermal CD8 T cells from depigmented vitiligo lesions co-expressed perforin and granzyme B as compared to the healthy skin, implicating a cytotoxic role of dermal $CD49a^+$ $T_{RM}$ cells in vitiligo (**PAPER I**, Figure 5). The presence of cytotoxic $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells might, thereby, target melanocytes *in situ*, and help to sustain the depigmented phenotype of vitiligo.

## 7.4 Active Psoriasis Lesions Consist of IL-17 and IL-22 Expressing Epidermal CD4 and CD8 T cells

Psoriasis pathogenesis is characterized by hyperproliferation of keratinocytes and infiltration of inflammatory cells in the skin lesions. In **PAPER II**, we quantified the T cell infiltration and analyzed gene expression of sorted epidermal and dermal T cells from active psoriasis lesions. Massive infiltration of both CD4 and CD8 T cells was noted in both epidermis and dermis of active psoriasis lesion (**PAPER II**, Figure 1). Gene expression profiling of sorted T cells showed that the differential gene expression between lesional and healthy skin was mainly confined in the T cells from the epidermal compartment, whereas the dermal populations showed only moderate alteration of gene expression in psoriasis as compared to the healthy state (Figure R3). Epidermal CD4 and CD8 T cells from psoriasis lesions showed a similar pattern of gene expression – up-regulation of the inflammatory genes - as compared to T cells sorted from the healthy epidermis (Figure R3 A,D). In particular, epidermal T cells from active lesions expressed significantly higher gene expression in *IL17A* and *IL22* as compared to T cells from healthy epidermis or lesional dermis (**PAPER II**, Figure 2). In accordance with gene expression data, intracellular cytokine expression analysis by flow cytometry confirmed that higher proportion of the epidermal CD4 or CD8 T cells expressed IL-17A and IL-22 in active lesions as compared to healthy skin (**PAPER II**, Figure 5); while only mild differences were noted in dermal T cells. In addition, comparisons of gene expression profiles between epidermal and dermal T cells revealed an interesting pattern in which epidermal T cells highly expressed inflammatory genes, whereas dermal T cell expressed a higher level of genes related to survival, cytokine receptors and Wnt signaling pathway (Figure R3 C,F).

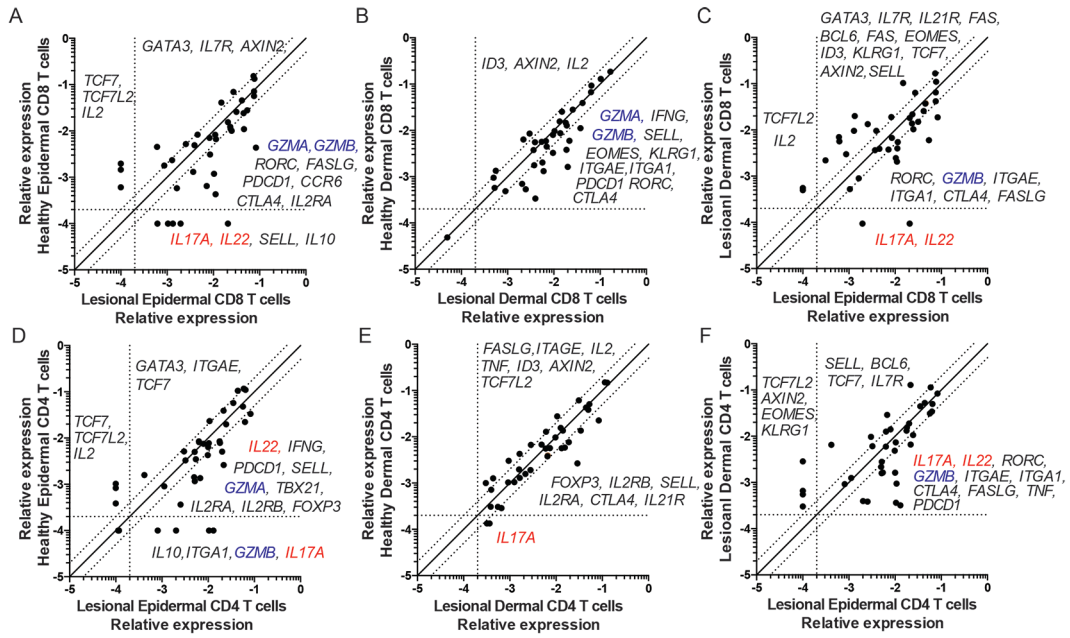


**Figure R3. Lesional epidermal T cells displayed upregulated inflammatory gene expression**
(A-F) Gene expression comparison between of CD8 (A-C) or CD4 (D-F) sorted from epidermis or dermis of lesional or healthy skin, groups compared were indicated in the x-axis and y-axis. Relative expression was calculated as: $2^{-(CT(\text{Target Gene})-CT(B2M))}$. Diagonal dashed lines depict 2-fold difference and diaponal soild lines depict equal expression. Vertical and horizontal dashed lines marked the arbitrary detection thresholds (relative expression = 0.0002)

## 7.5 Granzyme A Triggers Chemokine Expression In Inflammed Keratinocytes

Apart from the notable inflammatory genes, *IL17A* and *IL22*, our gene expression profiling showed CD8 T cells from dermis and epidermis of psoriasis lesion had significantly higher expression of cytotoxic-related genes, *GZMA*, *GZMB* and *PRF* (**PAPER II**, Figure 2). Previous histological studies showed that the numbers of granzyme B or perforin-expressing cells were significantly higher in lesional psoriasis skin compared with normal skin, but lower than that in atopic dermatitis lesions or allergic contact dermatitis (Yawalkar *et al.*, 2001a; Yawalkar *et al.*, 2001b). However, the cytotoxic role of granzymes in the pathogenesis of psoriasis is unclear, since keratinocytes from psoriasis lesions were shown to be resistant to apoptosis (Wrone-Smith *et al.*, 1997). In **PAPER III**, we revealed that a high proportion of CD8 T cells in psoriasis lesion, both epidermal and dermal, expressed granzyme A but not granzyme B or perforin (**PAPER III**, Figure 1).

Froelich and colleagues proposed that granzyme A is a noncytotoxic proinflammatory protease (Metkar *et al.*, 2008; Pardo *et al.*, 2009). Due to the high proportion of granzyme A expressing CD8 T cells in psoriasis lesion, we investigated the potential inflammatory effect of granzyme A on keratinocytes. Granzyme A treatment alone failed to trigger any cytokine or chemokine production in keratinocytes. However, when incubated together with IL-17A, granzyme A was able to enhance secretion of chemokines, CXCL1 (GROα), CXCL12 (SDF1), and CCL4 (MIP-1β) (**PAPER III**, Figure 2). These results implicated a novel proinflammatory role of granzyme A on keratinocytes. The enhanced chemokine production may lead to further recruitment of more inflammatory cells. It has previously been shown that granzyme A treatment triggered IL-1, IL-6 or IL-8 secretion by other cell types, such as monocytes, fibroblasts and lung epithelial cells, (Sower *et al.*, 1996; Metkar *et al.*, 2008; Pardo *et al.*, 2009). On the contrary, we showed that granzyme A specifically up-regulated chemokine expression in inflamed keratinocyte. Since large proportion of lesional CD8 T cells express granzyme A, these resident CD8 T cells may provide extra proinflammatory signals by granzyme A production. Given the prominent role of CD8 T cells in many inflammatory skin diseases, further study of the possible proinflammatory role of granzymes in other skin diseases would be of interest.

## 7.6 Th22 and Tc17 Cells Reside In The Epidermis of Clinically Healed Psoriasis Lesions

Psoriasis can be successfully treated but may relapse after withdrawal of treatment, and often at the previous site of inflammation. We and others hypothesized that a T cell-mediated localized disease memory may underlie this phenomenon (Clark, 2011; Suárez-Fariñas *et al.*, 2011) (**PAPER II**). To test this, we sampled and analyzed skin biopsies from patients who were successfully treated with narrowband (nb)-UVB, anti-TNF or anti-IL12/23 at sites of previous lesions. Upon successful treatment, the number of epidermal T cells in resolved lesions in all three treatment groups was normalized to the range of healthy skin (**PAPER II**, Figure 3). However, the number of epidermal CD8 T cells in resolved lesion of UVB treated patients was higher than their paired uninvolved skin (**PAPER II**, Figure 3). Gene

expressions of *GZMA*, *GZMB*, and *PRF* were fully resolved in CD8 T cells derived from both biologics and nb-UVB-treated resolved lesions (**PAPER II**, Figure 4). *IL17A* expression was also normalised in epidermal T cells from biologics treatment where residual expression was found in UVB treated patients (**PAPER II**, Figure 4). However, *IL22* expression was not resolved in epidermal CD4 and CD8 T cells from both the biologics or nb-UVB treatment (**PAPER II**, Figure 4). Therefore, skin resident T cells in resolved lesions resembled normal skin with minimal residual inflammatory gene expression.

Although transcriptional profile was largely resolved, the residual *IL17A* and *IL22* gene expression in sorted epidermal T cells from resolved lesions hinted towards a potential difference in their cytokine production capacity as compared to the healthy skin. Thus, we determined the cytokine expression of T cells from resolved lesions. In accordance to gene expression analysis, a population of epidermal CD4 T cells consistently expressed IL-22 (i.e. Th22) upon PMA and ionomycin stimualtion in cell suspesion derived from the resolved lesions from all three treatments studied (**PAPER II**, Figure 5; Figure R4). Higher proportion of IL-17 expressing in epidermal CD4 T cells was also noted in nb-UVB treated and anti-IL-12/23p40 treated lesions but not in anti-TNF treated lesion. In contrast, epidermal CD8 T cells showed higher IL-17A expression upon reactivation in all three studied treatments while IL-22 expression was only apparent in resolved epidermis from UVB treated patients (Figure R4). Previous studies found retention of dermal CD8 T cells within resolved lesions (Suárez-Fariñas *et al.*, 2011). While our result agreed with this (**PAPER II**, Supp. Figure 2), cytokine expression pattern found in the dermal T cells from resolved lesions was comparable to the healthy skin (**PAPER II**, Figure 5). These results revealed a population of CD8 T cells poised towards IL-17 production that resided in the epidermis of resolved lesions.

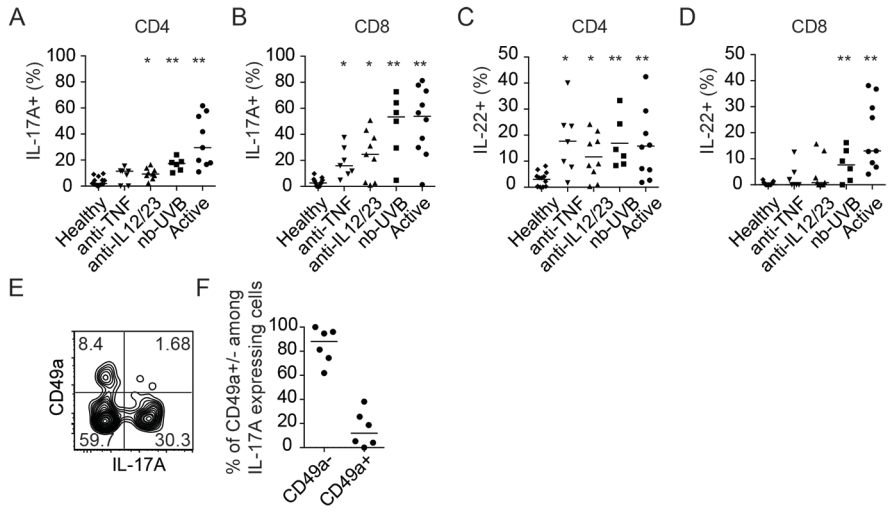


**Figure R4. IL-17 and IL-22 expression of epidermal T cells in resolved lesion**
(A-D) Proportion of IL-17A$^+$ (A,B) or IL-22$^+$ (C,D) cells among epidermal CD4 (A,C) or CD8 (B,D) T cells from healthy skin, resolved lesions treated with anti-TNF, anti-IL12/23 or nb-UVB, and active psoriasis lesions, upon PMA and ionomycin stimulation in the presence of Brefeldin A. Mann-Whitney U test against healthy control, corrected for multiple testing. * $p<0.05$, ** $p<0.01$. (E) Representative contour plot of epidermal CD8 T cells showing IL-17A and CD49a expression. (F) Proportion of CD49a- and CD49a$^+$ cells among IL-17A expressing epidermal CD8 T cells upon PMA and ionomycin stimulation.

A population of $CD103^+CCR6^+IL\text{-}23R^+$ CD8 $T_{RM}$ cells was highly enriched in resolved psoriasis (**PAPER II**, Figure 6), and IL-17A production was mainly produced by $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells (Figure R4, **PAPER II**, Figure 6). This finding echoed with the functional distinction between $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells and $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells from healthy epidermis in which IL-17A was produced mainly by the $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells (**PAPER I**). On the contrary, CD4 T cells in resolved epidermis did not show any preference for their IL-22 production regarding CD103 expression (**PAPER II**, Supplemental Figure 3). Thus, the epidermis of resolved psoriasis lesions upon successful treatment contain resident T cells poised for IL-17A or IL-22 expression, which are highly pathogenic cytokines in psoriasiform inflammation. We proposed that these pathogenic T cells resided in epidermis marked the region of previously inflamed skin and that their reactivation might lead to the relapse of psoriasis upon reactivation (**PAPER II**, Figure 7).

## 7.7 Genetic Variant in *IL22* Promoter Associates with Early Onset of Psoriasis

IL-22 from T cells sustains both keratinocyte hyperproliferation and epidermal antimicrobial responses in different settings(Wolk *et al.*, 2004; Eyerich *et al.*, 2009; Nograles *et al.*, 2009). Both CD4 and CD8 T cells expressed IL-22 in the lesional epidermis (**PAPER II**, Figure 2,5). Besides, Th22 cells resided in resolved lesions of anti-TNF, anti-IL12/23p40, and UVB-treated patients (**PAPER II**, Figure 5), but the inter-individual variation is high (Figure R4). The aryl hydrocarbon receptor (AHR) is a potent inducer of IL-22 response in CD4 T cells (Veldhoen *et al.*, 2008). Through stratification strategy, we found that four genetic variants (3 SNPs and one INDEL (insertion/deletion) on predicted AHR–ARNT binding sites of the *IL22* promoter were associated with psoriasis with onset before ten years of age in two independent Swedish cohorts (**PAPER IV**, Figure 1 and Table 2). Haplotype analysis revealed that the haplotype with all minor alleles for the four variations resulted in an odds ratio of 3.80, and the homozygousity for all minor alleles conferred an odds ratio of 4.03. The risk genotype led to higher *IL22* transcriptional activity (**PAPER IV**, Figure 2) and elevated IL-22 production from peripheral CD4 T cells in patients (**PAPER IV**, Figure 3). Specifically, these results identified a genetic association in patients with early onset of psoriasis that lead to higher expression of inflammatory cytokine related to the pathogenesis of psoriasis.

# 8 DISCUSSION

The complex interaction between genetic heterogeneity and environmental differences introduces large variations among human subjects as evident in the genetic variant of the *IL22* promoter that results in higher transcriptional activity and higher production of IL-22 protein in CD4 T cells (**PAPER IV**). In this thesis, the functional heterogeneity of skin-resident T cells was studied in the human system. The findings provided new insights into the role of $T_{RM}$ cell in skin immunology and dermatological diseases. At the same time, they have raised new questions to be further addressed.

## 8.1 Localized Disease Memory of Resolved Psoriasis

Relapsing psoriasis at the site of previous lesions upon treatment withdrawal is a major challenge when treating psoriasis (Clark, 2011). In **PAPER II**, we showed that pathogenic Th22 and Tc17 cells resided in successfully treated psoriasis lesions despite long-term clinical remission. Based on these results, we proposed a model of T-cell mediated localized disease memory in resolved psoriasis (**PAPER II**, Figure 7). After successful treatment, the number of epidermal T cells was normalized but $T_{RM}$ cells retained pathogenic potential within the previous lesions. Upon reactivation, epidermal CD4 T cells and CD8 T cells responded with IL-22 and IL-17A production respectively. We proposed that the pathogenic cytokines produced by reactivated $T_{RM}$ cells would activate surrounding keratinocytes, amplify the localized inflammation and, subsequently, recruit inflammatory cells into the skin (Figure D1). This model provides a T cell-mediated mechanism on how psoriasis relapses at sites of previous lesions.

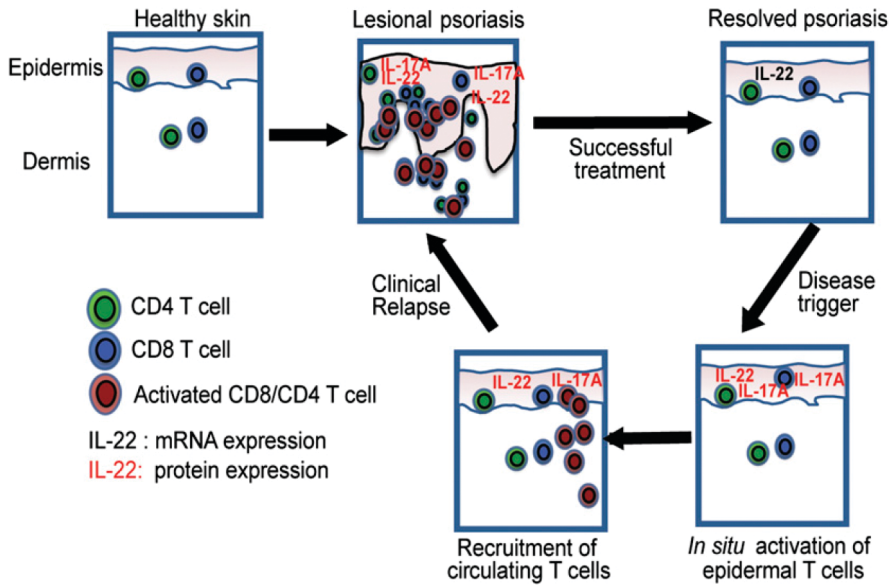


**Figure D1. A proposed model of localized disease memory in resolved psoriasis.**
In active psoriasis lesion, pathogenic T cells with high expression of IL-17 and IL-22 were enriched in the epidermis. Upon successful treatment, the number of T cells in skin is normalised but $T_{RM}$ cells retained pathogenic potential within the previous lesions. Upon reactivation, epidermal CD4 T cells respond with IL-22 production and epidermal CD8 T cells with IL-17A production; thereby, activating surrounding keratinocytes, amplifying the localized inflammation and, subsequently, recruiting inflammatory cells in to the skin. **PAPER II** (Cheuk *et al.*, 2014)

Whether pathogenic $T_{RM}$ cells remained in resolved epidermis were derived from the dominant clones during the active inflammation was not determined in **PAPER II**. Although oligoclonal expansion of T cells within psoriasis lesion has been reported (Chang *et al.*, 1994; Lin *et al.*, 2001), recent high-throughput TCR sequencing study suggested a rather polyclonal nature of T cell within the lesion (Harden *et al.*, 2015a). Lately, LL-37 and melanocyte-specific protein, ADAMTSL5, were proposed as autoantigens of psoriasis (Lande *et al.*, 2014; Arakawa *et al.*, 2015). The identification of LL-37 as autoantigen was particularly interesting and whether elevated production of LL-37 by stressed keratinocytes could trigger pathogenic $T_{RM}$ cell reactivation warrants further investigation. However, a variety of autoantigens could exist among a heterogeneous population of patients. Therefore, regardless of which autoantigens the pathogenic $T_{RM}$ cells are recognizing, understanding the developmental pathway of pathogenic $T_{RM}$ cells is of great importance. It is equally important to investigate whether the pathogenic $T_{RM}$ cells have different survival mechanisms as compared to the protective $T_{RM}$ cells. In this case, it would be possible to specifically target the pathogenic $T_{RM}$ and to preserve the protective $T_{RM}$ cells, which would be of great therapeutic interest.

## 8.2 Clinical Implications of the Presence of $CD8^+CD103^+CD49a^+$ $T_{RM}$ Cells in Vitiligo

$CD8^+CD103^+CD49a^+$ $T_{RM}$ cells were highly enriched in both epidermis and dermis of the vitiligo lesions (**PAPER I**). Although the antigen-specificity of $CD8^+CD103^+CD49a^+$ $T_{RM}$ cell from vitiligo lesion is not determined in **PAPER I**, previous studies showed that perilesional T cells from vitiligo were able to induce apoptosis in autologous melanocytes (van den Boorn *et al.*, 2009; Wu *et al.*, 2013). Therefore, it is likely that at least part of the $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells in vitiligo lesion could react to melanocyte-antigen. The epidermis of depigmented lesions lack melanocytes, but melanocytes can be preserved in hair follicles (Cui *et al.*, 1991) and their precursors can be found in the dermis (Li *et al.*, 2010). The presence of epidermal and dermal $CD8^+CD103^+CD49a^+$ T cells co-expressing perforin and granzyme B may, therefore, prevent repopulation of melanocytes from the reservoir in hair follicles or dermis. Interestingly, in autologous melanocyte transplantation treatment, a high density of CD8 T cells within the lesion led to poor repigmentation while effective repigmentation was noticed in patients with few lesional CD8 T cells (Zhou *et al.*, 2013). Therefore, the outcome of autologous melanocyte transplantation depends on the density of CD8 T cells within the lesion. Given the role of IL-15 in the cytotoxic function of $T_{RM}$ cells as illustrated in **PAPER I**, blocking of IL-15 signaling may be beneficial in vitiligo. Recently, a case study reported that treatment of Tofacitinib, which could antagonize JAK1/JAK3 mediated IFNs or IL-15 downstream signaling, resulted in significant repigmentation (Craiglow and King, 2015). Thus, the therapeutic effects of IL-15 signaling antagonist deserve further investigation. Also, relapses of vitiligo after treatment have been well documented (Shaffrali and Gawkrodger, 2000; Taieb *et al.*, 2012) but whether the relapses mainly occur at previously depigmented regions is less characterized. Nonetheless,

the potential role of $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells in this relapse process would be interesting to investigate.

## 8.3 Localized T Cell-mediated Immunologic Memory

T cell-mediated immunity is characterized by specialization in effector functions. Indeed, the classification of immune response according to their cytokine production, functional properties, and surface marker expression – the "Subsetology"- has become a cornerstone in contemporary immunology research. From the classical distinctions between Th1 and Th2 cells to the discovery of Th17 cells, research from the past decades showed that memory and effector T cells can be specialized in diverse immune responses. In addition to functional specialization, T cells can be recruited from the circulation to different tissues, and, thereafter, mediate localized cellular immunity. In this thesis, careful dissection of the resident T cell subpopulations in human skin provided further insights into their functional specialization. In **PAPER I**, functional distinctions of two epidermal $CD8^+$ $T_{RM}$ cell subpopulations were revealed, in which $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells were highly poised towards IFN-γ and cytotoxic function, whereas $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells preferentially produced IL-17. Such functional distinction was further illustrated in two common skin diseases with different pathogenic pathways. As shown in vitiligo, $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells were highly enriched in both epidermis and dermis of depigmented lesions, concurring the role of IFN-γ-mediated inflammation and cellular cytotoxicity during the pathogenesis. In psoriasis, both CD4 and CD8 epidermal T cells display inflammatory gene signature and heighted IL-17 or Il-22 expression. Upon successful treatment, $CD8^+CD103^+CD49a^-T_{RM}$ cells capable of producing IL-17 upon reactivation resided in resolved lesions (**PAPER II**). Together with epidermal CD4 capable of producing IL-22 upon restimulation, these $T_{RM}$ cells may form the cellular basis of the proposed molecular scar (Suárez-Fariñas *et al.*, 2011), or " localized disease memory" of psoriasis.

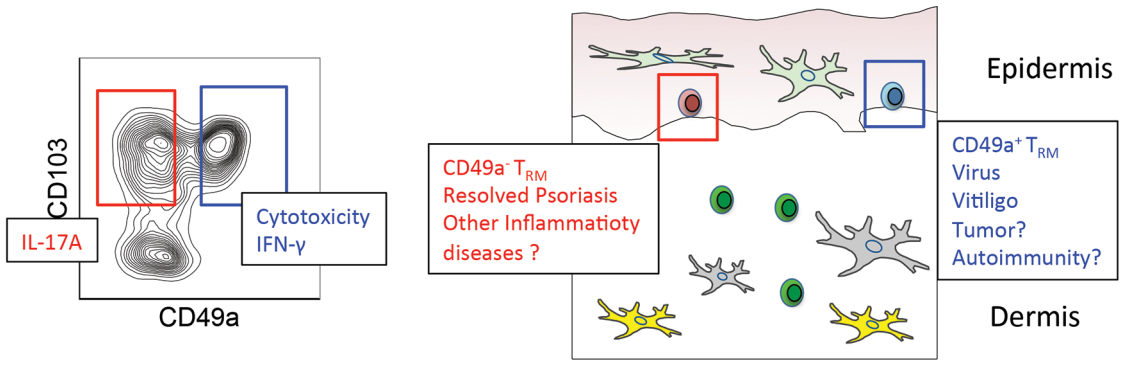


**Figure D2. Functional division of $CD8^+CD103^+CD49a^{+/-}$ $T_{RM}$ cells and their potential clinical relevance.** (Left) Functional specialization of $CD8^+CD103^+CD49a^+$ and $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells in which $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells are highly poised towards IFN-γ production and cellular cytotoxicity, whereas $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells are poised towards IL-17 production. (Right) Potential clinical relevance for $CD8^+CD103^+CD49a^+$ and $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells.

Due to topological differences and the diversity of microenvironments, various areas of the skin are exposed to different pathogens and may be at risk of repeated immunological insults. Therefore, placing $T_{RM}$ cells with specialized functionality acquired from previous infections

or inflammations may be advantageous to contain or eradicate the repetitive immunologic challenge. In resolved viral infection in mice, $T_{RM}$ cells were poised to IFN-γ production that protected against re-infection (Schenkel *et al.*, 2013; Ariotti *et al.*, 2014; Schenkel *et al.*, 2014). Likewise, upon clinical remission of IL-17-medaited inflammation, $T_{RM}$ cells produced IL-17 production in response to restimulation (**PAPER II**). Thus, it is plausible that $T_{RM}$ cells are programmed and poised towards specific responses associated with the previous immunologic insults. In this regard, the high variation of $CD49a^+$ $T_{RM}$ cells and the functional heterogeneity observed in **PAPER I** might reflect the complex history of the dermato-immunological events experienced locally at the site of investigation. For instance, a higher frequency of $CD49a^+$ $T_{RM}$ cells might imply a higher burden of previous anti-viral or cellular cytotoxicity response. In this context, it will be of great value to investigate how $T_{RM}$ cells with different functional capacities are generated by different infections and skin diseases.

Repeated antigenic stimulation enhanced the size of circulating memory T cell pool and subsequent recall response through consecutive recall rechallenge in murine infection model (Vezys *et al.*, 2009). Whether this is also true for the $T_{RM}$ cell population has not been determined. Of note, in murine models, there seems to be spatially limited epidermal niche for $T_{RM}$ cells (Zaid *et al.*, 2014). This poses an interesting question on how multiple challenges from different pathogens over time changes the landscape of $T_{RM}$ cells regarding both the clonality and the functional specialization. Interestingly, as compared to HSV infection model (Gebhardt *et al.*, 2009), vaccinia skin infection generates a globalized formation of $T_{RM}$ cells in skin epithelia during the primary effector phase (Jiang *et al.*, 2012). Further understanding of the underlying differences between these two models would provide valuable knowledge for vaccination program aiming at generating $T_{RM}$ at epithelial surfaces (Kupper, 2012). However, even in the vaccinia infection model, the number of virus-specific protective $T_{RM}$ cells is higher at the inoculation site as compared to the non-immunized sites, suggesting a quantitative difference. Through understanding how $T_{RM}$ cells are generated by various types of cutaneous inflammation, how different $T_{RM}$ subsets are formed and what the consequences of their reactivation are, the role of $T_{RM}$ cells in different disease contexts will be uncovered, and new therapeutic strategies could be identified.

### 8.4 Potential Roles of $CD49a^+$ $T_{RM}$ Cells in Anti-viral Protection, Tumor Surveillance and Anti-tumor Responses

Both IFN-γ production and cellular cytotoxicity are crucial effector mechanisms of T cell-mediated antiviral response. The skin is the target organ of common pathogenic viruses, such as HSVs, Human Papillomavirus, and Varicella zoster virus. $T_{RM}$ cells could provide localized defense and restrict viral propagation (Gebhardt *et al.*, 2009; Jiang *et al.*, 2012; Schenkel *et al.*, 2014). In particular, CD8 $T_{RM}$ cells exert their protective role through multiple mechanisms: (1) recruitment of circulating memory T cells; (2) induction of DC maturation; (3) bystander activation of cytotoxic lymphocytes; (4) induction of local tissue inflammation and anti-viral response by IFN-γ (Schenkel *et al.*, 2013; Ariotti *et al.*, 2014; Schenkel *et al.*, 2014). In **PAPER I**, functional and transcriptome data showed that

$CD8^+CD103^+CD49a^+$ $T_{RM}$ cells in healthy human skin fulfilled such functional profile; in addition, a preferential cytotoxic function was confirmed in $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells derived from healthy skin. Ariotti *et al* argued that the local anti-viral inflammatory response induced by IFN-γ from antigen specific $T_{RM}$ cell was sufficient to control asymptomatic viral re-infection or reactivation, whereas the contribution of subsequent recruitment of circulating lymphocytes and the direct killing capacity of $T_{RM}$ cell might be limited (Ariotti *et al.*, 2014). The first part of this argument is partially supported by Mackay *el al* where they showed that $T_{RM}$ cells could protect from reinfection even without the contribution of the recruitment of antigen specific circulating T cells (Mackay *et al.*, 2015b). Although, the role of *in situ* cellular cytolysis is unclear, in previously HSV infected human genital skin, resident CD8 T cell expressed perforin protein during asymptomatic viral shedding suggesting a possible cytolytic contribution to clearance of virally infected cells (Zhu *et al.*, 2013). Thus, it is highly plausible that effective $T_{RM}$ cell-mediated local defense requires the combination of IFN-γ and cytotoxic activity of $T_{RM}$ cells to constrain viral propagation and eradicate virally infected cells.

The skin is an organ with constant intense cell division and the human epidermis is turned over every 40-56 days (Halprin, 1972). With the exposure to chemical, UVB and HPV, the risk of tumorigenesis in the skin increases with time (Melnikova and Ananthaswamy, 2005; Akgül *et al.*, 2006). Indeed, skin cancers comprise some of the most prevalence forms of malignancy (Melnikova and Ananthaswamy, 2005). CD8 $T_{RM}$ cells are constantly crawling in the epidermis and can quickly recognize emergence of viral antigen (Ariotti *et al.*, 2012; Zaid *et al.*, 2014). In a similar manner, $T_{RM}$ cells may provide surveillance not only to pathogens but also to tumors. In physiological condition without prior priming to a tumor antigen, it is unlikely that $T_{RM}$ cells bearing TCR highly specific against tumor antigens would be stationed in the epithelia. Aside from the slight probability of cross-reactivity to tumor-antigen with the limited TCR- repertoire placed in the skin, it is plausible that $T_{RM}$ cells may recognize tumor cells through antigen-independent mechanisms, e.g. NKG2D- mediated activation (Nausch and Cerwenka, 2008; Zhang *et al.*, 2015), which has been reported in intraepithelial T cells in gut mucosa (Hüe *et al.*; Meresse *et al.*, 2004). Indeed, cutaneous tumor surveillance by DETC in mouse has been shown to be NKG2D-dependent (Girardi *et al.*, 2001). Therefore, it is possible that $T_{RM}$ cells can survey and contain initial tumorigenesis in epithelial layers. Alternatively, strategies generating tumor-specific $T_{RM}$ cells are worth exploring.

CD8 T cell plays a significant role in anti-tumor immunity. The numbers of tumor-infiltrating CD8 T cells show good prognosis for the vast majority of cancers (Fridman *et al.*, 2012). Tumor-infiltrating CD8 T cells shared the phenotypic characteristics of the $T_{RM}$ cells and it has been reported that, in ovarian, lung and bladder cancer, the density of CD103 expressing tumor-infiltrating CD8 T cells is strongly associated with patient survival rate (Webb *et al.*, 2014; Djenidi *et al.*, 2015; Wang *et al.*, 2015). In light of the highly specialized cytotoxic functions of the $CD103^+CD49a^+$ $T_{RM}$ cells in the epidermal tissue (**PAPER I**), it will be of great interest to determine whether tumor infiltrating T cells express CD49a and whether the

$CD49a^+$ tumor infiltrating T cells provide better immune protection as compared to other CD8 T cell subpopulations. Interestingly, $CD49a^+$ CD8 T cells have been identified in human mucosal tumors of epithelial origin, and CD49a blockade significantly reduced survival in a murine mucosa epithelial tumor model (Sandoval *et al.*, 2013). If a similar pattern can be observed in other forms of malignancy, detailed knowledge on the generation and activation of $CD49a^+$ $T_{RM}$ may improve the design of immunotherapies in the context of solid tumors.

## 8.5 The Multifaceted Roles of IL-15 in $T_{RM}$ Generation, Homeostasis and Effector Functions

**PAPER I** showed a critical role of IL-15 in augmenting $T_{RM}$ cell functions. Not only were IFN-γ production and cellular cytotoxicity strongly promoted in $CD49a^+$ $T_{RM}$ cells by IL-15, but also IL-17A expression in $CD49a^-$ $T_{RM}$ cells (**PAPER I**), suggesting a general role of IL-15 in enhancing multiple $T_{RM}$ cell-mediated effector mechanisms. On the contrary, the blood-borne effector-like $CD57^+$ CD8 cells did not require IL-15 for their cytolytic activity (PAPER I, Figure 4). This highlighted the difference in the regulation of effector functions between tissue resident cells and circulating effector cells. Placing highly cytotoxic $T_{RM}$ cells at skin epithelium may potentially lead to unwanted tissue damage. In this regard, IL-15 may provide an extra layer of regulation in $T_{RM}$ cell function apart from the antigenic stimulation.

Tissue stromal cells within the skin, including keratinocytes and fibroblasts, are able to produce IL-15 (Mohamadzadeh *et al.*, 1995). Keratinocytes, especially those within hair follicles, constitutively express IL-15, potentially maintaining $T_{RM}$ cell homeostasis (Adachi *et al.*, 2015). Expression of IL-15 and IL-15Rα in keratinocytes or epithelial cells can be elevated by interferon stimulation (Teunissen *et al.*, 1998; Sanda *et al.*, 2006). Also, it has been demonstrated that DCs and LCs could boost effector function or break T cell tolerance via IL-15 trans-presentation by IL-15Rα (McGill *et al.*, 2010; Romano *et al.*, 2012). Since $T_{RM}$ cell from healthy skin does not express perforin or granzyme prior to stimulation (**PAPER I**), the constitutive expression of IL-15 from keratinocyte is probably not sufficient to induce cytolytic protein expression in the $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells. It is, therefore, likely that the upregulation of granzymes and perforin in $T_{RM}$ cells *in vivo* requires a higher titer of IL-15 or trans-presentation of IL-15 by IL-15Rα (Figure D3). Since IL-15 provides both homeostatic and activating signals for $T_{RM}$ cells, misregulation of this delicate balance may cause pathology. Indeed, the role of IL-15 in skin diseases is well documented. Blocking of IL-15 in xenotransplant model dampened development of psoriasis (Villadsen *et al.*, 2003) and stromal cell production of soluble IL-15Rα restricted psoriasiform inflammation (Bouchaud *et al.*, 2013). Systemic blockade of IL-15Rβ prevented hair loss in an Alopecia areata mouse model of C3H/HeJ mice (Xing *et al.*, 2014). On the other hand, IL-15 signaling agonists may be favorable for tumor therapy (Rosalia *et al.*, 2014; Waldmann, 2015). Further understanding on how IL-15 regulates $T_{RM}$ cell homeostasis and function may lead to better modulation of their effector functions in different diseases.

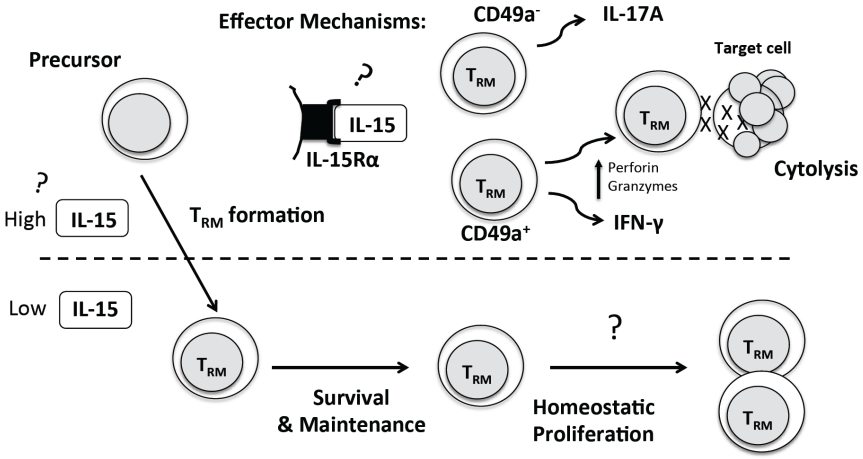


**Figure D3: Multifaceted roles of IL-15 in $T_{RM}$ cell biology.**
IL-15 is involved in $T_{RM}$ cell formation, survival and maintenance. Whether IL-15 also induces homeostatic proliferation of $T_{RM}$ cells *in vivo* is not clear. On the other hand (upper-right), IL-15 augments effector functions of $T_{RM}$ cells. These functions include IFN-γ and IL-17 production by different subsets of $T_{RM}$, and upregulating cytotytic proteins in $CD49a^+$, unleashing their cytotoxic potential. How these multifacet functions of IL-15 are regulated is unknown. We proposed that a higher concentration of IL-15 or trans-presentation by IL-15Rα is required for formation and effector function of $\mathbf{T_{RM}}$, whereas, lower concentration is sufficient for homeostatic survival of $\mathbf{T_{RM}}$. The differential stimulation between soluble IL-15 protein and trans-presentation by IL-15Rα deserves further investigation.

## 8.6 Psoriasis – What is Happening in the Dermis?

The functions of IL-17 and IL-22 have been highly implicated in the pathogenesis of psoriasis. We and others (Res *et al.*, 2010) (**PAPER II**) showed that the proportion of epidermal T cells capable of producing IL-17 or IL-22 was larger than that in dermal T cells in active psoriasis lesion. Since epidermal T cells are in close contact with keratinocytes, the cytokines they produced would have a great impact on the keratinocyte pathology, which is one of the hallmarks of psoriasis pathology. Indeed, Conrad et al. showed infiltration of epidermal T cells is crucial for the development of psoriasis (Conrad *et al.*, 2007).

Dermal cells showed a relatively low proportion of pathogenic-cytokine expressing T cells and expressed lower level of inflammatory genes (Figure R3). Due to the increase of vasculatures in active psoriasis, it is possible that circulating nonpathogenic T cells are included in the dermal T cells of our analysis, diluting the proportion of cytokine-producing T cells. However, the increase in vasculatures is associated with the emergence of large clusters of T cells and dendritic cells in the dermis of the active lesions. It is possible that only T cells receiving sufficient activation in the dermal compartment migrate into epidermis, leading to the gene expression and cytokine production profile observed in **PAPER II**. In this regard, the relatively low proportion of pathogenic-cytokine producing T cells in dermis may reflect a delicate regulatory or selection mechanism. The landmark studies by Boyman *et al.* and Conrad *et al.* showed the local expansion and epidermal infiltration of T cells led to spontaneous development of psoriasis in previously uninvolved skin of psoriasis transplanted on AGR mice (Boyman *et al.*, 2004; Conrad *et al.*, 2007). In this model, dermal T cells expanded within a week of transplantation, preceding the increase in the number of epidermal T cells (Conrad *et al.*, 2007), illustrating the expansion of dermal T cells before epidermal infiltration. In a recent report using the same model, proportion of IL-17 producing cells in

epidermal CD8 T cells was also significantly higher than the dermal CD8 T cells (Di Meglio *et al.*, 2016); corroborating with our findings in an experimental setting.

Laser capture microdissection microarray analysis revealed lymphoid tissue signature in these dermal inflammatory clusters of psoriasis (Mitsui *et al.*, 2012). It was postulated that antigen presentation might take place in these clusters (Mitsui *et al.*, 2012). Indeed, antigen presentation by dendritic cells in non-lymphoid tissue has been reported (Wakim *et al.*, 2008; McLachlan *et al.*, 2009; Macleod *et al.*, 2014). During HSV murine skin infection, migration of LCs out of epidermis and accumulation of DCs in dermis were observed at the foci of infection sites (Eidsmo *et al.*, 2009). Multiple DC subsets within infected skin were able to elicit CD4 T cell response (Macleod *et al.*, 2014). In secondary lymphoid organs, weak TCR signal is enough for activation and generation of effector and memory cells, but a strong TCR signal is required for sustained expansion (Zehn *et al.*, 2009). Therefore, secondary lymphoid organ selects the clone with the strongest TCR signals for expansion. Regarding cutaneous inflammation, it is plausible that the dermal T cell-DC clusters in psoriasis or the inducible Skin Associate Lymphoid Tissue (iSALT) structure, proposed by Kabashima and colleagues (Natsuaki *et al.*, 2014), may serve as a local "selection" site in a similar manner that only the clone(s) with sufficient activation signals would infiltrate the epidermis.

Taken the high prevalence of dermal inflammatory clusters in skin diseases, further understanding of this complex interaction between DCs and T cell in the dermis will be highly relevant for dermatology research. In delayed-type hypersensitivity reactions (DTH), Gaide *et al.* showed that hapten challenge to sensitized patients could lead to the formation of both $T_{RM}$ cell at the site of challenge as well as $T_{CM}$ cell in the circulation (Gaide *et al.*, 2015). In a similar setting, Gulati *et al.* illustrated the formation of DC-T cell clusters within 3 days and peak at 14 days after challenge (Gulati *et al.*, 2014). Taken together, the cellular process within inflammatory cluster may be involved in the generation of $T_{RM}$ cells. Spatial dissection of cellular process in sequential samples of DTH reactions will reveal dynamic process occurring in the clusters in the future.

During skin infection, the up-regulation of adhesion molecules and chemokine recruits memory and effector T cells from the circulation; non-pathogen specific cells are also recruited into the tissue (Chapman *et al.*, 2005; Ghani *et al.*, 2009). Indeed, even naïve T cells can be recruited to non-lymphoid tissue during chronic inflammation (Weninger *et al.*, 2003). It is, therefore, important to "select" the relevant clones to migrate to epidermis and fight pathogens. Misregulation of such "selection" process during infection may lead to epidermal infiltration and $T_{RM}$ formation of pathogenic T cell clones, potentially planting the seed for tissue autoimmunity or immune-mediated diseases.

# 9 CONCLUDING REMARKS

$T_{RM}$ cells provide immune surveillance against pathogen re-infection but can also cause unwanted tissue inflammation and may lead to the local recurrence of immune-mediated skin diseases. In this thesis, I have summarized and discussed my work on the functional heterogeneity of skin resident T cells, in healthy skin and two different immune-mediated diseases, vitligo and psoriasis.

The human skin contains heterogeneous populations of T cells. With a special emphasis on the CD8 T cells, a functional dichotomy of epidermal CD8 $T_{RM}$ cell in human skin was uncovered. $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells are highly poised towards IFN-γ production and cellular cytotoxicity. In healthy skin, $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells are relatively quiescent. IL-15 rapidly upregulates cytotoxic protein expression and unleashes efficient cytolytic killing capacity. In vitiligo, accumulation of perforin and granzyme B expressing $CD8^+CD103^+CD49a^+$ $T_{RM}$ cells are enriched and accumulated in the depigmented lesions, potentially preventing repigmentation of the lesion (**PAPER I**). On the other hand, $CD8^+CD103^+CD49a^-$ $T_{RM}$ cells are poised towards IL-17 production in healthy skin (**PAPER I**). A similar CD8 $T_{RM}$ cell subpopulation, with heightened IL-17 producing capacity, reside in epidermis of resolved psoriasis lesion, together with $CD4^+$ T cells preferentially expressing IL-22; thereby, forming a cellular basis for the proposed "localized disease memory" in resolved psoriasis (**PAPER II**).

Taken together, the results presented in this thesis illustrate that the functional commitment of $T_{RM}$ cell resided in the tissue could be decided by the local history of the dermato-immunological events, as exemplified by the CD8 $T_{RM}$ cells poised towards IL-17 production in resolved psoriasis. In order to provide a more comprehensive view on this concept, future studies should gather more data on the phenotypic and functional properties of $T_{RM}$ cells during active and resolved phases of various skin infections and inflammatory skin diseases while experimental models may further unravel the underlying mechanisms. What would be of most interest to me is to understand how consecutive local infections and inflammations shape or reshape the landscape of $T_{RM}$ cell population regarding both clonality and functional commitment.

For therapeutic measures, $T_{RM}$ cell research offers a promising vaccination strategy to create a localized adaptive defence against pathogens. However, it is vital to understand and distinguish the developmental pathways of protective or pathogenic $T_{RM}$ cells, in order to minimize the possibility of detrimental tissue inflammation or autoimmunity. At the epidermal layer, malignancy poses a larger threat to human health than viral infection nowadays. I anticipate and hope that increasing knowledge in the functional regulation of $T_{RM}$ cells will provide new insight to improve current anti-tumor immunotherapies.

Beside the main theme of the thesis, I have also explored an alternative effector mechanism of tissue resident T cells and determined the role of genetic control in T cell effector function.

These results broaden the scope of this thesis. **PAPER III** showed that granzyme A, highly expressed in lesional CD8 T cells in psoriasis, could enhance chemokine expression in inflamed keratinocytes, suggesting a potential inflammatory mechanism mediated by tissue-resident T cells during chronic inflammation. **PAPER IV** demonstrated that a genetic variant of *IL22* promoter associated with early onset of psoriasis confer increase *IL22* transcriptional activity and IL-22 production in CD4 T cells, underscoring the impact of genetic heterogeneity on the susceptibility of human diseases.

An excerpt from the research proposal to the Croucher Foundation:

*" Hypothesis: ....We proposed that pathogenic memory T cells reside in epidermis after acute/primary onset of psoriasis. Upon re-stimulation, these memory T cells will be activated in situ; thereby, giving rise to a local inflammatory response that further recruits immune cells to the lesion...."*

Stanley Cheuk, 2010

Back in 2010, the concept of tissue resident memory T cell was still budding. I entered the field, full of enthusiasm, to study the role of skin resident T cells in human skin with the special focus on resolved lesions in psoriasis. The project was challenging and we didn't know much about these cells in human skin. Five years later, different groups have confirmed the protective role of $T_{RM}$ cells in different murine models. During these exciting years in $T_{RM}$ cell research, much effort has been devoted to elucidating the formation, maintenance and effector mechanisms of $T_{RM}$ cells. In the same period, the role of $T_{RM}$ cells has been implicated in an increasing number of skin diseases in human. Hopefully, the data presented herein could contribute to fill some of the gaps of knowledge in the $T_{RM}$ cell and dermatology research.

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