Interaction and Immune Regulations of Innate Lymphoid Cells and Plasmacytoid Dendritic Cells in Skin Inflammatory Diseases



Yi-Ling Chen

Jesus College

University of Oxford

A thesis submitted for the degree of

Doctor of Philosophy

Trinity 2018

Contents

Contents **1**

Acknowledgements **9**

Abbreviations **11**

Abstract **16**

1 Introduction **17**

1.1 Skin 17

1.1.1 Barrier function of the skin 17

1.1.2 Immune system of the skin 18

1.2 Plasmacytoid dendritic cells 22

1.2.1 pDC markers 22

1.2.2 pDC subsets 23

1.2.3 pDC transcription factors 24

1.2.4 pDC in the skin 25

1.3 Type I Interferons 28

1.3.1 Type I IFN production 28

1.3.2 Type I IFN signalling 29

1.3.3 Therapeutic targeting of IFNAR pathway 31

2 Aims and Objectives **33**

3 Materials and Methods **34**

3.1 Isolation of immune cells from the blood or skin of human. 34

3.2 Antibodies, flow cytometry and cell isolation 34

3.3 Suction blister technique 36

3.4 Bulk RNA sequencing 36

3.5 Skin wound sample collection 37

3.6 Skin section and confocal microscopy analysis 37
3.7 Skin targeted RNA sequencing 38
3.8 *In vitro* pDC culture and T cell assay 39
3.9 CD1a-restricted T cell assay 39
3.10 Single-cell RNA sequencing 40
3.11 Single-cell RNA sequencing data analysis 41
3.12 ILC22 cell culture 41
3.13 Quantitative teal time-PCR (RT-qPCR) 42
3.14 Proliferation assays 42
3.15 IL-22 ELISA 43
3.16 Transwell assay 43
3.17 *In vitro* protein antigen presentation 43
3.18 Statistical analyses 44
4 Plasmacytoid DC Presentation of Self-Lipid Antigens to CD1a-reactive T Cells 45
4.1 Introduction and aims 45
4.1.1 Wound healing 45
4.1.2 CD1a antigen presentation 47
4.1.2.1 CD1 family 47
4.1.2.2 CD1a 49
4.1.2.3 CD1a ligands 51
4.1.2.4 CD1a ligand loading 53

4.1.3 CD1a-autoreactive IL-22 producing T cells 57

4.1.5 Aims.....59

4.2 Results 60

4.2.1 Two distinct subsets of BDCA-2-expressing DCs rapidly infiltrate skin wounds. 60

4.2.2 CD1a positive, BDCA-2 expressing DCs accumulated near skin wound sites…. 70

4.2.3 Induction of CD1a by pDCs in vitro 74

4.2.4 pDCs present autologous antigens to autoreactive CD1a-reactive T cells…. 79

4.3 Discussion.....81

5 Transcriptomic Profiling of Skin-infiltrating DC Subsets.....84

5.1 Introduction and aims.....84

5.1.1 cDC subsets in the blood and skin 84

5.1.2 Aims.....86

5.2 Results 87

5.2.1 PCA clustering analysis showed a closer relation between skin BDCA-2^{+} CD123^{int} DCs to pDCs.....87

5.2.2 Unbiased clustering of skin DCs revealed five clusters.....89

5.2.3 Skin BDCA-2^{+}CD123^{int} DCs were equipped with pDC-related markers and cDC-like functionality. 93

5.3 Discussion.....104

6 Plasmacytoid DCs Enhance the Capacity of ILC3 to Present Peptide Antigens to T Cells 110

6.1 Introduction and aims 110
6.1.1 Innate lymphoid cells 110
6.1.1.1 Group 3 ILC 112
6.1.1.2 ILC3 development 114
6.1.1.3 ILC3 functions and their role in skin inflammatory conditions 118
6.1.2 Psoriasis 121
6.1.2.1 Development of psoriatic plaques 122
6.1.2.2 Therapeutic targets of psoriasis 125
6.1.3 Aims 126
6.2 Results 127
6.2.1 ILC3 response to IFN-I 127
6.2.2 IFN-I regulated the survival, cytokine production, and marker expression of blood ILC22 130
6.2.3 IFN-I modulated skin ILC22 135
6.2.4 PDC produced IFN-I modulated blood ILC22 functions 136
6.2.5 IFN-I rescued the antigen presenting ability of ILC22 to $CD4^+$ T cells 138
6.2.6 Psoriatic ILC22 were more susceptible to IFN-I treatment 140
6.4 Discussion 143
7 Final Conclusions 148
8 References 156

List of figures and tables

Figure 1-1. Skin anatomy and immune system. 21

Figure 4-1. CD1a structure. 50

Figure 4-2. CD1a-lipid crystal structure. 55

Figure 4-3. Skin-infiltrating pDCs can be found in skin suction blisters. 61

Figure 4-4. Upregulated ISGs can be detected in skin-infiltrating cells. 62

Figure 4-5. Detection of pDC-relevant cytokines in the blister fluid. 64

Figure 4-6. BDCA-2^{+}CD123^{int} DC express CD1a 65

Figure 4-7. BDCA-2^{+}CD123^{int} DC express higher co-stimulatory markers. 66

Figure 4-8. FMO control using CD1a-expressing CD1c^{+}BDCA-2^{-} cDCs. 67

Figure 4-9. FMO controls of pDC specific markers. 68

Figure 4-10. LCs expressed CD1a. 69

Figure 4-11. CD1a-positive pDCs accumulated near skin wound sites. 71

Figure 4-12. Wound-related gene profile in the skin biopsies. 73

Figure 4-13. CD1a can be induced on blood pDC subsets. 75

Figure 4-14. Co-stimulatory markers on blood pDC subsets. 76

Figure 4-15. Expression levels of BDCA-2 and CD123 on cDC in skin and blood 77

Figure 4-16. Both blood pDC subsets can produce IFN-I when stimulated. 78

Figure 4-17. CD1a induced on blood pDC subsets is functionally active. 80

Figure 5-1. Workflow of experimental strategy of skin DC scRNA sequencing. 87

Figure 5-2. Human blood and skin DC heterogeneity in PCA analysis 88

Figure 5-3. t-SNE analysis incorporating all skin DC subsets. 89

Figure 5-4. t-SNE analysis revealed DC2 signature gene expressions on each cluster. 90

Figure 5-5. t-SNE analysis revealed pDC signature gene expressions on each cluster. 91

Figure 5-6. t-SNE analysis revealed DC1 signature gene expressions on each cluster. 91

Figure 5-7. Skin BDCA-2^{+}CD123^{int} DCs and AS DCs shared similar activation status. 92

Figure 5-8. Human blood and skin DCs heterogeneity analysis. 94

Figure 5-9. PDC signature gene profiles of human blood and skin DCs. 95

Figure 5-10. Human skin DCs gene expression profiles. 97

Figure 5-11. Human skin BDCA-2^{+}CD123^{int} DCs express genes relating to antigen presentation. 98

Figure 5-12. Pseudo-time ordering reveals a gradual transition of skin BDCA-2^{+}CD123^{int} DCs and cDCs. 101

Figure 5-13. Correlation between CD1a expression intensity on skin BDCA-2^{+}CD123^{int} DCs and DC-relevant genes. 103

Figure 5-14. Comparative human skin and blood DC scRNA sequencing analysis. 109

Figure 6-1. ILCs mirrors the diversity of T helper cells. 111

Figure 6-2. Summary of ILC development. 115

Figure 6-3. Summary of ILC3 functions. 119

Figure 6-4. Development of psoriatic Psoriasis. 124

Figure 6-5. ILC upregulated ISGs in the skin. 128

Figure 6-6. ILC3 upregulated ISGs in response to IFN-I. 129

Figure 6-7. IFN-I inhibited IL-2 induced ILC22 proliferation. 131

Figure 6-8. Blood ILC22 showed a reduce ability to produce IL-22 after IFN-I treatment. 132

Figure 6-9. Blood ILC22 showed an elevated activation marker expression after IFN-I treatment. 133

Figure 6-10. Blood ILC22 displayed an altered phenotype after IFN-I treatment. .. 134

Figure 6-11. The frequency of ex vivo skin ILC22 remained stable after IFN-I treatment. 135

Figure 6-12. Skin ILC22 displayed elevated markers after IFN-I treatment. 136

Figure 6-13. The scheme of transwell setup. 137

Figure 6-14. pDCs secreted IFN-I inhibited IL-22 secretion from blood ILC22. 137

Figure 6-15. Phenotypic profile of IFN -secreting $CD4^+$ T cells upon TT stimulation. 139

Figure 6-16. Psoriatic blood ILC22 showed a hyper activation in marker expression after IFN-I treatment. 141

Table 6-17. Age, sex, ethnicity and conditions of the donor in figure 6-12. 142

Figure 7-1. pDC effector functions in skin inflammatory conditions. 155

Acknowledgements

I would like to thank my supervisor Graham Ogg, for his constant support, patient guidance, and brilliant inspiration, and my cosupervisor Vincenzo Cerundolo, for his bright advice and encouragement, throughout my time as a DPhil student at the Human Immunology Unit at the Weatherall Institute of Molecular Medicine (WIMM). I have been extremely lucky to have had the opportunity to work in a such nurturing environment created by the department, and particularly the Ogg lab. I would also like thank my former supervisors, Chien-Kuo Lee of National Taiwan University and Shiow-Lian Catherine Jin of National Central University, who prepared me for this journey.

I am grateful to everyone in the lab past and present, particularly Clare who I worked very closely during this study, and also Maryam, Rachael, Danuta, Sumi, Janina, Anthony, Amina, Xaiopeng, Jiahe and Koshika, for all of their help. I am also very grateful to our collaborators, Drs. Felipe A Vieira Braga, Tomas Gomes and Prof. Sarah Teichmann at the Wellcome Trust Sanger Institute, and Dr. Gary Reynolds and Prof. Muzlifah Haniffa at Newcastle University, for their assistance and helpful insights in generating and analysing scRNA-seq data; Dr. David A. Duncan at Diamond Light Source for generating the heatmap; Dr. Mariolina Salio and Prof. Vincenzo Cerundolo at the WIMM for providing precious cells and advice; Jillian L. Barlow and Professor Andrew N. J. McKenzie at MRC Laboratory of Molecular Biology, Cambridge for reading the manuscript; Dr. David Johnson at John Radcliffe Hospital for sourcing skin materials.

My deep appreciation goes to all of the patients and donors for providing blood and blister samples, and research nurses, Melanie Westmoreland and Teena

Mackenzie, who made this research possible. My thanks also go out to the WIMM FACS facility, especially Craig Waugh who makes cell sorting an effortless experience; WIMM Wolfson Imaging Centre, especially Drs. Christoffer Lagerholm and Dominic Waithe who helped with confocal microscopy technique and image analysis; CBRG, especially Nicki Gray for analysing bulk RNA-seq and skin targeted RNA-seq data. In addition, I would like to acknowledge my funding bodies, Clarendon Scholarship, Jesus College Peter Jenks Award, and RDM Scholars Programme.

I would like to say thank you to all my friends back home in Taiwan, Ting, Rita and Sibyl, and here in my second home in the UK, for being my moral support. A special thank-you to my parents, who devoted their lives to support me in every way possible, so that I can accomplish so much in life. And finally, to my wonderful husband David, who has been by my side for the past three years, driving me to do better and giving me his never-ending support, and a constant source of love.

Abbreviations

AD: atopic dermatitis 29

AHR: Aryl hydrocarbon receptor 117

AP2: adaptor protein-2 50

APCs: antigen-presenting cells 20

AS DC: $Axl^{+}Siglec\text{-}6^{+}$ DCs.....24

BCL11A: B cell lymphoma 11A 25

BST2: Bone marrow stromal cell antigen 2 23

CCR4: C-C chemokine receptor.....20

cDCs: conventional DCs 20

CHILP: common helper ILC progenitor 115

CLA: cutaneous lymphocyte antigen.....20

CLP: common lymphoid progenitor 115

CTACK: cutaneous T-cell-attracting chemokine 64

CXCL10: chemokine (C-X-C motif) ligand 10.....23

DAMPs: damage-associated molecular patterns 47

DCs: dendritic cells.....20

DDM: didehydroxymycobactin.....52

DE: differentially expressed.....42

EBV: Epstein-Barr virus.....28

ECM: extracellular matrix 47

ELISA: enzyme linked immunoassay 44

EOMES: Eomesodermin 111

ER: endoplasmic reticulum.....49

FACS: fluorescence-activated cell sorting....44
FcεRIγ: Fc epsilon RI gamma....23
Flt3: fms-like tyrosine kinase 3....25
FMO: Fluorescence Minus One....68
FRCs: fibroblastic reticular cells....117
GAS: IFNγ activated site....30
GPP: generalised pustular psoriasis....124
HDM: house dust mite....54
HPC: hematopoietic progenitor cells....118
ICs: immune complexes....28
ICS: Intracellular staining....35
IFI16: IFNγ inducible protein 16....30
IFNAR: type I IFN receptor....30
IFN-I: type I interferons....17
IgG1: immunoglobulin G1....41
IL: interleukin....17
IL-23R: IL-23 receptor....27
IL-36Ra: IL-36 receptor antagonist....124
IL-3Rα: IL-3 receptor α-subunit....23
IL-7R: IL-7 receptor....25
ILC1: group 1 innate lymphoid cells....111
ILC2: group 2 innate lymphoid cells....50
ILC22: $Lin^{-}CD45^{+}CRTH2^{-}IL\text{-}7R\alpha^{+}c\text{-}Kit^{+}NKp44^{+}$ ILC3....131
ILC3: group 3 innate lymphoid cells....17

ILCp: ILC precursor 115
ILCs: innate lymphoid cells 21
ILT7: immunoglobulin-like transcript 7 23
iNKT: invariant natural killer T 120
IRFs: IFN-regulatory factors 30
ISGs: interferon-stimulated genes 28
ISRE: interferon-stimulated response element 30
ITAM: tyrosine-based cytoplasmic tail motif 50
JAK1: Janus kinase 1 30
LCs: Langerhans cells 19
LE: lupus erythematosus 28
LTE4: leukotriene E4 112
LTi cells: lymphoid tissue-inducer cells 113
M. tuberculosis: Mycobacterium tuberculosis 52
MACS: magnetic-activated cell sorting 36
MDA5: melanoma differentiation-associated protein 5 29
MFI: mean fluorescence intensity 75
MHC-II: major histocompatibility complex class II 23
MLR: mixed lymphocyte reaction 43
MxA: myxovirus resistance protein 1 28
NCR: natural cytotoxicity receptors 113
NK cells: natural killer cells 111
NRES: National Research Ethics Service 35
PBMCs: peripheral blood mononuclear cells 43

PCA: Principal Component Analysis 42

pDCs: plasmacytoid dendritic cells 17

PGD2: prostaglandin D2 112

PGE2: prostaglandin E2 105

PHA: phytohemagglutinin 43

PL: phospholipid 52

PLA2: phospholipase A2 54

PMM: pattern recognition receptors 114

PPD: purified protein derivative 27

PRRs: pattern-recognition receptors 29

RIG-I: retinoic acid inducible gene 1 29

ROS: reactive oxygen species 19

RT-qPCR: quantitative Real Time-PCR 43

RUNX2: Runt-related transcription factor 2 25

S. epidermidis: Staphylococcus epidermidis 19

S1PR1: sphingosine 1-phosphate receptor 1 32

scRNA-seq: single-cell RNA-sequencing 24

SEM: standard error of the mean 45

SLE: systemic LE 28

SLTs: secondary lymphoid tissues 118

STATs: signal transducer and activator of transcriptions 30

STING: stimulator of interferon genes protein 30

TCF4: Transcription Factor 4 25

TCR: T cell receptor 55, 56

TF: transcription factor....85
TLR: Toll-like receptor....23
TNFα: tumour necrosis factor-α....53
Treg: regulatory T cell....86
TSLP: thymic stromal lymphopoietin....112
t-SNE: t-distributed stochastic neighbour embedding....90
TST: tuberculin skin test....27
TT: tetanus toxoid....44
TTX: tetanus toxin....139
TYK2: tyrosine kinase 2....30
UMI: unique molecular identifier....108
UV: ultraviolet....28
β2m: β2 microglobulin....49

Abstract

Interaction and Immune Regulations of Innate Lymphoid Cells and Plasmacytoid Dendritic Cells in Skin Inflammatory Diseases

Yi-Ling Chen — Jesus College

Thesis submitted for the degree of Doctor of Philosophy — Trinity 2018

The skin is equipped with an immune network providing surveillance and effector function. Plasmacytoid dendritic cells (pDCs) are found to accumulate in skin wounds, yet the mechanisms of their role in skin wound healing are hitherto unstudied. Here, BDCA-2^{+} DCs were shown to infiltrate *in vivo* human wounds, showing heterogeneity based on differential expression of CD123, with a CD123^{int} DC subset expressing CD1a at high levels. The CD1a^{+}-bearing subsets could be derived from blood pDCs and presented self-lipid antigens to CD1a-reactive T cells, inducing interleukin (IL)-22 production. Using single-cell RNA sequencing, human skin wound CD123^{int}BDCA-2^{+} CD1a^{+} DCs were shown to acquire features compatible with a role in lymph node homing and antigen presentation.

Plasmacytoid DCs and group 3 innate lymphoid cells (ILC3) have emerged as important mediators of skin inflammation, but, the mechanisms of their interaction have remained elusive. Here, it was identified that type I interferons (IFN-I), produced by pDCs, negatively regulated IL-22 producing capability of ILC22, an ILC3 subset, and positively mediated their co-stimulatory marker expression. ILC22 also presented peptide antigens to CD4^{+} T cells. While exogenous IL-1β and IL-23 dampened the antigen presenting capacity of ILC22, addition of IFN-I rescued their ability, and the study of psoriatic ILC22 revealed a hyper responsiveness to IFN-I.

These data demonstrate early infiltration of a previously unrecognised subset of CD123^{int}BDCA-2^{+}CD1a^{+} DCs in human skin wounds which can present lipid antigens to autoreactive T cells, contributing to the wound healing process. Furthermore, the blood CD123^{hi}BDCA-2^{+} pDCs produce IFN-I and regulate ILC22 to promote T cell functions. Overall, this study reveals a new route for potential therapeutic manipulations in maintaining skin homeostasis: the pDC-IFN-I-ILC22-T cell axis.

1 Introduction

1.1 Skin

1.1.1 Barrier function of the skin

The skin comprises the outer epidermis and the underlying dermis, and is a complicated organ providing a physical and biological barrier against external environmental challenges. The outer layer of epidermis is called stratum corneum which is composed largely of cross-linked keratin, and tightly packed dead keratinocytes (corneocytes), functioning as a mechanical barrier to prevent water loss from the body to the environment, and the entry of foreign challenges into the host (Fig. 1-1) [1]. The stratum corneum normally maintains an acidic gradient, with an average pH of 5.4-5.9, which is essential for regulating the function of protease and lipid-producing enzymes [2, 3]. Under homeostatic conditions, proteases mostly degrade components of the outer layers of the stratum corneum, while lipid synthesis enzymes maintain the cohesion of the epidermal barrier, and hence preserve the thickness of the skin, preventing the penetration of foreign antigens. This acidic environment of the skin, together with the dry surface and lower temperature, create an unfavourable environment for the propagation of some bacteria [4]. The acidity originates largely from free fatty acids which are derived from microbial metabolites, sebaceous gland secretion, and enzymatic hydrolysis of phospholipid components of the cell membrane [5].

Normal skin surface is colonised by a diverse microbial community after birth, comprised of domestic and sometimes transient microorganisms, including bacteria, viruses, and fungi, which vary depending on age, sebum contents and the local milieu [4, 6-11]. These microorganisms are constantly in contact with host skin epithelial cells and the immune cells underneath which maintain a balance to shape the host immune system. Some bacteria have the ability to produce antimicrobial agents to suppress the distribution of adverse microbiota [12-14]. For instance, bacteriocin, lantibiotics, and epidermin produced by *Staphylococcus epidermidis* (*S. epidermidis*) have bactericidal activity by inhibiting bacterial adhesion and disrupting the integrity of microbial cytoplasmic membrane, restricting the colonisation of pathogenic bacteria on the skin [12-16]. The host skin epithelial cells can also produce chemicals, including antimicrobial peptides, reactive oxygen species (ROS), proteases, immune mediators, continuously or in response to stimuli that can inhibit microbial growth or activate host immune responses, which is essential when commensal microorganisms act as opportunistic pathogens when breaching the skin barrier [17-22].

1.1.2 Immune system of the skin

The skin contains multiple niches, in which numerous immune cells reside, in order to ensure physiological integrity of the skin and the host [23]. The most abundant immune cell types present in the epidermis are Langerhans cells (LCs) and to a lesser extent T cells. The dermis is rich in fibroblasts, collagen and elastin fibres, in which different populations of myeloid and lymphoid immune cells reside and can traffic through the blood and lymphatic vessels (Fig. 1-1) [24, 25].

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) and their main function is to communicate between the innate and adaptive immune system. DCs can be defined into conventional DCs (cDCs) and plasmacytoid dendritic cells (pDCs). PDCs are absent in the steady state skin, while cDCs are a major population in the skin. Human LCs express CD11c, langerin (CD207), E-cadherin, EpCAM, and CD1a, and equipped with a structure called Birbeck granules, thought to be an early endocytic compartment for antigen handling [26, 27]. Traditionally, dermal DCs can be subdivided into BDCA-3$^+$ cDCs, CD1c$^+$ cDCs and CD14$^+$ monocyte-derived cDCs, and are either skin-resident or circulating from peripheral sites [28-34]. However as discussed below, the field is undergoing rapid re-evaluation with the advent of single-cell analysis of heterogeneity. In steady state, DCs in the skin constantly probe their environment for self- and foreign stimuli that enable them to sense the tissue integrity and relay signals to the adaptive immune system.

Dermal macrophages express CD11b, F4/80, and factor XIIIa, and play an essential role in maintenance of the skin barrier functions by promoting fibroblast proliferation and angiogenesis [35-37]. Macrophages are involved in the wound healing process, help with human hair follicle regeneration, regulate salt balance in the skin, and play a key role in skin inflammation and autoimmune diseases [36-38]. Mast cells are part of immune surveillance of the skin, and react rapidly to stimuli, releasing pro-inflammatory mediators that are pre-made and stored within cytoplasmic granules [24]. T cells are also found in healthy human skin [39], expressing cutaneous lymphocyte antigen (CLA), C-C chemokine receptor type 4 (CCR4), CCR7 and CCR10 to migrate towards chemokines produced by fibroblasts in the dermis and towards keratinocytes in the epidermis. These skin-resident T cells have a diverse T cell

repertoire and can be polarised to produce a vast variety of cytokines in response to local stimulation [39-41]. Innate lymphoid cells (ILCs) have been recently appreciated to contribute to skin homeostasis and pathology and will be further discussed in greater detail in chapter six.

In summary, human skin is populated by numerous and diverse immune cells, responsible for local defence against foreign pathogens and malignancies, while developing self-tolerance. In addition, the physiological functions of these skin-resident immune cells are continuously shaped by the microbiota on the surface of the skin because of their close proximity. Dysregulation of the balance in the skin may lead to detrimental consequences to the host; therefore, understanding the complexity of these processes will allow the development of effective therapeutic approaches.

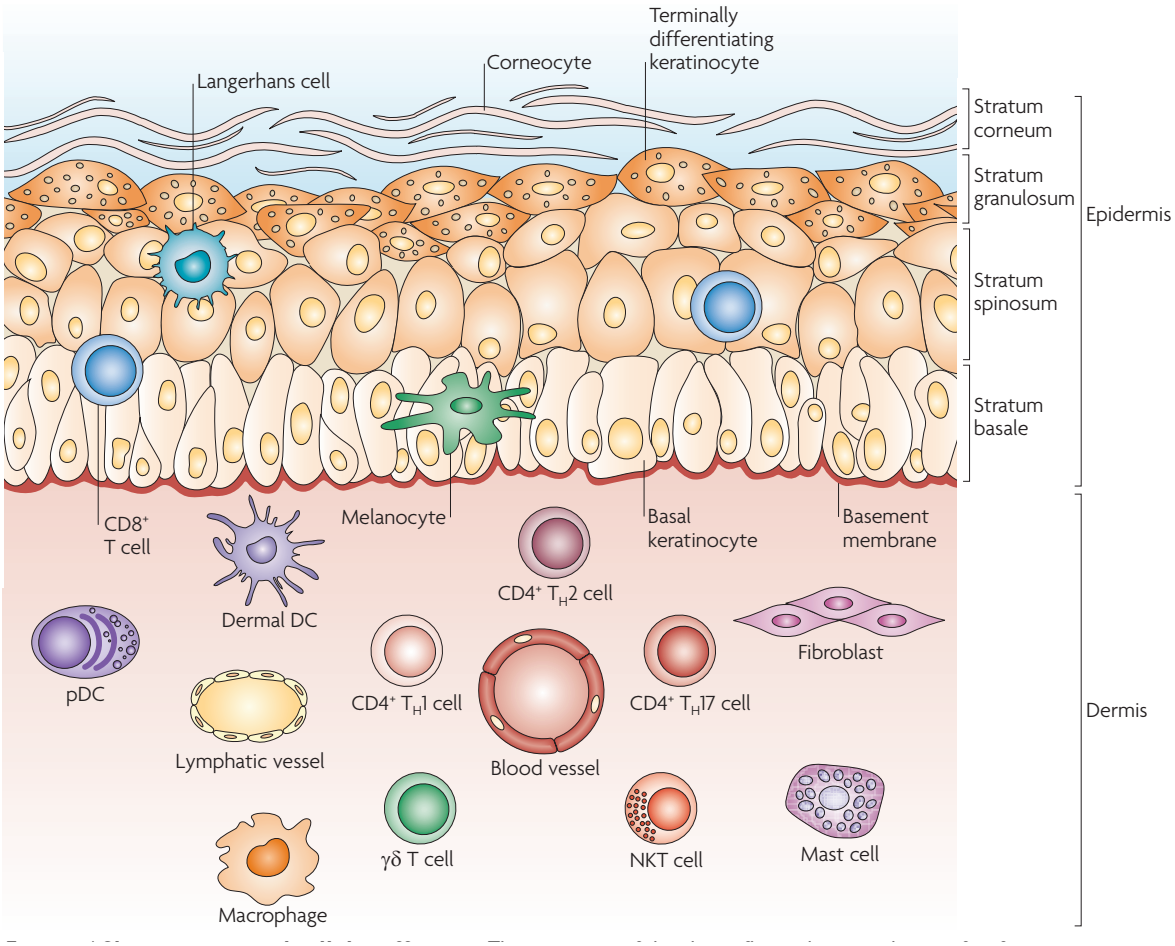


Figure 1-1. Skin anatomy and immune system.

Skin is a complex organ that functions as a protective barrier. The epidermis contains the stratum basale, the stratum spinosum, the stratum granulosum and the stratum corneum, working together to provide barrier functions of the skin, in order to maintain the body temperature, reduce the loss of water from the host, and prevent the penetration of undesired foreign intruders. The dermis is composed of collagen, elastic tissue and fibres. Immune cells can be found in both epidermis and dermis to sense environmental information. Figure was adapted and reprinted by permission from Springer Nature: Nestle, F.O., et al., *Skin immune sentinels in health and disease.* Nat Rev Immunol, 2009. **9**(10): p. 679-91.

1.2 Plasmacytoid dendritic cells

PDCs can secrete robust amounts of type I interferons (IFN-I) in response to stimuli through Toll-like receptor 7 (TLR7) and TLR9, and migrate to lymph nodes to induce T-cell activation, polarisation or T-cell tolerance. They can also secrete other pro-inflammatory cytokines and chemokines, including IL-6, IL-12, IL-8, and chemokine (C-X-C motif) ligand 10 (CXCL10) when stimulated with specific antigens [42-47], to regulate host immune responses.

1.2.1 pDC markers

Human pDCs selectively express the C‑type lectin BDCA-2, and neuropilin BDCA-4 [48-50]. In addition, they have surface receptors that link to their physiological function. The immunoglobulin superfamily receptor immunoglobulin-like transcript 7 (ILT7; LILRA4), which is restrictedly expressed on pDCs [51], can associate with Fc epsilon RI gamma (FcεRIγ) [51]. Bone marrow stromal cell antigen 2 (BST2) is a known ILT7 ligand and, when bound to ILT7, initiates signals through ILT7-FcεRIγ complex and strongly inhibits TLR-induced IFN-I production [52, 53]. IL-3 receptor α-subunit (IL-3Rα or CD123) is highly expressed on human pDCs and is crucial for their survival. After antigen up-take, pDCs express CD62L, and chemokine receptors, such as CCR7, CXCR4, CXCR3 and CCR5 [45, 54-58], which promote their migration to secondary lymphoid organs to drive T cell polarisation. PDCs also express human major histocompatibility complex class II (MHC-II) and co-stimulatory molecules, such

as CD86 and CD80, to present antigens to T cells, and have potential roles in the pathogenesis of autoimmune diseases, cancer, and virus infections [59-63].

1.2.2 pDC subsets

DC subsets are defined according to their surface markers, functional properties, and their developmental ancestry. However, it is widely speculated that the current classification underestimates the heterogeneity of tissue DC populations. Tissue-specific and disease-oriented studies reveal that ontogeny and microenvironment are essential for distinguishing DC subsets [64, 65]. pDCs were generally mapped as a homogeneous group, until recent reports noted the existence of phenotypically distinct blood pDC subsets in human and mouse systems [65-69]. A subset of pDC expressing CD56 has been shown to cluster close to myeloid DCs in a genome-wide transcriptional profiling study, and produced much less IFN-I but more proinflammatory cytokines [70, 71]. Another human blood $CD2^{high}$ pDC subset is reported to express lysozyme; however, whether they have the potential to lyse bacteria is unknown [72, 73]. Within $CD2^{high}$ pDCs, a $CD2^{hi}CD5^{+}CD81^{+}$ subset can be found to promote B cells to differentiate into plasma cells and antibody secretion, and trigger T-cell proliferation and regulatory T cell generation [67]. Recent studies using single-cell RNA-sequencing (scRNA-seq) and high-dimensional phenotypic mapping have revealed a new subtype of blood cells closely related to pDCs expressing Axl and Siglec-6, referred to as AS DCs, potentially exhibiting a continuum phenotype between cDCs and pDCs, perhaps reflecting the existence of pDC lineage plasticity. Further analysis of the frequency of the major DC subsets between organs,

showed that pDCs and AS DCs were not found in normal skin [65, 68]. In another *in vitro* study, human pDCs were challenged with various types of microbial or cytokine stimuli, resulting in the observation of multiple activation-induced pDC subpopulations with specific marker features, such as PD-L1 and CD80, and functions; the PD-L1^{+} pDCs can be found in the skin of psoriasis patients which potentially indicates their disease relevance [64].

1.2.3 pDC transcription factors

PDCs expresses high levels of Transcription Factor 4 (TCF4, also known as E2-2), which can directly bind to the promoter of ILT7 [52], to maintain their surface phenotypes [74-76]. TCF4 is negatively regulated by ID2, and studies have shown that there is a balance between TCF4 and ID2 regulation: in the event of losing the expression of TCF4, a higher level of ID2 occurred, leading to the expression of cDC genes. As a consequence, pDCs appear to be able to go through a cDC-like transition, and become potent APCs to induce T cell proliferation [77-79]. TCF4 controls several molecules, including SPIB [80, 81], B cell lymphoma 11A (BCL11A) [82], Runt-related transcription factor 2 (RUNX2) [83, 84] and IRF8 [85], and are involved in the regulation of pDC development, survival, and activation [86, 87]. SPIB controls the differentiation and survival of pDC progenitors [88]. Moreover, Spib-deficient pDCs exhibit lower IFN-I production following TLR7 or TLR9 stimulation [81]. In mice, Bcl11a maintains the expression of IL−7 receptor (IL-7R) and fms-like tyrosine kinase 3 (Flt3) in haematopoietic progenitor cells, which are essential for pDC development [89]. RUNX2 and its downstream signalling molecule, IRF7, are highly expressed in pDCs,

and are key regulators of IFN-I production [83, 84, 90, 91]. IRF8 is important for pDCs to retain their phenotype and gene-expression profile, but not for their development and survival. When mice pDCs loss their expression of Irf8, they display an upregulation of Irf4 level, and the genes linked to the MHC-II pathway of antigen presentation and the co-stimulatory marker CD80 [92], leading to decreased IFN-I production and shift to a more APC phenotype [93].

1.2.4 pDC in the skin

PDCs are generally absent in healthy human skin [94]. However, they can infiltrate skin during viral infection [95, 96], inflammatory diseases [94, 97], or skin injuries [98]. When skin is damaged, keratinocytes at the wound edge, as well as granulocytes, express increased levels of cathelicidin (also known as LL37), contributing to the inhibition of pathogen growth [17]. Cathelicidins can form complexes with DNAs and RNAs released from the dying cells, which then serve as TLR7 and TLR9 agonists to be captured by skin-infiltrated pDCs [99, 100], leading to a secretion of IFN-I [61, 101]. The importance of IFN-I in skin wound healing has been reported in murine models: depletion of pDCs or attenuating the IFN-I signalling impairs the subsequent T cells response and the reepithelisation of skin wounds [98]. IFN-I has been shown to be responsible for activating T cells to produce several effector cytokines, such as IFN-γ and IL-22, that helps promote the restoration of skin barrier function [97, 98].

Other than their roles in wound healing, pDCs have been proven to be relevant in several skin-related allergic reactions and inflammatory diseases. For example, in

the tuberculin skin test (TST), which is routinely used in clinic to evaluate reactivity to M. tuberculosis or its vaccine, an upregulation of cathelicidins is observed in the test site of individuals who exhibit a positive reaction [102]. The TST is performed by intradermally injecting purified protein derivative (PPD) into the forearm [103], and, in sensitive individuals, it results in a delayed-type hypersensitivity reaction, initiated by DCs uptaking of PPD and subsequently presenting the antigen to T cells [104, 105], and further investigation has revealed that a large pDC infiltration can be found in the TST site after PPD administration only in sensitive individuals [102].

Psoriasis is a complex inflammatory skin disease in which pDCs are also thought to play a role. Historically, some anti-tumour or antiviral drugs, such as IFN-Is or imiquimod, a TLR7 agonist that induces IFN-I production, have been clinically linked with the worsening of psoriasis [106-108]. Others reported that chemerin, which binds to ChemR23 receptor on pDCs, is expressed in the early psoriasis lesions, resulting the recruitment of pDC into the lesional skin [109-111]. In murine psoriasis models, inhibiting the IFN-I production from pDCs can prevent T cell-dependent development of psoriasis [97, 108, 112]. Moreover, IFN-I has been found to initiate plaque formation and trigger IL-23 accumulation in the skin [97, 113], which skews T helper cells to differentiate into IL-17-producing Th17 cells and help the maintenance of their phenotype [114-117]. Further evidence in the study of IL-23 receptor (IL-23R) gene variants and their association with the susceptibility to psoriasis further emphasises the involvement of the IL-23/Th17 axis in psoriasis [117, 118]. A more detailed introduction of this disease can be found in chapter six.

Lupus erythematosus (LE) is a chronic autoimmune disease with symptoms from confined skin lesions to systemic complications, which pDCs and their production of IFN-I are considered significant for the progression of the disease. Potential factors, including ultraviolet (UV) radiation exposure, skin trauma, and viral infection, can cause keratinocyte death and the release of TLR7 and 9 agonists to stimulate IFN-I production, which in turn induces the production of chemokines, such as CXCL9 and CXCL10 [119], leading to CXCR3-expressing pDC migration into the skin lesions [120-122]. Studies also report that, in the peripheral blood or lesional skin of patients suffering from systemic LE (SLE), interferon-stimulated genes (ISGs), such as human myxovirus resistance protein 1 (MxA) and IRF7 are upregulated [123, 124], and strongly expressed [125, 126]. Polymorphisms in genes involved in the IFN-I synthesis and IFN-I signalling have also been associated with an increased risk to develop SLE and other autoimmune diseases [127-129]. Possible triggers for autoantibodies are viral infections [130], perhaps due to molecular mimicry. For instance, Epstein-Barr virus (EBV) infection has been shown to associate with SLE by causing B cell hyperactivation and introducing autoantigens to give rise to autoantibodies [131-133]. The autoantibodies and the small nuclear agents form the interferogenic immune complexes (ICs) that are internalised via FcγRIIα on pDCs and triggers their IFN-I production in a TLR-dependent manner [134, 135]. In murine models of SLE, IFN-I plays a role in the onset and the progression of the disease, and the impairment of IFN-I receptor signalling rescues the SLE-like symptoms [136-138]. Depleting pDCs at the early stage of disease limits autoantibody production, decreases ISGs, and alleviates the pathology of the disease [139]. providing further evidence of the involvement of IFN-I in LE.

Atopic dermatitis (AD) is a chronic skin inflammatory disorder with disrupted skin-barrier function and elevated skin type 2 immune responses. AD patients are usually reactive to various airborne allergens, and show elevated IgE levels in the serum [140, 141]. Compared to psoriasis and SLE, there is less pDC recruitment into AD skin lesions [94, 142, 143]. However, pDCs have been shown to express FcεRIγ which binds to IgE, and can take up allergens by the FcεRIγ-IgE complex to promote Th2 responses [144].

1.3 Type I Interferons

1.3.1 Type I IFN production

Human type I IFNs are found universally expressed in vertebrates, and include 13 IFNαs, and IFNβ, IFNω, IFNκ and IFNε [145] that are highly specified and do not cross-react among different species [146]. Within this work, IFN-I refer to the two most immune relevant members of type I IFN family, namely IFNα and IFNβ. IFN-I function as essential immune modulators during infections and autoimmune diseases, inducing infected cells and their neighbouring cells to enter antimicrobial states, regulating of cell survival, boosting antitumor activities, promoting antigen presentation abilities of APCs, and modulating functions of effector cells in the adaptive immune system [147-152]. IFN-I production is inducible upon the detection of microbial derivatives or autolugous nucleic acids by pattern-recognition receptors (PRRs), including TLRs or cytosolic nucleic acid sensors, such as retinoic acid inducible gene 1 (RIG-I), melanoma differentiation-associated protein 5 (MDA5), stimulator of interferon genes

protein (STING), and nuclear protein IFNγ inducible protein 16 (IFI16) [153]. IFNα is prodominately secreted by pDCs, and to a lesser extent in other APCs; whereas IFNβ-producers are mainly stromal cells, such as fibroblasts and epithelial cells, in response to environmental stimuli [154, 155]. PDCs are equipped with a constantly high baseline of IRF7, which renders them ready to produce IFN-I upon signal engagements; however, stromal cells required a priming signal from low level of IFN-I in the environment to obtain a positive feedback expression of IRF7, subsequently triggering their IFN-I production [156]. In human endothelial cells, TNFα receptor signalling can augment the production of IFN-I, and these two cytokines work synergistically to induce recruitment and proinflammatory cytokine production from the surrounding effector cells [157, 158].

1.3.2 Type I IFN signalling

All IFN-I bind to the type I IFN receptor (IFNAR), which are composed of IFNAR1 and IFNAR2, that expresses across all nucleated cells, with IFNβ have higher binding affinity to IFNAR1 than IFNα. Upon IFN-I binding, the downstream molecules in its signal pathway, include Janus kinase 1 (JAK1), tyrosine kinase 2 (TYK2), signal transducer and activator of transcriptions (STATs), and IFN-regulatory factors (IRFs). There are seven known STATs, and IFN-I has been reported to activate all of them to form various homodimer or heterodimer complexes, inducing expression of ISGs by binding to DNA elements, termed interferon-stimulated response element (ISRE) and IFNγ activated site (GAS). ISGs contain genes encoding antiviral proteins, chemokines and antigen-presenting related molecules, and their expression profiles

are determined by the trascriptional network of the IFNAR signalling, which varies in different cell types, causing variable immune consequences [152, 159, 160]. In addition, the consequences of IFNAR signal cascades are highly depedent on the engagement of IFN-I subtypes, IFN-I concentration and their life span. For instance, engineering of IFN-I, which altered their binding affinity to IFNAR, and extended plasma lifetime, has been proven to be advantageous for anti-malignancy function [161]. Because of their multifecet functions, IFN-I has been considered as drug candidate against a wide range of diseases, such as SLE, hepatitic C, and cancers [162]; however, the manupulation of the activities of the IFN-I/IFNAR axis requires fine-tuning, as systemic blocking of this signal pathway may result in increased susceptibility to infectious diseases and cancer.

IFN-I signalling exhibits both positive and negative effects on immune cell functions, depending on the downstream signalling molecules, which in turn causes variable immune consequences [151, 152, 163]. IFN-I has been shown to have antiproliferative effects on solid tumors and their potencies are dependent on the IFNAR levels expressed on the cancer cells [164-166]. It has been reported that in T cells, activation of STAT1 leads to anti-proliferative and pro-apoptotic results, whereas, activation of STAT3 promotes cell proliferation and survival [160, 167]. The study also shows that the STAT1/3 balance is detemined by the timing between IFNAR signalling and other concomitant signalling pathways. Other than T cells, IFN-I appears to govern migration, function, and homeostasis of several other immune cells, such as NK cells [168, 169] and DCs [149-151], as well. Prolonged IFNAR activation can have adverse effects, such as uncontrolled inflammation and tissue diruption [162, 170, 171]; therefore, negative feedback mechanisms exist. Normally, several minutes

after IFN-I engagement, IFNAR are internalised, and then undergo ubiquitination and degradation that reduces the density of IFNAR on the cell surface, abrogating further activation [172-174]. Interestingly, there are kinetic discrepancies in recycling IFNAR1 and IFNAR2, with IFNAR1 showing slower recovery rates on the cell surface [175, 176]. Another negative regulation of the pathway is to upregulate interferon-inducible protiens which have the ability to interfere with the recruitment of IFNAR1 to IFNAR2, disrupting the formation of IFNAR complex [177-179]. IFNAR signalling can also elevate inhibitor proteins that suppress TYK2-mediated signal cascade even with a very low amount of IFNα, and can be present over a continuous period of time [180, 181].

1.3.3 Therapeutic targeting of IFNAR pathway

Several strategies have been implicated to modulate the IFN-I/IFNAR signalling pathway in diseases where IFN-I exacerbate the pathology, such as SLE and psoriasis [170, 182, 183]. The most common ones are utilising neutralising antibodies, either against the cytokine or to their receptor. Neutralising antibodies against IFN-I have shown only partially effectiveness in some diseases, as a low concentration of IFN-I is potent enough to trigger the signal cascade. On the other hand, attenuating IFNAR by antibodies has been established to almost completely abrogate their signalling [170, 184]. Another approach with sphingosine 1-phosphate receptor 1 (S1PR1) agonists is found to accelerate the recycling of IFNAR1, resulting in attenuated IFNAR signalling in pDCs [185]. Moreover, engineering IFN-I affinity to provide tighter binding

to one of the subunits of IFNAR, but not the other, has been found to block some of the effector function of IFN-I but leave other activity intact [186]. In addition, inhibitors targeting the downstream moleculars of the IFN-I signalling pathway have been reported to be effective in therapeutic situations. JAK1 and TYK2 are important mediators of IFNAR signalling, which transmit extracellular cytokine signals to activate various STATs, initiating responses against viral and non-viral pathogens. A number of JAK1 and TYK2 inhibitors have been approved or are in development. Notably, some JAK1 inhibitors have also shown specificity for other JAKs. Moreover, studies have shown that inhibition of pan-JAKs can occure in high doses due to the off-target activity of these inhibitors [187]. For instance, Tofacitinib, which is approved for the treatment of rheumatic disease in the USA, inhibits primarily JAK1 and JAK3; ABT-494 and Filgotinib, acting specificly on JAK1, are in phase II and phase III clinical trial, respectively, for both Crohn's disease and Ulcerative colitis; INCB018424 and Peficitinib have undergone phase II trial for treating psoriasis [187].

2 Aims and Objectives

It is increasingly becoming apparent that pDCs play an important role in both skin barrier restoration, and various skin disorders; therefore, understanding the dynamic mechanisms of pDCs in the skin will likely promote significant mechanistic and clinical progress. Exploiting skin suction blisters, which provides an easily accessible reservoir of skin wound infiltrating cells and interstitial fluid without further enzymatic digestion and prolonged manipulation process, and preserving the phenotypic integrity of the cells and blister fluid, has facilitated the investigation of cell-cell interactions and soluble mediators involved in maintaining skin homeostasis.

Here, it was hypothesised that pDCs, which influx early into skin wounds, act as upstream mediators, in both a direct and an indirect fashion, governing the subsequent immune responses to contribute to the wound healing process in humans. In chapter four and five, the heterogeneity and functionality of pDCs in human skin wounds was dissected, including through the use of bulk RNA-seq and scRNA-seq methods, and examine their functional capacity in skin wounds to communicate directly with the adaptive type 22 immune system. Next, in chapter six, the ability of pDCs to modulate ILC22 interactions with T cells was investigated, potentially through cell-cell interaction and soluble factors, indirectly shaping the adaptive immune response.

3 Materials and Methods

3.1 Isolation of immune cells from the blood or skin of human.

Human blood samples were obtained from healthy donors under local ethics approval [Oxford C, 09/H0606/71, National Research Ethics Service (NRES) Committee South Central]. PBMCs were isolated using Lymphoprep (Stem Cell Technologies) gradient isolation. Skin samples were obtained from healthy donors undergoing plastic surgery. Samples were taken under good clinical practice guidance with ethical approval of the NRES Committee South Central. Subcutaneous fat was removed completely before the sample was dissected and incubated in collagenase P (Roche) overnight at 37°C. Cold 10 mM EDTA solution was added to the sample to stop the digestion. The digested tissue was passed through a 70-μm strainer, washed with EDTA solution, and harvested mononuclear cells with Lymphoprep gradient isolation before further procedures.

3.2 Antibodies, flow cytometry and cell isolation

Surface staining was performed at 4 ^{o}C in cell staining buffer (0.5% FBS in PBS) for 15 min. Intracellular staining (ICS) was performed using Foxp3/Transcription Factor Staining Buffer Set (Invitrogen), following the manufacturer instructions. The following anti-human antibodies were used: Brilliant Violet 650™ anti-CD3 (OCT3; Biolegend), PE-Cy7 anti-CD123 (6H6; Biolegend), PerCP anti-CD11b (DCIS1/18; Biolegend), Brilliant Violet 421™ anti-CD11c (3.9; Biolegend), Brilliant Violet 650™

anti-CD11c (3.9; Biolegend), Brilliant Violet 785™- anti-HLA-DR (L243; Biolegend), PE anti-BDCA-2 (201A; Biolegend), Brilliant Violet 421 anti-BDCA-2 (201A; Biolegend), PE anti-BDCA-4 (12C2; Biolegend), PE/Dazzle 594 anti-CD86 (IT2.2; Biolegend), PE anti-Langerin (4C7; Biolegend), FITC anti-IFN alpha (MMHA-11, PBL Assay Science), PerCP anti-CD16 (3G8; Biolegend), PerCP anti-CD19 (SJ25C1; BD), PerCP anti-CD123 (FAB301C; R&D Systems), PerCP anti-CD11c (BU15; Abcam), PerCP anti-CD8 (RPA-T8; Biolegend), PerCP anti-FcεRIα (AER-37 [CRA-1]; Biolegend), PerCP anti-CD14 (MP9; BD), PerCP anti-CD4 (MEM-241; Biolegend), Pacific Blue anti-TCRα/β (IP26; Biolegend), AF700 anti-CD45 (H130; Biolegend), PE anti-CRTH2 (BM16; Miltenyi Biotec), PE-Cy7 anti-IL-7Rα (A019D5; Biolegend), APC anti-c-kit (104D2; Biolegend), Brilliant Violet 605™ anti-CD56 (5.1H11; Biolegend), Brilliant Violet 650™ anti-CD56 (5.1H11; Biolegend), PE anti-NKp44(P44-8; Biolegend), Brilliant Violet 605 anti-NKp46 (9E2; Biolegend), Brilliant Violet 605 anti-CD123 (6H6; Biolegend), AF700 anti-HLA-A,B,C (W6/32; Biolegend), Pacific Blue™ anti-CD54 (HA58; Biolegend), PE-Cy7 anti-CD40 (5C3; Biolegend), APC-Cy7 anti-CD69 (FN50; Biolegend), PE-Cy7-anti-CD4 (MEM-241; Biolegend), APC anti-IL-2 (MQ1-17H12; Biolegend), AF700 anti-IFNγ (4S.B3; Biolegend), PE anti-IL-22 (2G12A41; Biolegend), eFluor-450 anti-IL-17A (eBio17B7; ThermoFisher Scientific), PE-anti-RORγt (AFKJS-9; ThermoFisher Scientific), live/dead aqua (Invitrogen) and live/dead violet (Invitrogen). Flow data were acquired using FACSDiva on LSRFortessa and further analysed with FlowJo (Tree Star) software. Blister pDCs were sorted with the BD FACSAria IIIu as BDCA-2^{+}CD123^{hi} and BDCA-2^{+}CD123^{int} cells. Blood pDCs and T cells were isolated by magnetic-activated cell sorting (MACS) with anti-BDCA-4 and anti-CD3 MicroBeads (Miltenyi Biotec), respectively, according

to the manufacturer instructions. Blood and skin cDCs were isolated using BDCA-1$^+$ dendritic cell isolation kit (Miltenyi Biotec) with BDCA-4$^+$, CD19$^+$ and CD14$^+$ cells depleted from the sample before cDC separation. For pDC and cDC separation, the positively labeled cells underwent a second column run to ensure higher purity.

3.3 Suction blister technique

Saline was delivered to the upper arm of the volunteers by intraepidermal skin prick test and suction blister cups were applied to the sites with vacuum pressure of 200 mmHg to create blisters. Blisters were protected overnight, and the fluid was aspirated 24 hours later to collect skin-infiltrated cells and tissue fluid. The concentration of indicated cytokines was measured by multiplex array (multiplex bead array), and the cells were stained with cell surface antibodies for flow cytometric analysis of DC subsets or for bulk or single-cell RNA sequencing analysis.

3.4 Bulk RNA sequencing

Suction blister cells and PBMCs were harvested, and CD3$^+$CRTH2$^+$ T cells, CD45$^+$IL7Rα^-CRTH2$^+$ myeloid cells, and Lin$^-$CD45$^+$IL-7Rα^+CRTH2$^-$ ILC1/3 were sorted with the BD FACSAria IIIu and collected directly into TRIzol LS to obtain the RNA-containing phase. The purified RNA was extracted using Qiagen RNeasy Mini kit, followed by removing DNA contaminate with Ambion Turbo DNase. The mRNA extraction, RNA sequencing, and data analysis were performed as previously described [188]. NuGEN Ovation RNA-Seq system V2 (Ultralow DR Multiplex kit)

was used to amplify cDNA to subject to RNA-sequencing on an Illumina HiSeq 2000. FastQC package (www.bioinformatics.babraham.ac.uk/projects/fastqc) was used for quality control analysis, reads were aligned using STAR against the human genome assembly [NCBI (National Center for Biotechnology Information) build37 (hg19) UCSC (University of California, Santa Cruz) transcripts]. After necessary normalisation process, RPKM (reads per kilobase of transcript per million mapped reads) values were generated using the edgeR package.

3.5 Skin wound sample collection

Healthy donors were recruited, and each bilateral 3 mm baseline full thickness punch biopsies on the flank of the lower back (non UV-exposed site) were used to create skin wounds. Larger biopsies (6mm) to encompass the bilateral original wounds were taken between one to four days later. The study was under ethics approval [16/SC/0457, National Research Ethics Service (NRES) Committee South Central]

3.6 Skin section and confocal microscopy analysis

The skin biopsies were snap-frozen in OCT embedding compound and stored in -80°C. The tissues were cut into 4 μm sections and placed on slides to air-dried for 30 min before stored at -80°C. Slides were rehydrated in PBS for 10 min prior to staining. The endogenous peroxidase activity of the sample was quenched by adding 0.3% Hydrogen Peroxide Solution and incubate for 5 minutes at room temperature. The endogenous biotin was blocked with Avidin/Biotin Blocking Kit (Vector

Laboratories Ltd) and 10% goat serum was used to reduce non-specific binding of antibodies. Antibodies used for confocal microscopy include CD1a (1:100, OKT6; in-house production and conjugated with Alexa Fluor 647 dye) and BDCA2-Biotin (1:2, AC144; Miltenyi Biotec). Alexa Fluor™ 594 Tyramide SuperBoost™ kit (streptavidin, ThermoFisher Scientific) was used to enhance the signals. Slides were incubated at 4°C with primary antibodies overnight. After washing, HRP-conjugated streptavidin was added to the sections and incubated at 4°C overnight. Excess streptavidin was washed away, and the tissues were incubated with tyramide working solution for 8 minutes at room temperature and the reaction was stopped with Reaction Stop Reagent. Following staining, slides were mounted using antifade mounting medium with DAPI (Vector Laboratories Ltd), coverslips were applied, and slides were refrigerated in dark until analysed by confocal microscopy (Zeiss LSM 780 Confocal Microscope-Inverted Microscope).

3.7 Skin targeted RNA sequencing

The skin biopsies were subjected to targeted RNA sequencing analysis. Skin samples were collected directly into RNA Later reagent (Ambion) and frozen at -80°C until further use. Tissue disruption and homogenisation was carried out in RTL buffer (Qiagen) using TissueRuptor (Qiagen) on ice. RNA was extracted with RNeasy Fibrous Tissue Mini Kit (Qiagen) according to the manufacturer's instructions. Next Generation Sequencing was carried out with Illumina reagents (TruSeq Targeted RNA Index Kit and TruSeq Targeted RNA Custom Panel Kit) at the Deep Seq Facility (The University of Nottingham). Following QC analysis with the fastQC package, reads

were aligned using STAR against the human genome assembly (UCSC GRCh38/hg38). Gene expression levels were quantified as read counts using the featureCounts function from the Subread package with default parameters [189]. The read counts were used for the identification of global differential gene expression between specified populations using the edgeR package. RPKM values were also generated using the edgeR package.

3.8 *In vitro* pDC culture and T cell assay

Purified pDCs were seeded into 96-well U-bottom plates with 1-2.5x10^5 cells/well density in RPMI medium (supplied with 10% FCS, 2 mM l-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 100 μM sodium pyruvate, nonessential amino acids, 50 μM β-mercaptoethanol, 10 mM Hepes) and IL-3 (50 ng/ml, Biolegend) for 48 hours. Imiquimod (1 μg/ml, Invivogen) was used as stimulus in some conditions and Brefeldin A (5 μg/ml, Biolegend) was added for 12 hours to block cytokine transport process to enhance intracellular cytokine staining signals. Cells were then centrifuged at 1500 rpm for 5 min at 4°C, and used for flow cytometric analysis or T cell co-culture assays.

3.9 CD1a-restricted T cell assay

IL-22 ELISpot (Mabtech AB) were used to assess CD1a reactivity. The multiscreen-IP sterile filter plates with hydrophobic PVDF (Millipore) were coated with capture antibodies at 4°C overnight. *In vitro* cultured pDCs (1.5x10^4) were cocultured

with T cells ($5x10^4$) per well with anti-HLA-A,B,C (10 μg/ml; W6/32, Biolegend) and HLA-DR (10 μg/ml; L243, Biolegend) antibodies and incubate at 37°C and 5% CO_2 overnight. In some wells, pDCs were pretreated with anti-CD1a blocking antibody (OKT-6; 10 μg/ml, produced in house), or immunoglobulin G1 (IgG1) isotype control (10 μg/ml, Biolegend) an hour before the addition of T cells. PMA (5 ng/ml) and ionomycin (5 ng/ml) stimulation or T cells alone was set up as positive controls or negative controls, respectively. After incubation, plates were washed and incubated with biotin-linked anti-IL-22 monoclonal antibody for 2 hours. After washing, the plates were incubated for 2 hours with streptavidin-alkaline phosphatase and were developed using the alkaline phosphatase conjugate substrate kit (Bio-Rad). The numbers of IL-22-producing T cells were counted using the automated ELISpot reader (Autimmun Diagnostika gmbh ELISpot Reader Classic). The result graphs were calculated as the fold change of each condition to the anti-HLA/IgG1 isotype control treatment.

3.10 Single-cell RNA sequencing

Human PBMCs and blister cells were stained with antibodies in PBS for 10 minutes, and each targeted cell of interest was sorted into each well of 96-well plates containing 2 μl of 0.2% triton plus 1 U/μl RNaseOUT (ThermoFisher Scientific). Total of 182 blood cells and 273 skin cells were collected and single cell libraries were prepared using Smart-Seq2 protocol [190] with minor modifications. Reverse transcription using Smartscribe RT enzyme, 50 U/cell (Takara) was performed. For cDNA amplification, 25 PCR cycles were performed. All the other parameters were

as described in the original paper [190]. Not all the wells were successfully profiled, and some of the wells were excluded from the data analysis after applying QC filters.

3.11 Single-cell RNA sequencing data analysis

Gene expression for individual cells was quantified by transcriptome (GRCh38.p10 from Ensembl) pseudoalignment using Salmon [191]. Cells were kept if they had a mapping rate greater than 40%, expressed more than 1200 detected genes and contained less than 20% of their reads associated to ERCC spike-ins. Genes were removed from the analysis if they were expressed (more than 1 count) in less than 5 cells. Lastly, the total read count was regressed from the expression values to normalise the data. Principal Component Analysis (PCA) was performed using the prcomp function in the base R package. Differentially expressed (DE) genes were obtained by fitting a linear model with a term accounting for the cell types being compared. This model was compared with a reduced model only including an intercept term via a likelihood-ratio test. Finally, the p-value was corrected using FDR. Genes were considered DE if the corrected p-value was below 0.05 and their absolute log2 fold change was above 1. Monocle was used to generate pseudotime figures. Some figures were generated base on the methods described by Villani et al [68, 192].

3.12 ILC22 cell culture

Blood ILC22 were sorted from healthy or psoriasis individuals with the BD FACSAria IIIu as Lineage (CD3, CD4, CD8, CD14, CD19, CD16, CD11c, CD11b,

CD123, and FcεRIα)-negative, $CD94^{-}CD45^{+}IL\text{-}7R\alpha^{+}CRTH2^{-}c\text{-}Kit^{+}NKp44^{+}$ cells into 96-well U-bottom plates with 100 cells/well density and cocultured with mixed lymphocyte reaction (MLR) of γ-irradiated peripheral blood mononuclear cells (PBMCs) from three healthy donors ($2x10^6$ cells/ml) supplied with IL-2 (250 IU/ml; Preprotech) and phytohemagglutinin (PHA; ThermoFisher Scientific) at 37°C and 5% CO_2. Equal volume of fresh medium containing IL-2 was replenished every 3-5 day. After 4 to 6 weeks, the growing lines were tested by flow cytometry staining, and pure ILC22 populations were used for experiments. To further expand the population, some ILC22 lines were re-fed with MLR mix.

3.13 Quantitative teal time-PCR (RT-qPCR)

The mRNA of the cells was prepared using TurboCapture kit (QIAGEN). The cDNA was synthesised with M-MLV Reverse Transcriptase (Invitrogen) and then subjected to RT-qPCR using TaqMan Probe (Life Technologies) to *OAS2* (Hs00942643_m1) and *GAPDH* (Hs99999905_m1). Reactions were performed in a 7500 Fast Thermal Cycler (Applied Biosystems). Data were normalised to *GAPDH*.

3.14 Proliferation assays

ILC22 were stained with 0.5 μM CellTrace violet (Life Technologies), according to manufacturer's instructions, and stimulated with 200 IU/ml of IL-2 at 37°C and 5% CO_2. The cells were collected and analysed with flow cytometry at the indicated time points.

3.15 IL-22 ELISA

ILC22 ($1x10^6$) were seeded in U-bottom 96-well plates and treated with IL-1β and IL-23 mixture, in addition of Roferon-A (500 IU/ml, Roche, human IFNα-2a, termed IFN-I), or IFN-I alone for 72 hours at 37°C and 5% CO_2, and supernatants were collected and stored at -80°C before assays. The concentrations of IL-22 was were measured by sandwich enzyme linked immunoassay (ELISA) to manufacturer's instructions (ThermoFisher Scientific). Corning 96 Well Half Area Clear Flat Bottom Microplate were used to coat IL-22 captured antibodies overnight at 4°C.

3.16 Transwell assay

HTS-Transwell-96 Well Plates with 0.4 μm pore filters (Corning Costar) were used. The lower chambers were seeded with 200 μl of $0.5x10^6$ ILC22, and the upper chambers were loaded with $1.5\text{-}2x10^5$ blood pDCs in 100 μl of medium, treated with or without Imiquimod (1 μg/ml, Invivogen) for 16-hour at 37°C and 5% CO_2. The pDCs were then removed, and the remaining ILC22 were treated with IL-1β and IL-23 for 72 hours (Fig. 10-9) at 37°C and 5% CO_2. The supernatant was collected for IL-22 ELISA, and the cells were subjected to fluorescence-activated cell sorting (FACS) analysis.

3.17 *In vitro* protein antigen presentation

To determine antigen-specific $CD4^+$ T cell activation by ILC22, human tetanus toxoid (TT)-reactive $CD4^+$ T cell clones were used. The $CD4^+$ T cell clones were

generated by Dr. Mariolina Salio. ILC22 ($2x10^4$) were seeded in U-bottom 96-well plates and treated with IL-1β and IL-23 mixture, in addition of IFN-I, or IFN-I alone for 72 hours. The cells were washed thoroughly to remove cytokines and incubated with TT protein (10^1-10^{-1} μg/ml) for 4 hours at 37°C and 5% CO_2. Another set of thorough washes was carried to remove excess proteins before coculturing with $CD4^+$ T cell clones (3-5 $x10^4$). Brefeldin A (5 μg/ml, Biolegend) was added after 4 hours and followed by another 12-hour incubation at 37°C and 5% CO_2. Cells were then centrifuged at 1500 rpm for 5 min at 4° C, and used for surface stainings of $CD4^+$ T cells ($CD4^+CD69^+$) and ICS stainings of IFNγ, IL-22, IL-2 and IL-17A.

3.18 Statistical analyses

All values are shown as mean. Error bars represent the standard error of the mean (SEM). Paired two-tailed t tests, one-way Anova, and Pearson correlation were performed using GraphPad Prism version 7.00 (GraphPad Software) to assess statistical significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; and ****$P < 0.0001$.

4 Plasmacytoid DC Presentation of Self-Lipid Antigens to CD1a-reactive T Cells

4.1 Introduction and aims

4.1.1 Wound healing

As an outer physical interface, the skin frequently encounters a variety of external challenges, potentially associating with barrier compromise. Traumatic injury to the skin can serve as an entrance for previously sequestered microorganisms to the underlying tissue, leading to local and systemic infection. In addition to unintended trauma, routine medical procedures create surgical wounds and represent a major avenue for complications in recovery; therefore, facilitating tissue restoration of the skin wounds remains a major concern. Most skin injuries can heal well in healthy individuals; however, patients who suffer from diabetes, vascular insufficiency, skin inflammatory diseases, and cancer can experience a poor skin wound healing process, resulting in chronic wounds [193, 194]. Sensing and restoring skin tissue integrity is a complex process and involves intensive crosstalk between epithelial cells, stromal cells, and hematopoietic cells.

Wound healing can be separated into three major stages: inflammatory, proliferative, and remodeling [195]. The inflammatory stage includes a robust infiltration of the wound site by immune cells [196]; upon skin injury initiation, platelets aggregate to form a fibrin network, reestablishing the homeostasis and barrier integrity, and release immune mediators [197-199]. Local resident mast cells degranulate and

release a variety of mediators to trigger the recruitment and activation of immune effector cells. Neutrophils are recruited into the wound site by damage-associated molecular patterns (DAMPs) released from damaged and necrotic cells [200, 201], and secrete pro-inflammatory cytokines, lysosomal enzymes, cationic peptides, and ROS. Other immune cells, such as monocytes, T cells, ILCs and DCs, are subsequently drawn into the skin wound in response to chemokines and growth factors that have accumulated locally [202-211]. Together, the aforementioned cells provide the host protection against microbial invasion and the clearance of cell debris.

The proliferative stage involves the proliferation and migration of keratinocytes, and extracellular matrix (ECM) deposition, and angiogenesis. At the wound edge, keratinocytes proliferate and migrate to restore the barrier function of epidermis. In addition, keratinocytes induce fibroblasts to undergo fibroplasia and secrete growth factors, which in turn help keratinocyte proliferation. During the process of fibroplasia, fibroblasts synthesise ample amounts of ECM, providing a scaffold for cellular adhesion which facilitates epithelial cells to migrate into the injury site, resulting in angiogenesis [212-215]. In the course of restoring the architecture of the skin, angiogenesis is essential for developing new capillaries to supply blood, nutrients, and oxygen to the site [216-220].

The remodeling stage involves barrier restoration and scar formation. The repair process first enters the tissue maturation stage where scar tissues are formed and the re-epithlialisation and inflammation processes are regulated. After its formation, ECM continuously undergoes remodeling from the initially disorganised structures by displacing fibrin with hyaluronan, proteoglycans and fibronectin, and

eventually depositing with collagen fibers [221-223]. For proper wound closure, the balance between type III collagen and type I collagen is crucial. Normally type III collagen undergoes degradation, and type I collagen synthesis increases, resulting in the collagen fibers becoming thicker and being deposited in a parallel manner to enhance the strength of the skin tissue [195].

Immune cells are involved in both physiological and pathological aspects of wound healing. These cells play a key role in pathogen clearance and necrotic tissue removal at the injury sites. Other than their protective role, through secretory mediators, such as cytokines and growth factors, immune cells also help with re-epithelialisation, angiogenesis, and scar formation during the process of the wound healing [213, 224]. Dysregulation of immune responses during this process contributes to delayed or improper wound formation and excessive scarring [225]; hence, understanding the underlying mechanisms of skin wound sensing and repair, and modulation of the local immune response, is of therapeutic relevance.

4.1.2 CD1a antigen presentation

4.1.2.1 CD1 family

Other than peptides, it has been recently appreciated that a proportion of the T cell repertoire can recognise lipid antigens that are presented by APCs. CD1 molecules, which are relatively non-polymorphic and structurally related to the MHC-I molecule, are responsible for lipid presentation [226]. There are five CD1 isoforms that can separate into three groups: group 1 contains CD1a, CD1b, CD1c;

group 2 includes CD1d; and group 3 includes CD1e [227]. Humans express all five isoforms, but only CD1d is expressed by mice. Lipid antigen recognition is now appreciated to be an essential part of host defence: when the host is under stress, such as during infection or tissue damage, the display of foreign- or self-lipids could provide a trigger to the adaptive immune response. It has been reported that intracellular mycobacteria-infected APCs can present lipid and glycolipid antigens derived from the mycobacteria to group 1 CD1-restricted T cells [228]. In addition, lipid components of human sebum can be presented by CD1 to T cells and are thought to play a role in facilitating skin wound healing [229, 230] which I will discuss in further details in §4.1.2.3.

Like MHC-I, CD1 contains a heavy chain which forms heterodimeric complexes in the endoplasmic reticulum (ER) by pairing non-covalently with β2 microglobulin (β2m) light-chain subunit [231-234]. The heavy chain of CD1 consists of α1, α2, and α3 domains, and shortly after translation into the ER, it starts to fold and, by bringing the hydrophobic amino acids of the α1 and α2 domains together, a CD1 antigen-binding groove with hydrophobic surface is formed [235, 236], and can be loaded naturally with lipids [236-238].

Other than to MHC-I, CD1 molecules also share some similarities to MHC-II: CD1 localises in the endosomal compartments, and sometimes co-localises with MHC-II [239-241]. Many foreign lipid antigens do not engage CD1 at the cell surface, but undergo intracellular processing and loading, similar to the process of MHC-I/II molecules taking up some peptides in cytosolic and endosomal compartments. Another parallel pathway is identified for loading self-lipids, which load onto CD1a in

lower efficiency without the assistance of endosomal factors, because self-lipids can be derivatives from cell membranes or extracellular oil secretion, and are of great abundance; therefore, do not always require the more efficient intracellular endosomal pathway [239].

4.1.2.2 CD1a

CD1a can present autologous and foreign lipids to T cells circulating in the peripheral and residing in the skin. CD1a is expressed predominantly on epidermal LCs [242], some tissue-specific DCs [243], monocyte-derived DCs [244], thymocytes [245], and inducible on group 2 innate lymphoid cells (ILC2) [188]. The antigen-binding groove of CD1 is rigid and has two interconnecting pockets, with an A' pocket immersed inside and covered by a roof-like structure (A' roof), and an F' pocket at the protein surface which provides access for lipids [246, 247] (Fig. 4-1). Unlike other CD1 isoforms in the family, CD1a has a short cytoplasmic tail that lacks the tyrosine-based cytoplasmic tail motif (ITAM) which allows other CD1 isoforms to bind to adaptor protein-2 (AP2) in the cytosol to mediate the delivery of CD1-lipid complex to late endosomes [239, 248, 249]; therefore, CD1a is not found expressed in late endosomes. However, CD1a can be detected in plasma membrane–associated clathrin-coated pits and vesicle, early endocytic vesicles, Birbeck granules of LCs, as well as on the plasma membrane [250-252]. Further evidence shows that, for CD1a to present lipid to T cells, it requires the internalisation of CD1a, but not the lowering of pH in the intracellular compartments which is a feature of the late endosome. Kinetic studies also show that the CD1a-lipid complexes are detectable approximately twenty

minutes after antigen priming, which is not sufficient for antigen loading through late endosomes [239, 253]. The aforementioned research concluded that the antigen loading and presentation of CD1a occur on the cell surface or in the early endosomal compartments. Yet current knowledge of how CD1a is internalised into cells requires further studies.

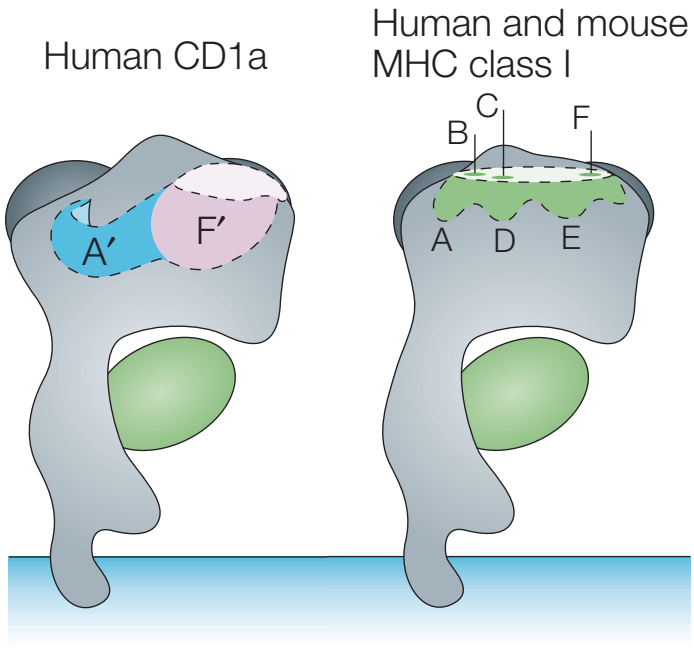


Figure 4-1. CD1a structure.

The grooves of CD1a are deeper than those of the MHC-I molecules. The antigen-binding groove of CD1a has two interconnecting pockets, with an A' pocket immersed inside and covered by a roof-like structure (A' roof), and an F' portal at the protein surface so that solvent or ligands can enter the CD1a grooves. Figure was modified and reprinted by permission from Springer Nature: Moody, D.B., D.M. Zajonc, and I.A. Wilson, *Anatomy of CD1-lipid antigen complexes.* Nat Rev Immunol, 2005. **5**(5): p. 387-99.

4.1.2.3 CD1a ligands

Studies have demonstrated that many CD1a ligands contain a hydrophilic headgroup made of polar or charged moieties with various sizes, and hydrophobic tails formed of one to three alkyl chains between C18 and C23 in length [254]. The structure of microbial glycolipids and mammalian-derived lipids is vastly diverse, yet studies have shown that human T cells can directly recognise surface CD1a molecules loaded with endogenous lipids, while can also identify exogenously acquired lipid antigens [255]. This indicates that CD1a plays a multifaceted role in maintaining physiological balance, repertoire selection, the pathology of autoimmune diseases, and host defence. Many lipid antigens have been identified, including lipopeptide, sulfolipid, glycolipid, lysophospholipid, phospholipid (PL), and glycophospholipid.

One of the first foreign lipids was identified in *Mycobacterium tuberculosis* (*M. tuberculosis*), a lipopeptide, called didehydroxymycobactin (DDM) [256, 257]. DDM is a precursor of the siderophore mycobactin, which helps mycobacteria to scavenge iron from the host cell and promote mycobacterial growth inside macrophages [258, 259]. DDM-specific CD1a activation of T cells induces IL-2 secretion, and the level of IL-2 reduces in response to different synthetic DDM analogues [260], which suggests that hosts are capable of distinguishing bacterial components responsible for virulence.

Urushiol, a poison-ivy-derived lipid, is one of the exogenous inducers of toxicodendron dermatitis, a T cell-mediated, delayed contact dermatitis. Kim and colleagues identified that unsaturated pentadecylcatechol (C15:2) is the responsible lipid antigen among urushiol analogues presented by CD1a [261]. Urushiol-CD1a-T

cell axis promotes Th17-mediated skin immune response, as CD1a-restricted memory T cells from the blood of individuals who had suffered moderate-to-severe poison-ivy rash have higher capacity of IL-17A and IL-22 production when stimulated with urushiol.

Agea and colleagues have reported that PLs originated from the pollen are another plant-derived CD1a ligand. PLs are composed of approximately 40% of the pollen membrane lipids and they are aligned in nonbilayer arrangements with their acyl side chains exposed for making contact with APCs [262]. CD1a-PL-restricted T cells exhibit some functions of regulatory T cells, as well as displaying both Th1 and Th2 cytokine profiles, plus the ability to promote IgE production by B cells.

Sulfatide, an endogenous glycolipid of the sphingolipid family, is a known CD1a self-ligand, and can be found in neuron, kidney and pancreas, and is inducible in bacterial infected DCs [263-266]. Interestingly, sulfatide can be presented by all group 1 CD1 [267, 268]. It has been shown that CD1a interaction with sulfatide is primarily restricted in the early endocytic network and this intracellular colocalisation is essential for the subsequent activation of CD1a-autoreactive T cells to produce IL-4, IFNγ, and tumour necrosis factor-α (TNFα) secretion [269]. Notably, as the production of sulfatide is highly linked with bacterial or viral infection caused tissue damage, this host reaction to self-lipid may contribute to the predisposition of individuals to autoimmune diseases [269, 270].

Another major group of endogenous CD1a antigen is skin oils. As CD1a is constitutively expressed at high levels on skin-resident LCs, this tissue-specific distribution suggests the involvement CD1a in skin physiology. De Jong and

colleagues discovered that lipid extracts prepared with chloroform and methanol from human epidermis can activate CD1a-restricted T cells [230]. Further analysis concludes that epidermis-enriched lipids, such as triacylglycerol, squalene, wax esters, lysophospholipids and fatty acids are all CD1a ligands [230]. Moreover, recent studies by the Ogg group define phospholipase-derived lipid products and their roles in skin inflammatory conditions. Phospholipase A2 (PLA2) are enzymes that are found to be highly expressed in allergens, such as bee/wasp venom and house dust mite (HDM) [271, 272], as well as in activated immune cells, including macrophages, neutrophils, DCs, mast cells and ILC2s [188, 273-277]. Both exogenous and endogenous PLA2 can generate small neo-antigens, such as free fatty acids and lysophopholipids from common phospholipids which are presented in abundance in cell membranes, as a rich source for autologous lipids. These PLA2-generated small neo-antigens can activate T cells through a CD1a-dependent manner in the skin inflammatory situation [188, 271, 272, 277]. In transgenic murine models for studying psoriatic and dermatitis skin inflammation, CD1a blockade led to the alleviation of the diseases, indicating therapeutic relevance of CD1a and skin-related oils [261].

4.1.2.4 CD1a ligand loading

Despite the narrowness of its binding groove, CD1a can harbour various chemically diverse lipids. For instance, DDM is composed of a single alkyl chain and a headgroup formed by amino acids and organic acids; sulfatide is composed of sulfated galactose headgroup, two alkyl chains, a sphingosine backbone and a fatty acid [254]; and squalene contains no headgroup [230]. The crystal structure of CD1a-

lipid complex revealed that, broadly, the flexible alkyl chains or the sphingosine backbone of the lipids can bend and sit inside of the A' pocket of CD1a antigen-binding groove, while the headgroup protrudes out of the F' pocket, allowing T cell receptor (TCR) recognition [246, 254]. Lipids without a polar head-group ("headless") can be buried almost completely inside of the CD1a, masked by the A' roof, and lack the protruding polar moieties to interact with the TCR, which granted them with the name of "permissive" lipids [230]. Birkinshaw and colleagues have investigated the crystal structures of TCR-CD1a-permissive lipid complexes, and revealed that direct contact of TCR to lipids is not required, as interaction between TCR and the A' roof of the CD1a is sufficient for T cell activation [278] (Fig. 4-2).

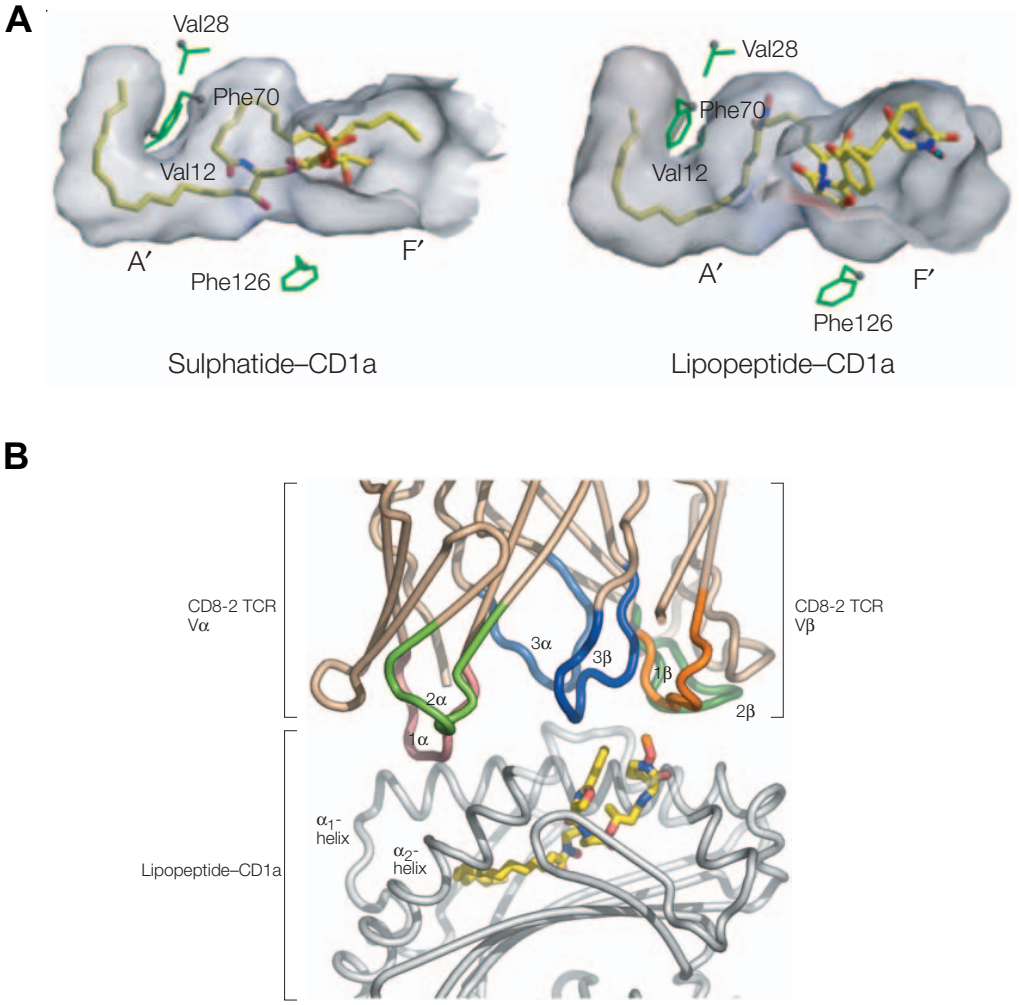


Figure 4-2. CD1a-lipid crystal structure.

(A)The crystal structure of CD1a-lipid complex showed that alkyl chains or the sphingosine backbone of the lipids can fit inside of the A' pocket of CD1a antigen-binding groove, while the headgroup remains out of the F' pocket, allowing TCR recognition (B). In addition, headless lipids can be buried deep inside of the CD1a. Figure was adapted and reprinted by permission from Springer Nature: Moody, D.B., D.M. Zajonc, and I.A. Wilson, *Anatomy of CD1-lipid antigen complexes.* Nat Rev Immunol, 2005. **5**(5): p. 387-99.

Interestingly, a crystal structure study of the CD1a-urushiol complex provides a distinct binding possibility of the antigen. It shows that urushiol can be non-covalently bound in the CD1a antigen-binding groove with its headgroup anchored deeply inside the A' pocket, and acyl chain positioned toward the F' pocket. The majority of urushiol is hidden underneath the A' roof; however, this reverse direction of the ligand positioning may provide a potential direct contact of TCR with the acyl chain exposed at the F' cleft [261].

Notably, CD1a molecules can exchange antigens at the cell surface. Manolova and colleagues have established that surface CD1a can unfold readily and become functionally inactive, yet remain expressed on the cell surface. Upon administration of exogenous lipids, the architectural stability and functionality of CD1a is restored by displacing with the new CD1a ligand, without *de novo* synthesis of CD1a [279, 280].

While some CD1a-antigenic lipids complexes engage the TCR to activate T cells, others are antagonistic to the TCR. These inhibitory lipids usually contain large carbohydrate or charged headgroups which interfere with the CD1a and TCR contact [278]. The polar headgroups of these lipids disrupt the interaction between amino acid residues of CD1a which causes the conformational readjustments of the A' roof, resulting some uncompensated charges of the amino residues. Together, the charges of the headgroups and the amino acid residues hamper binding to the hydrophobic site of TCR with CD1a. In addition, in some cases, the length of the acyl chain causes the polar headgroup to extend further out of the F' pocket of CD1a, which subsequently alters the structural properties of the A' roof and impede TCR engagement of CD1a [278].

Studies on other CD1 isoforms suggested that the possibility of loading multiple lipid ligands on a single CD1 molecules [281]. The first small lipid immerses deeply in the binding groove, and a second lipid then fit into the remaining space with its headgroup overhang from the surface of CD1 to interact with TCR. However, this is less likely to be a mechanism applicable for CD1a since it has a limited volume of antigen binding. Several reports on other CD1 isoform have proposed that the modification of the fatty acid tails of the lipid can revise the CD1 antigen presentation capacity [282]. This, most likely, is due to fitting the structurally modified fatty acid tails into the CD1 binding groove causing the conformational changes of the CD1 molecules or the positional alteration of the polar headgroup.

4.1.3 CD1a-autoreactive IL-22 producing T cells

CD1a-restricted T cell repertoire contains T cells that express diverse TCR $\alpha\beta$ chains (and possibly some $\gamma\delta$ chains), and can recognise self and foreign lipid antigens, and have both pathological and protective roles. As mentioned in the previous sections, mycobacterial and plant-derived lipid antigens have been identified as CD1a ligands; but many CD1a-restricted T cells are considered autoreactive [283-285], and are presented at a higher frequency in the blood of healthy individuals than other CD1 isoform-restricted T cells [283]. These CD1a-autoreactive T cells express skin homing receptors, including CCR4, CCR6, CCR10 and CLA, suggesting a potential recruitment into the skin, where they encounter CD1a-expressing cells [283]. Comparing the frequency of CD1a-reactive T cells from umbilical cord blood and adult blood reveal that CD1a-autoreactive T cells accumulate in the memory compartment

through life [284]. A phenomenon of dual recognition has been reported for these CD1a-autoreactive TCRs: they are able to interact with CD1a molecules loaded with self-lipids as well as microbial lipids, with self-antigens priming a weaker T cell response than foreign antigens [285] which may be essential for establishing immune tolerance to self-antigens.

When activated by self-antigens, these T cells have the ability to secrete a broad spectrum of cytokines, including IL-2, IFN-γ, TNF-α, IL-13, IL-22, and IL-17A [283]. De Jong et al. [283] demonstrated the presence of IL-22-producing CD1a-restricted T cells in the skin, suggesting IL-22 is a key effector in maintaining skin homeostasis. During the reconstruction of the skin barrier, IL-22 acts on keratinocytes, which leads to up-regulation of the expression of proinflammatory genes, promoting the production of antimicrobial peptides, and enhancing re-epithelialisation [286-289]. In patients who suffer from chronic diabetic ulcers, the wounds can fail to re-epithelialise, but after supplying IL-22, the altered behaviour of these keratinocytes is recovered [290]. In addition, IL-22 also contributes to the immune dysregulation of inflammatory diseases, such as AD [291, 292] and psoriasis [287]. In psoriatic skin, excess IL-22 production induces hyperplasia and inhibits keratinocyte differentiation, resulting in skin thickening and formation of psoriatic plaques.

4.1.5 Aims

Despite the circumstantial evidence supporting a role for pDC in contributing to human skin integrity, the underlying mechanisms remain ill-defined in humans. Here, I aim to test the hypothesis that pDCs contribute to the wound healing process in humans. Specifically, I will dissect the longitudinal presence and phenotype of pDC during human skin wounds. I will also investigate the functional capacity of skin wound pDC to amplify an adaptive type 22 immune response. The study will help establish a novel role of pDCs in sensing wounded skin and could be a potential therapeutic target in inflammation and wound repair.

4.2 Results

4.2.1 Two distinct subsets of BDCA-2-expressing DCs rapidly infiltrate skin wounds.

PDCs, characterised as $CD123^{+}BDCA\text{-}2^{+}$ leukocytes, have been observed to accumulate in skin wounds, but are rarely observed in normal skin. Indeed, it was confirmed that healthy human skin had undetectable pDCs, although $CD11c^{+}HLA\text{-}DR^{+}BDCA\text{-}2^{-}$ cDCs were present (Fig. 4-3A, 0% $BDCA\text{-}2^{+}$ pDCs; 3.413±0.6133% cDCs). Human skin suction blisters were used as an *in vivo* human skin traumatic wound model [272, 293]. Cells isolated from the blisters do not require processing or prolonged enzymatic treatments required by conventional skin biopsies and so while being labor-intensive, do offer the ability to capture tissue cells directly *ex vivo*. In contrast to healthy skin, detectable levels of $BDCA2^{+}$ pDCs and cDCs were found in the skin blister fluid taken from 3 healthy donors (Fig. 4-3B, 13.23±8.583% $BDCA\text{-}2^{+}$ pDCs; 10.76±4.557% cDCs).

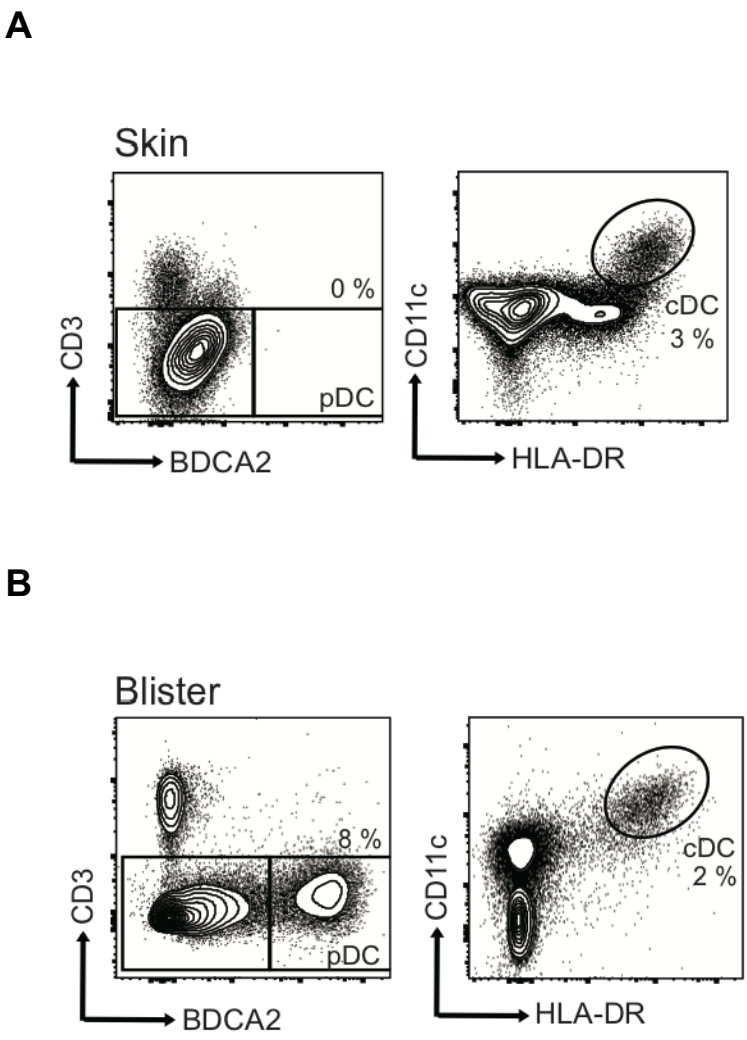


Figure 4-3. Skin-infiltrating pDCs can be found in skin suction blisters.

(A) Mononuclear cells isolated from normal skin were analysed for DC subsets by flow cytometry. Representative of three experiments. (B) Mononuclear cells isolated from skin suction blisters were analysed for DC subsets by flow cytometry. Representative of three experiments.

In order to determine whether there was evidence of an IFN-I response in the skin wounds, bulk RNA-sequence analysis was undertaken on $CD45^{+}IL7R\alpha^{-}CRTH2^{+}$ myeloid cells and T cells isolated directly ex vivo from the skin blisters. This showed a significant increase in ISGs, such as *IFITM1*, *IFITM2*, *IFIT3*, *MX1*, and *OAS2* (Fig. 4-4), confirming that IFN-I was present at functionally relevant levels.

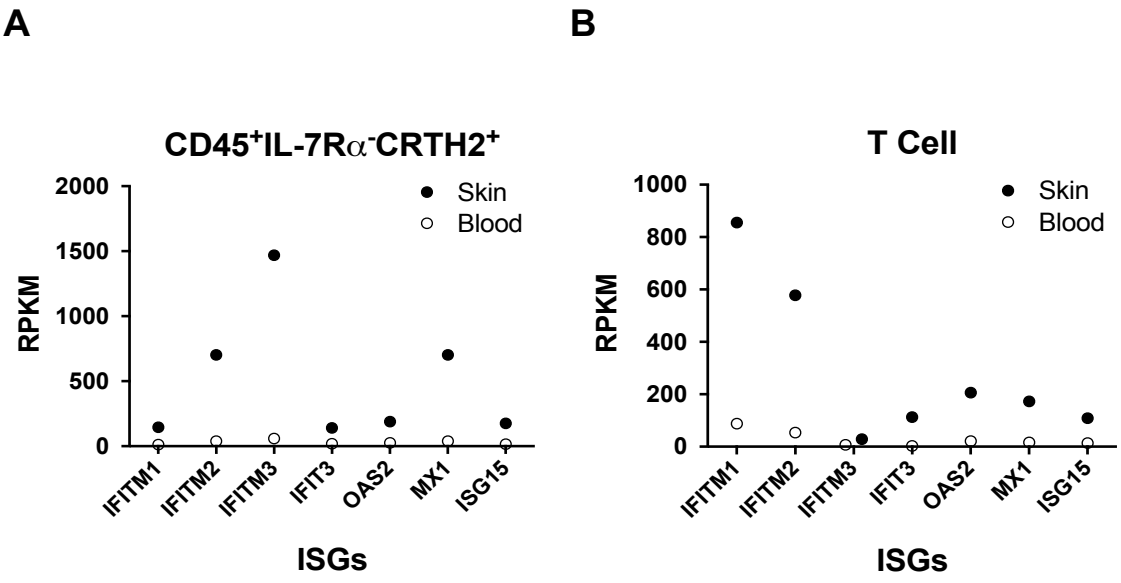


Figure 4-4. Upregulated ISGs can be detected in skin-infiltrating cells.

Upregulated ISGs can be detected in skin-infiltrating cells. (A) $CD45^{+}IL7R\alpha^{-}CRTH2^{+}$ myeloid cells and (B) T cells were isolated from skin suction blisters or blood, and subjected to RNA-sequencing analysis. Reads of skin-infiltrated cells were compared with those of blood counterparts. RPKM as Reads Per Kilobase of transcript per Million mapped reads. A single donor was recruited for this data set.

In the work of Mathan *et al* [294], exposure of pDCs to IL-3, produced by activated T cells and epithelial cells, resulted in the increased expression of CCR10 and induced pDC recruitment to inflamed murine skin lesion sites. Furthermore, IL-3 has also been shown to be an essential cytokine involved in pDCs survival and activation, with a high density of IL-3R on the surface [295]. Therefore, concentrations of the CCR10 ligand, cutaneous T-cell-attracting chemokine (CTACK), and IL-3 were measured in the skin blister fluid isolated from healthy donors, compared to their levels in the serum. Both CTACK and IL-3 were significantly upregulated in the interstitial blister fluid, potentially contributing to pDC recruitment to the skin wound site (Fig. 4-5). Overall, the aformentioned data showed that pDCs infiltrate into skin wounds where there are high levels of CTACK and IL-3, and responding cells show evidence of IFN-I stimulation.

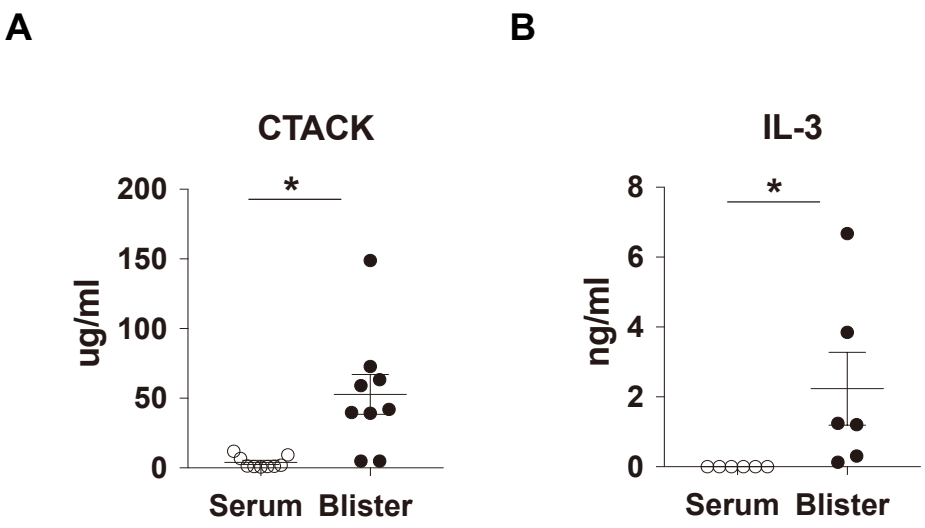


Figure 4-5. Detection of pDC-relevant cytokines in the blister fluid.

Concentration of (A) CTACK and (B) IL-3 in the blister fluid or serum was evaluated by multiplex bead array. Each dot represents individual donor. *P < 0.05, Paired student's t test.

However, on further analysis of the skin-infiltrating BDCA2^{+} DC population, two distinct subsets were observed: CD123^{hi} and CD123^{int} populations (Fig. 4-6). Unexpectedly, it was observed that the CD123^{int} DC subset had high levels of surface CD1a expression which hitherto has been shown to be restricted to cDC populations, LCs, innate lymphoid cells and thymocytes.

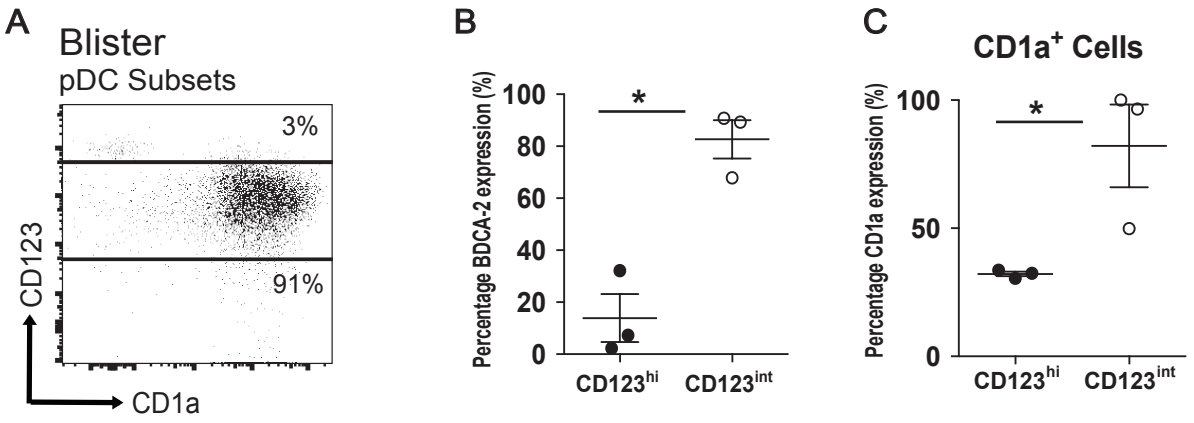


Figure 4-6. BDCA-2^{+}CD123^{int} DC express CD1a

(A) CD123 and CD1a expression on skin suction blister BDCA-2^{+} DCs were analysed by flow cytometry. Representative of three experiments. (B) Percentages of CD123^{hi} and CD123^{int} subpopulations of the BDCA2^{+} cells in skin blisters. (C) Percentage of CD1a-positive cells in either CD123^{hi} or CD123^{int} DCs from blisters. Lines represent the mean$\pm$SEM. *P < 0.05, Paired student's t test.

In terms of co-stimulatory marker expression of both subsets, $CD123^{int}CD1a^{+}$ DCs expressed higher CD86 and HLA-DR levels, in comparison to $CD123^{hi}CD1a^{-}$ DCs (Fig. 4-7). In all, the newly identified $BDCA\text{-}2^{+}CD123^{int}$ $CD1a^{+}$ DCs in the skin wound showed a more active phenotype and expressed molecules associated with antigen presentation. However, while offering *ex vivo* analysis, skin suction blisters represent a specific form of wounding associated with dermo-epidermal separation. Therefore, complementary approaches were used to further explore the observations and underlying mechanisms.

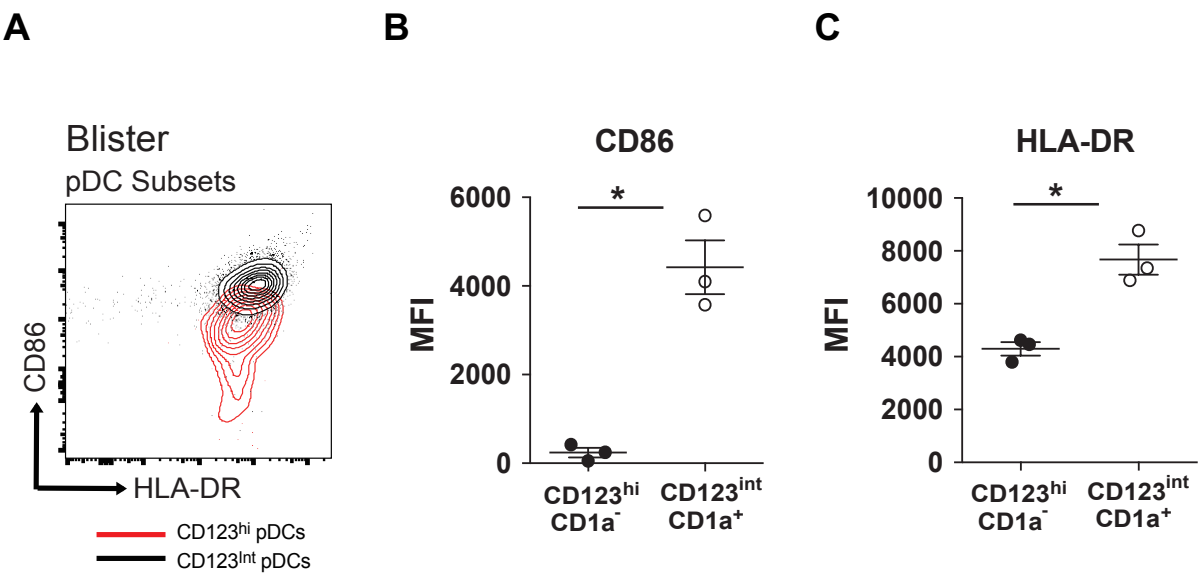


Figure 4-7. $BDCA\text{-}2^{+}CD123^{int}$ DC express higher co-stimulatory markers.

(A) Flow cytometry of CD86 and HLA-DR expression $CD123^{hi}$ and $CD123^{int}$ subpopulations from skin suction blisters. Representative of three experiments. Mean fluorescence intensity of (B) CD86 and (C) HLA-DR expression on $CD123^{hi}$ $CD1a^{-}$ and $CD123^{int}$ $CD1a^{+}$ pDCs from skin suction blister. Lines represent the mean±SEM. *P < 0.05, Paired student's t test.

Due to the limited cell numbers in the blisters, the specificities of each antibody were tested later using the Fluorescence Minus One (FMO) control on *ex vivo* blood pDCs or cDCs. First, APC anti-CD1a-APC was tested. To do so, blood $CD1c^+$ cDCs were isolated and cultured in FCS-containing medium for 48 hours and checked for their CD1a expression (Fig. 4-8). PE anti-BDCA-2, PE-Cy7 anti-CD123, PE/Dazzle 594 anti-CD86, and BV785-HLA-DR were next tested (Fig. 4-9). Collectively, FMO controls showed a low background in each channel, while cells stained with specific antibodies resulted in a distinct shift, suggesting all the antibodies have satisfactory performance (Fig. 4-8 and 9). Skin mononuclear cells were analysed for LCs and their CD1a and HLA-DR expression to ensure the antibodies work well in staining skin cells. LCs were defined as $CD45^+Langerin^+HLA\text{-}DR^+$ cells, and they all expressed a high level of CD1a (Fig 4-10).

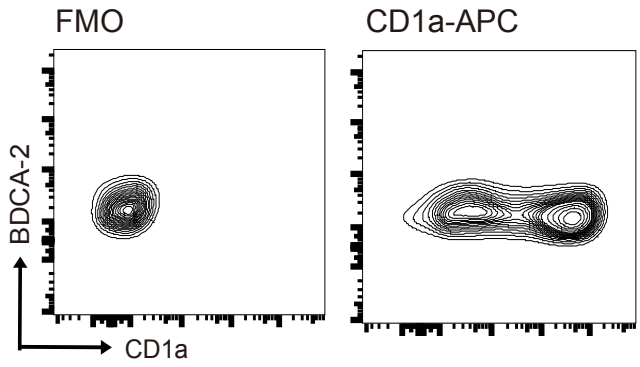


Figure 4-8. FMO control using CD1a-expressing $CD1c^+BDCA\text{-}2^-$ cDCs.

Flow cytometry of CD1a expression of $CD1c^+$ cDCs from blood. FMO control was stained with the same antibody mix except for CD1a.

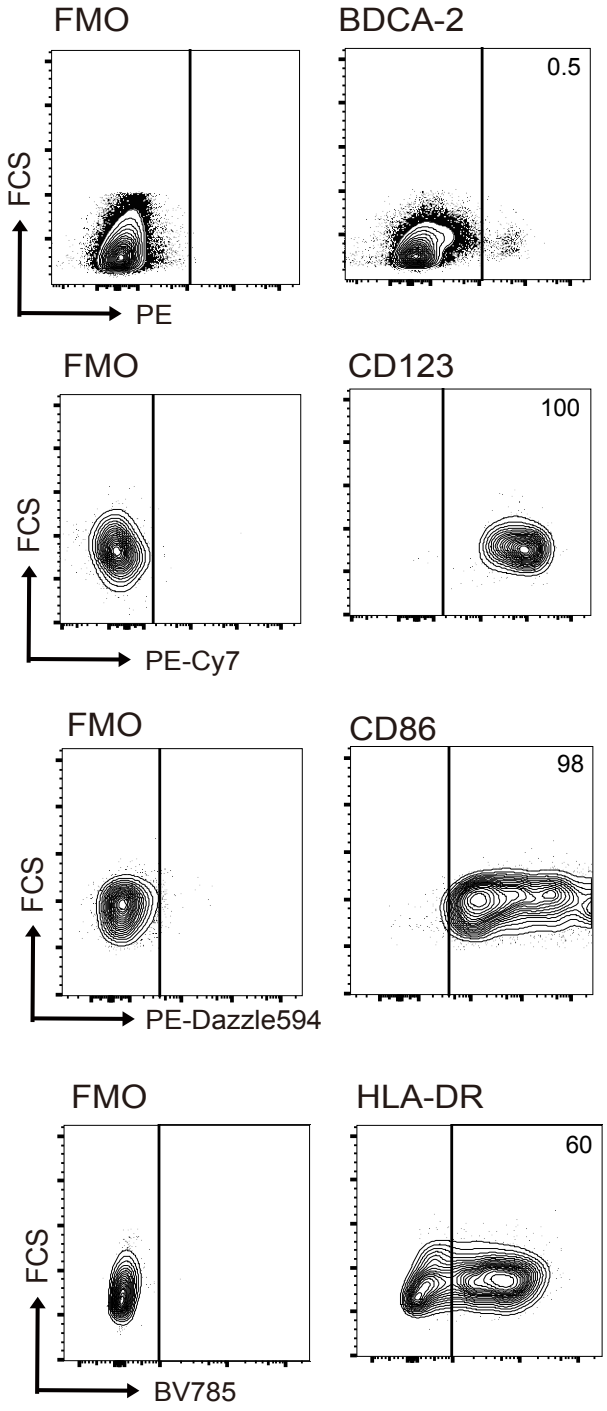


Figure 4-9. FMO controls of pDC specific markers.

Flow cytometry of BDCA-2, CD123, CD86, and HLA-DR expression of blood pDCs. FMO controls were stained with the same antibody mix except for indicated markers.

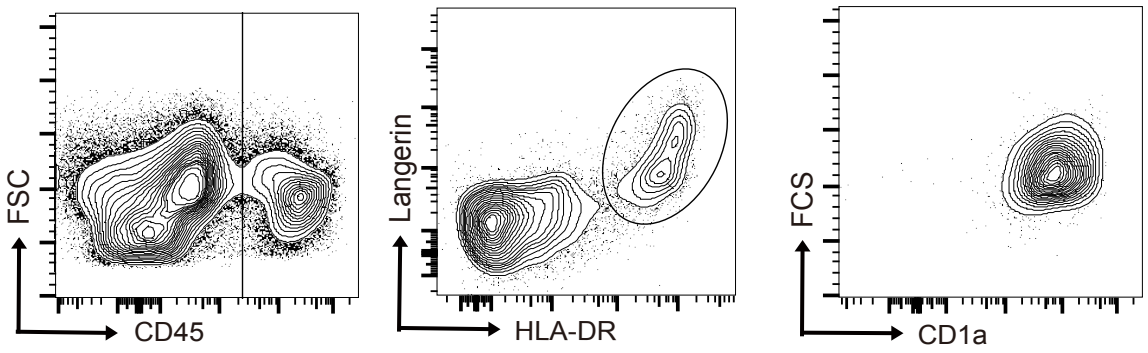


Figure 4-10. LCs expressed CD1a.

Mononuclear cells isolated from normal skin were analysed for LC subsets by flow cytometry. Analysis of CD1a expression on $CD45^{+}Langerin^{+}HLA\text{-}DR^{+}$ LCs from skin. Representative of three experiments.

4.2.2 CD1a positive, BDCA-2 expressing DCs accumulated near skin wound sites.

In order to investigate the architectural response of BDCA-2$^+$CD1a$^+$ DC to wounding over time, the longitudinal frequency and distribution of the population after skin wounding were investigated. Twelve healthy adult donors were recruited and received bilateral 3 mm baseline full thickness punch biopsies on the flank (UV-protected) to create skin wounds. This was followed by larger biopsies (6mm) to encompass the bilateral original wounds taken between one to four days later. The frozen tissue sections were cut, and stained with anti-CD1a, anti-BDCA-2, and DAPI. In pre-wound skin, CD1a$^+$ cells can be found aligning in the epidermis where the CD1a-expressing LCs were enriched. As predicted, there were no BDCA-2$^+$ cells in baseline skin samples, which further confirmed that, in steady-state conditions, pDCs were not a significant population in human skin (Fig. 4-11A). One-day post wounding, an accumulation of BDCA-2$^+$CD1a$^+$ cells was detected in the dermis along the wound margin (Fig. 4-11B). The abundant amounts of BDCA-2$^+$CD1a$^+$ DCs infiltrating into the skin wounds was sustained up to 48 hours and then reduced (Fig. 4-11C). The results provided supporting evidence that BDCA-2$^+$CD1a$^+$ DCs may contribute to the early inflammatory response during skin wound healing.

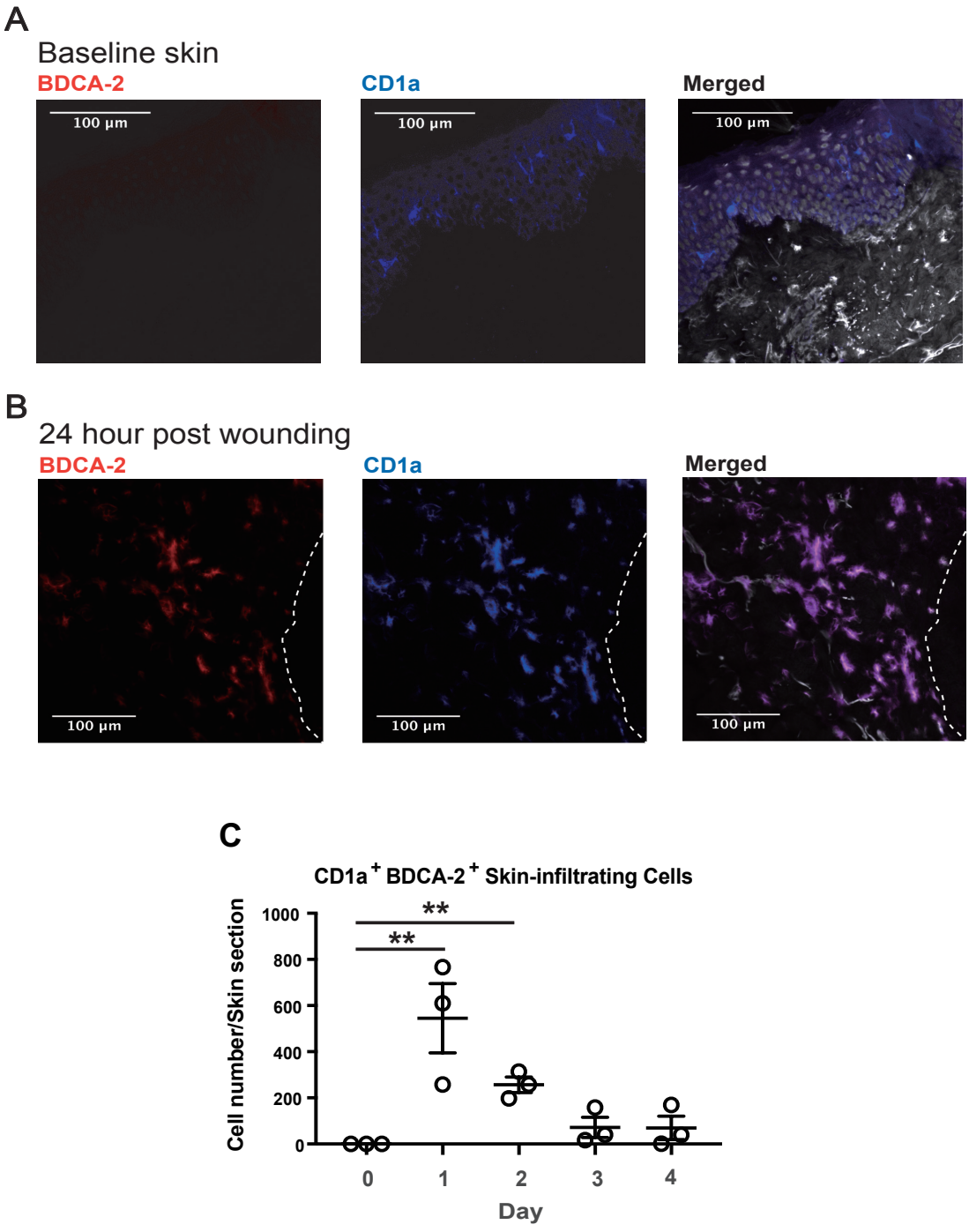
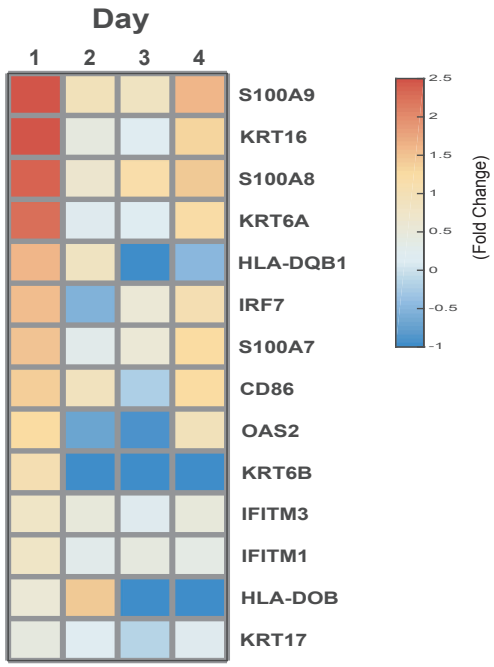


Figure 4-11. CD1a-positive pDCs accumulated near skin wound sites.

(A-B) Immuno-histology of skin biopsies collected at day 0 to day 4 after bilateral skin wounds on the flank from healthy volunteers. DAPI (Grey), $CD1a^+$ (Blue), $BDCA\text{-}2^+$ (Red), and $CD1a^+BDCA\text{-}2^+$ cells (purple). Dashed white lines represent the edge of the wound. One representative result of three is shown. (C) Frequency of $CD1a^+BDCA\text{-}2^+$ DC per section. $^{**}P < 0.01$; one-way Anova; Tukey's multiple comparisons post hoc test was performed.

Targeted RNA sequencing analysis was performed on the skin biopsies to determine wound-related gene profiles. Fold changes of gene expression from each day compared to wounding at day 0 are shown in figure 4-12. Here, high induction of keratin proteins *KRT6*, *KRT16*, and *KRT17*, and antimicrobial peptides *S100A7*, *S100A8*, and *S100A9* in early skin wounds were detected. Activated keratinocytes in skin wounds have been reported to express these keratin proteins and antimicrobial peptides [287, 296, 297], which are involved in the re-epithelialisation stage of the wound healing process. Moreover, several ISGs, such as *OAS2*, *IFITM3* and *IFITM1*, and co-stimulatory markers, including *HLA-DQB*, *HLA-DOB* and *CD86*, were upregulated one-day post wounding, suggesting the potential involvement of IFN-I and DCs in the early stage of the skin wound healing process.



Data analysed together with Nicki Gray, CBRG, Weatherall Institute of Molecular Medicine, Oxford, UK

Figure generated together with David A. Duncan, Diamond Light Source, Didcot, UK.

Figure 4-12. Wound-related gene profile in the skin biopsies.

Targeted RNA sequencing analysis was performed on the skin biopsies collected at day 0 to day 4 after skin wounds. Heat map coloured by log fold-change values for genes on each day post wounding. Due to limited amount mRNA extracted from the skin of donors, only a single donor per day was incorporated into the figure.

4.2.3 Induction of CD1a by pDCs in vitro

Next, it was investigated whether the CD1a-expressing BDCA-2^{+}CD123^{int} DC represented a distinct lineage or whether they acquired their phenotype in response to skin-derived environmental cues. Blood pDCs, identified as BDCA-2^{+}CD123^{hi}, were incubated with IL-3 which had been observed to be present within the skin blister fluid. The untreated pDCs in the blood were predominantly the BDCA-2^{+}CD123^{hi} subpopulation with low levels of CD1a on the surface (Fig. 4-13A). However, after 48 hours of culture, both BDCA-2^{+}CD123^{hi} and BDCA-2^{+}CD123^{int} populations were identified and were capable of expressing CD1a on their surface, with CD123^{int} cells having significantly higher CD1a density (Fig 4-13B-C). Freshly isolated blood pDCs were largely CD86^{dim}, and HLA-DR^{int}; however, after culture, both CD123^{hi} and CD123^{int} subsets expressed elevated levels of co-stimulatory markers (Fig. 4-14A-B). There was no significant difference in the expression level of CD86 between CD123^{hi} and CD123^{int} subsets, and a minor decrease in HLA-DR mean fluorescence intensity (MFI) in the CD123^{int} population was observed (Fig. 4-14B). Meanwhile, blood and skin cDCs were isolated and treated with IL-3 *in vitro* for 48 hours, and upregulated CD123 expression was observed; however, neither population expressed BDCA-2 after culture (Fig. 4-15).

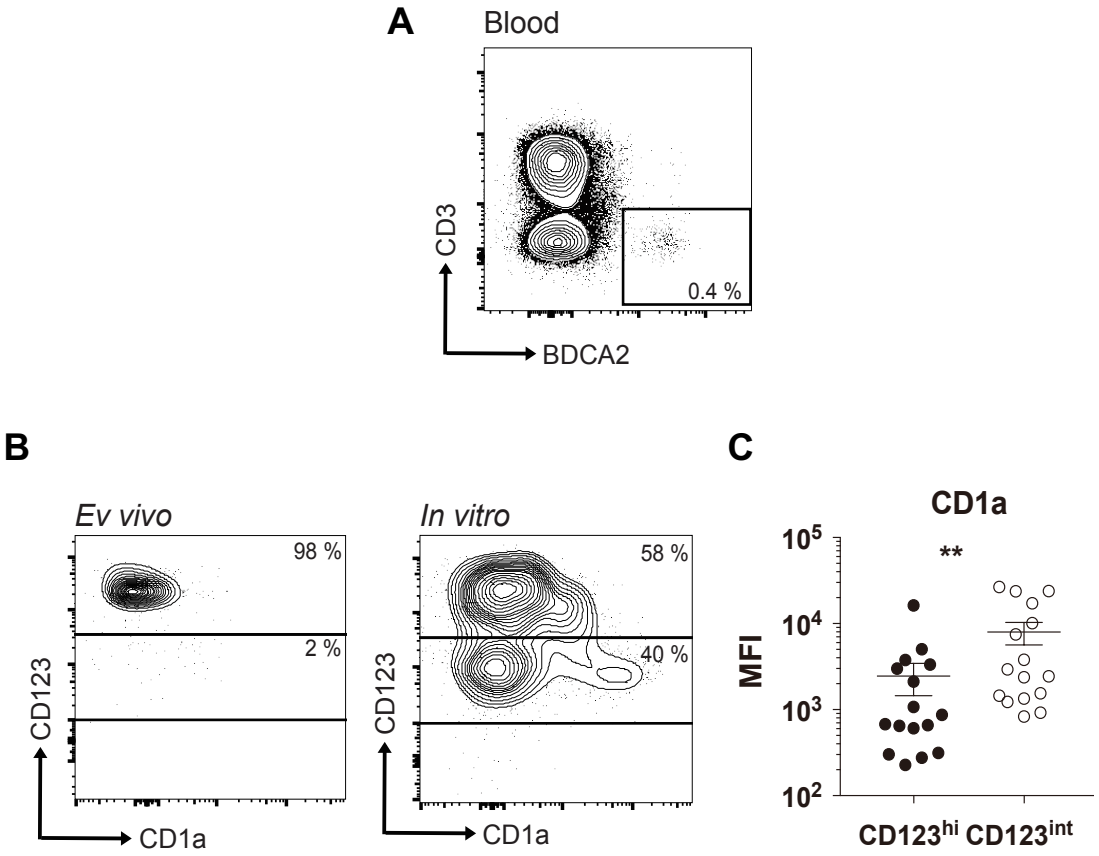


Figure 4-13. CD1a can be induced on blood pDC subsets.

(A) Peripheral blood mononuclear cells (PBMC) were analysed for pDC by flow cytometry. (B) Expression of CD123 and CD1a on freshly isolated pDCs (*ex vivo*) or blood pDCs treated with IL-3 (50 ng/ml) for 48 hours (*in vitro*). One representative result of sixteen donors is shown. (C) CD1a expression level on $CD123^{hi}$ and $CD123^{int}$ pDCs after *in vitro* culture. Lines represent the mean±SEM. **P < 0.01, Paired student's t test.

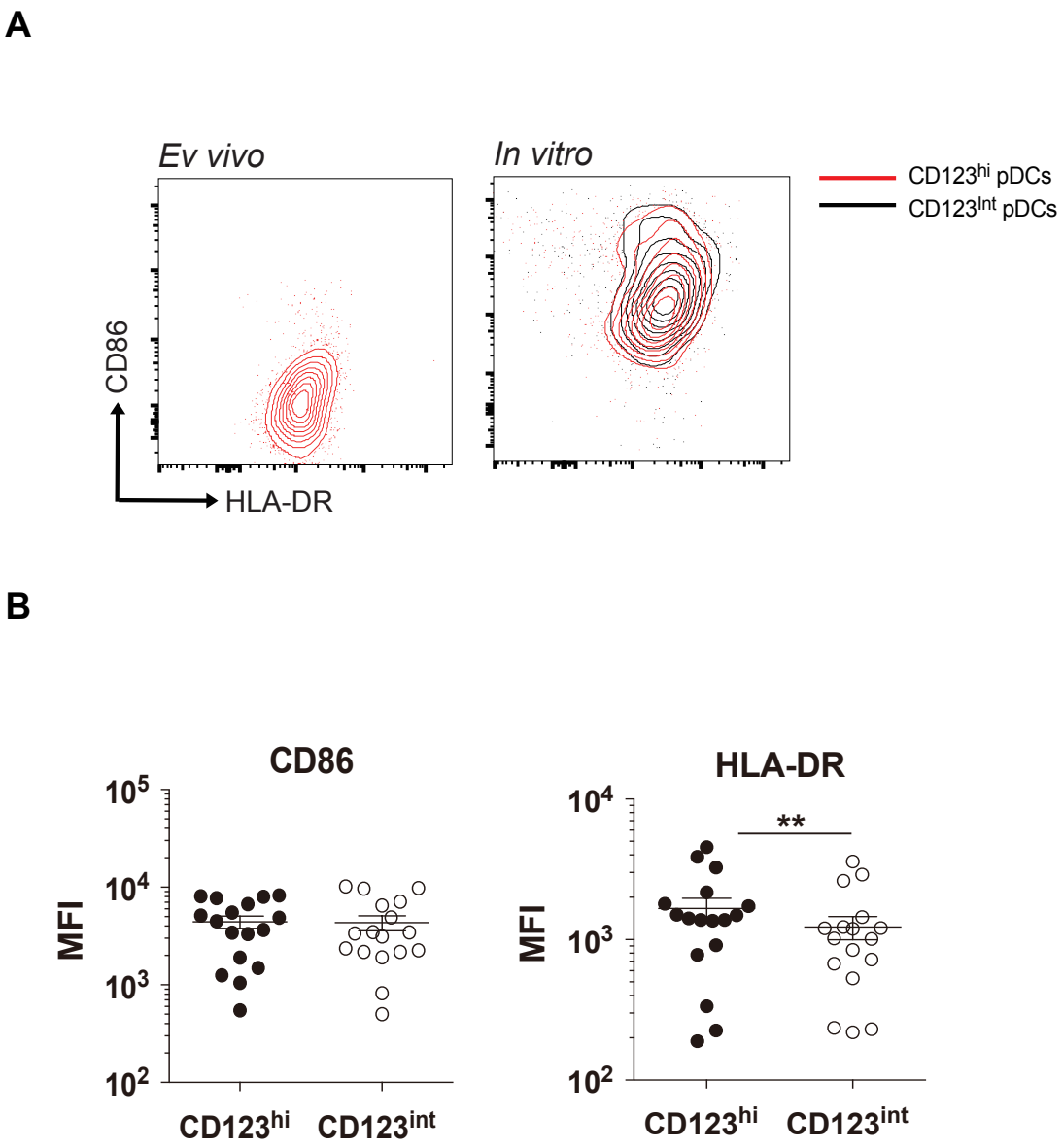


Figure 4-14. Co-stimulatory markers on blood pDC subsets.

(A) CD86 and HLA-DR expression on *in vitro* pDCs analysed by flow cytometry. One representative result of seventeen donors is shown. (B) CD86 and HLA-DR expression level on CD123^{hi} and CD123^{int} pDCs after *in vitro* culture with IL-3. Lines represent the mean±SEM. **P < 0.01, Paired student's t test.

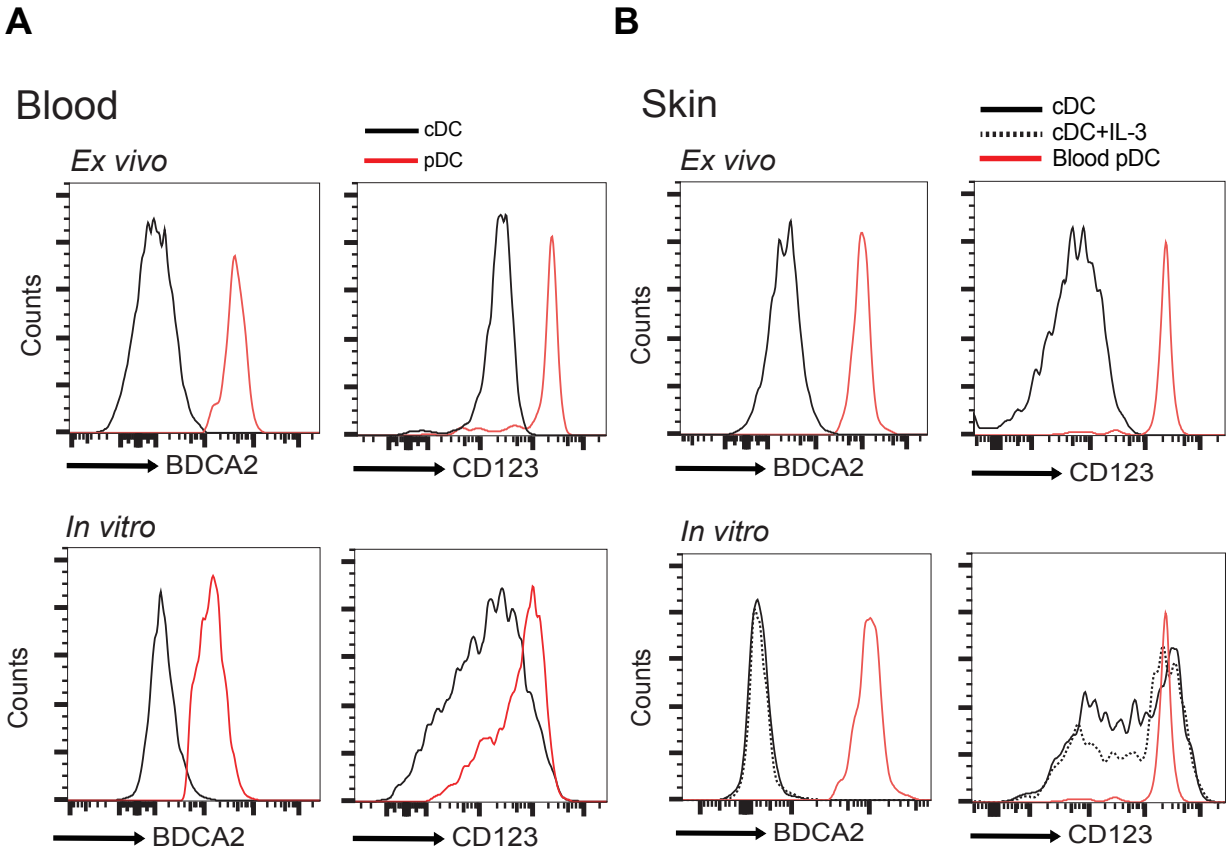


Figure 4-15. Expression levels of BDCA-2 and CD123 on cDC in skin and blood.

BDCA-2 and CD123 expression was analysed by flow cytometry on (A) *ex vivo* and *in vitro* IL-3-cultured blood cDCs and blood pDCs, and (B) *ex vivo* and *in vitro* IL-3-cultured skin cDCs and blood pDCs. One representative result of three donors is shown.

In vitro cultured blood pDCs and cDCs were stimulated with Imiquimod (TLR7 agonist) for 12 hours, and similar levels of IFNα production were detected from both BDCA-2^{+}CD123^{hi} and BDCA-2^{+}CD123^{int} subsets, in contrast to undetectable IFNα production by *in vitro* cultured cDCs (Fig. 4-16). The data are consistent with recruitment of blood pDC being a predominant source of skin BDCA-2^{+}CD123^{int} DC.

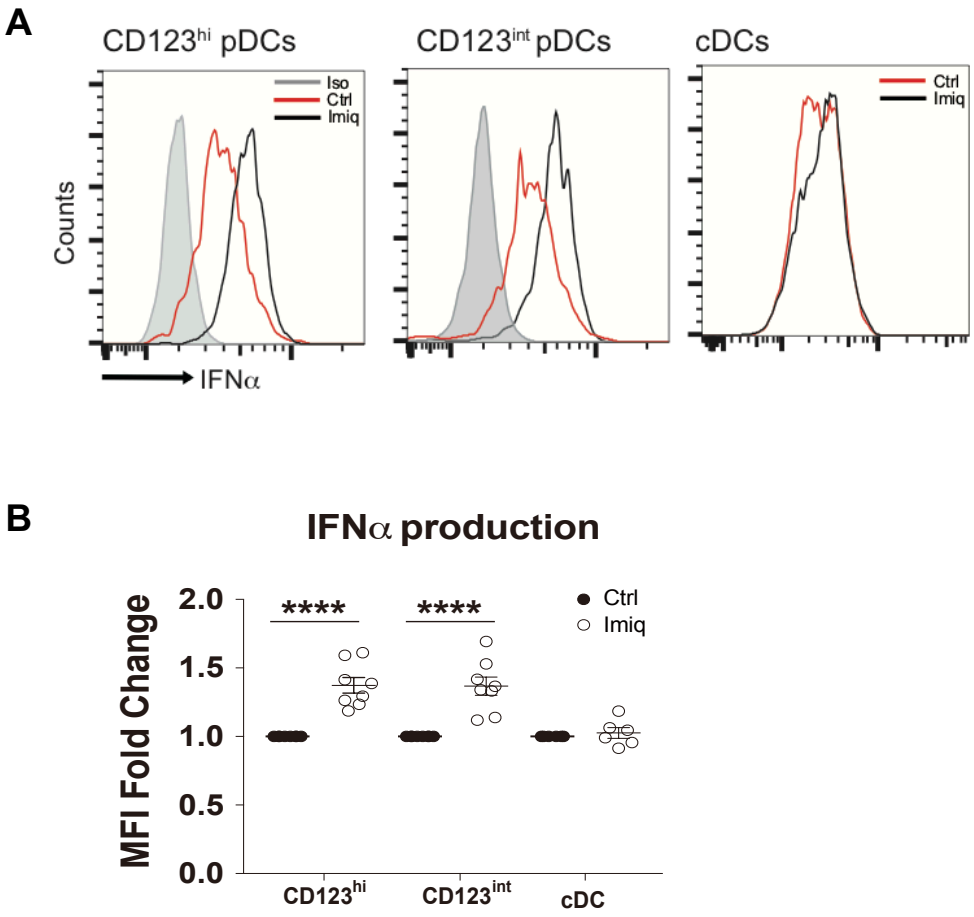


Figure 4-16. Both blood pDC subsets can produce IFN-I when stimulated.

(A) Expression of IFNα production by each pDC subset and cDC analysed by flow cytometry. (B) MFI fold-change of IFNα by CD123^{hi} and CD123^{int} pDCs and cDCs after *in vitro* Imiquimod stimulation. Representative of eight donors. Lines represent the mean±SEM. ****P < 0.0001, one-way Anova; Tukey's multiple comparisons post hoc test was performed.

4.2.4 pDCs present autologous antigens to autoreactive CD1a-reactive T cells.

It has been reported that the skin infiltrating pDCs trigger and perpetuate the activation of autoimmune T cells to secrete Th1 cytokines and contribute to epidermal hyperproliferation through secreting IFN-I in mouse models [97]. However, it remains unclear if pDCs and T cells could interact via CD1a antigen presentation. Blood polyclonal T cells from healthy individuals were incubated with *in vitro* cultured autologous $CD1a^+$ pDCs for 24 hours. IL-22 and IFNγ cytokine production was detected in the culture, even after blocking human MHC-I and II signalling. Neutralising CD1a signalling with anti-CD1a antibodies attenuated IL-22 secretion from T cells, but not IFNγ (Fig. 4-17), suggesting the dependence on CD1a for IL-22 secretion. In summary, these results show that CD1a expression by pDCs was functionally active and could present endogenous lipid antigens to CD1a-reactive IL-22-producing T cells.

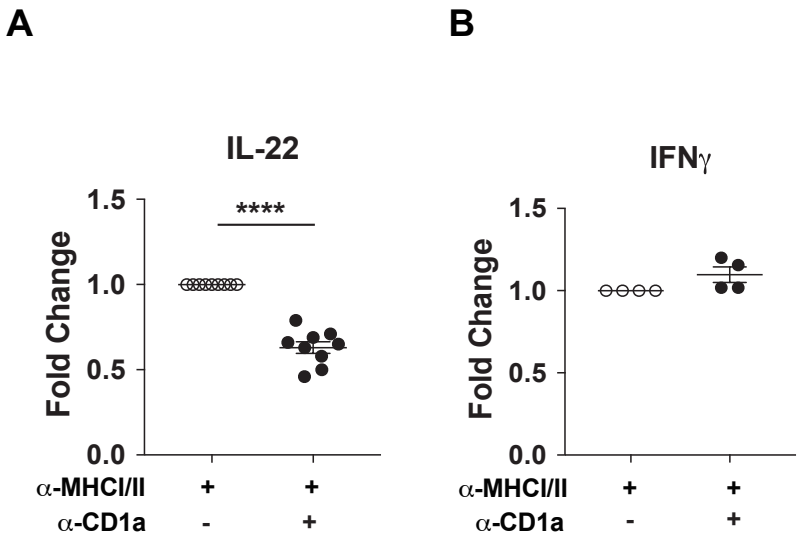


Figure 4-17. CD1a induced on blood pDC subsets is functionally active.

(A) IL-22 and (B) IFNγ production from blood T cells co-cultured overnight with CD1a positive blood pDCs. Cytokines were measured by enzyme-linked immunospot (ELISpot) in the absence or presence of anti-CD1a antibody and anti-MHC I/II antibodies. Data represent the fold reduction. Lines represent the mean±SEM. ****P < 0.0001, Paired student's t test.

4.3 Discussion

Here, a skin pDC-like CD123^{int} DC subset was identified that associates with functional specialisation during wound healing, and could be derived from blood pDCs. These CD1a-bearing DCs infiltrated early after wounding and were able to present lipid antigen to T cells and induce IL-22 production. The expression of CD1a by these pDC-like subsets in the skin prompts a re-evaluation of cells previously considered to be dermal DCs or LCs in skin pathology, including psoriasis and AD.

LCs are enriched in atopic skin lesions [272], and express langerin (CD207), a C-type lectin, which supports lipid loading of CD1a [298]. It is of interest that CD207 polymorphisms are strongly associated with AD [299]. Other CD1a^{+} dermal DCs exist in both steady-state skin as well as in inflammatory skin conditions consistent with a functional role for CD1a in skin [300-303]. Angel and colleagues previously identified that in healthy human skin, CD1a^{+} dermal APCs localised closely to lymphatic vessels with chemotactic abilities [300]. Kissenpfennig and colleagues used a model of dermatitis to show that dermal DCs arrived in cutaneous lymph nodes earlier than LCs [304]. Klechevsky et al. reported that there are functional differences between human LCs and CD1a^{+} dermal APCs in cytokine production and inducing naive T cell responses [301]. In addition, a subset of CD123^{+}CD5^{+}CD1a^{dim} DCs has been defined in both healthy and psoriatic skin, that can stimulate T cells to produce IL-22 [305]. Lastly, Wollenberg et al. have detected a CD1a-expressing DC population lacking LC signature features in the lesional skin of AD patients [27]. These reports indicate the importance of having multiple CD1a-expressing cell types during skin inflammation. The robust infiltration, rapid turnover rate, and specialised features of skin CD123^{int}

DCs defined herein, which are not found in healthy skin, suggest that they also contribute to the skin immune network and could potentially have a broad role in disease pathology.

CD1a antigen presentation to T cells has been demonstrated by LC, monocyte-derived DC and innate lymphoid cells, and shown to contribute to the inflammation of AD, venom allergy, contact dermatitis and psoriasis [188, 261, 271, 272, 277]. Increasing evidence indicates that pDCs contribute to skin wound healing; nonetheless, the underlying mechanisms remain largely unstudied. Thus far, there have been no studies to address the potential ability of pDC to express CD1a. In a recent study [188], Hardman et al. described the upregulation of CD1a expression on ILC2 in the skin, which supports the existence of a CD1a-inducible event given appropriate stimuli in the skin. It is not clear whether the CD1a-expressing $CD123^{int}$ DCs represent a distinct subset, or whether CD1a can be induced on all pDCs given a suitable tissue-specific environment. Nevertheless, the ability to induce CD1a expression on peripheral blood-derived pDCs, cultured *in vitro*, suggests that the environmental milieu plays a key role.

To maintain the acidity of the skin stratum corneum layer of epidermis, free fatty acids are continuously generated from commensal bacterial metabolites, human sebum, and phospholipid degradation. These free fatty acids, along with wax esters, triglycerides, TAG, and squalene, are all components of human sebum and have been identified to be CD1a ligands. They normally accumulate extracellularly in the outer layer of epidermis [230, 306], and, in steady state conditions, the CD1a-bearing cells residing in the suprabasal spinous layer of epidermis have limited accessibility to skin

lipids in the outer stratum corneum. However, in the event of skin trauma, these skin oils act as natural autoantigens after being transferred deep into the epidermis where they encounter $CD1a^{+}$ antigen presenting cells. The results provide a new perspective, namely the transient and robust infiltration of $CD123^{int}$ DC subsets during the beginning of a skin breach event which contribute to the CD1a-autoreactive T cell response to skin lipids, helping to initiate the wound healing process.

5 Transcriptomic Profiling of Skin-infiltrating DC Subsets

5.1 Introduction and aims

5.1.1 cDC subsets in the blood and skin

DCs are heterogeneous and multiple subsets have been defined in human blood [64, 68, 307]. Despite sharing some surface marker features and transcription factors (TFs), subsets are likely to be functionally specialised [28, 32]. Human blood DCs express CD11c and HLA-DR and can be subclassified based on their morphology, physiological properties, markers, and developmental origins [308-310]. Villani and colleagues used scRNA-seq to examine the heterogeneity of blood DCs and monocytes [68], identifying new subtypes among these cells, and further refining their classification. Six DC populations are now identified: BDCA-3^{+} cDCs correspond to DC1; CD1c^{+} cDCs are subdivided into DC2 and DC3; BDCA-3^{-}CD1c^{-} cDCs, which shared monocytes gene signature, correlate as DC4; a newly identified DC subset, AS DCs, are called DC5; and pDCs correspond to DC6. Four monocyte subsets are described: CD14^{+}CD16^{-} Mono1, CD14^{+}CD16^{++} Mono2, and two minor monocyte subtypes, Mono3 and Mono4. These cells have all shown distinct, yet interchangeable functions in providing host defence against pathogens or allergen, as well as shaping host tolerance.

In steady state skin, several subtypes of DCs are identified, including LCs and dermal DCs. LCs reside in epidermis where they regularly elongate and retract their dendrites to sense environmental stimuli [311]. When required, they can be self-

replenished locally or repopulated from blood progenitors [26, 312-314]. When activated, they upregulate MHC-II and co-stimulatory markers, and migrate toward T cell areas of lymph nodes [315-317]. Functionally, LCs are able to cross-present antigens to $CD8^+$ T cells, and expand regulatory T cell (Treg) populations by secreting IL-15 and IL-10 or expressing specific surface markers [318-324]. Human LCs can also initiate Th1, Th2, Th22 and Th17 responses [283, 324-327]. In addition, CD1a are continuously express at high frequency on LCs, facilitating the presentation of lipid antigens to skin-resident T cells [283, 298]. Dermal DCs, in general, are immature and express TLR2 and TLR4 [328]. when activated, they undergo maturation procedure and express co-stimulatory molecules, such as CD83, and produce TNFα and iNOS [329]. Human dermal $CD1c^+$ cDCs co-expressed lower level CD1a, compared to LCs, and are potent in inducing T cell activation [31, 330, 331], and can produce IL-15 and IL-10 [332, 333]. BDCA-3^{hi} cDCs lack the expression of CD14 and exert low level of CD11c, and they are capable of cross-presentation and promote Treg functions [28, 32]. The dermal $CD14^+$ DCs have been shown to be monocyte-derived, and can secrete IL-12, IL-10, and TGF-β, which skew Th1 and Treg responses, but with limited $CD8^+$ T cell priming ability [302, 334]. In addition, $CD14^+$ DCs can activated B cells to differentiate into plasma cells [33, 335, 336].

The plasticity of dermal DC subsets greatly depends on their function, location, microenvironment, and the presence of stimulus [29, 337, 338]. To this day, there is still a controversy about which DC subsets play key roles in skin wound healing or sterile inflammatory conditions. Therefore, the understanding of effector functions, plasticity, and cell-cell interactions of each DC subsets in the skin requires further exploration.

5.1.2 Aims

Here, I aimed to investigate gene expression profiles of the BDCA-2^{+} subpopulations isolated from the skin suction blister to understand their niche roles in skin trauma. The relationship of the skin CD123^{int} DCs, which exhibited both pDC and cDC markers, to skin CD123^{hi} DCs and skin cDCs was examined at the single cell level. To answer this question, scRNA-seq methods for each cell subset was adopted to study the possibility of BDCA-2^{+}CD123^{int} DCs representing a tissue-specific DC subtype which held features related, but not identical, to cDCs or BDCA-2^{+}CD123^{hi} DCs. By knowing their origins and potential effector functions, it is possible to inspire therapeutic modulation of their role in dysregulated skin wounds or other inflammatory diseases.

5.2 Results

5.2.1 PCA clustering analysis showed a closer relation between skin BDCA-2^{+} CD123^{int} DCs to pDCs

Blood- and skin suction blister-derived cDCs, BDCA-2^{+}CD123^{hi} DCs, and BDCA-2^{+}CD123^{int} DCs were directly stained with antibodies and isolated by FACS based on the surface expression of DC markers (Fig. 5-1). This was followed by scRNA-seq and RNA expression quantification (see methods §2.10 and §2.11).

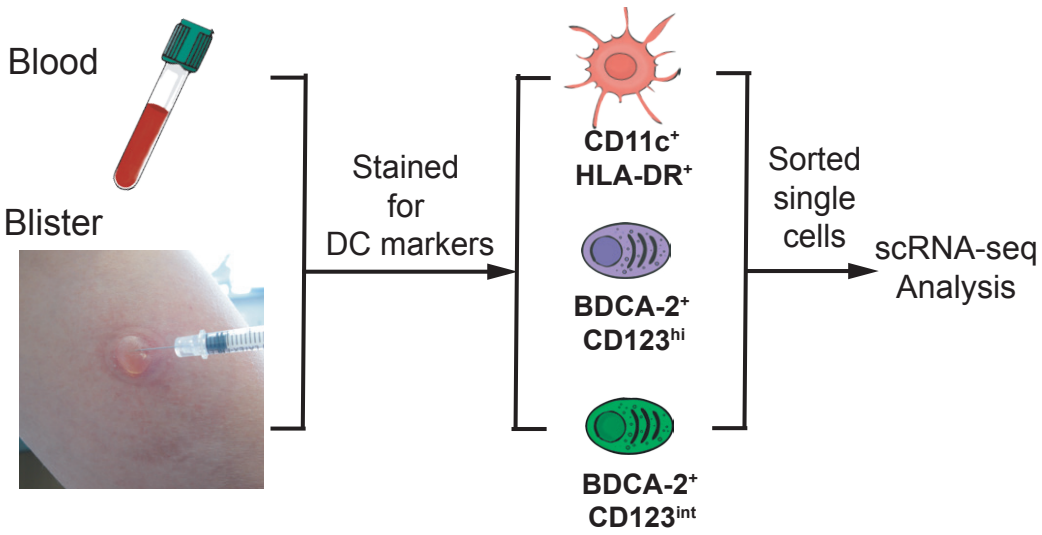
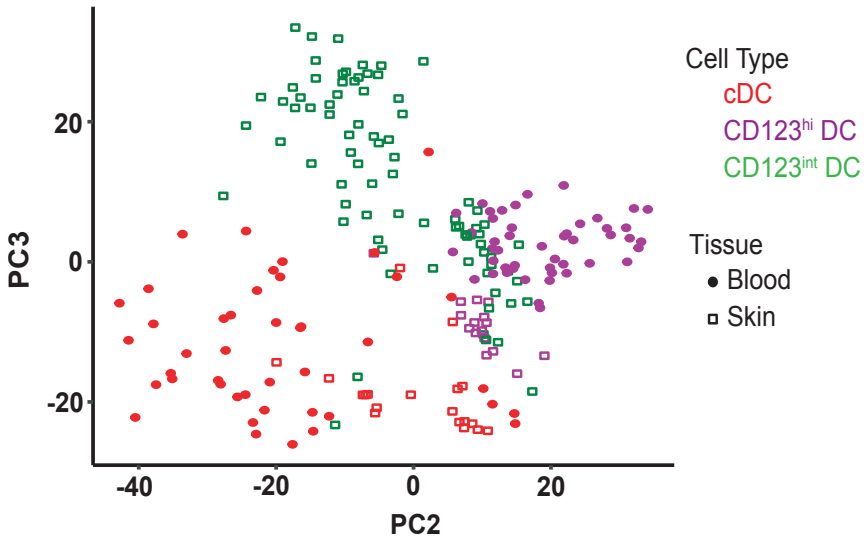


Figure 5-1. Workflow of experimental strategy of skin DC scRNA sequencing.

43 blood cDCs, 47 blood BDCA-2^{+}CD123^{hi} DCs, 19 skin cDCs, 16 skin BDCA-2^{+}CD123^{hi} DCs and 81 skin BDCA-2^{+}CD123^{int} DCs were successfully profiled. One single donor was recruited for this data set.

Dimensionality reduction of the scRNA-seq data with PCA analysis identified that blood CD123^{hi} pDCs and blood cDCs formed two distinct clusters, whereas skin DC subsets situated closely together. Notably, skin BDCA-2^{+}CD123^{hi} DCs were more closely associated with skin BDCA-2^{+}CD123^{int} DCs and clustered closely to blood pDC (Fig. 5-2). Lastly, PC2 shows an organisation of the DC subset phenotypes that places skin BDCA-2^{+}CD123^{int} DCs between blood BDCA-2^{+}CD123^{hi} DCs and blood cDCs.



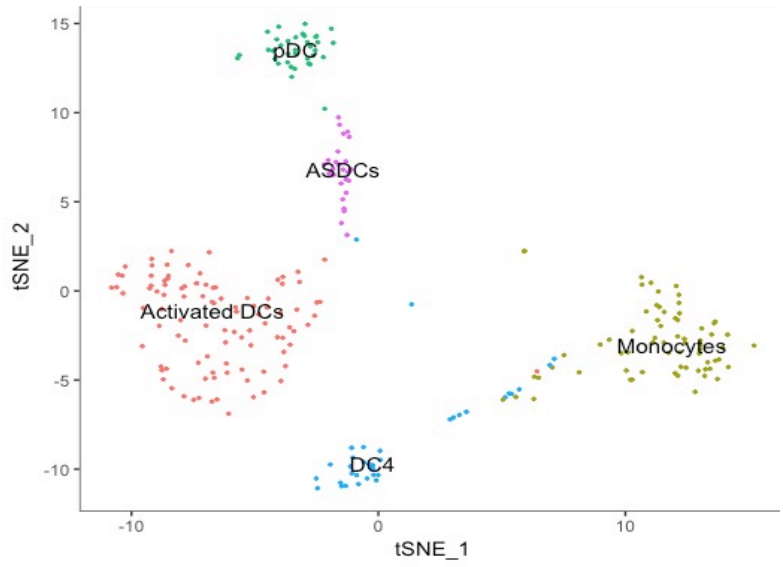
Data analysis performed together with Tomas Gomes, Wellcome Sanger Institute, Hinxton, Cambridge UK

Figure 5-2. Human blood and skin DC heterogeneity in PCA analysis

PCA analysis of all DC subsets. Each dot represents an individual cell.

5.2.2 Unbiased clustering of skin DCs revealed five clusters

Next, unbiased classification used by Villani [68] et al. was adopted to generate t-distributed stochastic neighbour embedding (t-SNE) for visualisation of all the populations isolated from the skin. The clustering analysis was performed without reference to the original sorting index and five clusters were identified within the blisters. $CD11c^{+}HLA\text{-}RA^{+}$ cells were mixtures of monocytes and cDCs, while $BDCA\text{-}2^{+}CD123^{hi}$ DCs contained previously validated pDCs and AS DCs, suggesting that AS DCs were part of the skin wound repertoire which has not been previously described. The majority of $BDCA\text{-}2^{+}CD123^{int}$ DCs, on the other hand, were clustered in the position of the previously validated DC2 population (termed "Activated DCs", Fig 4-3).

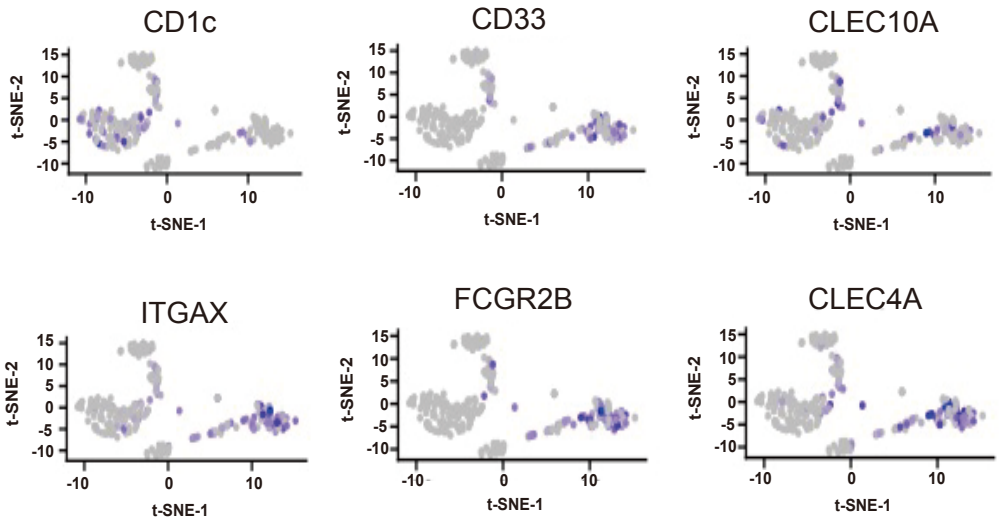


Data analysis performed by Gary Reynolds, Institute of Cellular Medicine, Newcastle University, UK

Figure 5-3. t-SNE analysis incorporating all skin DC subsets.

Five clusters were generated, and colours indicate potential DC subsets. Each dot represents an individual cell.

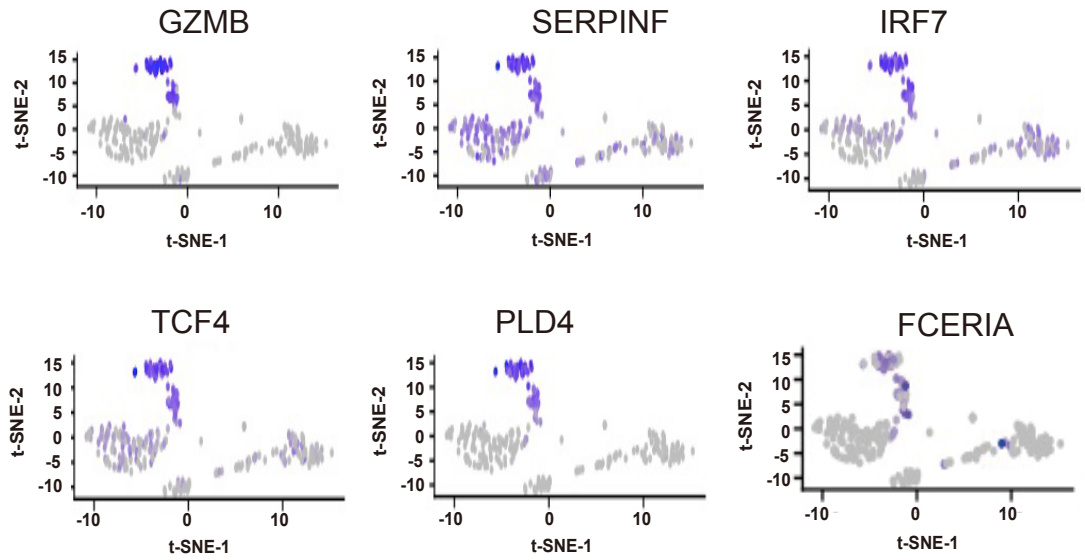
Despite the close clustering of skin BDCA-2^{+}CD123^{int} DCs to blood DC2 and skin pDCs, they failed to express certain signature DC2 or pDC markers, such as *ITGAX* (CD11c), *ITGAM*, and *FCGR2B*, or *GZMB*, *IRF7*, and *PLD4*, respectively (Fig. 5-4 and 5-5). Unsurprisingly, skin BDCA-2^{+}CD123^{int} DCs did not express any DC1 signature genes, consistent with the clustering results (Fig. 5-6). Interestingly, skin BDCA-2^{+}CD123^{int} DCs, expressing no *AXL* and *SIGLEC6*; and AS DCs shared a degree of similarity in activation status, expressing high levels of *CCR7*, *LAMP3*, and *CD83* (Fig. 5-7)



Data analysis performed by Gary Reynolds, Institute of Cellular Medicine, Newcastle University, UK

Figure 5-4. t-SNE analysis revealed DC2 signature gene expressions on each cluster.

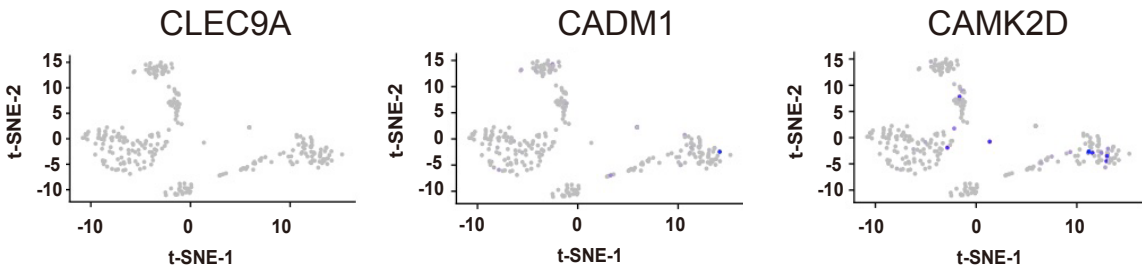
DC2 signature gene expressing cells (purple) were overlaid onto the t-SNE map. Each dot represents an individual cell. The darker colour correlated to higher gene expression level.



Data analysis performed by Gary Reynolds, Institute of Cellular Medicine, Newcastle University, UK

Figure 5-5. t-SNE analysis revealed pDC signature gene expressions on each cluster.

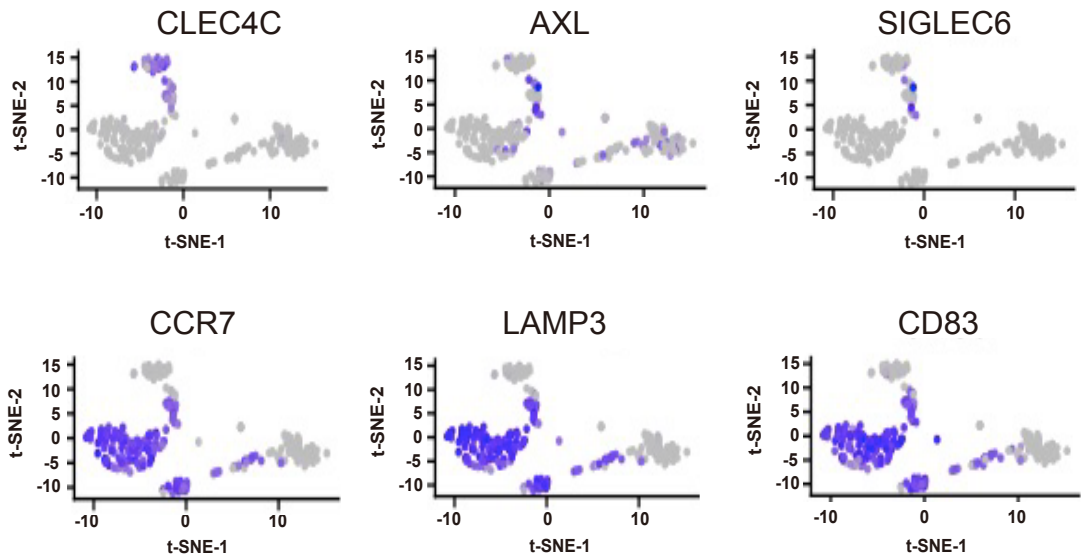
PDC signature gene expressing cells (purple) were overlaid onto the t-SNE map. Each dot represents an individual cell. The darker colour correlated to higher gene expression level.



Data analysis performed by Gary Reynolds, Institute of Cellular Medicine, Newcastle University, UK

Figure 5-6. t-SNE analysis revealed DC1 signature gene expressions on each cluster.

DC1 signature gene expressing cells (purple) were overlaid onto the t-SNE map. Each dot represents an individual cell. The darker colour correlated to higher gene expression level.



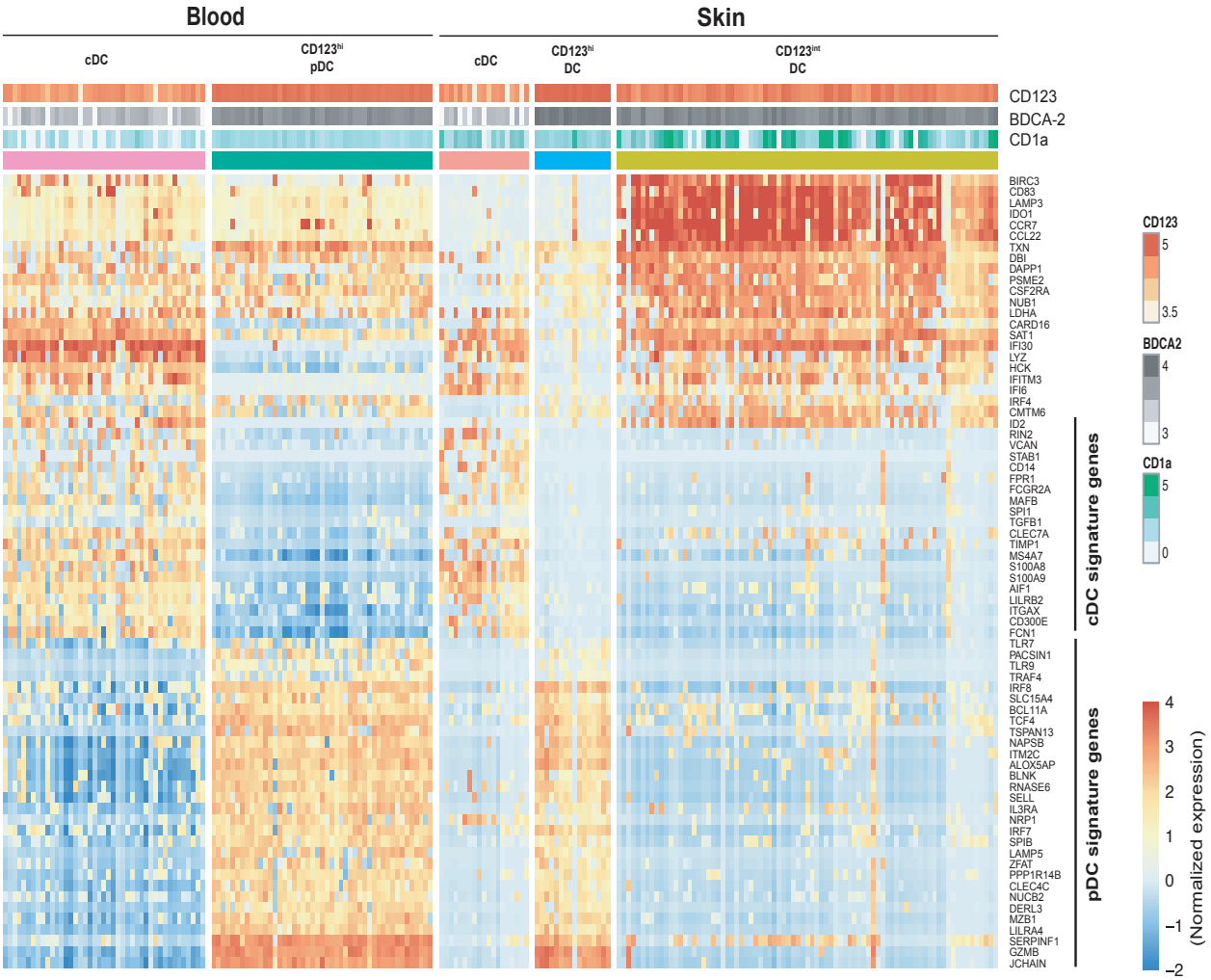
Data analysis performed by Gary Reynolds, Institute of Cellular Medicine, Newcastle University, UK

Figure 5-7. Skin BDCA-2^{+}CD123^{int} DCs and AS DCs shared similar activation status.

Indicated gene expressing cells (purple) were overlaid onto the t-SNE map. Each dot represents an individual cell. The darker colour correlated with higher gene expression level.

5.2.3 Skin BDCA-2^{+}CD123^{int} DCs were equipped with pDC-related markers and cDC-like functionality.

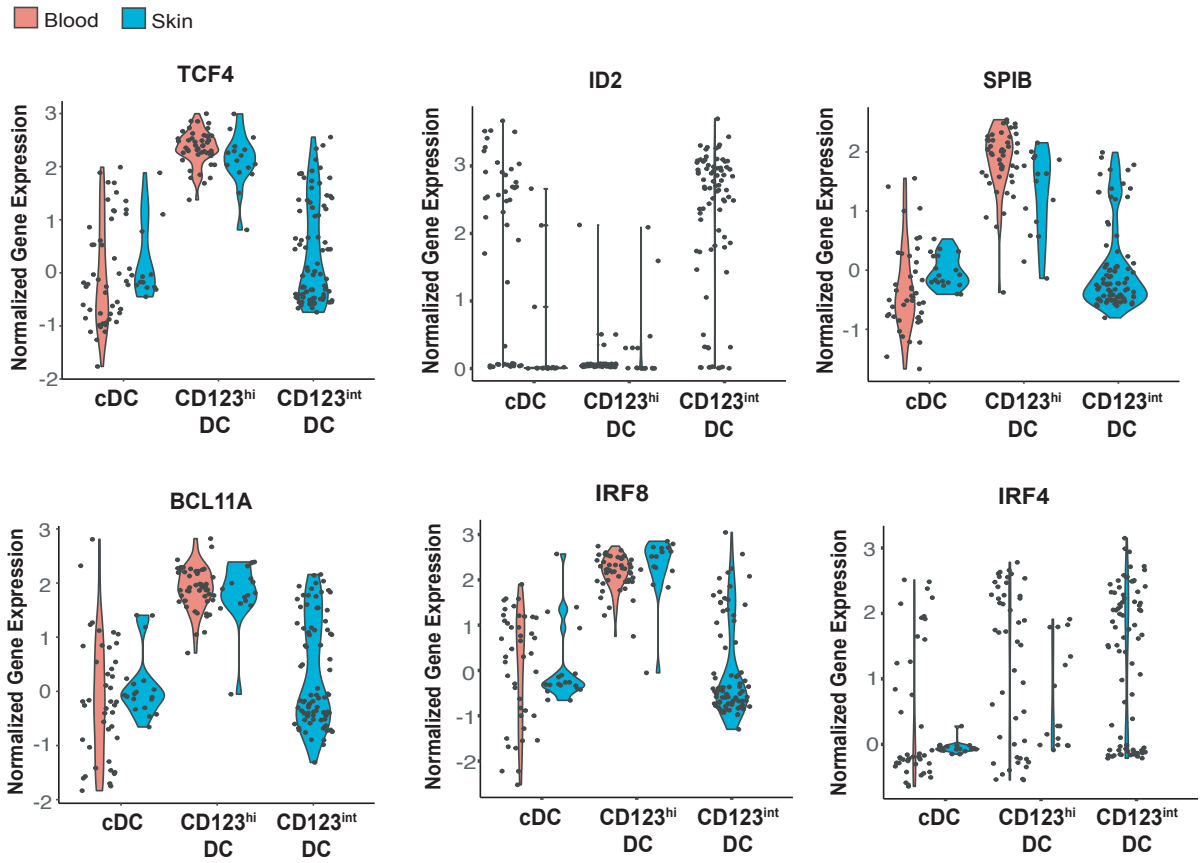
After further comparing gene expression profiles between each DC subsets, 72 differentially expressed genes were identified to be DC-related, with some of the top cDC and pDC signature genes shown in Figure 5-8. The data supported the hypothesis that heterogeneity existed in the skin BDCA-2^{+}CD123^{+} population. Skin BDCA-2^{+}CD123^{int} DCs lack certain cDC and pDC signature genes, but have a highly increased expression of DC functional genes. Notably, *TCF4* expression level was lower in skin BDCA-2^{+}CD123^{int} DCs compared to the skin BDCA-2^{+}CD123^{hi} DCs, while the ID2 level had increased (Fig. 5-9). In addition, skin BDCA-2^{+}CD123^{int} DCs expressed reduced levels of *SPIB*, *BCL11A* and *IRF8*. Meanwhile, *IRF4*, which is highly expressed in pDCs, remained at a high degree of expression in skin BDCA-2^{+}CD123^{int} DCs (Fig. 5-9). The data indicated that skin BDCA-2^{+}CD123^{int} DCs potentially represented an intermediate phenotype between pDCs and a more cDC-like population.



Data analysis performed together with Tomas Gomes, Wellcome Sanger Institute, Hinxton, Cambridge UK

Figure 5-8. Human blood and skin DCs heterogeneity analysis.

Heat map coloured by normalised gene expression in individual cells of each subset. Top margin colour bars report the index fluorescent values of CD123, BDCA-2, and CD1a.

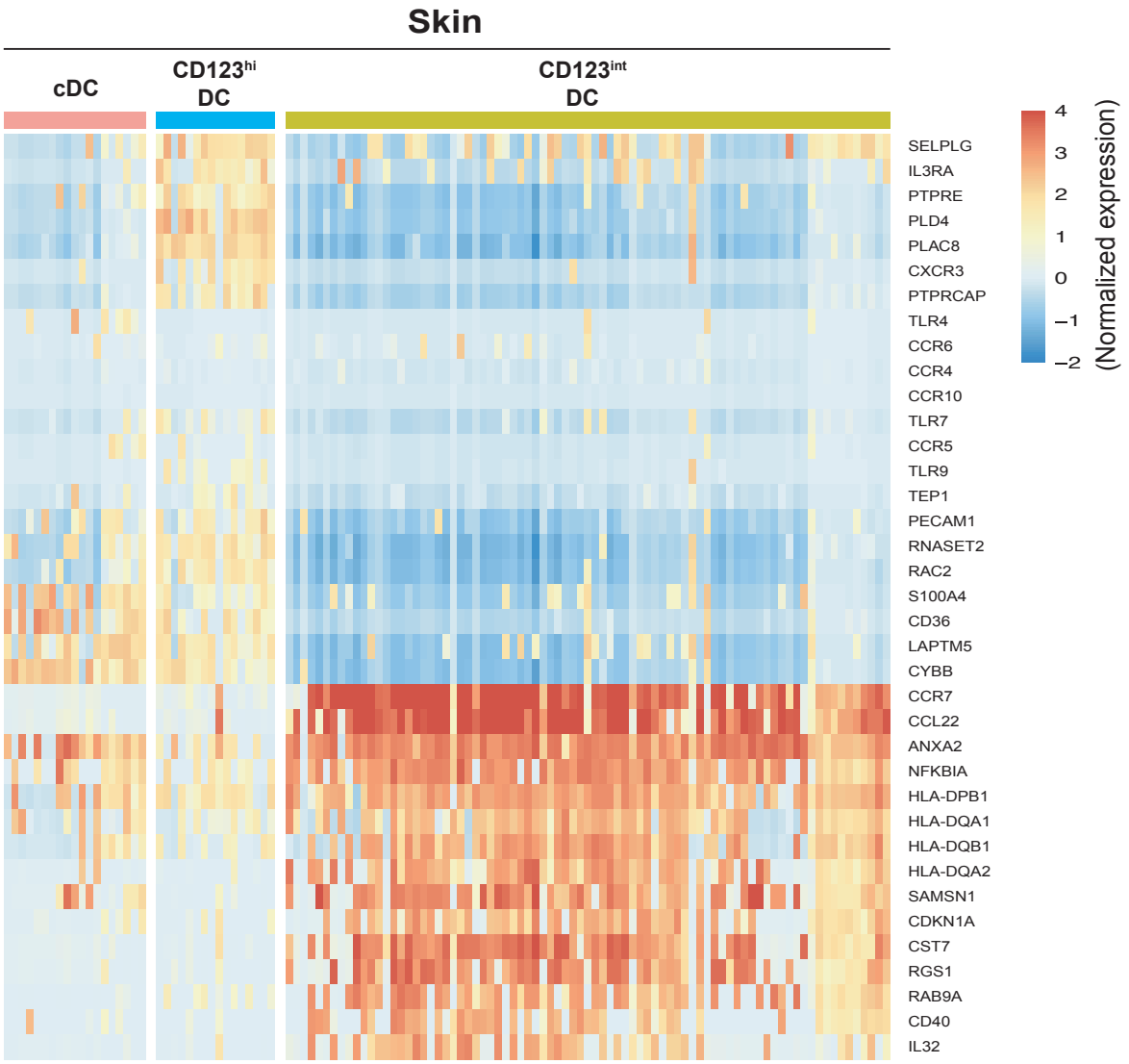


Data analysis performed together with Tomas Gomes, Wellcome Sanger Institute, Hinxton, Cambridge UK

Figure 5-9. PDC signature gene profiles of human blood and skin DCs.

Violin plots demonstrate the expression distribution of indicated genes across DC subsets. Each dot represented an individual cell. Genes with adjusted p-value < 0.01 between skin CD123^{hi} and CD123^{int} DC were plotted.

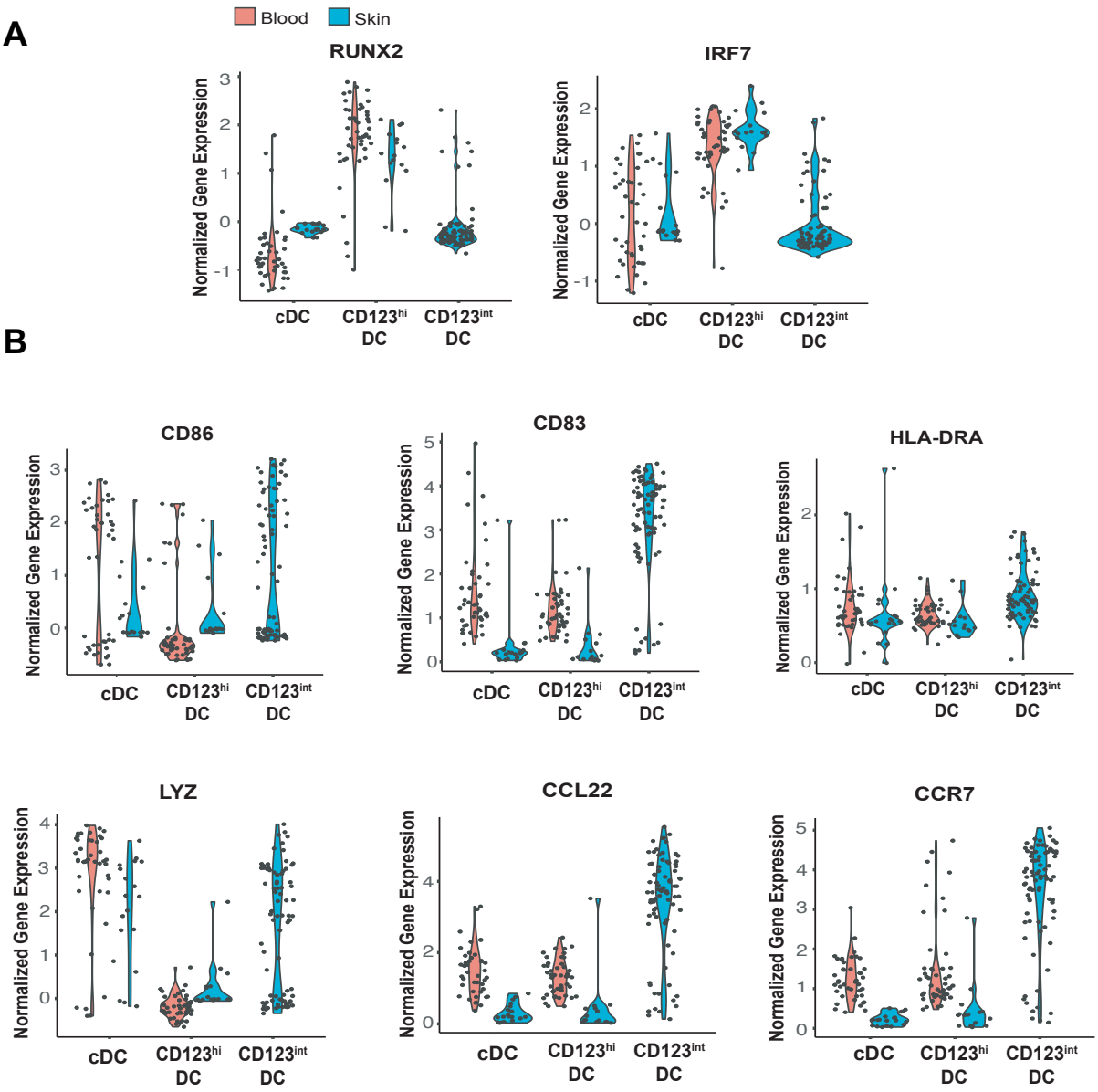
In terms of genes relating to pDC functions, skin BDCA-2^{+}CD123^{int} DCs had an elevated presence of genes linked to antigen-presentation and tissue-homing (Fig. 5-10). RUNX2 and its downstream signalling molecule, IRF7, are key regulators of IFN-I production [83, 84], and their gene expression was decreased in skin BDCA-2^{+}CD123^{int} DCs (Fig. 5-11A). Co-stimulatory marker genes, such as *CD86*, *CD83*, and *HLA-DR*, and antigen processing and loading genes, *LYZ* [72, 339, 340], were elevated in skin BDCA-2^{+}CD123^{int} DCs (Fig. 5-11B). Notably, skin BDCA-2^{+}CD123^{int} DCs, but not skin cDCs and skin BDCA-2^{+}CD123^{hi} DCs, expressed very high levels of CCL22, a chemoattractant for CCR4-expressing activated T cells and FoxP3^{+} regulatory T cells into the skin [341-344]. After uptake of antigens, one of the essential functions of DCs is to migrate toward draining lymph nodes, where they initiate the adaptive immune response. CCR7 directs the lymph node homing of DCs and was one of the most significantly upregulated transcripts in skin BDCA-2^{+}CD123^{int} DCs (Fig. 5-11B).



Data analysis performed together with Tomas Gomes, Wellcome Sanger Institute, Hinxton, Cambridge UK

Figure 5-10. Human skin DCs gene expression profiles.

scRNA-seq analysis of human skin DC subsets. Heat map coloured by normalised gene expression in individual cells from the skin DC subsets.



Data analysis performed together with Tomas Gomes, Wellcome Sanger Institute, Hinxton, Cambridge UK

Figure 5-11. Human skin BDCA-2^{+}CD123^{int} DCs express genes relating to antigen presentation.

scRNA-seq analysis of human skin DC subsets. Violin plots demonstrate the expression distribution of (A) *RUNX2* and *IRF7*, (B) *CD86*, *CD83*, *HLA-DR*, and *LYZ*, and (C) *CCL22* and *CCR7* across DC subsets. Each dot represents an individual cell. Genes with adjusted p-value < 0.01 between skin CD123^{hi} and CD123^{int} DC were plotted.

Pseudo-time analysis using Monocle was exploited to dissect cell state transitions, based on the differential gene expression profiles between each DC subpopulations, reconstructing cellular trajectories of the skin BDCA-2^{+}CD123^{int} DCs. Monocle was designed to order cells based on their transcriptional profiles, generating single-cell trajectories in a tree-like structure, potentially aligned accordingly to the time series. This method can analyse the "switch point", where a set of genes are off and other set of genes are on during cell transitions [345]. The analysis of single-cell trajectories of blood and skin DC populations resulted in three branches, A, B, and C (Fig. 5-12A). Mapping of each DC subtype onto the cell trajectories indicated that each branch accommodated key lineage-specific genes which enabled pDCs and cDCs originating from the blood to locate near the tips of the tree branches, while the skin cells were distributed amongst the branches, with skin BDCA-2^{+}CD123^{int} DCs settled mostly across branch C (Fig. 5-12B-C). Monocle is a powerful tool to study cells in transitional status in responding to biological events when intermediate states exist, which pseudotemporal ordering produces meaningful biological interpretations. Of note, Monocle method fails to identify the priori of the trajectory, hence the term "pseudo-time" [346]. However, in the skin DCs wound healing study, the *bona fide* pDCs and cDCs were terminally differentiated cells known to have little hierarchical relationship; therefore, this analysis could only be performed on the basis of key

lineage-specific transcriptomic profiles that were differentially expressed across each DC population; this explained why cDCs were separated into two branches, as gene profile analysis showed that CD11c^{+}HLA-RA^{+} cells contained both monocytes and cDCs (§ 5.2.2). To conclude, skin BDCA-2^{+}CD123^{int} DCs were considered a DC subset but expressed a spectrum of genes different from both pDCs and cDCs, which was consistent with the findings in figure 5-4 and 5-5, yet shared some transitional continuum with cDCs (Fig. 5-12C). Nonetheless, the developmental root of the skin BDCA-2^{+}CD123^{int} DCs remained unresolved.

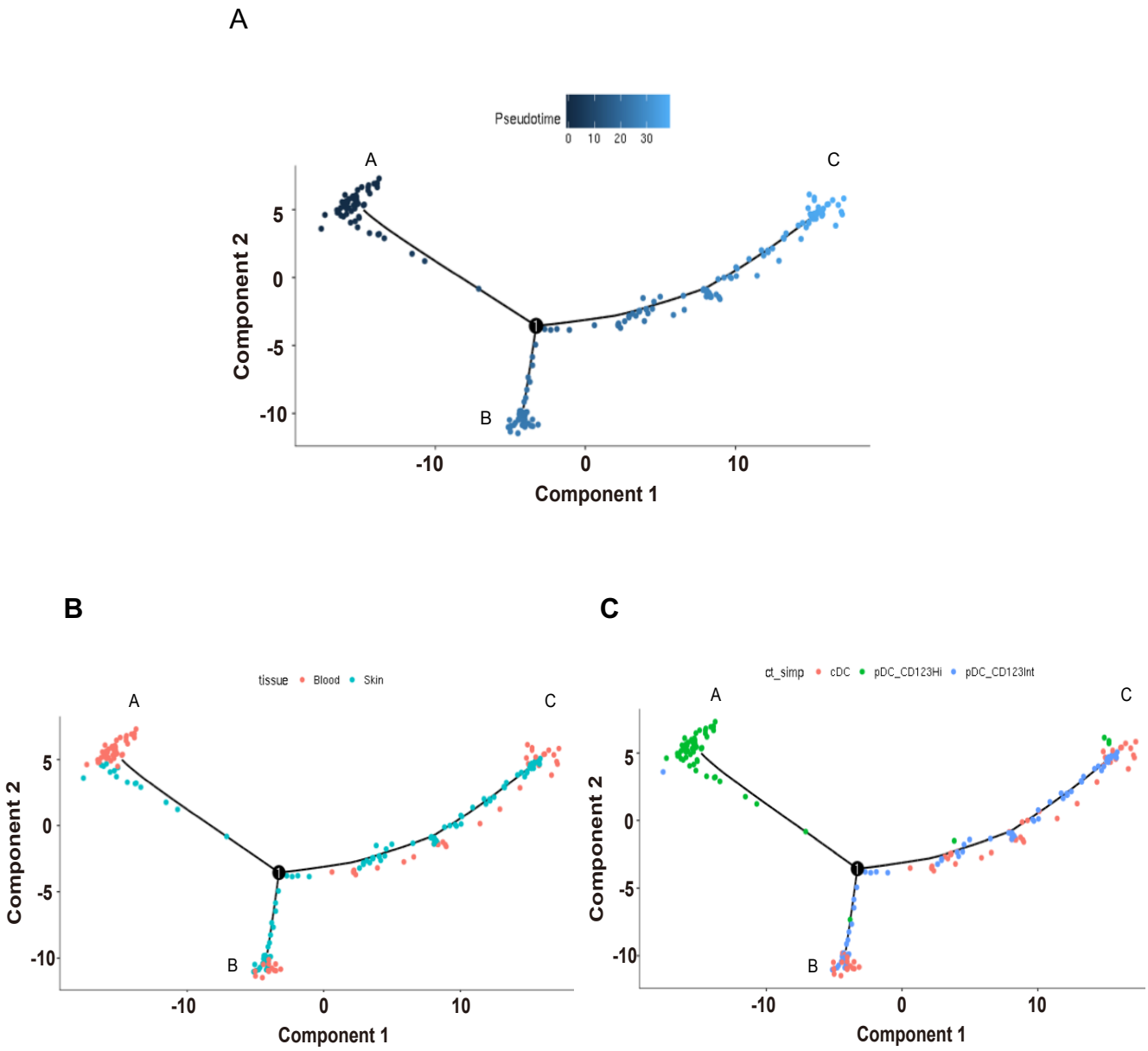


Figure 5-12. Pseudo-time ordering reveals a gradual transition of skin BDCA-2^{+}CD123^{int} DCs and cDCs.

Gene expression profiles for individual cell were organised into trajectories. Three cell states were predicted using temporal analyses of single-cell transcriptomes. (A) The plot shows the time series in a two-dimensional component space. (B-C) Cell types assigned are colour-coded accordingly. Each dot represents an individual cell.

While a widespread spectrum of CD1a expression level was observed on the skin BDCA-2^{+}CD123^{+} DCs, it was speculated that the CD1a expression may be linked to expression of related families of molecules involved in antigen presentation and recruitment of T cells. The correlations between gene expression and CD1a MFI level of the cells showed that CD1a expression was positively correlated with *CCL22*, *HLA class II* and *CD86* expression (Fig. 5-13). These findings suggested that the CD1a-expressing BDCA-2^{+} DC subset in human skin wounds had the potential to migrate to lymph nodes, recruit T cells and present antigen. Collectively, the skin wound BDCA-2^{+}CD123^{int} DCs were demonstrated to be equipped with pDC-related markers and cDC-like functionality, acquiring the ability to present antigens.

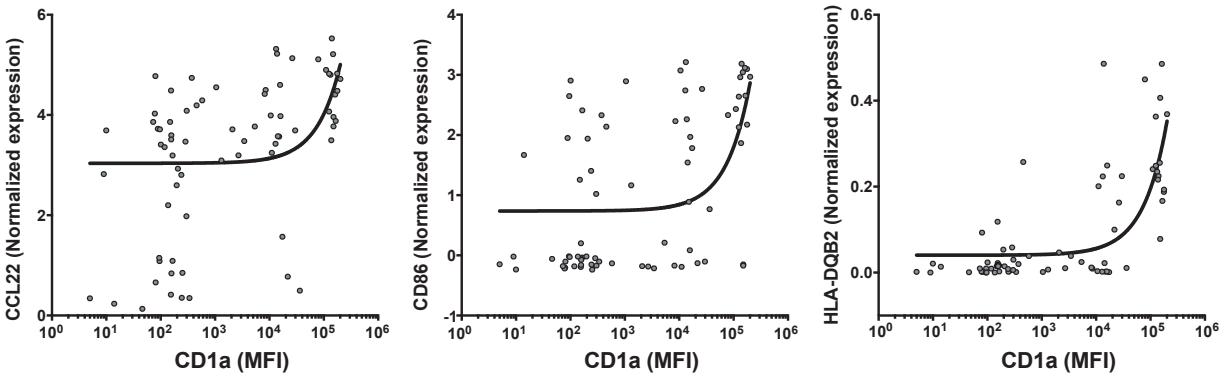


Figure 5-13. Correlation between CD1a expression intensity on skin BDCA-2^{+}CD123^{int} DCs and DC-relevant genes.

CD1a expression level of each BDCA-2^{+}CD123^{int} DC was analysed against its indicated gene expression. Correlations between *CCL22* and CD1a (R^2=0.383, P=0.004), *CD86* and CD1a (R^2=0.4685, P<0.0001), and *HLA-DQB2* and CD1a (R^2=0.6976, P<0.0001) are shown. Each dot represents an individual cell. Pearson correlation was performed.

5.3 Discussion

As there is a growing body of evidence that supports the existence of pDC plasticity in humans in which the pDC signatures are modified according to environmental stimuli [64], it is of current interest to study the underlying mechanisms of how a single stimulus or a combined set of stimuli, including microbiome- or cytokine-derived factors, induces the corresponding activated pDC states. It has been shown that these stimuli are capable of shaping the transcriptional signatures, molecular features, and functions of pDCs; however, how this is achieved is still not fully understood. In this study, the first single cell transcriptomic analysis of DC subsets in human skin wounds was established without artefacts associated with tissue dispersion, and two previously undetected DC subtypes were revealed in the skin wound: $CD123^{int}$ DCs and AS DCs. The unprecedented observation of CD1a expression on pDC-like cells drove the study of their roles in the skin wound. The lipid recognition capacity of T cells through the CD1a molecule constitutively expressed on LCs has been established for some time now; however, the understanding of inducible CD1a presentation system is only just recently appreciated. Seshadri and colleagues have reported that a common genetic mutation in 5' UTR of CD1a is associated with the level of CD1a surface expression. This CD1a expression variation is independent of the expression of other CD1 molecules, such as CD1c; and the cytokine production and endocytic capacity of these DCs [347]. Others reported that inflammatory cytokines treatment and exogenous stimuli can induce a dynamic expression of CD1a, on mature APCs or during the course of their development. Aquino and colleagues reviewed that lipid mediators and cytokines, such as prostaglandin E2 (PGE2), IFNα,

IL-6, and IL-10 can downregulate CD1a expression, while IL-3, IL-13 and TGFβ show upregulating effects [348]. Hardman et al. have demonstrated that TSLP and other skin-derived inflammatory mediators, such as IL-33, PGD2 and LTE4, could elevate CD1a expression on blood ILC2 [188]. Wirths et al. revealed the effect of IFNγ-secreted from $CD8^+$ T cells on CD1a upregulation of monocyte-derive DCs *in vitro* [349]. Nascimento et al. reported that TLR2 agonist can increase CD1a expression, while the short chain fatty acid, such as sodium butyrate, inhibits the TLR- or cytokine-induced CD1a upregulation during the course of monocyte-derived DC development [350]. The discovery of $CD123^{int}$ DCs in the skin wound highlights the adaptability of the CD1a system in accordance to tissue milieu.

The transcriptomic analysis of this novel skin DC population showed that they carried both pDC and cDC gene signatures, as well as surface markers. The clustering results clarified their relationships with blood cDC subtypes, yet showed a perceptible number of discrepancies in gene expression profiles, especially those related to DC activation status. These $CD123^{int}$ DCs appeared to be in an "activated" state, in which genes linked with antigen presentation and migratory functions were greatly expressed, whereas those associated with IFN-I production were dampened. This coincided with the functionality of CD1a-bearing blood pDCs *in vitro* described in §4.2.3 and §4.2.4, which were able to present lipid antigens to T cells. Even though IFN-I secretion is recognised as a distinct effector function of pDCs, the concept of pDCs attenuating their IFN-I-producing ability and transforming into adaptive immune mediators, depends largely on the environmental influences, and has been described previously [64]. In addition, the expression of CD1a was highly correlated with the activation status of these $CD123^{int}$ DCs, which can potentially be used as a biomarker

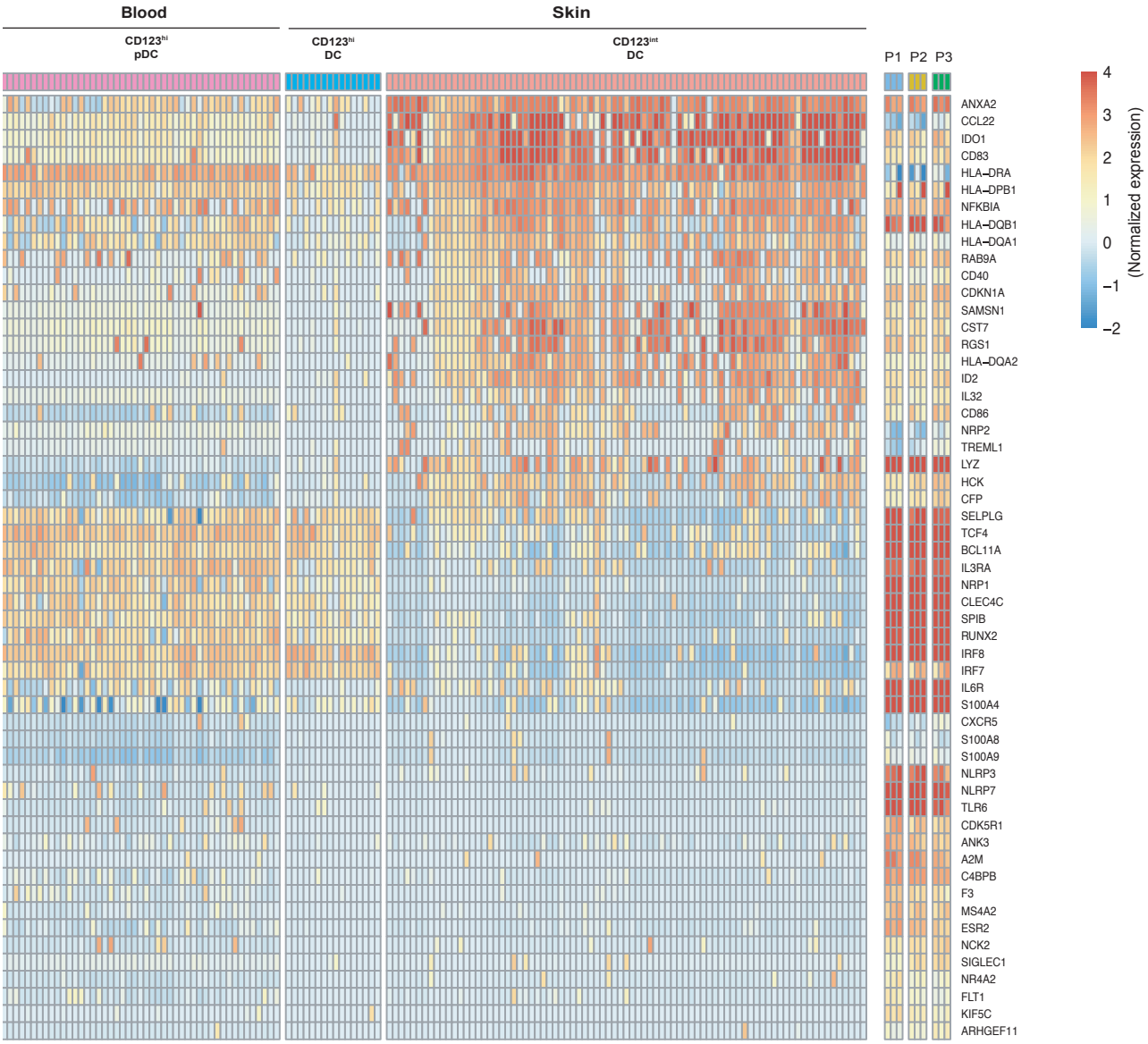
to trace this population in various inflammatory skin conditions *in vivo*. AS DCs, on the other hand, that were previously found to be absent in healthy skin [65], were detected in skin blisters, expressing genes closely related to *bone fide* pDC subsets and did not express CD1a. On the genetic level, skin AS DCs were equipped with both innate and adaptive functionality; however, their specific roles in the skin wound remains an open question. Collectively, how these pDC-like cells obtained their phenotypes in response to the skin micro-environment, and if they played a role beyond the wound healing process and in skin inflammatory pathologies requires further investigation.

Other than presenting lipid antigens, the scRNA-seq data suggest that skin $CD123^{int}$ DCs express lysozyme. Lysozyme, known to be produced by neutrophils, monocytes and epithelial cells, can hydrolytically disrupt the structure of peptidoglycan, an essential ingredient of the bacterial cell wall that maintains the architectural integrity and osmotic balance of the bacteria [340]. Therefore, these cells were thought to have the potential to be involved in lysozyme-mediated bacterial cell wall processing and antigen presentation. Matsui and colleagues have reported that human blood $CD2^{high}$ pDCs express lysozyme, and proposed this as a marker to distinguish blood pDC subsets [72]. However, CD2 transcript was not detected in $CD123^{int}$ DCs in our system; therefore, the origin of these skin-infiltrating $CD123^{int}$ DCs remains unclear and awaits further study. Alculumbre and colleagues recently report a PD-$L1^{-}CD80^{+}CCR7^{+}$ pDC subset (named as P3-pDCs) that arises during specific culture conditions and expresses high levels of CCL22 transcript with potential antigen-presenting ability [64]. However, the subset was not identified in psoriatic lesional skin. In addition, after comparing the gene expression profiles of their pDC subsets with skin-infiltrating

CD123^{int} DCs (Fig. 5-14), there is little phenotypic overlap. Moreover, the skin CD123^{int} DCs cluster excluded genes related to the AS DC and pre-DC reported by Villani and colleagues [68]. These collective comparisons suggest that the skin CD123^{int} DCs represent a novel tissue-specific DC subset.

Powerful technological advances in scRNA-seq methodology enables the study of gene expression to a higher resolution on the basis of a single cell and associated networks. This allows the detection of previously masked cell subtypes in the bulk RNA-seq analysis, and reveals intricate details relating to their phenotypes and behaviours. Therefore, the scRNA-seq method can be used to improve the understanding of cell heterogeneity and plasticity, as well as the underlying molecular mechanisms, in steady state, activation conditions, or in diseases. However, several challenges and shortcomings still exist in this technique. The first step of the process is to isolate the cell of interest, often using FACS sorting, in which each individual cell can be seeded in 96-well plates. The quality of the RNA isolated from these cells is correlated with the precision of cell seeding, duration of cell sorting, homogeneity of cell sizes, nature of the cells, and setting of the FACS. Ensuring cells are obtained under optimum conditions is essential for subsequent steps [351]. The availability of the starting material is limited as an individual cell contains roughly 0.1 pg of mRNA on average, varying between different cell types [352]. An amplification step is required for generating sufficient cDNA for sequencing; however, this amplification step is a nonlinear reaction which results in disproportional expansion of certain sets of genes, leading to potentially biased interpretation of gene expression levels. Several strategies are implemented to solve this issue, including inserting a unique molecular identifier (UMI), or oligonucleotides barcodes, which serve as internal

controls, enabling the bioinformaticians to account for the varied cDNA products of reverse transcription procedure, and diminishing the undesired bias [353]. In addition, among the scarce cDNA, only 10-20% of the molecules can be actually detected, resulting in poor sensitivities of genes expressed in low levels within the cell [353]. Another limitation lies in the nature of the sporadic fashion of gene expression pattern: most genes do not express in a continuous fashion, but display temporal variability, resulting a diverse gene expression profile even in relatively homogeneous populations [353]. Lastly, a discrepancy is often observed in transcriptomic profiles and protein expression levels. This is partly caused by the time-course difference in mRNA expression and protein translation and transportation. While the biological aim of utilising scRNA-seq is to understand the relationship between genotype and phenotype, it is of great importance to validate the data with subsequent functional assays [354]. A potential restriction of this study lies on the limited number of the skin $CD123^{int}$ DCs, which renders functional validations impossible. Therefore, blood pDCs and cDCs were used to study the mechanistic insights (Fig. 4-13-17); nonetheless, the risk of oversimplifying the complexity of tissue-specific environment is present.



Data analysis performed together with Tomas Gomes, Wellcome Sanger Institute, Hinxton, Cambridge UK

Figure 5-14. Comparative human skin and blood DC scRNA sequencing analysis.

Heatmap comparing normalised gene expression of individual cells of each DC subset, and the P1-, P2-, and P3-pDCs data reported by Alculumbre and colleagues [64].

6 Plasmacytoid DCs Enhance the Capacity of ILC3 to Present Peptide Antigens to T Cells

6.1 Introduction and aims

6.1.1 Innate lymphoid cells

ILCs are groups of multifunctional hematopoietic cells that lack known lineage markers associated with T cells, B cells, myeloid cells, or granulocytes, but display lymphoid cell morphologies. These cells often reside at mucosal interfaces and epithelial barriers, such as the gastrointestinal tract, lung, adipose tissue and skin, where they are involved in various immune responses against microorganisms, environmental challenges, and tumors [355-361]. ILCs lack re-arranged T cell receptors or B cell receptors, which render their activation in an antigen non-specific manner; yet, they mirror T cell populations on the basis of their developmental requirements, transcription factors, and effector cytokine-producing capacity, which enable them to be classified into three groups, namely group 1 ILCs (ILC1), ILC2 and ILC3 (Fig 6-1). ILCs do not require polarisation to activate, instead they act rapidly to alarmins, antigens, and cytokine signals by producing effector cytokines [355-361].

ILC1, which include *bona fide* ILC1 and classical natural killer cells (NK cells), express the transcription factor T-bet and Eomesodermin (EOMES); respond to IL-12, IL-15 and IL-18, and secrete IFNγ and TNFα [362]. ILC1 and NK cells share some functional degree of functional similarities in their surface marker profiles, transcriptional, and effector functions; however, NK cells are involved in a more

cytotoxic pathway, and develop from distinct progenitors compared to ILC1 [363, 364]. ILC2 express GATA-3 and RORα; respond to IL-25, IL-33, thymic stromal lymphopoietin (TSLP), prostaglandin D2 (PGD2), leukotriene E4 (LTE4), and produce IL-5, IL-4 and IL-13. ILC3 are heterogeneous populations which depend on the transcription factor RORγt for their development and function, and can produce IFNγ, GM-CSF, IL-22 and/or IL-17A upon stimulation by IL-1β and IL-23 [361, 365-368].

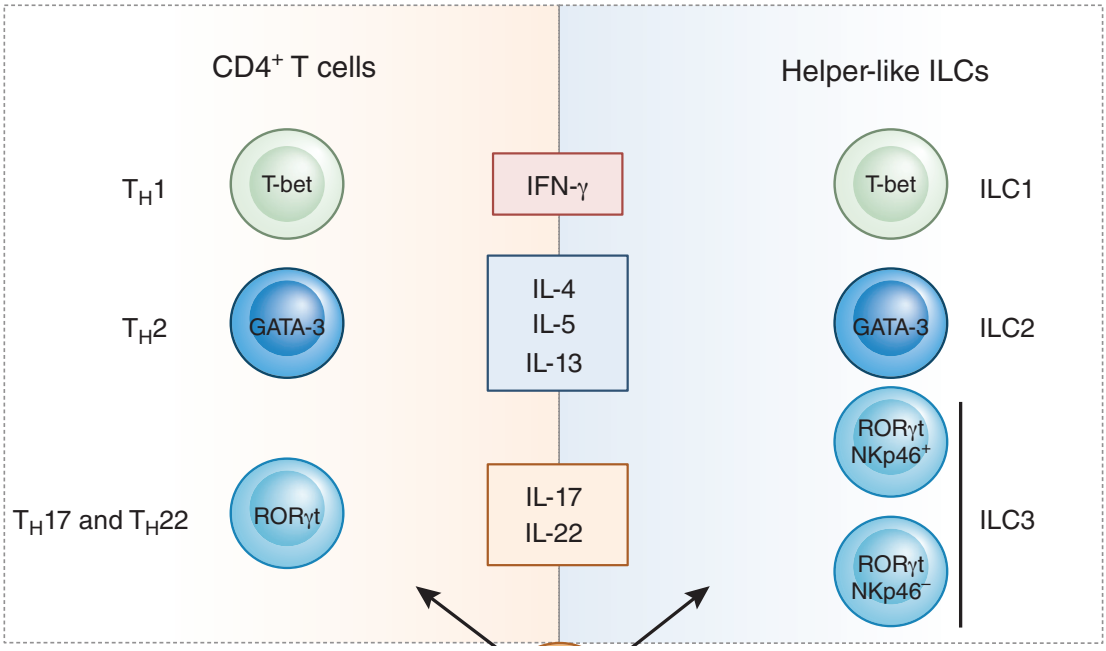


Figure 6-1. ILCs mirrors the diversity of T helper cells.

The main transcription factors and cytokine profiles that differentiate the functions of T helper cell subsets are shared with specific ILC subsets. Figure was modified and reprinted by permission from Springer Nature: Eberl, G., J.P. Di Santo, and E. Vivier, *The brave new world of innate lymphoid cells.* Nat Immunol, 2015. **16**(1): p. 1-5.

6.1.1.1 Group 3 ILC

Recent high dimensional transcriptomic studies of human and murine ILC3 have revealed the plasticity and heterogeneity of ILC3 [362, 367, 369-373], and at least two *bona fide* subsets are identified. In adult mice and human, ILC3 are defined into CCR6-expressing ILC3, which phenotypically mirror fetal lymphoid tissue-inducer cells (LTi cells), termed LTi-like ILC3; and natural cytotoxicity receptors (NCR)-expressing ILC3, named NCR^+ ILC3 [371, 374]. Despite sharing many overlapping features, such as the expression of RORγt and IL-23R, LTi-like ILC3 and NCR^+ ILC3 exhibit appreciable differences in subset-restricted gene expression, chemokine receptor profiles, tissue residency, and effector functions [362, 371, 375, 376].

Tissue-resident ILC3 act as sentinels to sense a wide range of environmental signals locally, and relay the information to surrounding cells through cytokine secretion or cell-cell interaction in a challenge-specific manner. LTi-like ILC3 inhabit dominantly in the colon and lymphoid tissues, while NCR^+ ILC3 mostly reside in the tonsils, small intestine, lamina propria and Peyer's patches [377]. LTi-like ILC3 can secrete both IL-22 and IL-17A, while NCR^+ ILC3 population produce IL‑22 but not IL-17A, thus are referred to as NCR22 or ILC22 cells in the literature [368, 376, 378-380]. LTi-like ILC3 express molecules, such as CD4, MHC-II and CD1d, that allow them to indirectly or directly attune the adaptive immune responses in lymphoid tissues [371]. Moreover, LTi-like ILC3 specifically express Neuropilin-1 (BDCA-4), a receptor expressed by regulatory T cells and pDCs [49, 381-384]; however, it is unclear if Neuropilin-1 on LTi-like ILC3 is functionally active and plays any subset-restricted

roles for these cells [362, 371]. In contrast, NCR$^+$ ILC3 express *Il12rb2*, *Tbx21* and *Notch1* which are required for their development and maturation [385, 386]. In addition, NCR$^+$ ILC3 act as an innate immune modulator by interacting with myeloid and stromal cells in the tissue through Notch receptor and GM-CSF. Recent evidence indicates that NCR can serve as a novel pattern recognition molecule [387], or act as an activating receptor [388]. Notably, ILC3 are not found to express TLRs or pattern recognition receptors (PMM) in the steady state, but are shaped by signals from APCs or environmental intermediates which will be addressed in detail in later sections. Even though the NCR$^+$ ILC3 are considered to possess little classical cytotoxic functions, their expression of *Gzmc* nevertheless suggests cytolytic potential [389].

Several groups have reported that the transcriptional overlap between NCR$^+$ ILC3 and ILC1 is observed more than that of the NCR$^+$ ILC3 and LTi-like ILC3, and there may be multiple intermediate/transitional activation statuses presented in ILC3 subsets in response to microenvironment-specific regulation [390, 391]. Gury-BenAri and colleagues have combined multiple transcriptomic methods to analyse all ILC subsets in mice small intestinal lamina propria, and have identified five distinct functional states of ILC3 in reaction to microbial stimuli [391]. They suggested that a potential interchangeable plasticity between each activation state exists in coordination with cell-cell interactions and soluble mediator exposure. Namely, without homeostatic commensal bacterial signals, the ILC subsets in the lamina propria acquired ILC3-like expression profiles, suggesting that microbiome provide inhibitory signals in ILC3 fate determination [366, 378, 386, 391, 392]. The complex heterogeneity among tissue-resident ILC3 is a call for a more comprehensive and stringent classification of ILC3.

6.1.1.2 ILC3 development

All mouse ILCs can be derived from a common lymphoid progenitor (CLP) [393-397], and there are ILC lineage-restricted progenitors that exist downstream of the CLP. Common helper ILC progenitor (CHILP) express high levels of Id2 [395] which heterodimerises with E proteins to repress their binding to DNA and sequesters the transcriptional activating ability. Such CHILP express few ILC lineage-defining transcription factors, such as RORγt, RORα, EOMES and T-bet, suggesting that they retain a certain degree of flexibility [398, 399]. The transcription factor GATA-3 is vital for the development of all ILC subsets by controlling IL-7Rα expression. Notably, CHILP has the ability to differentiate into non-NK ILC1, ILC2, both ILC3 subsets, but not NK cells [395]. Moreover, a common ILC precursor (ILCp) subset develops downstream of CHILP that expresses transcription factor PLZF and PD-1 [364, 400], which can give rise to ILC1, ILC2, and NCR^+ ILC3, but not LTi-like ILC3, indicating a developmental bifurcation process during ILC3 development. LTi-like ILC3, on the other hand, develop through a separate progenitor expressing CXCR5 that diverges upstream of ILCp [394, 401-403] (Fig 6-2).

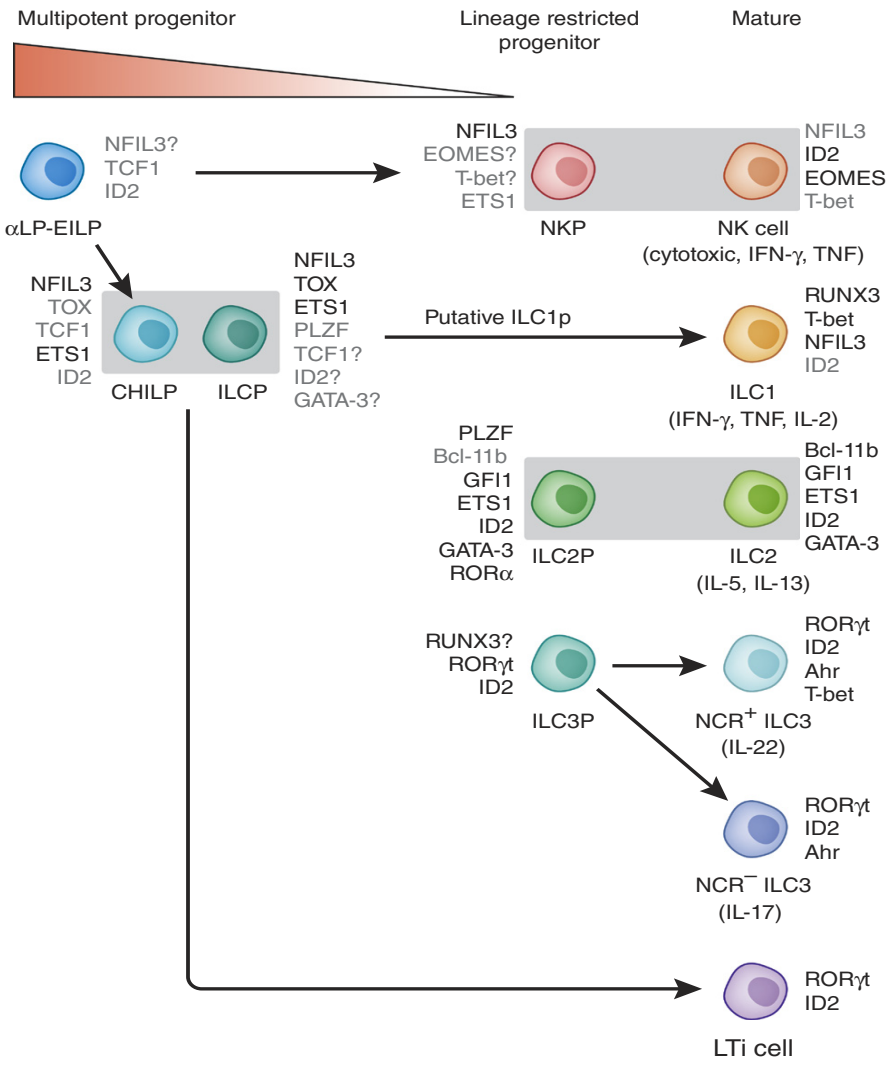


Figure 6-2. Summary of ILC development.

All ILCs can be developed from multipotent CHILP and ILCP. Multipotent progenitors develop into lineage restricted progenitors and then mature ILCs. The figure summarised specific transcription factors and the stages at which they are expressed. Figure was modified and reprinted by permission from Springer Nature: Zook, E.C. and B.L. Kee, *Development of innate lymphoid cells.* Nat Immunol, 2016. **17**(7): p. 775-82.

In addition, as mentioned in previous sections, plasticity exists between ILC3 and ILC1 or within ILC3 subsets that is determined by the distinct tissue

microenvironment, such as bacterial metabolite composition, dietary cues and host-derived signals from the immune system or stroma, that dictates the transcriptional decisions of mature ILC3 through the modification of intracellular signalling and epigenetic modification. For example, butyrate, short-chain fatty acids-generated by colonic bacteria, can negatively regulate the numbers of NCR^+ ILC3 in the Peyer's patches, leading to a reduced regulatory T cell numbers, and enhanced antigen-specific T-cell proliferation [404]. Another intestinal bacteria derivative, Aryl hydrocarbon receptor (AHR) ligands, can regulate the development, migration, and cytokine production pattern of ILC3 [405-409]. In addition to microbial metabolites, dietary milieu can modulate ILC3 development in the gut. For instance, vitamin A-derived retinoids is required for ILC3 maintenance [410-412]; vitamin D is reported to lessen IL-23R expression level which renders the diminished ILC3 number and IL-22 production in both human and mice [413, 414].

Other than the exogenous modulators, host-derived signals also direct ILC3 development and transformation. ILC3 are relatively long-lived in adults, and can be self-replenished or renewed from tissue-resident precursors expressing RORγt [415, 416]. After receiving cytokine signals and the Notch signal, these $ROR\gamma t^+$ progenitors in tissues can give rise to both ILC3 subsets, with lesser LTi-like ILC3 potency [366, 386, 417, 418]. ILC3 and their progenitors express common cytokine γ-chain receptors comprising IL-2R, IL-7Rα and IL-15R. In committed ILC3, the transcription factor GATA-3 positively regulates IL-7Rα expression [419]. The loss of IL-7Rα signal leads to reduced numbers of ILC3 in the adult [420, 421], and elevated LTi-like ILC3 numbers are observed when IL-7Rα signalling is potentiated [420, 422]. Additionally,

TSLP and IL-15, produced by stromal cells and fibroblastic reticular cells (FRCs) for example, appear to support the development and survival of NCR^+ ILC3, but not LTi-like ILC3, in the intestinal tract [423]. Strikingly, LTi-like ILC3 remain abundant even without the IL-7Rα signal, by expressing anti-apoptotic transcript Bcl2 [423].

In humans, $CD34^+$ hematopoietic progenitor cells (HPC) from neonatal umbilical cord blood (UCB) can give rise to ILC3 [424]. In addition, in adult human secondary lymphoid tissues (SLTs), ILC-restricted, RORγt-expressing progenitors are found, termed $Lin^-CD34^+CD45RA^+CD117^+IL\text{-}1R1^+ROR\gamma t^+$ progenitors, and can give rise to ILC3 subsets in vitro [377, 415, 416, 425]. They are exclusively found in tonsils, lymph nodes, lamina propria and the spleen, indicating these are the preferential sites for ILC3 development [415]. These progenitors express a range of ILC3 signature genes, including CXCR6, NCRs, ID2, KIT, IL23R, and IL1R, but not ILC3-related cytokine genes, such as IL-22, IL-2, CCL20, and CSF2. Interestingly, when activated, $CD34^+ROR\gamma t^+$ HPC display high IL-17F transcripts and can produce IL-17F and IL-17A, but little IL-2 and IL-22. Differentiation of ILC3s from $CD34^+ROR\gamma t^+$ HPC was supported by a cytokine mix containing combinations comprised of IL-7, IL-15 and SCF which maintain the expression of RORγt. AHR is expressed in these precursors and the impairment of the AHR signal leads to the loss of, CD117, NCR, and IL-22 expression, while promoting NK-related markers, including Eomes and CD94 [415]. Moreover, IL-1β inhibits ILC3 proliferation, RORγt expression, and IL-22 production, but drives HPC to NK cell differentiation with increased expression of EOMES, LFA-1, CD94, and cytolytic granules, and the secretion of IL-8 and IFNγ was also detected

[426]. Human ILC3 also display plasticity: upon IL-12 and IL-15 stimulation, ILC3 acquire features of early differentiated NK and increase their cytotoxicity [427].

6.1.1.3 ILC3 functions and their role in skin inflammatory conditions

NCR^+ ILC3 can be activated by extrinsic and intrinsic signals through the expression of cytokine receptors, including IL1β, IL-23, IL-12, IL-2 and IL-15 [428], and their signalling regulates the proliferation and effector functions of ILC3. IL-1β and IL-23 are essential for promoting IL-22 cytokine production in mature NCR^+ ILC3 [380, 388, 429]; however, the impairment of IL-1R1 signal diminishes IL-23-induced IL-22 [429]. Additional evidence in studies using NCR^+ ILC3 from human tonsils have showed that IL-1β alone cannot induce IL-22 production, but, in addition to IL-23, there is augmented IL-22 and IFNγ secretion detected *in vitro.* [430]. Furthermore, IL-1β acts synergistically with IL-7-induced NCR^+ ILC3 expansion, and induces constitutive production of IL-22. Some proportion of NCR^+ ILC3 cultured in IL-1β plus IL-7 gain the ability to produce IL-17. Other than IL-1β, IL-2 can work together with IL-7 to promote NCR^+ ILC3 proliferation and induce RORγt expression. The combination of IL-23, IL-1β and IL-7 in the culture amplifies IL-22 and IFNγ of NCR^+ ILC3 [430] (Fig 6-3).

Next, studies showed that ILC3 possess the ability to modulate adaptive immunity through antigen presentation [431]. LTi-like ILC3 express MHC-II and can present antigens, yet cause bidirectional effector functions. One study has shown that this interaction does not cause T cell proliferation: intrinsic depletion of MHC-II on LTi-

like ILC3 results in imbalanced $CD4^+$ T cell functions in mice, exhibiting spontaneous intestinal inflammatory response toward commensal bacteria [432]. Remarkably, MHC-II expressed on LTi-like ILC3 can serve functionally as thymic epithelial cells to negatively select commensal bacteria–specific T cells by inducing cell death, and establish tolerance [433]. A second study on mice, on the other hand, has indicated that peripheral LTi-like ILC3 can up-take and process protein antigens, and up-regulate surface MHC-II molecules and other co-stimulatory molecules upon IL-1β stimulation, inducing $CD4^+$ T cell proliferation and priming T cell-dependent B cell responses [434]. Collectively, these reports suggest LTi-like ILC3 either directly keep $CD4^+$ T cell function at bay, or induce their activation, through MHC-II. Further, murine ILC3 from a variety of lymphoid tissues express CD1d with LTi-like ILC3 displayed an incremental amount of CD1d. The CD1d molecules on these ILC3 are functionally active to present lipids to invariant natural killer T (iNKT) cells. Other than lipid antigen presentation, loading of lipid antigens to CD1d is capable of promoting ILC3 to produce IL-22 to modulate immune responses [435].

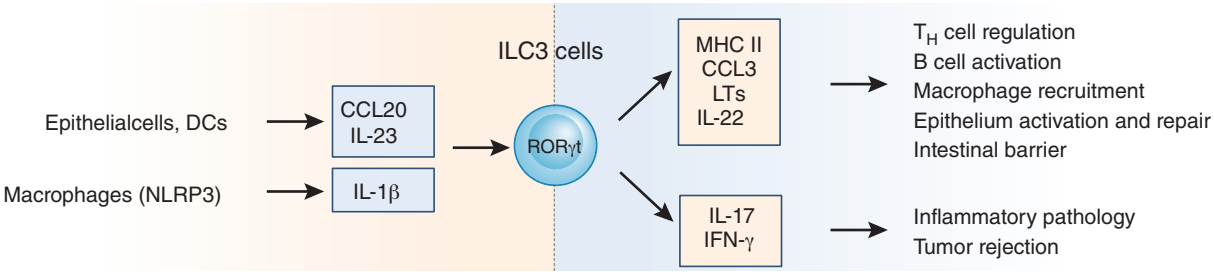


Figure 6-3. Summary of ILC3 functions.

ILC3 serve pleiotropic roles. Figure was modified and reprinted by permission from Springer Nature: Eberl, G., J.P. Di Santo, and E. Vivier, *The brave new world of innate lymphoid cells.* Nat Immunol, 2015. **16**(1): p. 1-5.

Both subsets of ILC3 cells are involved in maintaining the barrier function of the skin, with higher frequency of NCR^- ILC3 present in steady-state skin [436, 437]. Teunissen et al. and Villanova et al. have described the composition and functional changes of ILC3 in blood and skin of psoriasis patients. Their data showed that NCR^- ILC3 can convert into NCR^+ ILC3 through *in vitro* culturing with IL-23 and IL-1β. These blood NCR^+ ILC3 produce IL-22, the cytokine known to be involved in psoriasis pathology, and express CLA, implying they have skin homing potential. Notably, even in the non-lesional psoriatic skin, NCR^+ ILC3 frequency is elevated compared to skin from healthy donors. A therapeutic treatment of psoriasis with anti-TNF has showed a correlation between psoriasis disease severity with decreased peripheral NCR^+ ILC3 frequency, reflecting that the peripheral NCR^+ ILC3 might be the key player/responder in psoriasis inflammation [437, 438]. Bruggen and colleagues also reported AD skin harboured a larger frequency of ILC3 residing in close proximity to T cells next to the epidermis in comparison to healthy human skin [439]. An inducible psoriasis model was established using a humanised mouse on which healthy human skin was transplanted and revealed further understanding of the disease. The characteristic psoriatic phenotypes were induced on the xenotransplants by intradermal injection of purified pre-activated autologous $ROR\gamma t^+$ ILC3 only, indicating that ILC3 alone are able to drive the development of the psoriasis, even in the absence of T cells and other effector cells [440].

Other than inflammatory diseases, ILC3, particularly LTi-like ILC3, play a role in skin wounds. In human skin punch biopsy wounds, ILC3 appear to infiltrate into the wound site. In mice, LTi-like ILC3 are recruited near to the wound site by upregulated danger signal Notch1 in the epidermis in a TNFα-dependent fashion. These LTi-like ILC3 express CCR6, CXCR4 and CXCR5 for chemotaxis, and produce predominately IL-17F and CCL3 which promote keratinocyte proliferation and macrophage migration, respectively. Further, RORγt$^+$ ILC3-deficient mice have a defective wound healing process [441].

6.1.2 Psoriasis

Psoriasis is a chronic inflammatory skin disease with signature plaque-like, scaly lesions and thickened epidermis which are caused by hyperproliferation of the epidermal cells, infiltration of leukocytes and altered vasculature [112, 442, 443]. The plaques are characterised with silvery dry scales and well-defined borders, and are usually located on elbows, knees, and back [444]. The immunopathogenesis of psoriasis is a complicated process, at both the cellular and genetic levels [445], and can be linked to environmental factors, including the Koebner phenomenon (physical injury to the skin), allergen induced inflammation, drugs, bacterial and viral infections [107, 446-448].

6.1.2.1 Development of psoriatic plaques

The commencement of psoriatic lesion initiates with a robust infiltration of leukocyte into the skin in response to signals thought to be released by injured keratinocytes [444]. Neutrophils are the first wave of cell infiltrate [449, 450]. Mature DCs and T cells accumulate near the blood vessels in the early skin lesion, and form a unique structure similar to that of the T-cell zone of lymph nodes. PDCs also come into the early psoriasis lesion and drive the onset of the disease through their ability to release IFN-I as mention in §1.2.4 [112, 451]. Injured keratinocytes can proliferate vigorously in response to the leukocyte infiltrates and their products, forming psoriatic plaques that can persist for years. The epidermal modification of the skin in psoriasis patients is considered as a T cell-mediated autoimmune event [452]. Immunohistological features of chronic psoriasis lesions showed predominately $CD4^+$ T cells in the dermis, whereas, in the lesional epidermis, the majority of T cells are CD8 positive [453, 454].

Recent studies suggest an essential involvement of the IL-23 and IL-22 axes [455, 456]. In human psoriatic skin lesions, keratinocytes are the source of IL-23 [457, 458] which triggers the production of IL-22 from T cells or ILC3 [459]. IL-22 is found to elevate the expression of S100A7, S100A8 and S100A9 in keratinocytes, and induces keratinocyte proliferation and migration [286]. In addition, anti-bacterial peptide defensins are upregulated by IL-22 in skin epithelial cells, which modulate normal keratinocyte differentiation [286]. Dysregulated production of IL-22 can lead to exacerbation of psoriasis with unrestrained proliferation of keratinocytes which leads to hyperplasia [458, 460, 461]. In the serum of psoriasis patients, IL-22

correlates with the severity of the disease [289]. IFNγ, secreted by Th1 cells and other cells, is also thought to contribute to psoriasis pathology [454]; it is detected in psoriatic plaques and increased in the serum of psoriasis patients [462]. It has been shown to program DCs to produce IL-1β and IL-23 which in turns activates T cells, and induces DCs to produce CCL20 to recruit T cells. Working together with IL-17A, both cytokines synergise the production of defensins [454]. Growing evidence has demonstrated the importance of IL-17 family members in psoriasis as well [114, 463]. IL-17A and IL-17F are all detected in increased level in psoriatic lesional skin, and IL-17-producing T cells are found localised in the dermis of psoriatic lesions [464-467]. Notably, the expression of IL-22 is in parallel with both IL-17A and IL-17F; and IL-22 combined with either IL-17A or IL-17F synergistically induces antimicrobial peptide production by keratinocytes [468]. On the genetic level, it has been shown that mutations in alleles involving IL-17/IL-17R and IL-23/IL-23R signalling pathway are associated with psoriasis and other autoimmune diseases [117, 469-474], which further strengthen the relevance of IL-23-IL-17 axis in psoriasis (Fig. 6-4). Evidence supporting a role of these pathways also comes from the effects of specific therapeutic intervention which is discussed below. A less common, but potentially lethal form of psoriasis is called generalised pustular psoriasis (GPP) which is characterised by widespread pus-filled blisters, accompanied by repeated developments of fever [475, 476]. The triggers of the disease are unclear, but potentially associate to viral infections or drug exposures [476]. Genetic studies have revealed that mutations of *IL36RN*, coded an anti-inflammatory cytokine IL-36 receptor antagonist (IL-36Ra), result in the pathology of GPP [476-479]. The identification of IL36RN mutations highlights the importance of IL-36 pathway in some forms of psoriasis and identifies this pathway as a potential

therapeutic target. Recent studies have also confirmed that, besides T cells and DCs, other cell types, such as ILC3 and mast cells [439, 480-483], can be responsible for producing the aforementioned cytokines and are important in psoriasis pathogenesis which warrants further investigation of their therapeutic relevance.

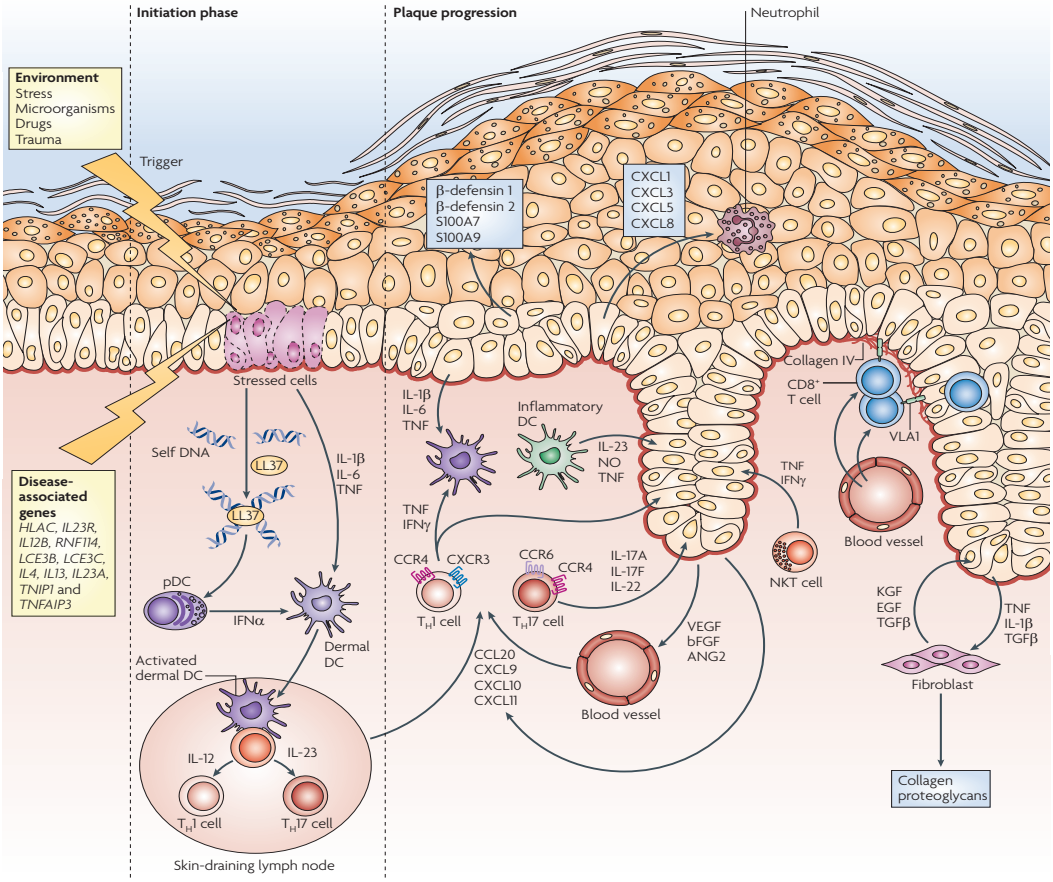


Figure 6-4. Development of psoriatic plaque.

Environmental factors were thought to be one of the triggers of psoriasis in genetically predisposed individuals. Self DNA/RNA released from the dying keratinocytes that forms complexes with the cathelicidin LL37 to induce IFN-I production from pDCs. IFN-I, IL-1β, and TNFα activate dermal DCs to release inflammatory cytokines and migrate to the skin-draining lymph nodes to promote T cell differentiation into Th1 and Th17 cells. These T cells migrate into skin and secrete IFNγ or IL-17A, IL-17F and IL-22, which stimulate keratinocyte proliferation and the release of antimicrobial peptides, leading to the formation of psoriatic plaques. Figure was adapted and reprinted by permission from Springer Nature: Nestle, F.O., et al., *Skin immune sentinels in health and disease.* Nat Rev Immunol, 2009. **9**(10): p. 679-91.

6.1.2.2 Therapeutic targets of psoriasis

Several therapeutic drugs have been developed to target the immune cells or their effector cytokines in order to treat psoriasis [463]. Neutralising antibodies against IL-12/23p40 have proven to be effective modalities for treating psoriasis with most patients achieving clinically meaningful results; however, a small group of patients exert only a partial improvement [484-486]. Treatment of patients with the AHR agonists reduces the inflammatory response in the skin of psoriasis patients [487]. Anti-TNFα in psoriasis treatment has been shown to be capable of reducing inflammatory cell infiltrates and cytokines, including IL-22 and IL-17, in psoriasis skin, with a delayed effect on IFNγ production. Collectively, psoriasis disease is improved after anti-TNFα treatment [488-490]. Several antagonists toward IL-17A or its receptor, such as secukinumab, brodalumab and ixekizumab [466, 491-500], and human monoclonal antibodies targeting IL-23 p19, such as risankizumab, ruselkumab, and tildrakizumab [498, 501-503], have demonstrated promising results in psoriasis clinical trials. Together, these clinical studies provide further evidence that T cell-related cytokines play a central role in sustaining psoriasis pathology.

Despite the effectiveness of anti-TNFα in the treating of psoriasis, a small percentage of the treated patients develop paradoxical psoriasis exacerbation. This side-effect is characterised by a prolonged skin accumulation of pDCs and their product, IFN-I, while displaying a reduced frequency of T cells in the skin lesions, which is different from classic psoriasis where T cells are highly present. Studies have

shown that blocking of TNFα results in delayed pDC maturation, causing exaggerated IFN-I production to trigger the psoriasis-like side-effect [504]. Collectively, this further emphasised the complexity of psoriasis; therefore, a thorough and comprehensive understanding of the mechanism behind this disease is of therapeutic interest.

6.1.3 Aims

As has been described in the previous chapters, pDCs and IFN-I were important components of skin immune compartments. Therefore, I aimed to study whether communications are established between pDCs and other immune cells in the system. Duerr and colleagues reported the case of IFN-I regulating the cytokine production and the survival of ILC2 in viral respiratory tract infections, indicating the potential interaction between ILCs and pDCs [505]. Here, the hypothesis was investigated that ILC3 can be regulated by pDCs through cell surface molecules or soluble mediators to contribute to skin inflammation, such as psoriasis. This study will provide new therapeutic insights for skin disease modulation.

6.2 Results

6.2.1 ILC3 response to IFN-I

Under homeostatic conditions, pDCs are absent in human skin [98] (Figure 3-1); however, they can infiltrate into psoriasis lesions sites at an early stage and their production of IFN-I contributes to the onset of the disease [97]. We first investigated the involvement of IFN-I in skin inflammatory conditions. By analysing the bulk RNA-sequencing data previously generated in the lab, in which an ILC1/3 mixed population was extracted *ex vivo* from HDM pre-treated skin blisters. Compared with blood ILC1/3, there were significant upregulation in ISGs, such as *IFITM1*, *IFITM2*, *IFIT3*, and *OAS2* (Fig. 6-5). To validate, ILC3, defined as $Lin^{-}CD45^{+}CRTH2^{-}IL\text{-}7R\alpha^{+}c\text{-}Kit^{+}$, were isolated and expanded from the blood of healthy donors (Fig. 6-6A). After stimulated with human IFN-I, the mRNA of *OAS2* was elevated in these cells, suggesting IFN-I can act on blood ILC3 (Fig. 6-6B).

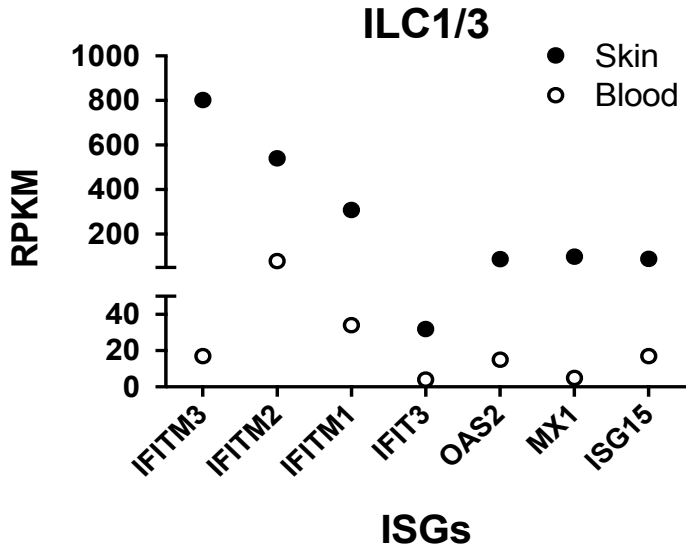
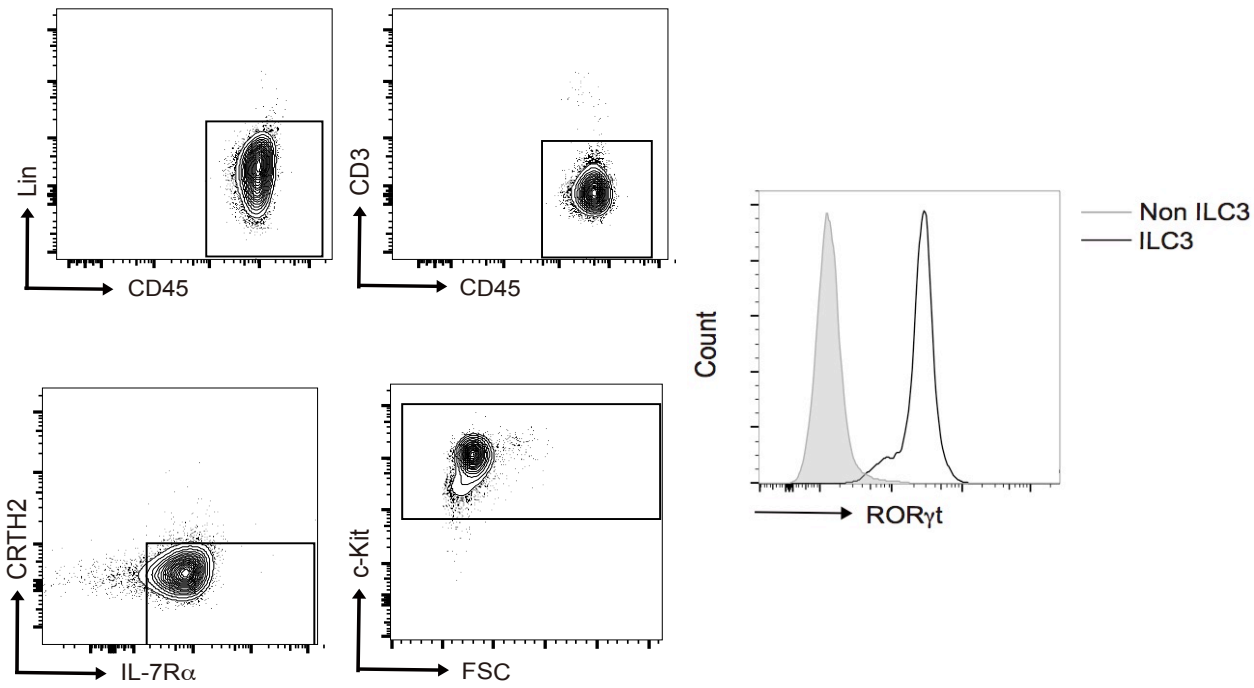


Figure 6-5. ILC upregulated ISGs in the skin.

(A) ILC1/3s isolated from blood or HDM-provoked blisters and subjected to RNA-sequencing analysis. ISGs reads of skin-infiltrated ILC1/3 were compared with those of blood counterparts. RPKM as Reads Per Kilobase of transcript per Million mapped reads. A single donor was recruited for this data set.

A



B

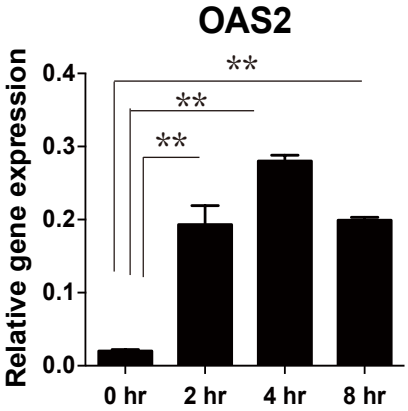


Figure 6-6. ILC3 upregulated ISGs in response to IFN-I.

(A) Cultured blood ILC3 were investigated for their surface marker phenotype. The ILC3 were $Lin^{-}CD45^{+}CRTH2^{-}IL\text{-}7R\alpha^{+}c\text{-}Kit^{+}$ and expressed high levels of RORγt. (B) Cultured blood ILC3 were stimulated with IFN-α (Roferon-A, 500 IU/ml) at indicated time-points, and subjected to quantitative RT-PCR analysis. Expression of *OAS2* was evaluated. Representative of three ILC3 lines. **P < 0.01, one-way Anova; Tukey's multiple comparisons post hoc test was performed.

6.2.2 IFN-I regulated the survival, cytokine production, and marker expression of blood ILC22

As Lin$^-$CD45$^+$CRTH2$^-$IL-7Rα^+c-Kit$^+$ ILC3 contained both NCR$^+$ ILC3 and LTi-like ILC3, and the NCR$^+$ ILC3 subset have been reported to play essential roles in skin inflammatory conditions [437, 438], the following experiments were pursued using Lin$^-$CD45$^+$CRTH2$^-$IL-7Rα^+c-Kit$^+$NKp44$^+$ ILC3 (ILC22), which were found to only produce IL-22, but not IL-17A. To determine whether IFN-I influenced IL-2-induced ILC22 proliferation, blood ILC22 were cultured in IL-2-supplemented culture, with or without additional IFN-I. A reduced proliferation rate of ILC22 after IFN-I treatment was observed (Fig. 6-7), indicating a negative effect of IFN-I on ILC22. Next, it was examined whether the effector functions, such as cytokine producing ability and surface marker expression, of ILC22 can be altered by IFN-I. Blood ILC22 were stimulated with IL-1β and IL-23 cytokine mixture for 72 hours, and the IL-22 protein concentration in the culture was measured. The addition of IFN-I to the cell culture inhibited the IL-22 secretion. Notably, IFN-I alone had no effect on the cytokine production (Fig. 6-8). In terms of surface activation markers, IFN-I can induce an upregulation of co-stimulatory markers including CD69, CD40, HLA-DR and HLA-A,B,C, and IFN-I worked synergistically with IL-1β and IL-23 to increase a further elevation of all markers, while ICAM-1 showed an opposite trend (Fig. 6-9). Interestingly, IL-1β and IL-23 cytokine mixture could only significantly upregulate CD69, HLA-DR and ICAM-1, but not CD40 and HLA-A,B,C. Moreover, IFN-I altered the phenotype of ILC22 by enhancing the expression of c-Kit and NKp44 (Fig. 6-10);

however, whether this phenomenon has an effect on the function of ILC22 remains unclear.

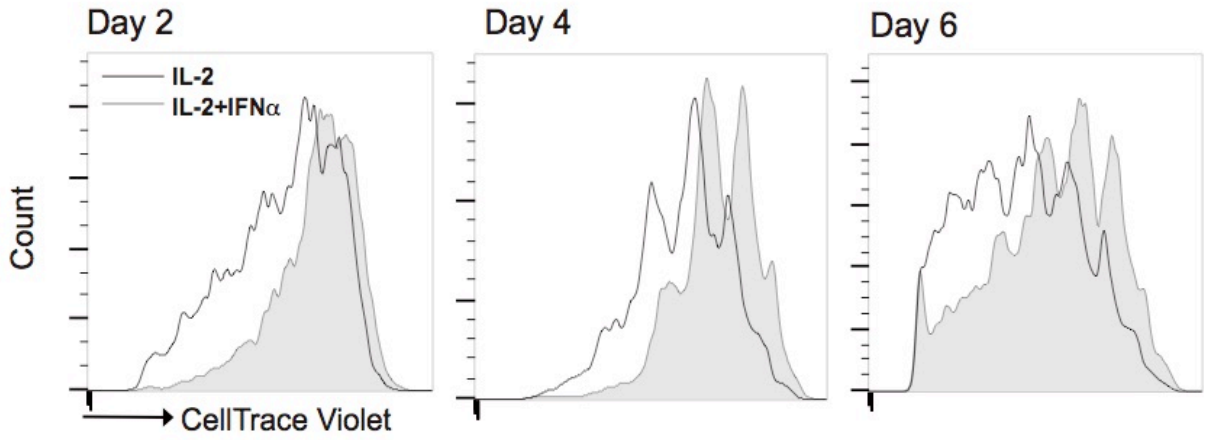


Figure 6-7. IFN-I inhibited IL-2 induced ILC22 proliferation.

Cultured blood ILC22 labelled with CellTrace violet and stimulated with or without IFN-α, and analysed by flow cytometry at the indicated time points. Representative of five ILC22 lines. Black line represented IL-2 alone, and grey line with filled histogram represented IL-2 plus IFN-I.

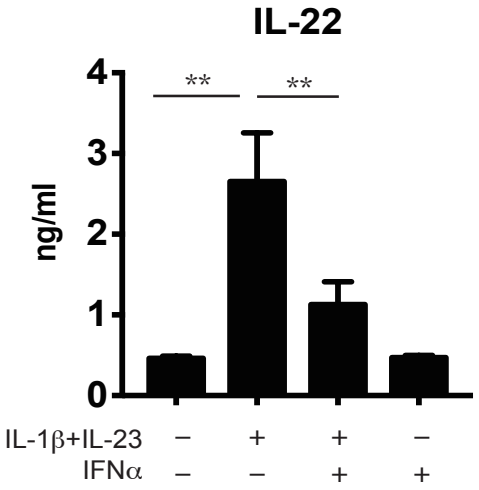


Figure 6-8. Blood ILC22 showed a reduce ability to produce IL-22 after IFN-I treatment.

Cultured blood ILC22 treated with IL-1β (50 ng/ml) and IL-23 (50 ng/ml) for 72 hours in the presence or absence of IFN-I (500 IU/ml). Supernatant was collected, and IL-22 concentration was measured by ELISA. Representative of eleven ILC22 lines. **P < 0.01, one-way Anova; Tukey's multiple comparisons post hoc test was performed.

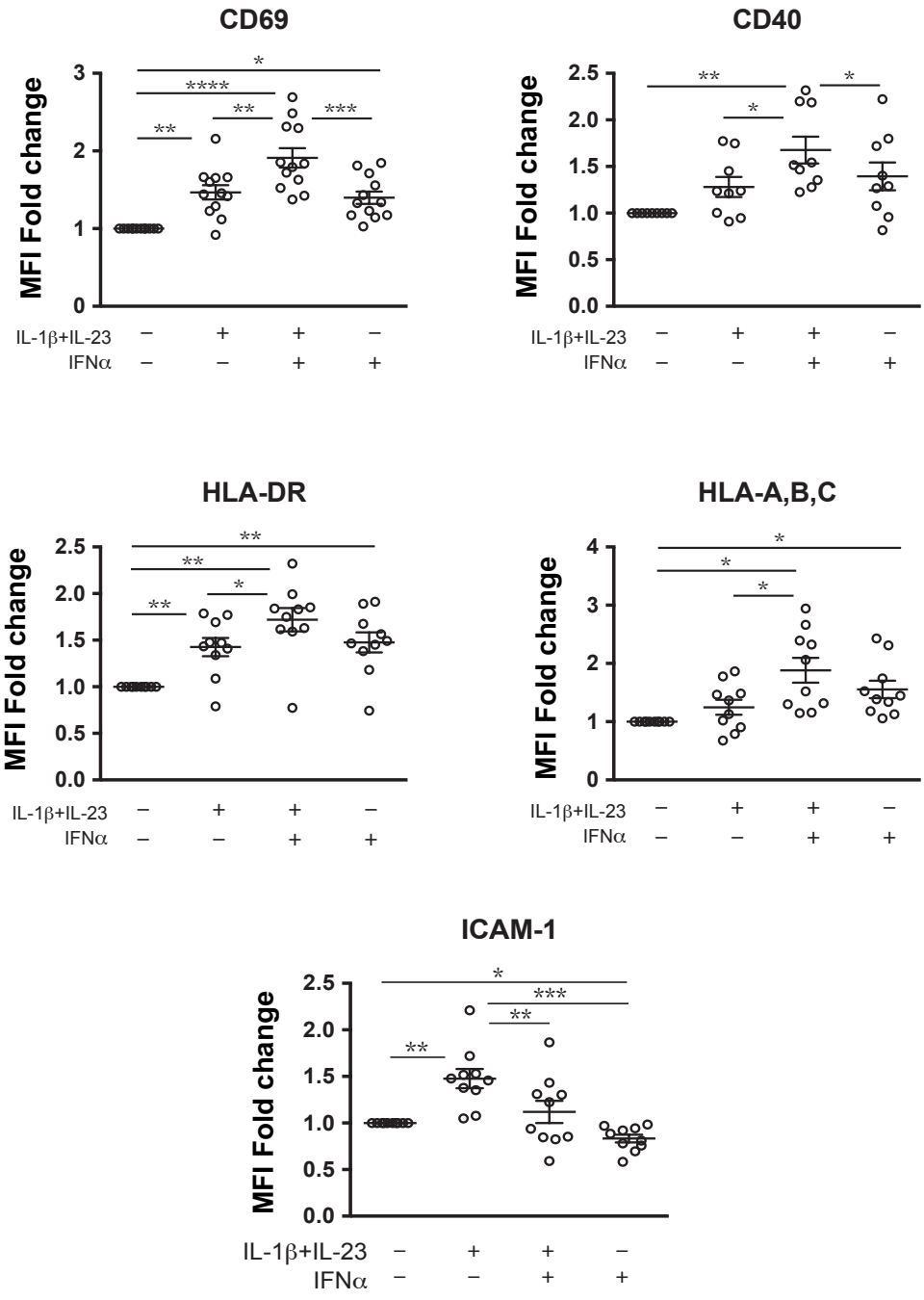


Figure 6-9. Blood ILC22 showed an elevated activation marker expression after IFN-I treatment.

Cultured blood ILC22 treated with IL-1β (50 ng/ml) and IL-23 (50 ng/ml) for 72 hours in the presence or absence of IFN-I (500 IU/ml). MFI fold-change of CD69, CD40, HLA-DR, HLA-A,B,C, and ICAM-1 expression were analysed by flow cytometry. All treatments were normalised with control group. *P<0.05; **P < 0.01; ***P < 0.005; ****P < 0.0001, one-way Anova; Tukey's multiple comparisons post hoc test was performed.

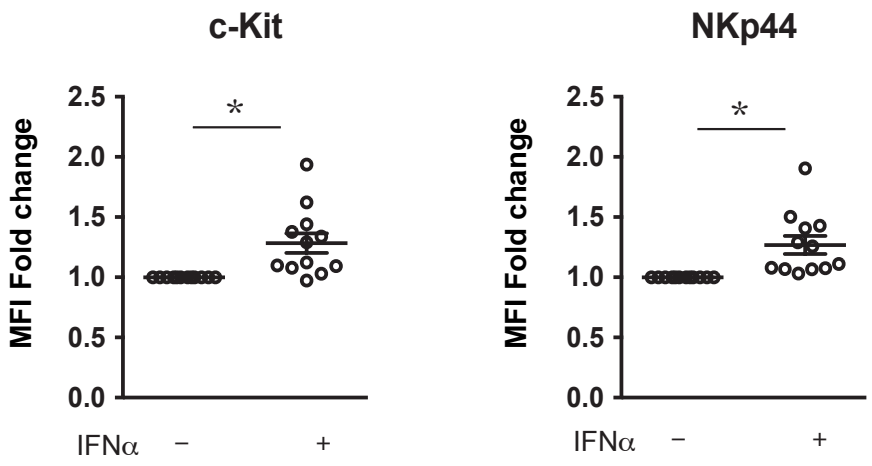


Figure 6-10. Blood ILC22 displayed an altered phenotype after IFN-I treatment.

Cultured blood ILC22 cultured with or without IFN-I (500 IU/ml). MFI fold-change of c-Kit and NKp44 expression were analysed by flow cytometry. All treatments were normalised with control group. *P<0.05, **P < 0.01; ***P < 0.005; ****P < 0.0001, Paired student's t test.

6.2.3 IFN-I modulated skin ILC22

Skin mononuclear cells were next isolated from normal skin, and treated with IFN-I. The frequency of ILC22 was not altered by IFN-I, suggesting IFN-I failed to transform ILC22 from other ILC subsets (Fig. 6-11A). However, IFN-I increased the expression of CD69, consistent with the findings of the blood ILC22 (Fig. 6-11B). Due to the limited number of skin cells available in skin mononuclear cells, for further investigation, skin ILC22 were sorted and expanded *in vitro*. In line with the observation on blood ILC22, IFN-I enhanced the expression of c-Kit and NKp44 expression on skin ILC3 (Fig. 6-12A). In addition, IL-1β, IL-23 and IFN-I synergised to induce a further upregulation of CD69 (Fig. 6-12B).

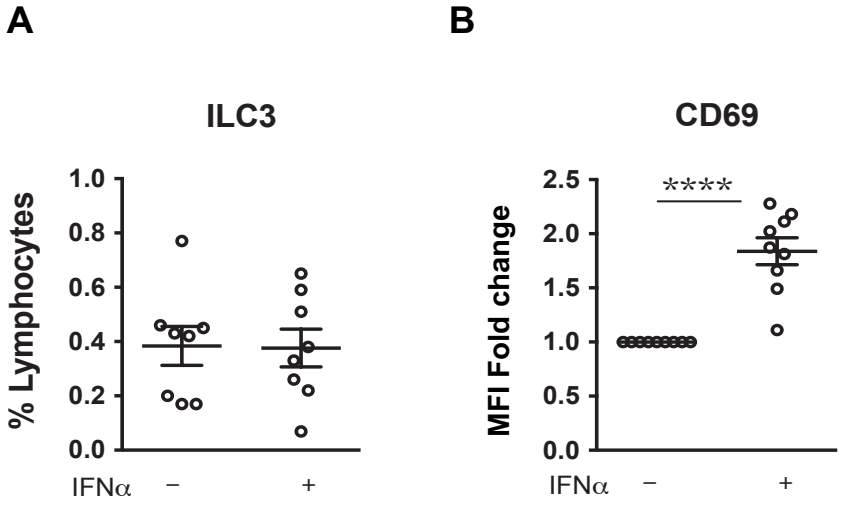


Figure 6-11. The frequency of ex vivo skin ILC22 remained stable after IFN-I treatment.

Skin mononuclear cells treated with IFN-I for 48 hours in the presence or absence of IFN-I (500 IU/ml). (A) Frequency of ILC22 and (B) MFI fold-change of CD69 expression by ILC22 were analysed by flow cytometry. All treatments were normalised with control group. ****P < 0.0001, Paired student's t test.

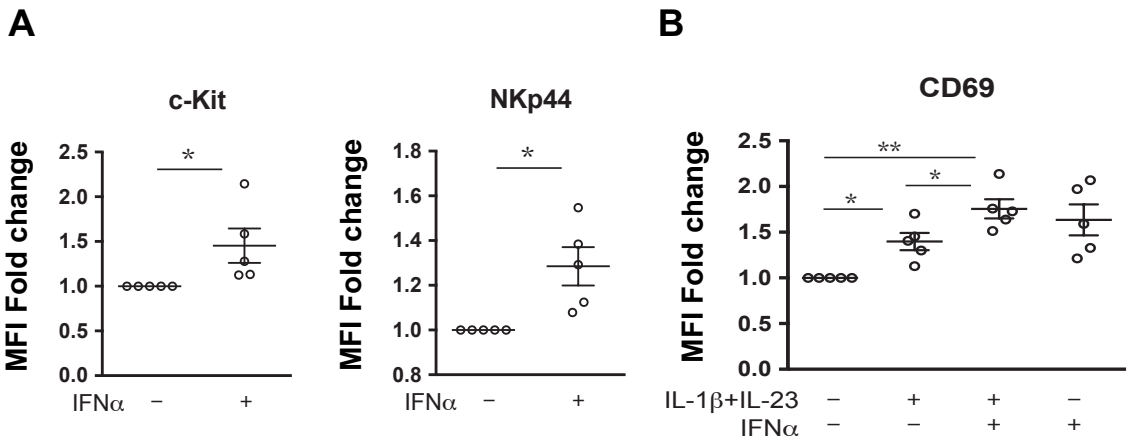


Figure 6-12. Skin ILC22 displayed elevated markers after IFN-I treatment.

Cultured skin ILC22 were treated with indicated cytokines for 72 hours. MFI fold-change of (A) c-Kit, NKp44, and (B) CD69 expression were analysed by flow cytometry. All treatments were normalised with control group. *P<0.05, **P < 0.01; ***P < 0.005; ****P < 0.0001, Paired student's t test or one-way Anova; Tukey's multiple comparisons post hoc test was performed.

6.2.4 PDC produced IFN-I modulated blood ILC22 functions

To determine if pDCs were the main source of IFN-I, blood pDCs were placed onto the upper chamber of the transwell system and imiquimod was used as a stimulus. ILC22 were seeded into the lower chamber under the permeable membrane. Following a 16-hour incubation period, the pDCs were removed, leaving ILC22 to be treated with IL-1β and IL-23 for 72 hours (Fig. 6-13). The IL-22 production was inhibited (Fig. 6-14A) and CD69 expression was upregulated (Fig. 6-14B) on the ILC22, which were primarily mediated by soluble factors secreted by imiquimod-stimulated pDCs. Concomitantly, neutralisation of IFNAR1 restored the IL-22 (Fig. 6-14A) and CD69 (Fig. 6-14B) levels. The results supported the hypothesis that pDCs can produce IFN-I to regulate ILC22 functions.

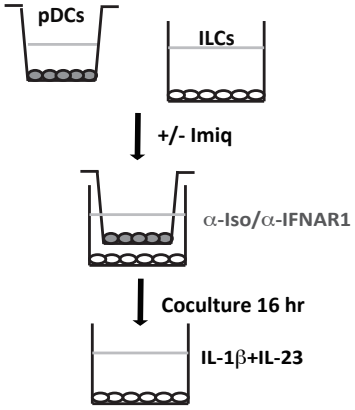


Figure 6-13. The scheme of transwell setup.

Blood pDCs seeded onto the upper chamber of the transwell system and treated with or without imiquimod (1 μg/ml). ILC22 were seeded into the lower chamber below the permeable membrane. After coculturing for 16 hours, the pDCs were removed. ILC22 were then treated with IL-1β (50 ng/ml) and IL-23 (50 ng/ml) for 72 hours.

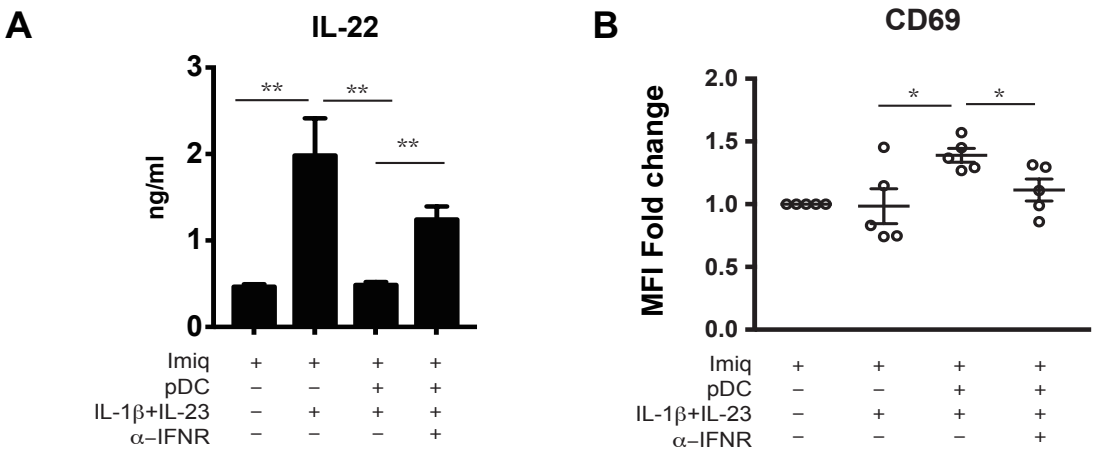


Figure 6-14. pDCs secreted IFN-I inhibited IL-22 secretion from blood ILC22.

(A) pDC-stimulated ILC22 were cultured with IL-1β (50 ng/ml) and IL-23 (50 ng/ml) for 72 hours. (A) IL-22 was measured from the supernatant, and (B) MFI fold-change of CD69 expression were analysed by flow cytometry. All treatments were normalised with imiq alone treatment. *P<0.05; **P < 0.01, one-way Anova; Tukey's multiple comparisons post hoc test was performed.

6.2.5 IFN-I rescued the antigen presenting ability of ILC22 to CD4$^+$ T cells

As it has been recently appreciated that ILC3 can mediate adaptive immunity through antigen presentation; it was therefore investigated whether IFN-I can alter the antigen presenting ability of ILC3. Tetanus toxoid (TT) is a nontoxic form of tetanus toxin (TTX), a soluble protein antigen derived from *Clostridium tetani*. TT is broadly used as a carrier in vaccines, and is regularly exploited in experimental conditions to identify human CD4$^+$ T cell epitopes [506-513]. Here, TT proteins were used as a tool to study the antigen presenting ability of ILC3. The common strategy undertaken in laboratory setting to study antigen-specific CD4$^+$ T cell response is to coculture T cells with APCs treated with different antigen loads which governs the functional and phenotypic profiles of these T cells. ILC22 were treated with IL-1β and IL-23 for 72 hours to induce the expression of co-stimulatory markers, then followed by different concentrations of TT stimulation, and their ability to present antigens to IFNγ-producing CD4$^+$ T cell clones were monitored by cytokine profiles of the T cells (Fig. 6-15A). These CD4$^+$ T cell clones do not express IL-17A after TT stimulation (Fig. 6-15A). The results showed that ILC22 were able to present antigens to CD4$^+$ T cells in a dose dependent manner. IL-1β and IL-23 mixture exhibit a negative effect on the antigen presenting ability of ILC22, whereas, the addition of IFN-I to the cytokine mixture exhibited a rescue effect (Fig. 6-15B). However, the underlying mechanism of this IFN-I-dependent regulation of antigen presentation requires further investigation.

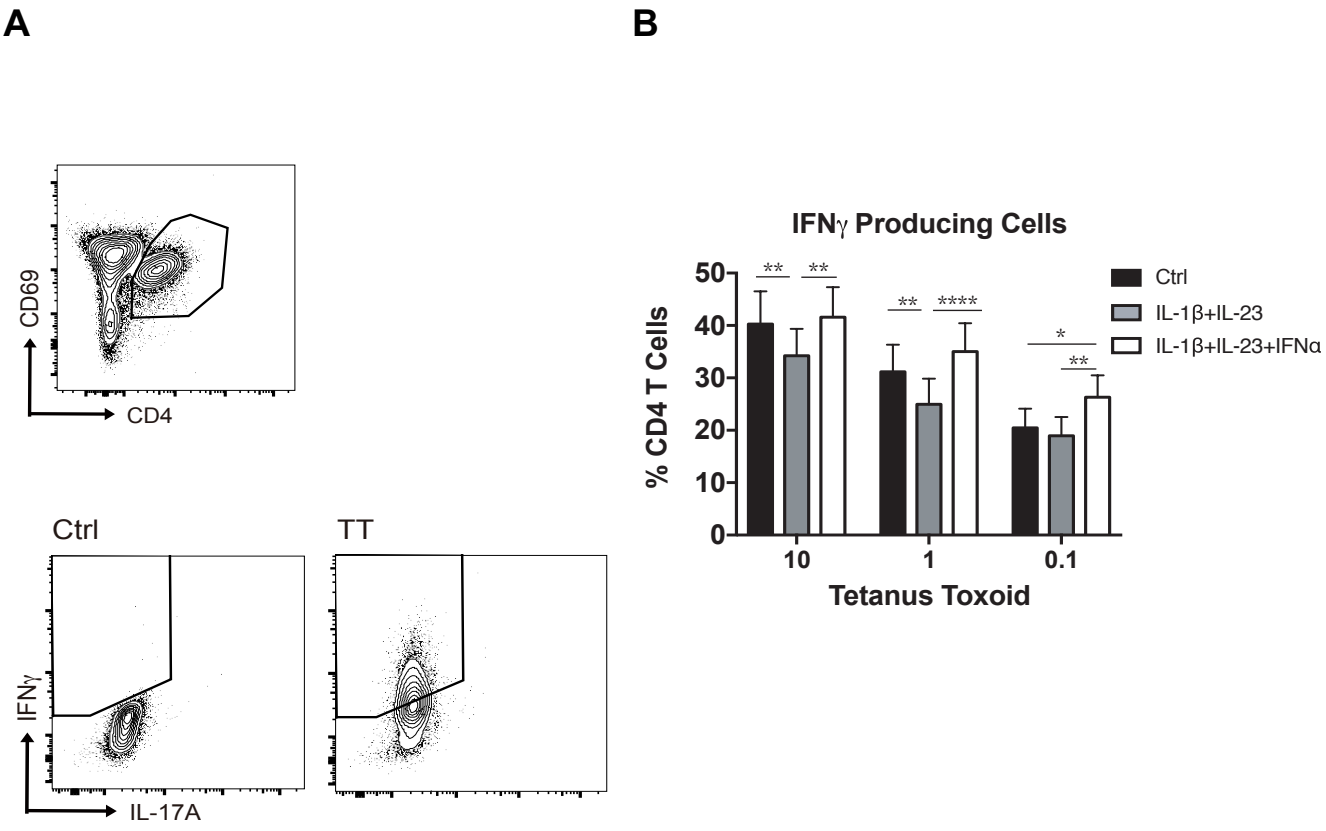


Figure 6-15. Phenotypic profile of IFNγ-secreting CD4⁺ T cells upon TT stimulation.

ILC22 were treated with indicated cytokines for 72 hours, and followed by different concentrations of TT stimulation (10-0.1 μg/ml). These ILC22 were cocultured with $CD4^+$ T cell clones (generous gift of Dr. Mariolina Salio), and surface markers were stained to define $CD4^+$ T cells to measure their IFNγ production by ICS after 24 hours. (A) Representative FACS plot. $CD4^+$ T cells were identified as $CD4^+CD69^+$ cells. IFNγ was detected in these cells after TT-stimulation of ILC22. (B) Percentage of TT-specific IFNγ-secreting $CD4^+$ T cells at the indicated TT concentration. Representative of five experiments. *P<0.05; **P < 0.01; ****P < 0.001, one-way Anova; Tukey's multiple comparisons post hoc test was performed.

6.2.6 Psoriatic ILC22 were more susceptible to IFN-I treatment

As described above, ILC22 and pDC are both elevated in the skin of psoriasis patients, and IFN-I are reported to contribute to aggravation of the disease. Here, ILC22 were isolated and expanded from blood of adult psoriasis patients, who suffered from stable moderate-severity plaque psoriasis, but not yet on systemic therapy. One to three lLC22 lines were generated per donor, and the psoriatic ILC22 were treated with IFN-I, and the variety of marker expression on psoriatic ILC22 were tested, and compared to healthy controls. Indeed, the addition of IFN-I induced a higher upregulation of activation markers CD69 and CD40, compared to healthy individuals. However, psoriatic ILC22 failed to elevate HLA-DR in respond to IFN-I. Remarkably, IL-1β and IL-23 increased the expression of ICAM-I on psoriasis ILC22, and IFN-I alone also shared a similar effect (Fig. 6-16), while the opposite was observed in control lLC22. Table 6-17 summarised the information of healthy and psoriasis donors, and it showed a diversity in age and sex, but not in ethnicity group, suggesting a fair comparison was conducted in this experiment. Collectively, psoriatic lLC22 displayed an overall heightened response toward lFN-I signal; nevertheless, whether these hyperreactive phenotypes of psoriatic ILC22 were accountable for their contribution in psoriasis pathology remains a subject of interest and warrants the development of suitable psoriatic mouse models.

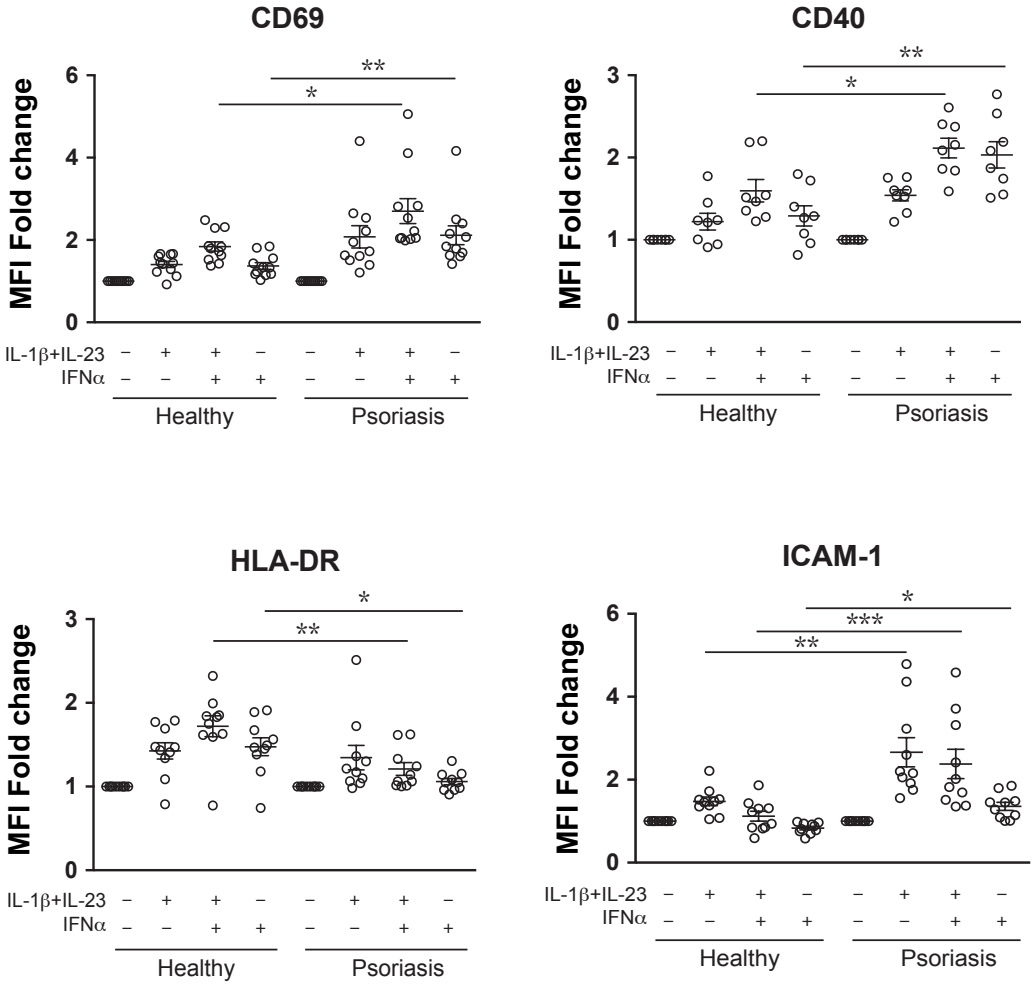


Figure 6-16. Psoriatic blood ILC22 showed a hyper activation in marker expression after IFN-I treatment.

Cultured healthy and psoriatic blood ILC22 were treated with IL-1β (50 ng/ml) and IL-23 (50 ng/ml) for 72 hours in the presence or absence of IFN-I (500 IU/ml). MFI fold-change of CD69, CD40, HLA-DR, and ICAM-I expression were analysed by flow cytometry. All treatments were normalised with each control group. *P<0.05; **P < 0.01; ***P < 0.005, one-way Anova; Tukey's multiple comparisons post hoc test was performed.

Patient ID	Age	Sex	Ethnicity	Condition
W059	34	F	Caucasian	Healthy
W036	49	M	Caucasian	Healthy
C1943	74	F	Caucasian	Healthy
C1945	81	F	Caucasian	Healthy
PS1912	40	M	Caucasian	Psoriasis, without systemic treatments
PS1949	44	M	Caucasian	Psoriasis, without systemic treatments
PS1959	69	F	Caucasian	Psoriasis, without systemic treatments
PS1980	55	F	Caucasian	Psoriasis, without systemic treatments

Table 6-17. Age, sex, ethnicity and conditions of the donor in figure 6-12.

6.4 Discussion

Overall, an experimental system has been established to examine the cross-talk between pDCs and ILC22. Initial data have shown that IFN-I secreted by pDCs attenuated the proliferation and cytokine secreting capacity of ILC22, yet enhanced some co-stimulatory marker expression and their ability to present specific antigens to $CD4^+$ T cells. In addition, the data collected from psoriasis patients supports the possibility of the direct interaction of pDCs and ILC22 contributing to the inflammation status of the disease.

Compelling evidence has shown that IFN-I have essential roles in immune regulation of ILCs. Duerr and colleagues reported that IFN-I inhibits the cytokine production of human ILC2. Using a mouse intranasal challenge model, IFN-I reduced IL-33-induced proliferation and type 2 cytokine production from ILC2, resulting downregulated numbers of eosinophils and DCs recruiting into the lung, which suggests that IFN-I are upstream regulators of ILC2-mediated immunity [505]. Other than induced inflammatory conditions, the role of ILC2 during viral infection in humans has been reported. For example, studies of HIV-1 Infection demonstrated that ILCs are depleted from the blood during acute HIV-1 infection and the phenomenon persists in chronic infection, which is caused by altering compositions and survival rates of ILC subsets. These peripheral ILCs express increased level of CD69, tissue homing markers, or death receptors, leading to tissue redistribution or increased apoptosis rate of ILCs, which explained the reduced frequency in the blood. The disappearance of ILCs has been shown to correlate with an elevated IFN-I level corresponding to viral replication [514]. In a humanised mouse model, persistent HIV-I infection depletes

human ILC3 from lymphoid organs in a pDC-IFN-I dependent cell apoptosis process, whereas antiviral treatment rescued the phenotype [515]. Notably, the loss of ILCs from the blood seems to be restricted to viral infection. For instance, ILC3 expansion instead were found in the filarial-infected individuals [516]. These aforementioned studies indicate a likelihood of immunological consequences of IFN-I on ILC populations. However, it has been a hitherto unappreciated possibility that IFN-I and ILC3 can interact in sterile skin inflammatory condition; therefore, this study provided potential implications for mechanisms of some skin diseases.

Losing cytokine producing ability and gaining antigen presenting capacity of ILC22 could potentially be explained by a similar observation found in NK cells. NK cells have been known to play important roles in both innate and adaptive immunity [517-521]. While conventional NK cells are shown to express no MHC-II; however, a subset of activated NK cells have been found to be MHC-II expressing and can direct $CD4^+$ T cell response to specific antigens [522-524]. It is speculated that, through cytokine production and direct cell-cell contact from other immune cells, NK cells are shaped to establish appropriate immune responses. Of note, impairment of IFN-I signalling results in defects in proliferation, cytotoxicity and antitumor activity of NK cells [147, 148]. It is plausible that ILC22 can be regulated in a similar manner, as ILC22 were converted from cytokine producers into APCs that can present antigens through MHC-II after IFN-I treatment. Nonetheless, the HLA-DR expression pattern of ILC22 after IFN-I treatment was not consistent with their antigen-presenting capacity, which attests the possibility of the involvement of other co-stimulatory markers, such as ICAM-1 (Fig. 6-9). Moreover, some ILCs survived the depletion process in viral infection end up showing enhanced anti-viral responses by upregulating CD69 [514].

This is consistent with my finding of elevating CD69 on ILC22 after IFN-I treatment, and a further shift on psoriatic ILC22 in which persistent IFN-I signals are presented in the skin of patients with psoriasis. This will potentially be investigated in future studies utilising the mouse model of imiquimod-induced psoriasiform disease.

Human keratinocytes release a spectrum of alarmins in response to damage, and among these, IL-1β has been recognised to be a key factor for inflammatory skin diseases [525-527]. As a result of scratching, IL-1β is released in uninvolved psoriatic skin, which synergised with IFNγ and TNFα to increase various chemokines, including CCL5, CCL20, CXCL9 and CXCL10, recruiting T cells to the wound which contributes to the pathogenesis of the inflammatory state [528]. In addition, IL-1β can directly regulate *IL23r* expression by ILCs, which acts together with IL-23 to induce the production of IL-22 [529]. Similarly to IFN-I, dysregulation of IL-1β can cause harm to the host; therefore, impeding excessive IL-1 signalling is necessary. Studies have shown that there is cross-talk between the IL-1 and IFN-I pathways in both directions [530-532]. Some have shown that IL-1β is able to inhibit the production of IFN-I and the phosphorylation of STAT1 which leads to the attenuation of subsequent activation of the IFNAR pathway [533-535]. On the other hand, without functioning IFNAR signalling, an upregulated IL-1-dependent IL-17 response can be observed in mice during infection [536]. In various immune cells, IFN-I can directly inhibit the transcription and translation of both IL-1α and IL-1β and the activation of inflammasome, while upregulating the IL-1 receptor antagonist (IL-1Ra) and IL-10 which in turn act negatively on IL-1 signalling *in vitro* and *in vivo* [535, 537-544]. In this study, another antagonistic system of IFN-I/IFNAR pathway on IL-1 driven immune

response is established, in which IFN-I impair IL-1β and IL-23 induced IL-22 production of ILC22, resulting in modulation of excessive IL-22 production in early skin wounds before a more specialised and controlled adaptive immune mechanism is initiated. Maintaining the balance between IFN-I and IL-1 function in contextual differences in disease pathogenesis can be the key to timely regulation of the wound healing process or the development of promising therapeutic approaches for inflammatory diseases, such as psoriasis where both IFN-I and IL-1β play a role.

In most skin inflammatory conditions, T cell functions are required for achieving skin barrier recovery or the development of disease pathology, and cellular cross-talks are the necessary hallmark to initiate T cell functions. PDCs, and their ability to engage in a complex dialog with T cells, have been broadly reported. They have been shown, in the literature and the chapter four of this thesis, to directly influence T cell activation through IFN-I secretion and antigen presentation [545]. Here, an indirect route was discovered that other innate immune compartments, such as ILC22, can be converted into efficient APCs by pDCs, bridging the innate and adaptive immunity in an alternative pathway. As addressed in chapter five, three DC populations resembling blood pDCs were identified in the skin wound: $CD123^{hi}$ DCs, AS DCs, and $CD123^{int}$ DCs. Among these subtypes, $CD123^{hi}$ pDCs and AS DCs still expressed high level of *IRF7* (Fig. 5-5 and 5-11), suggesting that they possess the ability to produce IFN-I. These two populations were potentially responsible for the ISG induction in the blister cells (Fig. 4-4 and 6-5). As attenuating IFN-I/IFNAR signalling often results in systemic infection and the development of malignancy, it is controversial approach in treating skin inflammatory diseases. Hence, the generation of $CD123^{int}$ DCs can be advantageous as they lose the ability to produce IFN-I, instead,

they can act in an antigen-specific fashion to assist the host to react in a timely manner to the wound and help clear associated pathogens. The identification of these previously unrecognised pathways provides new therapeutic targets for treatment of skin diseases in which both pDCs and ILC3 have a role.

7 Final Conclusions

Current understanding of the skin as a peripheral immune tissue highlights the role of immunity in barrier sensing. Compromising the skin barrier homeostasis, by external challenges or intrinsic dysregulation, can result barrier dysfunction. Skin wounds occur constantly in the host, either caused by unintentional means or by necessary surgeries, and so it is of high importance to the host to repair the damage rapidly and effectively, in order to prevent the invasion of microorganisms. Sensing and restoring skin tissue integrity has been proven to be a complex process and has prompted the development of appropriate model systems for study. Human skin suction blisters provide an opportunity to examine the immune mechanisms underlying the skin wound healing process. When the skin barrier function is breached by separating the epidermal plane from the dermis to form a skin suction blister, a robust number of immune cells, along with tissue fluid, infiltrate into the injury sites. The blister forms a reservoir which allow immune cells to interact with other hematopoietic cells, epithelial cells, and stromal cells. Without introducing a physical opening on the skin surface, the skin suction blister diminishes the likelihood of infection; therefore, skin suction blisters create an ideal human *in vivo* sterile wound model, minimising the complexity caused by foreign antigens in the skin wound healing studies. Furthermore, the cells and tissue fluid isolated from skin blisters, do not require further dispersion which may be associated with introduction of artefacts.

Another advantage of the blister system is that it allows a snap-shot of the dynamic milieu of the skin where skin resident immune cells and infiltrating cells can coexist. When the skin wound occurs, the skin resident cells can act rapidly to

stimulation and initiate the subsequent events, before either undergoing cell death or migrating out of the skin toward skin-draining lymph nodes. In the meantime, infiltrating cells from the circulation are recruited into the injury sites, where they transform into specialised effector cells, allowing a detailed phenotypic analysis of how the immune system is shaped by distinct tissue milieu. Here, the skin-infiltrating or resident DCs and ILC3 are captured and profiled in early skin wounds. Three pDC-like subpopulations were identified in this sterile skin wound: $CD123^{hi}$ DCs, AS DCs, and $CD123^{int}$ DCs which were CD1a-bearing. Transcriptomic analysis of the first two pDC-like subpopulation indicates that they were most likely to have originated from blood. A question remains about the ancestry of these $CD123^{int}$ DCs, as pseudo-time analysis was inconclusive (§ 5.2.3). It is speculated that these cells are recruited from the blood to the trauma site, or they are converted from skin-resident cDCs as the transcriptomic profile displayed similarity between these subsets. Phenotypic analysis of skin-related cytokine effects on both blood-derived pDCs, blood- and skin-derived $CD1c^{+}$ DCs shows that neither cDCs can be induced to display pDC-like surface markers in culture. It is recognised that an *in vitro* system may overlook the complexity of the cytokine profile of interstitial fluids [546]. The ILC3 are detected in the healthy skin biopsies and as well as in the blisters, and they can be recruited to the skin lesions. In chapter six, both blood and skin isolated ILC22 could be activated in a similar manner, suggesting some skin-mediated functions can be driven by blood migratory ILC3 subsets; however, whether these ILC3 subsets have specific capabilities which enable tissue-specialised roles requiring a more thorough high-throughput strategy.

The Koebner phenomenon was described in some psoriasis patients who developed psoriasiform lesions in the previously uninvolved skin after wounding,

including burns, scratches, allergic reactions, animal/insect bites, and surgical incision [547]. These wound-induced psoriasis lesions can develop in any part of the body, and it usually takes about 10 to 20 days for them to develop throughout the injury site, with some of them then resolving spontaneously later [548, 549]. Several environmental and intrinsic factors triggering this phenomenon have also been observed in psoriasis, including sun exposure, therapy/medication stimulation, scar formation and emotional stress, yet the underlying mechanisms of this phenomenon are still obscure. Interestingly, suction blister wounds only undergo Koebnerisation when the epidermis cap is removed from the wound [550]. In the event of skin injury, pDCs can infiltrate into the trauma site and produce IFN-I, which may be a potential explanation for the Koebner phenomenon, as IFN-I can initiate a signal cascade to govern the T cell-dependent psoriatic pathogenesis in patients with genetic disposition. This further emphasises the importance of effective immune regulation during the skin wound healing process.

IL-22 is produced by immune cells, such as T cells, NK cells, and ILC3, and its responders are mostly non-hematopoietic cells, such as epithelial cells in the skin. During the wound healing process, IL-22 exhibits opposing functions: helping with tissue remodeling or inducing keratinocyte hyperplasia [460, 551, 552]. Genetic defects in the IL-22/IL-22R axis also correlate with host's susceptibility to infections at barrier sites [553]. On the other hand, dysregulation of the IL-22 signal inhibits appropriate differentiation of keratinocytes, resulting in psoriasiform changes [286]. In chapter four, it was reported that communication between pDCs and T cells via CD1a can promote IL-22 production from T cells, while in chapter six, pDCs secreting IFN-I were found to exhibit a negative effect on the IL-22 producing capacity of ILC22. Thus,

pDCs appear to act as a double-edged sword, encouraging or hampering wound healing by controlling IL-22 concentration at the barrier surface. When pDCs were recruited to the site in the first wave of immune infiltrates, their production of IFN-I is required to establish appropriate immune responses of acute inflammatory cytokines and increased MHC-II expression on APCs to boost the host defence against microorganisms and clear cell debris [554-556], commencing subsequent T cell mediated immune consequences [98]. However, prolonged IFN-I signalling has been proven to be harmful to the host; IFN-I can also inhibit the proliferation of keratinocytes and delayed angiogenesis process of the wound, impairing timely wound closure [557-559]. Therefore, a well-balanced negative feedback system should be established. Several mechanisms have been addressed in §1.3.2, here, a novel regulatory route is presented: pDCs act according to tissue-specific signals and shift from cytokine-producers to potent APCs. This phenomenon can be related to the timing of the wound healing or the nature of the wounds, for instance, sterile or infected wounds. IFN-I production is reduced by $CD123^{int}$ DCs which leads to an increase of IL-22 production via ILC22, rapidly initiating the re-epithelialisation process. In addition, $CD123^{int}$ DCs can trigger T cells to produce IL-22 in a lipid antigen-dependent manner 1-2 days after wounding, which ensures a prolonged but controlled IL-22 release at the wound site, in response to a specific type of wound and associated pathogens.

Therapeutic manipulation of immune response is an intricate process. Despite the establishment of the extensive and interconnected involvement of IFN-I and IL-22 in psoriasis, drugs targeting these targets do not show clinical improvements in the disease [560, 561]. MEDI-545 or sifalimumab is a human monoclonal antibody that neutralises IFNα, which can block most highly expressed ISGs in blood and skin

lesions of SLE patients [562-566]. However, there were no significant differences in the clinical measures, and no changes of ISG profile in the lesion skin after MEDI-545 treatment in chronic psoriasis patients. The suggested that IFNα is not involved in chronic psoriatic skin lesions, which could potentially have been triggered by other IFN-I subtypes, such as IFNβ or that redundancy exists [566]. To escape this redundancy effect, a neutralising antibody targeting IFNAR, instead of a subtype of IFN-I, should perhaps be considered in the future for psoriasis treatment. Several drugs targeting IL-22 have also been tested in psoriasis patients. As IL‑22 does not act on immune cells, manipulating its signalling can be advantageous compared to attenuating IFN-I pathway which has proven to result in immune-related side effects [567]. Yet, a neutralising antibody against IL-22 did not show improvements in most psoriasis patients either, while targeting its upstream cytokines IL-12 and IL-23 reveals great results [502, 568, 569]. This lack of effectiveness of anti-IL-22 is considered to be related to the existence of redundant cytokines, such as IL-20 and IL-24, that can bind to IL-22R; ergo, blocking of IL‑22R could be a more appropriate approach. Furthermore, it may be possible that IL-22 acts early during lesional onset, but once disease is established then redundancy becomes more relevant. Fezakinumab, a human monoclonal antibody against IL-22, was given to adult patients with moderate-to-severe AD who failed to respond to conventional treatments. Interestingly, the results of the trial suggest that IL-22 inhibition appears to show efficacy in the setting of severe AD, implicating this pathway in disease pathogenesis [570]. Collectively, this study has established a pDC-governed modulation of adaptive immune system, in which pDCs can serve both directly as antigen-presenting cells and indirectly by producing mediators to prompt other immune cells to equip with appropriate effector

phenotypes. In the skin traumatic blisters, pDCs sensed the skin barrier defect, and infiltrated into the injury site, where they recognised the self-DNA/RNA containing ICs and secrete IFN-I. A novel CD1a$^+$ pDC-like cell subset was identified and expressed an array of genes related to antigen presentation and activation, and displayed both pDC and cDC associated gene signatures, yet failed to express several essential pDC transcription factors. In addition, these cells exhibit chemokines that allow them to recruit other immune cells to the skin. Surface protein analysis revealed that the CD1a$^+$ pDC-like cells were equipped with receptors essential for presenting both peptides and lipid antigen, through the expression of HLA-DR, and CD1a. These previously unrecognised CD1a-expressing pDC-like cells in human skin, which infiltrated early on in the wound healing process, were considered to drive T cell-derived IL-22 production. Despite the possibility of CD1a induction on blood pDCs, the lack of lineage tracing approach in human subjects limited the investigation of the ancestry of these skin CD1a$^+$ pDC-like cells. Next, ILC22, which are key cells in maintaining barrier function and promoting tissue repair, are proposed to have a nonredundant role in skin inflammation. ILC22, that are resident in the skin or have migrated into the skin from the blood, can respond to IFN-I, and revise their functionality accordingly. Moreover, the degree of IFN-I-induced activation of ILC22 correlates with the psoriatic phenotype, which is consistent with reports stating that ILC3 alterations are associated with the severity of inflammatory diseases. Together, a previously unrecognised pathway has been identified in which pDCs represent an upstream regulator to influence skin homeostasis and disease pathology (Fig. 7-1). This work suggests that regulating the numbers and function of CD1a$^+$ pDC-like cells may provide a novel target for therapeutic intervention in conditions of aberrant tissue

repair, yet, the regulation of pDC effector functions in the skin may prove to be both beneficial or detrimental; therefore, a Goldilocks principle applies when it comes to therapeutic intervention of pDC/IFN-I signalling in skin inflammatory conditions. Of note, the recognition of $CD1a^{+}$ pDC-like cells and AS DC in skin wounds opens up a new avenue of research: for example, whether these populations have roles in skin inflammatory diseases, such as AD, contact hypersensitivity and psoriasis. Overall, this study informed a new avenue for potential therapeutic manipulation through cells and pathways in the pDC-IFN-I-ILC22-T cell axis.

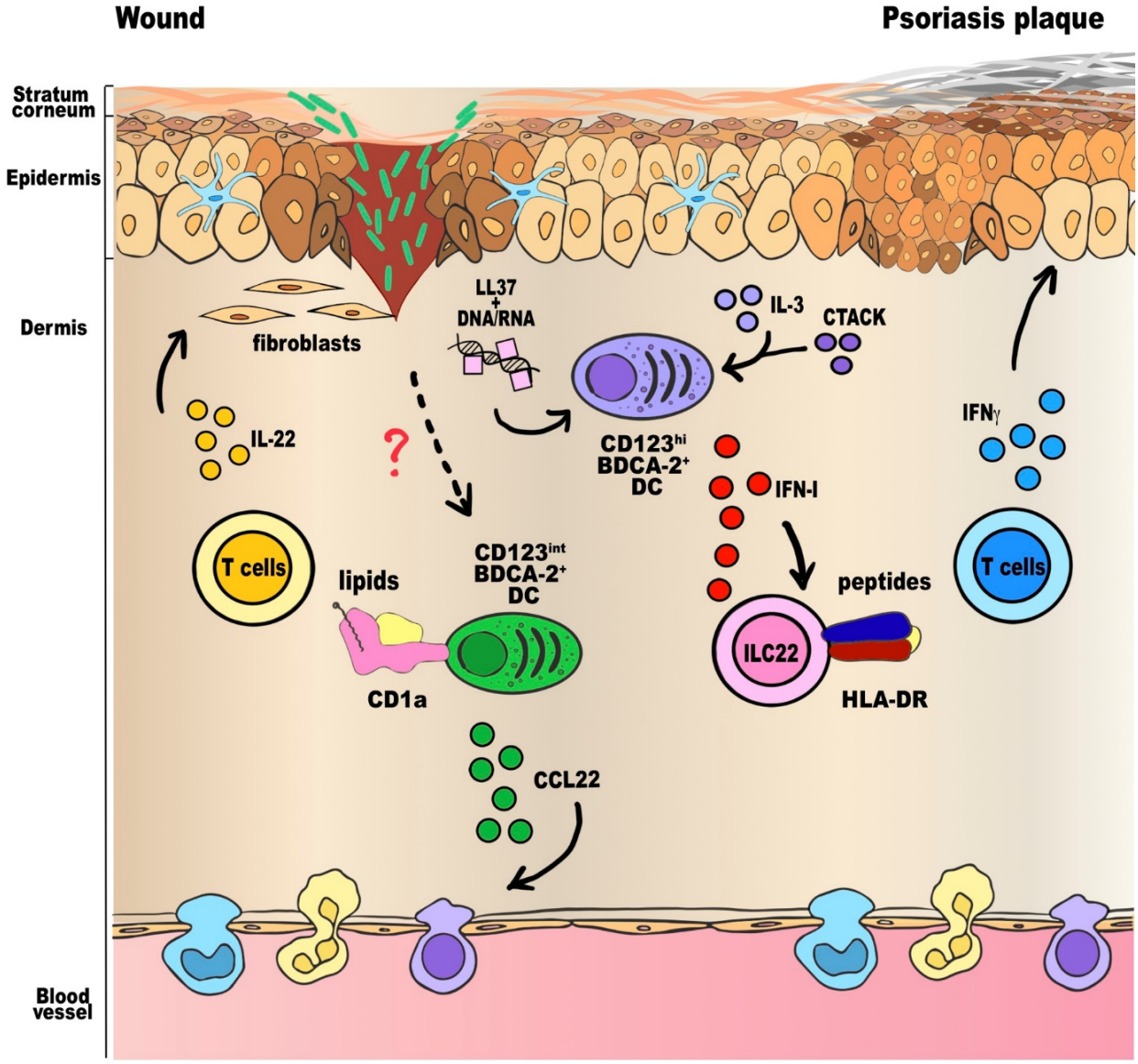


Figure 7-1. pDC effector functions in skin inflammatory conditions.

In the skin, pDCs are recruited in to the wound by CTACK and their survival is maintained by IL-3. They are activated by immune complex containing self-DNA/RNA and produce IFN-I, which in turns promote ILC22 to enter the antigen presentation status to prompt T cell activation through MHC-II. Unknown factors give rise to an "activated" CD1a-bearing pDC-like cells, which can present lipid antigens to T cells, initiating the reepithelialisation process, and recruit immune cells from the blood through CCL22 secretion.

8 References

1. Urmacher, C., *Histology of normal skin.* Am J Surg Pathol, 1990. **14**(7): p. 671-86.

2. Brunner, P.M., D.Y.M. Leung, and E. Guttman-Yassky, *Immunologic, microbial, and epithelial interactions in atopic dermatitis.* Ann Allergy Asthma Immunol, 2018. **120**(1): p. 34-41.

3. Cork, M.J., et al., *Epidermal barrier dysfunction in atopic dermatitis.* J Invest Dermatol, 2009. **129**(8): p. 1892-908.

4. Christensen, G.J. and H. Bruggemann, *Bacterial skin commensals and their role as host guardians.* Benef Microbes, 2014. **5**(2): p. 201-15.

5. Hachem, J.P., et al., *pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion.* J Invest Dermatol, 2003. **121**(2): p. 345-53.

6. Rosenthal, M., et al., *Skin microbiota: microbial community structure and its potential association with health and disease.* Infect Genet Evol, 2011. **11**(5): p. 839-48.

7. Bek-Thomsen, M., H.B. Lomholt, and M. Kilian, *Acne is not associated with yet-uncultured bacteria.* J Clin Microbiol, 2008. **46**(10): p. 3355-60.

8. Coelho, M.A., J.P. Sampaio, and P. Goncalves, *Living and thriving on the skin: Malassezia genomes tell the story.* MBio, 2013. **4**(2): p. e00117-13.

9. Grice, E.A. and J.A. Segre, *The Human Microbiome: Our Second Genome*, in *Annual Review of Genomics and Human Genetics, Vol 13*, A. Chakravarti and E. Green, Editors. 2012. p. 151-170.

10. Grice, E.A. and J.A. Segre, *The skin microbiome.* Nat Rev Microbiol, 2011. **9**(4): p. 244-53.

11. Grice, E.A., et al., *Topographical and temporal diversity of the human skin microbiome.* Science, 2009. **324**(5931): p. 1190-2.

12. Bastos, M.C., et al., *Staphylococcal antimicrobial peptides: relevant properties and potential biotechnological applications.* Curr Pharm Biotechnol, 2009. **10**(1): p. 38-61.

13. Fontana, M.B., C. de Bastos Mdo, and A. Brandelli, *Bacteriocins Pep5 and epidermin inhibit Staphylococcus epidermidis adhesion to catheters.* Curr Microbiol, 2006. **52**(5): p. 350-3.

14. Nascimento, J.S., et al., *Bacteriocins as alternative agents for control of multiresistant staphylococcal strains.* Lett Appl Microbiol, 2006. **42**(3): p. 215-21.

15. Cotter, P.D., R.P. Ross, and C. Hill, *Bacteriocins - a viable alternative to antibiotics?* Nat Rev Microbiol, 2013. **11**(2): p. 95-105.

16. Sahl, H.G. and G. Bierbaum, *Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from gram-positive bacteria.* Annu Rev Microbiol, 1998. **52**: p. 41-79.

17. Dorschner, R.A., et al., *Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus.* J Invest Dermatol, 2001. **117**(1): p. 91-7.

18. Handfield, C., J. Kwock, and A.S. MacLeod, *Innate Antiviral Immunity in the Skin.* Trends Immunol, 2018. **39**(4): p. 328-340.

19. Krishna, S. and L.S. Miller, *Innate and adaptive immune responses against Staphylococcus aureus skin infections.* Semin Immunopathol, 2012. **34**(2): p. 261-80.

20. Schroder, J.M., *The role of keratinocytes in defense against infection.* Curr Opin Infect Dis, 2010. **23**(2): p. 106-10.

21. Gilliet, M. and R. Lande, *Antimicrobial peptides and self-DNA in autoimmune skin inflammation.* Curr Opin Immunol, 2008. **20**(4): p. 401-7.

22. Zanetti, M., *Cathelicidins, multifunctional peptides of the innate immunity.* J Leukoc Biol, 2004. **75**(1): p. 39-48.

23. Matejuk, A., *Skin Immunity.* Arch Immunol Ther Exp (Warsz), 2018. **66**(1): p. 45-54.

24. Pasparakis, M., I. Haase, and F.O. Nestle, *Mechanisms regulating skin immunity and inflammation.* Nat Rev Immunol, 2014. **14**(5): p. 289-301.

25. Spetz, A.L., J. Strominger, and V. Groh-Spies, *T cell subsets in normal human epidermis.* Am J Pathol, 1996. **149**(2): p. 665-74.

26. Merad, M., F. Ginhoux, and M. Collin, *Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells.* Nat Rev Immunol, 2008. **8**(12): p. 935-47.

27. Wollenberg, A., et al., *Immunomorphological and ultrastructural characterization of Langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema.* J Invest Dermatol, 1996. **106**(3): p. 446-53.

28. Haniffa, M., et al., *Human tissues contain CD141hi cross-presenting dendritic cells with functional homology to mouse CD103+ nonlymphoid dendritic cells.* Immunity, 2012. **37**(1): p. 60-73.

29. Malissen, B., S. Tamoutounour, and S. Henri, *The origins and functions of dendritic cells and macrophages in the skin.* Nat Rev Immunol, 2014. **14**(6): p. 417-28.

30. Lenz, A., et al., *Human and murine dermis contain dendritic cells. Isolation by means of a novel method and phenotypical and functional characterization.* J Clin Invest, 1993. **92**(6): p. 2587-96.

31. Nestle, F.O., et al., *Characterization of Dermal Dendritic Cells Obtained from Normal Human Skin Reveals Phenotypic and Functionally Distinctive Subsets.* Journal of Immunology, 1993. **151**(11): p. 6535-6545.

32. Chu, C.C., et al., *Resident CD141 (BDCA3)+ dendritic cells in human skin produce IL-10 and induce regulatory T cells that suppress skin inflammation.* J Exp Med, 2012. **209**(5): p. 935-45.

33. Klechevsky, E., *Human dendritic cells - stars in the skin.* Eur J Immunol, 2013. **43**(12): p. 3147-55.

34. Boltjes, A. and F. van Wijk, *Human dendritic cell functional specialization in steady-state and inflammation.* Front Immunol, 2014. **5**: p. 131.

35. Nestle, F.O., et al., *Skin immune sentinels in health and disease.* Nat Rev Immunol, 2009. **9**(10): p. 679-91.

36. Yanez, D.A., et al., *The role of macrophages in skin homeostasis.* Pflugers Arch, 2017. **469**(3-4): p. 455-463.

37. Willenborg, S. and S.A. Eming, *Macrophages - sensors and effectors coordinating skin damage and repair.* J Dtsch Dermatol Ges, 2014. **12**(3): p. 214-21, 214-23.

38. Muller, S., et al., *Salt-dependent chemotaxis of macrophages.* PLoS One, 2013. **8**(9): p. e73439.

39. Bos, J.D., et al., *Predominance of Memory T-Cells (Cd4+, Cdw29+) over Naive T-Cells (Cd4+, Cd45r+) in Both Normal and Diseased Human-Skin.* Archives of Dermatological Research, 1989. **281**(1): p. 24-30.

40. Homey, B., et al., *CCL27-CCR10 interactions regulate T cell-mediated skin inflammation.* Nat Med, 2002. **8**(2): p. 157-65.

41. Clark, R.A., *Skin-resident T cells: the ups and downs of on site immunity.* J Invest Dermatol, 2010. **130**(2): p. 362-70.

42. Irla, M., et al., *MHC class II-restricted antigen presentation by plasmacytoid dendritic cells inhibits T cell-mediated autoimmunity.* J Exp Med, 2010. **207**(9): p. 1891-905.

43. Sage, A.P., et al., *MHC Class II-restricted antigen presentation by plasmacytoid dendritic cells drives proatherogenic T cell immunity.* Circulation, 2014. **130**(16): p. 1363-73.

44. Martin-Gayo, E., et al., *Plasmacytoid dendritic cells resident in human thymus drive natural Treg cell development.* Blood, 2010. **115**(26): p. 5366-75.

45. Cella, M., et al., *Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization.* Nat Immunol, 2000. **1**(4): p. 305-10.

46. Colonna, M. and M. Cella, *Crosspresentation: plasmacytoid dendritic cells are in the business.* Immunity, 2007. **27**(3): p. 419-21.

47. Tel, J., et al., *Human plasmacytoid dendritic cells efficiently cross-present exogenous Ags to CD8+ T cells despite lower Ag uptake than myeloid dendritic cell subsets.* Blood, 2013. **121**(3): p. 459-67.

48. Boiocchi, L., et al., *BDCA-2 (CD303): a highly specific marker for normal and neoplastic plasmacytoid dendritic cells.* Blood, 2013. **122**(2): p. 296-7.

49. Dzionek, A., et al., *BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood.* J Immunol, 2000. **165**(11): p. 6037-46.

50. Dzionek, A., et al., *BDCA-2, a novel plasmacytoid dendritic cell-specific type IIC-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction.* Journal of Experimental Medicine, 2001. **194**(12): p. 1823-1834.

51. Cao, W., et al., *Plasmacytoid dendritic cell-specific receptor ILT7-Fc epsilonRI gamma inhibits Toll-like receptor-induced interferon production.* J Exp Med, 2006. **203**(6): p. 1399-405.

52. Cao, W. and L. Bover, *Signaling and ligand interaction of ILT7: receptor-mediated regulatory mechanisms for plasmacytoid dendritic cells.* Immunol Rev, 2010. **234**(1): p. 163-76.

53. Cao, W., et al., *Regulation of TLR7/9 responses in plasmacytoid dendritic cells by BST2 and ILT7 receptor interaction.* J Exp Med, 2009. **206**(7): p. 1603-14.

54. Diacovo, T.G., et al., *Adhesive mechanisms governing interferon-producing cell recruitment into lymph nodes.* J Exp Med, 2005. **202**(5): p. 687-96.

55. Yoneyama, H., et al., *Evidence for recruitment of plasmacytoid dendritic cell precursors to inflamed lymph nodes through high endothelial venules.* Int Immunol, 2004. **16**(7): p. 915-28.

56. Hadeiba, H., et al., *Plasmacytoid dendritic cells transport peripheral antigens to the thymus to promote central tolerance.* Immunity, 2012. **36**(3): p. 438-50.

57. Sozzani, S., *Dendritic cell trafficking: more than just chemokines.* Cytokine Growth Factor Rev, 2005. **16**(6): p. 581-92.

58. Seth, S., et al., *CCR7 essentially contributes to the homing of plasmacytoid dendritic cells to lymph nodes under steady-state as well as inflammatory conditions.* J Immunol, 2011. **186**(6): p. 3364-72.

59. Siegal, F.P., et al., *The nature of the principal type 1 interferon-producing cells in human blood.* Science, 1999. **284**(5421): p. 1835-7.

60. Cella, M., et al., *Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon.* Nat Med, 1999. **5**(8): p. 919-23.

61. Gilliet, M., W. Cao, and Y.J. Liu, *Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases.* Nat Rev Immunol, 2008. **8**(8): p. 594-606.

62. Blasius, A.L. and B. Beutler, *Intracellular toll-like receptors.* Immunity, 2010. **32**(3): p. 305-15.

63. Kawai, T. and S. Akira, *Toll-like receptors and their crosstalk with other innate receptors in infection and immunity.* Immunity, 2011. **34**(5): p. 637-50.

64. Alculumbre, S.G., et al., *Diversification of human plasmacytoid predendritic cells in response to a single stimulus.* Nat Immunol, 2018. **19**(1): p. 63-75.

65. Alcantara-Hernandez, M., et al., *High-Dimensional Phenotypic Mapping of Human Dendritic Cells Reveals Interindividual Variation and Tissue Specialization.* Immunity, 2017. **47**(6): p. 1037-1050 e6.

66. Matsui, T., et al., *CD2 Distinguishes Two Subsets of Human Plasmacytoid Dendritic Cells with Distinct Phenotype and Functions.* Journal of Immunology, 2009. **182**(11): p. 6815-6823.

67. Zhang, H., et al., *A distinct subset of plasmacytoid dendritic cells induces activation and differentiation of B and T lymphocytes.* Proc Natl Acad Sci U S A, 2017. **114**(8): p. 1988-1993.

68. Villani, A.C., et al., *Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors.* Science, 2017. **356**(6335).

69. Rodrigues, P.F., et al., *Distinct progenitor lineages contribute to the heterogeneity of plasmacytoid dendritic cells.* Nat Immunol, 2018.

70. Osaki, Y., et al., *Characterization of CD56+ dendritic-like cells: a normal counterpart of blastic plasmacytoid dendritic cell neoplasm?* PLoS One, 2013. **8**(11): p. e81722.

71. Yu, H., et al., *Human BDCA2+CD123+CD56+ dendritic cells (DCs) related to blastic plasmacytoid dendritic cell neoplasm represent a unique myeloid DC subset.* Protein Cell, 2015. **6**(4): p. 297-306.

72. Matsui, T., et al., *CD2 distinguishes two subsets of human plasmacytoid dendritic cells with distinct phenotype and functions.* J Immunol, 2009. **182**(11): p. 6815-23.

73. Bryant, C., et al., *A CD2 high-expressing stress-resistant human plasmacytoid dendritic-cell subset.* Immunol Cell Biol, 2016. **94**(5): p. 447-57.

74. Cisse, B., et al., *Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development.* Cell, 2008. **135**(1): p. 37-48.

75. Cheng, M., et al., *Characterization of species-specific genes regulated by E2-2 in human plasmacytoid dendritic cells.* Sci Rep, 2015. **5**: p. 10752.

76. Esashi, E. and Y.J. Liu, *E-box protein E2-2 is a crucial regulator of plasmacytoid DC development.* Eur J Immunol, 2008. **38**(9): p. 2386-8.

77. Jackson, J.T., et al., *Id2 expression delineates differential checkpoints in the genetic program of CD8alpha+ and CD103+ dendritic cell lineages.* EMBO J, 2011. **30**(13): p. 2690-704.

78. Li, H.S., et al., *The signal transducers STAT5 and STAT3 control expression of Id2 and E2-2 during dendritic cell development.* Blood, 2012. **120**(22): p. 4363-73.

79. Spits, H., et al., *Id2 and Id3 Inhibit Development of Cd34+Stem Cells into Predendritic Cell (Pre-Dc)2 but Not into Pre-Dc1.* The Journal of Experimental Medicine, 2000. **192**(12): p. 1775-1784.

80. Karrich, J.J., et al., *The transcription factor Spi-B regulates human plasmacytoid dendritic cell survival through direct induction of the antiapoptotic gene BCL2-A1.* Blood, 2012. **119**(22): p. 5191-200.

81. Sasaki, I., et al., *Spi-B is critical for plasmacytoid dendritic cell function and development.* Blood, 2012. **120**(24): p. 4733-43.

82. Ippolito, G.C., et al., *Dendritic cell fate is determined by BCL11A.* Proc Natl Acad Sci U S A, 2014. **111**(11): p. E998-1006.

83. Chopin, M., et al., *RUNX2 Mediates Plasmacytoid Dendritic Cell Egress from the Bone Marrow and Controls Viral Immunity.* Cell Rep, 2016. **15**(4): p. 866-878.

84. Sawai, C.M., et al., *Transcription factor Runx2 controls the development and migration of plasmacytoid dendritic cells.* J Exp Med, 2013. **210**(11): p. 2151-9.

85. Esashi, E., et al., *The signal transducer STAT5 inhibits plasmacytoid dendritic cell development by suppressing transcription factor IRF8.* Immunity, 2008. **28**(4): p. 509-20.

86. Ghosh, H.S., et al., *Continuous expression of the transcription factor e2-2 maintains the cell fate of mature plasmacytoid dendritic cells.* Immunity, 2010. **33**(6): p. 905-16.

87. Robbins, S.H., et al., *Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling.* Genome Biol, 2008. **9**(1): p. R17.

88. Schotte, R., et al., *The ETS transcription factor Spi-B is required for human plasmacytoid dendritic cell development.* J Exp Med, 2004. **200**(11): p. 1503-9.

89. Wu, X., et al., *Bcl11a controls Flt3 expression in early hematopoietic progenitors and is required for pDC development in vivo.* PLoS One, 2013. **8**(5): p. e64800.

90. Honda, K., et al., *IRF-7 is the master regulator of type-I interferon-dependent immune responses.* Nature, 2005. **434**(7034): p. 772-7.

91. Kawai, T., et al., *Interferon-alpha induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6.* Nat Immunol, 2004. **5**(10): p. 1061-8.

92. Vander Lugt, B., et al., *Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation.* Nat Immunol, 2014. **15**(2): p. 161-7.

93. Sichien, D., et al., *IRF8 Transcription Factor Controls Survival and Function of Terminally Differentiated Conventional and Plasmacytoid Dendritic Cells, Respectively.* Immunity, 2016. **45**(3): p. 626-640.

94. Wollenberg, A., et al., *Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases.* J Invest Dermatol, 2002. **119**(5): p. 1096-102.

95. Gerlini, G., et al., *Massive recruitment of type I interferon producing plasmacytoid dendritic cells in varicella skin lesions.* J Invest Dermatol, 2006. **126**(2): p. 507-9.

96. Donaghy, H., et al., *Role for plasmacytoid dendritic cells in the immune control of recurrent human herpes simplex virus infection.* J Virol, 2009. **83**(4): p. 1952-61.

97. Nestle, F.O., et al., *Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production.* J Exp Med, 2005. **202**(1): p. 135-43.

98. Gregorio, J., et al., *Plasmacytoid dendritic cells sense skin injury and promote wound healing through type I interferons.* J Exp Med, 2010. **207**(13): p. 2921-30.

99. Ganguly, D., et al., *Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8.* J Exp Med, 2009. **206**(9): p. 1983-94.

100. Lande, R., et al., *Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide.* Nature, 2007. **449**(7162): p. 564-9.

101. Reizis, B., et al., *Plasmacytoid dendritic cells: recent progress and open questions.* Annu Rev Immunol, 2011. **29**(1): p. 163-83.

102. Bond, E., et al., *Plasmacytoid dendritic cells infiltrate the skin in positive tuberculin skin test indurations.* J Invest Dermatol, 2012. **132**(1): p. 114-23.

103. Magnusson, M. and M.W. Bentzon, *Preparation of purified tuberculin RT 23.* Bull World Health Organ, 1958. **19**(5): p. 829-43.

104. Poulter, L.W., et al., *Immunohistological analysis of delayed-type hypersensitivity in man.* Cell Immunol, 1982. **74**(2): p. 358-69.

105. Scheynius, A., L. Klareskog, and U. Forsum, *Insitu Identification of Lymphocyte-T Subsets and Hla-Dr Expressing Cells in the Human-Skin Tuberculin Reaction.* Clinical and Experimental Immunology, 1982. **49**(2): p. 325-330.

106. Kim GK and D.R. JQ, *Drug-Provoked Psoriasis: Is It Drug Induced or Drug Aggravated?* J Clin Aesthet Dermatol, 2010. **3**: p. 32–38.

107. Gilliet, M., et al., *Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors.* Arch Dermatol, 2004. **140**(12): p. 1490-5.

108. Yao, Y., et al., *Type I interferon: potential therapeutic target for psoriasis?* PLoS One, 2008. **3**(7): p. e2737.

109. Vermi, W., et al., *Role of ChemR23 in directing the migration of myeloid and plasmacytoid dendritic cells to lymphoid organs and inflamed skin.* J Exp Med, 2005. **201**(4): p. 509-15.

110. Wittamer, V., et al., *Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids.* J Exp Med, 2003. **198**(7): p. 977-85.

111. Albanesi, C., et al., *Chemerin expression marks early psoriatic skin lesions and correlates with plasmacytoid dendritic cell recruitment.* J Exp Med, 2009. **206**(1): p. 249-58.

112. Nestle, F.O., D.H. Kaplan, and J. Barker, *Psoriasis.* N Engl J Med, 2009. **361**(5): p. 496-509.

113. van der Fits, L., et al., *In psoriasis lesional skin the type I interferon signaling pathway is activated, whereas interferon-alpha sensitivity is unaltered.* J Invest Dermatol, 2004. **122**(1): p. 51-60.

114. Di Cesare, A., P. Di Meglio, and F.O. Nestle, *The IL-23/Th17 axis in the immunopathogenesis of psoriasis.* J Invest Dermatol, 2009. **129**(6): p. 1339-50.

115. Nakajima, K., *Critical role of the interleukin-23/T-helper 17 cell axis in the pathogenesis of psoriasis.* J Dermatol, 2012. **39**(3): p. 219-24.

116. van der Fits, L., et al., *Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis.* J Immunol, 2009. **182**(9): p. 5836-45.

117. Nair, R.P., et al., *Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways.* Nat Genet, 2009. **41**(2): p. 199-204.

118. Blauvelt, A., *T-helper 17 cells in psoriatic plaques and additional genetic links between IL-23 and psoriasis.* J Invest Dermatol, 2008. **128**(5): p. 1064-7.

119. Antonelli, A., et al., *Interferon-alpha, -beta and -gamma induce CXCL9 and CXCL10 secretion by human thyrocytes: modulation by peroxisome proliferator-activated receptor-gamma agonists.* Cytokine, 2010. **50**(3): p. 260-7.

120. Wenzel, J., et al., *Type I interferon-associated recruitment of cytotoxic lymphocytes: a common mechanism in regressive melanocytic lesions.* Am J Clin Pathol, 2005. **124**(1): p. 37-48.

121. Wenzel, J., et al., *CXCR3-mediated recruitment of cytotoxic lymphocytes in lupus erythematosus profundus.* J Am Acad Dermatol, 2007. **56**(4): p. 648-50.

122. Asselin-Paturel, C., et al., *Type I interferon dependence of plasmacytoid dendritic cell activation and migration.* J Exp Med, 2005. **201**(7): p. 1157-67.

123. Baechler, E.C., et al., *Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus.* Proc Natl Acad Sci U S A, 2003. **100**(5): p. 2610-5.

124. Bennett, L., et al., *Interferon and granulopoiesis signatures in systemic lupus erythematosus blood.* J Exp Med, 2003. **197**(6): p. 711-23.

125. Fah, J., J. Pavlovic, and G. Burg, *Expression of MxA protein in inflammatory dermatoses.* J Histochem Cytochem, 1995. **43**(1): p. 47-52.

126. Xu, W.D., et al., *IRF7, a functional factor associates with systemic lupus erythematosus.* Cytokine, 2012. **58**(3): p. 317-20.

127. Sigurdsson, S., et al., *Polymorphisms in the tyrosine kinase 2 and interferon regulatory factor 5 genes are associated with systemic lupus erythematosus.* Am J Hum Genet, 2005. **76**(3): p. 528-37.

128. Remmers, E.F., et al., *STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus.* N Engl J Med, 2007. **357**(10): p. 977-86.

129. Sigurdsson, S., et al., *Comprehensive evaluation of the genetic variants of interferon regulatory factor 5 (IRF5) reveals a novel 5 bp length polymorphism as strong risk factor for systemic lupus erythematosus.* Hum Mol Genet, 2008. **17**(6): p. 872-81.

130. Hunziker, L., et al., *Hypergammaglobulinemia and autoantibody induction mechanisms in viral infections.* Nat Immunol, 2003. **4**(4): p. 343-9.

131. Chen, C.J., et al., *High prevalence of immunoglobulin A antibody against Epstein-Barr virus capsid antigen in adult patients with lupus with disease flare: case control studies.* J Rheumatol, 2005. **32**(1): p. 44-7.

132. James, J.A., et al., *Systemic lupus erythematosus in adults is associated with previous Epstein-Barr virus exposure.* Arthritis and Rheumatism, 2001. **44**(5): p. 1122-1126.

133. James, J.A., et al., *An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus.* J Clin Invest, 1997. **100**(12): p. 3019-26.

134. Ronnblom, L., M.L. Eloranta, and G.V. Alm, *The type I interferon system in systemic lupus erythematosus.* Arthritis Rheum, 2006. **54**(2): p. 408-20.

135. Ronnblom, L., *The type I interferon system in the etiopathogenesis of autoimmune diseases.* Ups J Med Sci, 2011. **116**(4): p. 227-37.

136. Braun, D., P. Geraldes, and J. Demengeot, *Type I Interferon controls the onset and severity of autoimmune manifestations in lpr mice.* J Autoimmun, 2003. **20**(1): p. 15-25.

137. Santiago-Raber, M.L., et al., *Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice.* J Exp Med, 2003. **197**(6): p. 777-88.

138. Santiago-Raber, M.L., L. Baudino, and S. Izui, *Emerging roles of TLR7 and TLR9 in murine SLE.* J Autoimmun, 2009. **33**(3-4): p. 231-8.

139. Rowland, S.L., et al., *Early, transient depletion of plasmacytoid dendritic cells ameliorates autoimmunity in a lupus model.* J Exp Med, 2014. **211**(10): p. 1977-91.

140. Leung, D.Y., *Role of IgE in atopic dermatitis.* Curr Opin Immunol, 1993. **5**(6): p. 956-62.

141. Liu, F.T., H. Goodarzi, and H.Y. Chen, *IgE, mast cells, and eosinophils in atopic dermatitis.* Clin Rev Allergy Immunol, 2011. **41**(3): p. 298-310.

142. Tomasini, D., et al., *Plasmacytoid dendritic cells: an overview of their presence and distribution in different inflammatory skin diseases, with special emphasis*

on Jessner's lymphocytic infiltrate of the skin and cutaneous lupus erythematosus. J Cutan Pathol, 2010. **37**(11): p. 1132-9.

143. Hashizume, H., et al., *Compartmental imbalance and aberrant immune function of blood CD123+ (plasmacytoid) and CD11c+ (myeloid) dendritic cells in atopic dermatitis.* J Immunol, 2005. **174**(4): p. 2396-403.

144. Novak, N., et al., *Characterization of FcepsilonRI-bearing CD123 blood dendritic cell antigen-2 plasmacytoid dendritic cells in atopic dermatitis.* J Allergy Clin Immunol, 2004. **114**(2): p. 364-70.

145. Krause, C.D. and S. Pestka, *Cut, copy, move, delete: The study of human interferon genes reveal multiple mechanisms underlying their evolution in amniotes.* Cytokine, 2015. **76**(2): p. 480-495.

146. Harari, D., et al., *Bridging the species divide: transgenic mice humanized for type-I interferon response.* PLoS One, 2014. **9**(1): p. e84259.

147. Nguyen, K.B., et al., *Coordinated and distinct roles for IFN-alpha beta, IL-12, and IL-15 regulation of NK cell responses to viral infection.* J Immunol, 2002. **169**(8): p. 4279-87.

148. Lee, C.K., et al., *Distinct requirements for IFNs and STAT1 in NK cell function.* J Immunol, 2000. **165**(7): p. 3571-7.

149. Li, H.S., et al., *Cell-intrinsic role for IFN-alpha-STAT1 signals in regulating murine Peyer patch plasmacytoid dendritic cells and conditioning an inflammatory response.* Blood, 2011. **118**(14): p. 3879-89.

150. Swiecki, M., et al., *Type I interferon negatively controls plasmacytoid dendritic cell numbers in vivo.* J Exp Med, 2011. **208**(12): p. 2367-74.

151. Chen, Y.L., et al., *A type I IFN-Flt3 ligand axis augments plasmacytoid dendritic cell development from common lymphoid progenitors.* J Exp Med, 2013. **210**(12): p. 2515-22.

152. Platanias, L.C., *Mechanisms of type-I- and type-II-interferon-mediated signalling.* Nat Rev Immunol, 2005. **5**(5): p. 375-86.

153. Ori, D., M. Murase, and T. Kawai, *Cytosolic nucleic acid sensors and innate immune regulation.* Int Rev Immunol, 2017. **36**(2): p. 74-88.

154. Muskardin, T.L.W. and T.B. Niewold, *Type I interferon in rheumatic diseases.* Nat Rev Rheumatol, 2018. **14**(4): p. 214-228.

155. Hall, J.C. and A. Rosen, *Type I interferons: crucial participants in disease amplification in autoimmunity.* Nat Rev Rheumatol, 2010. **6**(1): p. 40-9.

156. Prakash, A., et al., *Tissue-specific positive feedback requirements for production of type I interferon following virus infection.* J Biol Chem, 2005. **280**(19): p. 18651-7.

157. Yarilina, A., et al., *TNF activates an IRF1-dependent autocrine loop leading to sustained expression of chemokines and STAT1-dependent type I interferon-response genes.* Nat Immunol, 2008. **9**(4): p. 378-87.

158. Venkatesh, D., et al., *Endothelial TNF receptor 2 induces IRF1 transcription factor-dependent interferon-beta autocrine signaling to promote monocyte recruitment.* Immunity, 2013. **38**(5): p. 1025-37.

159. Brierley, M.M. and E.N. Fish, *Stats: Multifaceted regulators of transcription.* Journal of Interferon and Cytokine Research, 2005. **25**(12): p. 733-744.

160. Nguyen, T.P., et al., *Interferon-alpha inhibits CD4 T cell responses to interleukin-7 and interleukin-2 and selectively interferes with Akt signaling.* J Leukoc Biol, 2015. **97**(6): p. 1139-46.

161. Zhang, K.J., et al., *A Potent In Vivo Antitumor Efficacy of Novel Recombinant Type I Interferon.* Clin Cancer Res, 2017. **23**(8): p. 2038-2049.

162. Trinchieri, G., *Type I interferon: friend or foe?* J Exp Med, 2010. **207**(10): p. 2053-63.

163. Brierley, M.M. and E.N. Fish, *Stats: multifaceted regulators of transcription.* J Interferon Cytokine Res, 2005. **25**(12): p. 733-44.

164. Katlinskaya, Y.V., et al., *Suppression of Type I Interferon Signaling Overcomes Oncogene-Induced Senescence and Mediates Melanoma Development and Progression.* Cell Rep, 2016. **15**(1): p. 171-180.

165. Chiantore, M.V., et al., *Interferon-beta induces cellular senescence in cutaneous human papilloma virus-transformed human keratinocytes by affecting p53 transactivating activity.* PLoS One, 2012. **7**(5): p. e36909.

166. Booy, S., et al., *Influence of type-I Interferon receptor expression level on the response to type-I Interferons in human pancreatic cancer cells.* J Cell Mol Med, 2014. **18**(3): p. 492-502.

167. van Boxel-Dezaire, A.H.H., M.R.S. Rani, and G.R. Stark, *Complex modulation of cell type-specific signaling in response to type I Interferons.* Immunity, 2006. **25**(3): p. 361-372.

168. Nguyen, K.B., et al., *Coordinated and distinct roles for IFN-alpha/beta, IL-12, and IL-15 regulation of NK cell responses to viral infection.* Journal of Immunology, 2002. **169**(8): p. 4279-4287.

169. Lee, C.K., et al., *Distinct requirements for IFNs and STAT1 in NK cell function.* Journal of Immunology, 2000. **165**(7): p. 3571-3577.

170. Crow, M.K., M. Olferiev, and K.A. Kirou, *Targeting of type I interferon in systemic autoimmune diseases.* Transl Res, 2015. **165**(2): p. 296-305.

171. Bhattacharya, S., et al., *Triggering ubiquitination of IFNAR1 protects tissues from inflammatory injury.* EMBO Mol Med, 2014. **6**(3): p. 384-97.

172. Kumar, K.G., et al., *Site-specific ubiquitination exposes a linear motif to promote interferon-alpha receptor endocytosis.* J Cell Biol, 2007. **179**(5): p. 935-50.

173. Kumar, K.G., et al., *Basal ubiquitin-independent internalization of interferon alpha receptor is prevented by Tyk2-mediated masking of a linear endocytic motif.* J Biol Chem, 2008. **283**(27): p. 18566-72.

174. Liu, J., et al., *Ligand-independent pathway that controls stability of interferon alpha receptor.* Biochemical and Biophysical Research Communications, 2008. **367**(2): p. 388-393.

175. Kalie, E., et al., *The stability of the ternary interferon-receptor complex rather than the affinity to the individual subunits dictates differential biological activities.* J Biol Chem, 2008. **283**(47): p. 32925-36.

176. Marchetti, M., et al., *Stat-mediated signaling induced by type I and type II interferons (IFNs) is differentially controlled through lipid microdomain association and clathrin-dependent endocytosis of IFN receptors.* Mol Biol Cell, 2006. **17**(7): p. 2896-909.

177. Francois-Newton, V., et al., *USP18-based negative feedback control is induced by type I and type III interferons and specifically inactivates interferon alpha response.* PLoS One, 2011. **6**(7): p. e22200.

178. Malakhova, O.A., et al., *UBP43 is a novel regulator of interferon signaling independent of its ISG15 isopeptidase activity.* EMBO J, 2006. **25**(11): p. 2358-67.

179. Arimoto, K.I., et al., *STAT2 is an essential adaptor in USP18-mediated suppression of type I interferon signaling.* Nat Struct Mol Biol, 2017. **24**(3): p. 279-289.

180. Piganis, R.A., et al., *Suppressor of cytokine signaling (SOCS) 1 inhibits type I interferon (IFN) signaling via the interferon alpha receptor (IFNAR1)-associated tyrosine kinase Tyk2.* J Biol Chem, 2011. **286**(39): p. 33811-8.

181. Levin, D., et al., *Multifaceted activities of type I interferon are revealed by a receptor antagonist.* Sci Signal, 2014. **7**(327): p. ra50.

182. Sandling, J.K., et al., *A candidate gene study of the type I interferon pathway implicates IKBKE and IL8 as risk loci for SLE.* Eur J Hum Genet, 2011. **19**(4): p. 479-84.

183. Gui, J., et al., *Therapeutic Elimination of the Type 1 Interferon Receptor for Treating Psoriatic Skin Inflammation.* J Invest Dermatol, 2016. **136**(10): p. 1990-2002.

184. Oon, S., N.J. Wilson, and I. Wicks, *Targeted therapeutics in SLE: emerging strategies to modulate the interferon pathway.* Clin Transl Immunology, 2016. **5**(5): p. e79.

185. Teijaro, J.R., et al., *S1PR1-mediated IFNAR1 degradation modulates plasmacytoid dendritic cell interferon-alpha autoamplification.* Proc Natl Acad Sci U S A, 2016. **113**(5): p. 1351-6.

186. Pan, M., et al., *Mutation of the IFNAR-1 receptor binding site of human IFN-alpha2 generates type I IFN competitive antagonists.* Biochemistry, 2008. **47**(46): p. 12018-27.

187. Winthrop, K.L., *The emerging safety profile of JAK inhibitors in rheumatic disease.* Nat Rev Rheumatol, 2017. **13**(4): p. 234-243.

188. Hardman, C.S., et al., *CD1a presentation of endogenous antigens by group 2 innate lymphoid cells.* Sci Immunol, 2017. **2**(18).

189. Ramos, E., et al., *Evidence for distinct pathways of hepcidin regulation by acute and chronic iron loading in mice.* Hepatology, 2011. **53**(4): p. 1333-41.

190. Picelli, S., et al., *Full-length RNA-seq from single cells using Smart-seq2.* Nat Protoc, 2014. **9**(1): p. 171-81.

191. Patro, R., et al., *Salmon provides fast and bias-aware quantification of transcript expression.* Nat Methods, 2017. **14**(4): p. 417-419.

192. Shalek, A.K., et al., *Single-cell RNA-seq reveals dynamic paracrine control of cellular variation.* Nature, 2014. **510**(7505): p. 363-9.

193. Brem, H. and M. Tomic-Canic, *Cellular and molecular basis of wound healing in diabetes.* J Clin Invest, 2007. **117**(5): p. 1219-22.

194. Sen, C.K., et al., *Human skin wounds: a major and snowballing threat to public health and the economy.* Wound Repair Regen, 2009. **17**(6): p. 763-71.

195. Gonzalez, A.C., et al., *Wound healing - A literature review.* An Bras Dermatol, 2016. **91**(5): p. 614-620.

196. Wilgus, T.A., *Immune cells in the healing skin wound: influential players at each stage of repair.* Pharmacol Res, 2008. **58**(2): p. 112-6.

197. Martin, P., *Wound healing--aiming for perfect skin regeneration.* Science, 1997. **276**(5309): p. 75-81.

198. Nurden, A.T., et al., *Platelets and wound healing.* Front Biosci, 2008. **13**: p. 3532-48.

199. Barrientos, S., et al., *Growth factors and cytokines in wound healing.* Wound Repair Regen, 2008. **16**(5): p. 585-601.

200. Egozi, E.I., et al., *Mast cells modulate the inflammatory but not the proliferative response in healing wounds.* Wound Repair and Regeneration, 2003. **11**(1): p. 46-54.

201. Puxeddu, I., et al., *Mast cells in allergy and beyond.* Int J Biochem Cell Biol, 2003. **35**(12): p. 1601-7.

202. Lucas, T., et al., *Differential roles of macrophages in diverse phases of skin repair.* J Immunol, 2010. **184**(7): p. 3964-77.

203. Smigiel, K.S. and W.C. Parks, *Macrophages, Wound Healing, and Fibrosis: Recent Insights.* Curr Rheumatol Rep, 2018. **20**(4): p. 17.

204. Kratofil, R.M., P. Kubes, and J.F. Deniset, *Monocyte Conversion During Inflammation and Injury.* Arterioscler Thromb Vasc Biol, 2017. **37**(1): p. 35-42.

205. Hesketh, M., et al., *Macrophage Phenotypes Regulate Scar Formation and Chronic Wound Healing.* Int J Mol Sci, 2017. **18**(7).

206. Bonefeld, C.M. and C. Geisler, *The role of innate lymphoid cells in healthy and inflamed skin.* Immunol Lett, 2016. **179**: p. 25-28.

207. Wynn, T.A. and K.M. Vannella, *Macrophages in Tissue Repair, Regeneration, and Fibrosis.* Immunity, 2016. **44**(3): p. 450-462.

208. Hieronymus, T., et al., *The clash of Langerhans cell homeostasis in skin: Should I stay or should I go?* Semin Cell Dev Biol, 2015. **41**: p. 30-8.

209. Das, A., et al., *Monocyte and macrophage plasticity in tissue repair and regeneration.* Am J Pathol, 2015. **185**(10): p. 2596-606.

210. Gause, W.C., T.A. Wynn, and J.E. Allen, *Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths.* Nat Rev Immunol, 2013. **13**(8): p. 607-14.

211. Rak, G.D., et al., *IL-33-Dependent Group 2 Innate Lymphoid Cells Promote Cutaneous Wound Healing.* J Invest Dermatol, 2016. **136**(2): p. 487-496.

212. Werner, S., T. Krieg, and H. Smola, *Keratinocyte-fibroblast interactions in wound healing.* J Invest Dermatol, 2007. **127**(5): p. 998-1008.

213. Greaves, N.S., et al., *Current understanding of molecular and cellular mechanisms in fibroplasia and angiogenesis during acute wound healing.* J Dermatol Sci, 2013. **72**(3): p. 206-17.

214. Gurtner, G.C., et al., *Wound repair and regeneration.* Nature, 2008. **453**(7193): p. 314-21.

215. Macri, L. and R.A. Clark, *Tissue engineering for cutaneous wounds: selecting the proper time and space for growth factors, cells and the extracellular matrix.* Skin Pharmacol Physiol, 2009. **22**(2): p. 83-93.

216. Bauer, S.M., R.J. Bauer, and O.C. Velazquez, *Angiogenesis, vasculogenesis, and induction of healing in chronic wounds.* Vasc Endovascular Surg, 2005. **39**(4): p. 293-306.

217. Eming, S.A., et al., *Regulation of angiogenesis: wound healing as a model.* Prog Histochem Cytochem, 2007. **42**(3): p. 115-70.

218. Hartlapp, I., et al., *Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis in vivo.* FASEB J, 2001. **15**(12): p. 2215-24.

219. Folkman, J. and P.A. D'Amore, *Blood Vessel Formation: What Is Its Molecular Basis?* Cell, 1996. **87**(7): p. 1153-1155.

220. Risau, W., *Mechanisms of angiogenesis.* Nature, 1997. **386**(6626): p. 671-4.

221. Clark, R.A.F., *Fibrin and wound healing*, in *Fibrinogen*, W. Nieuwenhuizen, M.W. Mosesson, and M.P.M. DeMaat, Editors. 2001. p. 355-367.

222. Welch, M.P., G.F. Odland, and R.A. Clark, *Temporal relationships of F-actin bundle formation, collagen and fibronectin matrix assembly, and fibronectin receptor expression to wound contraction.* J Cell Biol, 1990. **110**(1): p. 133-45.

223. Clark, R.A., et al., *Collagen matrices attenuate the collagen-synthetic response of cultured fibroblasts to TGF-beta.* J Cell Sci, 1995. **108 (Pt 3)**: p. 1251-61.

224. Eming, S.A., P. Martin, and M. Tomic-Canic, *Wound repair and regeneration: mechanisms, signaling, and translation.* Sci Transl Med, 2014. **6**(265): p. 265sr6.

225. Guo, S. and L.A. Dipietro, *Factors affecting wound healing.* J Dent Res, 2010. **89**(3): p. 219-29.

226. Van Rhijn, I., et al., *Lipid and small-molecule display by CD1 and MR1.* Nat Rev Immunol, 2015. **15**(10): p. 643-54.

227. Haeryfar, S.M.M. and T. Mallevaey, *CD1- and MR1-restricted T Cells in Antimicrobial Immunity*. Frontiers Research Topics. 2016.

228. Stenger, S., et al., *Differential effects of cytolytic T cell subsets on intracellular infection.* Science, 1997. **276**(5319): p. 1684-7.

229. Colonna, M., *Skin function for human CD1a-reactive T cells.* Nat Immunol, 2010. **11**(12): p. 1079-80.

230. de Jong, A., et al., *CD1a-autoreactive T cells recognize natural skin oils that function as headless antigens.* Nat Immunol, 2014. **15**(2): p. 177-85.

231. Beckman, E.M., et al., *Recognition of a lipid antigen by CD1-restricted alpha beta+ T cells.* Nature, 1994. **372**(6507): p. 691-4.

232. Sieling, P.A., et al., *CD1-restricted T cell recognition of microbial lipoglycan antigens.* Science, 1995. **269**(5221): p. 227-30.

233. Kawano, T., et al., *CD1d-restricted and TCR-mediated activation of V(alpha)14 NKT cells by glycosylceramides.* Science, 1997. **278**(5343): p. 1626-1629.

234. Moody, D.B., et al., *Structural requirements for glycolipid antigen recognition by CD1b-restricted T cells.* Science, 1997. **278**(5336): p. 283-6.

235. Zeng, Z., et al., *Crystal structure of mouse CD1: An MHC-like fold with a large hydrophobic binding groove.* Science, 1997. **277**(5324): p. 339-45.

236. Gadola, S.D., et al., *Structure of human CD1b with bound ligands at 2.3 A, a maze for alkyl chains.* Nat Immunol, 2002. **3**(8): p. 721-6.

237. Karadimitris, A., *Human CD1d–glycolipid tetramers generated by in vitro oxidative refolding chromatography.* Proceedings of the National Academy of Sciences of the United States of America, 2000. **98**: p. 3294-3298.

238. Altamirano, M.M., et al., *Ligand-independent assembly of recombinant human CD1 by using oxidative refolding chromatography.* Proc Natl Acad Sci U S A, 2001. **98**(6): p. 3288-93.

239. Moody, D.B. and S.A. Porcelli, *Intracellular pathways of CD1 antigen presentation.* Nat Rev Immunol, 2003. **3**(1): p. 11-22.

240. Sullivan, B.A., N.A. Nagarajan, and M. Kronenberg, *CD1 and MHC II find different means to the same end.* Trends Immunol, 2005. **26**(5): p. 282-8.

241. Watts, C., *The exogenous pathway for antigen presentation on major histocompatibility complex class II and CD1 molecules.* Nat Immunol, 2004. **5**(7): p. 685-92.

242. Caux, C., et al., *GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells.* Nature, 1992. **360**(6401): p. 258-61.

243. Tazi, A., et al., *Evidence that granulocyte macrophage-colony-stimulating factor regulates the distribution and differentiated state of dendritic cells/Langerhans cells in human lung and lung cancers.* J Clin Invest, 1993. **91**(2): p. 566-76.

244. Grassi, F., et al., *Monocyte-derived dendritic cells have a phenotype comparable to that of dermal dendritic cells and display ultrastructural granules distinct from Birbeck granules.* Journal of Leukocyte Biology, 1998. **64**(4): p. 484-493.

245. Sotzik, F., A. Boyd, and K. Shortman, *Surface antigens of human thymocyte populations defined by CD3, CD4 and CD8 expression: CD1a is expressed by mature thymocytes but not peripheral T cells.* Immunology Letters, 1993. **36**(1): p. 101-106.

246. Zajonc, D.M., et al., *Crystal structure of CD1a in complex with a sulfatide self antigen at a resolution of 2.15 A.* Nat Immunol, 2003. **4**(8): p. 808-15.

247. Moody, D.B., D.M. Zajonc, and I.A. Wilson, *Anatomy of CD1-lipid antigen complexes.* Nat Rev Immunol, 2005. **5**(5): p. 387-99.

248. Calabi, F. and C. Milstein, *A novel family of human major histocompatibility complex-related genes not mapping to chromosome 6.* Nature, 1986. **323**(6088): p. 540-3.

249. Jackman, R.M., et al., *The tyrosine-containing cytoplasmic tail of CD1b is essential for its efficient presentation of bacterial lipid antigens.* Immunity, 1998. **8**(3): p. 341-351.

250. Sugita, M., et al., *Separate pathways for antigen presentation by CD1 molecules.* Immunity, 1999. **11**(6): p. 743-52.

251. Sugita, M., et al., *Failure of trafficking and antigen presentation by CD1 in AP-3-deficient cells.* Immunity, 2002. **16**(5): p. 697-706.

252. Salamero, J., et al., *CD1a molecules traffic through the early recycling endosomal pathway in human Langerhans cells.* J Invest Dermatol, 2001. **116**(3): p. 401-8.

253. Moody, D.B., et al., *Lipid length controls antigen entry into endosomal and nonendosomal pathways for CD1b presentation.* Nat Immunol, 2002. **3**(5): p. 435-42.

254. Zajonc, D.M., et al., *Molecular mechanism of lipopeptide presentation by CD1a.* Immunity, 2005. **22**(2): p. 209-19.

255. Porcelli, S., et al., *Recognition of cluster of differentiation 1 antigens by human CD4-CD8-cytolytic T lymphocytes.* Nature, 1989. **341**(6241): p. 447-50.

256. Moody, D.B., et al., *T cell activation by lipopeptide antigens.* Science, 2004. **303**(5657): p. 527-31.

257. Pena-Cruz, V., et al., *Epidermal Langerhans cells efficiently mediate CD1a-dependent presentation of microbial lipid antigens to T cells.* J Invest Dermatol, 2003. **121**(3): p. 517-21.

258. Wooldridge, K.G. and P.H. Williams, *Iron uptake mechanisms of pathogenic bacteria.* FEMS Microbiol Rev, 1993. **12**(4): p. 325-48.

259. De Voss, J.J., et al., *The salicylate-derived mycobactin siderophores of Mycobacterium tuberculosis are essential for growth in macrophages.* Proc Natl Acad Sci U S A, 2000. **97**(3): p. 1252-7.

260. Young, D.C., et al., *Synthesis of dideoxymycobactin antigens presented by CD1a reveals T cell fine specificity for natural lipopeptide structures.* J Biol Chem, 2009. **284**(37): p. 25087-96.

261. Kim, J.H., et al., *CD1a on Langerhans cells controls inflammatory skin disease.* Nat Immunol, 2016. **17**(10): p. 1159-66.

262. Bedinger, P.A., K.J. Hardeman, and C.A. Loukides, *Travelling in style: the cell biology of pollen.* Trends Cell Biol, 1994. **4**(4): p. 132-8.

263. Vos, J.P., M. Lopes-Cardozo, and B.M. Gadella, *Metabolic and functional aspects of sulfogalactolipids.* Biochim Biophys Acta, 1994. **1211**(2): p. 125-49.

264. Sandhoff, R., et al., *Kidney sulfatides in mouse models of inherited glycosphingolipid disorders: determination by nano-electrospray ionization tandem mass spectrometry.* J Biol Chem, 2002. **277**(23): p. 20386-98.

265. Fredman, P., et al., *The glycosphingolipid sulfatide in the islets of Langerhans in rat pancreas is processed through recycling: possible involvement in insulin trafficking.* Glycobiology, 2000. **10**(1): p. 39-50.

266. De Libero, G., et al., *Bacterial infections promote T cell recognition of self-glycolipids.* Immunity, 2005. **22**(6): p. 763-72.

267. Shamshiev, A., et al., *Presentation of the same glycolipid by different CD1 molecules.* J Exp Med, 2002. **195**(8): p. 1013-21.

268. Zajonc, D.M., et al., *Structural basis for CD1d presentation of a sulfatide derived from myelin and its implications for autoimmunity.* J Exp Med, 2005. **202**(11): p. 1517-26.

269. Cernadas, M., et al., *Early recycling compartment trafficking of CD1a is essential for its intersection and presentation of lipid antigens.* J Immunol, 2010. **184**(3): p. 1235-41.

270. Takahashi, T. and T. Suzuki, *Role of sulfatide in normal and pathological cells and tissues.* J Lipid Res, 2012. **53**(8): p. 1437-50.

271. Bourgeois, E.A., et al., *Bee venom processes human skin lipids for presentation by CD1a.* J Exp Med, 2015. **212**(2): p. 149-63.

272. Jarrett, R., et al., *Filaggrin inhibits generation of CD1a neolipid antigens by house dust mite-derived phospholipase.* Sci Transl Med, 2016. **8**(325): p. 325ra18.

273. Chock, S.P., et al., *The localization of phospholipase A2 in the secretory granule.* Biochem J, 1994. **300 (Pt 3)**: p. 619-22.

274. Granata, F., et al., *Secreted phospholipases A(2): A proinflammatory connection between macrophages and mast cells in the human lung.* Immunobiology, 2009. **214**(9-10): p. 811-21.

275. Rosenthal, M.D., et al., *Human neutrophils store type II 14-kDa phospholipase A2 in granules and secrete active enzyme in response to soluble stimuli.* Biochem Biophys Res Commun, 1995. **208**(2): p. 650-6.

276. Murakami, M., et al., *Release of 14-kDa group-II phospholipase A2 from activated mast cells and its possible involvement in the regulation of the degranulation process.* Eur J Biochem, 1992. **209**(1): p. 257-65.

277. Cheung, K.L., et al., *Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a.* J Exp Med, 2016. **213**(11): p. 2399-2412.

278. Birkinshaw, R.W., et al., *alphabeta T cell antigen receptor recognition of CD1a presenting self lipid ligands.* Nat Immunol, 2015. **16**(3): p. 258-66.

279. Manolova, V., et al., *Functional CD1a is stabilized by exogenous lipids.* Eur J Immunol, 2006. **36**(5): p. 1083-92.

280. Manolova, V., et al., *CD1a and CD1b surface expression is independent from de novo synthesized glycosphingolipids.* Eur J Immunol, 2003. **33**(1): p. 29-37.

281. Garcia-Alles, L.F., et al., *Endogenous phosphatidylcholine and a long spacer ligand stabilize the lipid-binding groove of CD1b.* EMBO J, 2006. **25**(15): p. 3684-92.

282. Wang, J., et al., *Lipid binding orientation within CD1d affects recognition of Borrelia burgorferi antigens by NKT cells.* Proc Natl Acad Sci U S A, 2010. **107**(4): p. 1535-40.

283. de Jong, A., et al., *CD1a-autoreactive T cells are a normal component of the human alphabeta T cell repertoire.* Nat Immunol, 2010. **11**(12): p. 1102-9.

284. de Lalla, C., et al., *High-frequency and adaptive-like dynamics of human CD1 self-reactive T cells.* Eur J Immunol, 2011. **41**(3): p. 602-10.

285. Vincent, M.S., et al., *CD1a-, b-, and c-restricted TCRs recognize both self and foreign antigens.* Journal of Immunology, 2005. **175**(10): p. 6344-6351.

286. Boniface, K., et al., *IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes.* J Immunol, 2005. **174**(6): p. 3695-702.

287. Wolk, K., et al., *IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis.* Eur J Immunol, 2006. **36**(5): p. 1309-23.

288. Gutowska-Owsiak, D., et al., *Interleukin-22 downregulates filaggrin expression and affects expression of profilaggrin processing enzymes.* Br J Dermatol, 2011. **165**(3): p. 492-8.

289. Wolk, K., et al., *IL-22 increases the innate immunity of tissues.* Immunity, 2004. **21**(2): p. 241-54.

290. Avitabile, S., et al., *Interleukin-22 Promotes Wound Repair in Diabetes by Improving Keratinocyte Pro-Healing Functions.* J Invest Dermatol, 2015. **135**(11): p. 2862-2870.

291. Nograles, K.E., et al., *IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells.* J Allergy Clin Immunol, 2009. **123**(6): p. 1244-52 e2.

292. Gittler, J.K., et al., *Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis.* J Allergy Clin Immunol, 2012. **130**(6): p. 1344-54.

293. Salimi, M., et al., *A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis.* J Exp Med, 2013. **210**(13): p. 2939-50.

294. Sisirak, V., et al., *CCR6/CCR10-mediated plasmacytoid dendritic cell recruitment to inflamed epithelia after instruction in lymphoid tissues.* Blood, 2011. **118**(19): p. 5130-40.

295. Mathan, T.S., C.G. Figdor, and S.I. Buschow, *Human plasmacytoid dendritic cells: from molecules to intercellular communication network.* Front Immunol, 2013. **4**: p. 372.

296. Freedberg, I.M., et al., *Keratins and the keratinocyte activation cycle.* J Invest Dermatol, 2001. **116**(5): p. 633-40.

297. Aragona, M., et al., *Defining stem cell dynamics and migration during wound healing in mouse skin epidermis.* Nat Commun, 2017. **8**: p. 14684.

298. Hunger, R.E., et al., *Langerhans cells utilize CD1a and langerin to efficiently present nonpeptide antigens to T cells.* J Clin Invest, 2004. **113**(5): p. 701-8.

299. Paternoster, L., et al., *Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis.* Nat Genet, 2015. **47**(12): p. 1449-1456.

300. Angel, C.E., et al., *Cutting edge: CD1a+ antigen-presenting cells in human dermis respond rapidly to CCR7 ligands.* J Immunol, 2006. **176**(10): p. 5730-4.

301. Klechevsky, E., et al., *Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells.* Immunity, 2008. **29**(3): p. 497-510.

302. Haniffa, M., M. Gunawan, and L. Jardine, *Human skin dendritic cells in health and disease.* J Dermatol Sci, 2015. **77**(2): p. 85-92.

303. Katou, F., et al., *Immunological activation of dermal Langerhans cells in contact with lymphocytes in a model of human inflamed skin.* Am J Pathol, 2000. **156**(2): p. 519-27.

304. Kissenpfennig, A., et al., *Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells.* Immunity, 2005. **22**(5): p. 643-54.

305. Korenfeld, D., et al., *A type of human skin dendritic cell marked by CD5 is associated with the development of inflammatory skin disease.* JCI Insight, 2017. **2**(18).

306. Nicolaides, N., *Skin Lipids - Their Biochemical Uniqueness.* Science, 1974. **186**(4158): p. 19-26.

307. Srivatsan, S., et al., *CD2-associated protein regulates plasmacytoid dendritic cell migration, but is dispensable for their development and cytokine production.* J Immunol, 2013. **191**(12): p. 5933-40.

308. Haniffa, M., V. Bigley, and M. Collin, *Human mononuclear phagocyte system reunited.* Semin Cell Dev Biol, 2015. **41**: p. 59-69.

309. Mildner, A. and S. Jung, *Development and function of dendritic cell subsets.* Immunity, 2014. **40**(5): p. 642-56.

310. Schraml, B.U. and C. Reis e Sousa, *Defining dendritic cells.* Curr Opin Immunol, 2015. **32**: p. 13-20.

311. Kubo, A., et al., *External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers.* J Exp Med, 2009. **206**(13): p. 2937-46.

312. Merad, M., et al., *Langerhans cells renew in the skin throughout life under steady-state conditions.* Nat Immunol, 2002. **3**(12): p. 1135-41.

313. Ginhoux, F., et al., *Langerhans cells arise from monocytes in vivo.* Nat Immunol, 2006. **7**(3): p. 265-73.

314. Rozis, G., et al., *Human Langerhans' cells and dermal-type dendritic cells generated from CD34 stem cells express different toll-like receptors and secrete different cytokines in response to toll-like receptor ligands.* Immunology, 2008. **124**(3): p. 329-38.

315. Larsen, C.P., et al., *Migration and maturation of Langerhans cells in skin transplants and explants.* J Exp Med, 1990. **172**(5): p. 1483-93.

316. Ohl, L., et al., *CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions.* Immunity, 2004. **21**(2): p. 279-88.

317. Pierre, P., et al., *Developmental regulation of MHC class II transport in mouse dendritic cells.* Nature, 1997. **388**(6644): p. 787-92.

318. Bigley, V., et al., *Langerin-expressing dendritic cells in human tissues are related to CD1c+ dendritic cells and distinct from Langerhans cells and CD141high XCR1+ dendritic cells.* J Leukoc Biol, 2015. **97**(4): p. 627-34.

319. Igyarto, B.Z. and D.H. Kaplan, *Antigen presentation by Langerhans cells.* Curr Opin Immunol, 2013. **25**(1): p. 115-9.

320. Romano, E., et al., *Human Langerhans cells use an IL-15R-alpha/IL-15/pSTAT5-dependent mechanism to break T-cell tolerance against the self-differentiation tumor antigen WT1.* Blood, 2012. **119**(22): p. 5182-90.

321. Ruckert, R., et al., *Dendritic cell-derived IL-15 controls the induction of CD8 T cell immune responses.* Eur J Immunol, 2003. **33**(12): p. 3493-503.

322. Polak, M.E., et al., *CD70-CD27 interaction augments CD8+ T-cell activation by human epidermal Langerhans cells.* J Invest Dermatol, 2012. **132**(6): p. 1636-44.

323. van der Aar, A.M., et al., *Cutting edge: virus selectively primes human langerhans cells for CD70 expression promoting CD8+ T cell responses.* J Immunol, 2011. **187**(7): p. 3488-92.

324. Seneschal, J., et al., *Human epidermal Langerhans cells maintain immune homeostasis in skin by activating skin resident regulatory T cells.* Immunity, 2012. **36**(5): p. 873-84.

325. Mathers, A.R., et al., *Differential capability of human cutaneous dendritic cell subsets to initiate Th17 responses.* J Immunol, 2009. **182**(2): p. 921-33.

326. Penel-Sotirakis, K., et al., *Differential capacity of human skin dendritic cells to polarize CD4+ T cells into IL-17, IL-21 and IL-22 producing cells.* PLoS One, 2012. **7**(11): p. e45680.

327. Fujita, H., et al., *Human Langerhans cells induce distinct IL-22-producing CD4+ T cells lacking IL-17 production.* Proc Natl Acad Sci U S A, 2009. **106**(51): p. 21795-800.

328. Angel, C.E., et al., *CD14+ antigen-presenting cells in human dermis are less mature than their CD1a+ counterparts.* Int Immunol, 2007. **19**(11): p. 1271-9.

329. Serbina, N.V., et al., *TNF/iNOS-Producing Dendritic Cells Mediate Innate Immune Defense against Bacterial Infection.* Immunity, 2003. **19**(1): p. 59-70.

330. Furio, L., et al., *Human langerhans cells are more efficient than CD14(-)CD1c(+) dermal dendritic cells at priming naive CD4(+) T cells.* J Invest Dermatol, 2010. **130**(5): p. 1345-54.

331. Meunier, L., A. Gonzalez-Ramos, and K.D. Cooper, *Heterogeneous populations of class II MHC+ cells in human dermal cell suspensions. Identification of a small subset responsible for potent dermal antigen-presenting cell activity with features analogous to Langerhans cells.* J Immunol, 1993. **151**(8): p. 4067-80.

332. Nizzoli, G., et al., *IL-10 promotes homeostatic proliferation of human CD8(+) memory T cells and, when produced by CD1c(+) DCs, shapes naive CD8(+) T-cell priming.* Eur J Immunol, 2016. **46**(7): p. 1622-32.

333. Banchereau, J., et al., *The differential production of cytokines by human Langerhans cells and dermal CD14(+) DCs controls CTL priming.* Blood, 2012. **119**(24): p. 5742-9.

334. McGovern, N., et al., *Human dermal CD14(+) cells are a transient population of monocyte-derived macrophages.* Immunity, 2014. **41**(3): p. 465-477.

335. Briere, F., et al., *Human interleukin 10 induces naive surface immunoglobulin D+ (sIgD+) B cells to secrete IgG1 and IgG3.* J Exp Med, 1994. **179**(2): p. 757-62.

336. Dullaers, M., et al., *A T cell-dependent mechanism for the induction of human mucosal homing immunoglobulin A-secreting plasmablasts.* Immunity, 2009. **30**(1): p. 120-9.

337. Henri, S., et al., *CD207+ CD103+ dermal dendritic cells cross-present keratinocyte-derived antigens irrespective of the presence of Langerhans cells.* J Exp Med, 2010. **207**(1): p. 189-206.

338. Tamoutounour, S., et al., *Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin.* Immunity, 2013. **39**(5): p. 925-38.

339. Escola, J.M., et al., *Characterization of a lysozyme major histocompatibility complex class II molecule-loading compartment as a specialized recycling endosome in murine B lymphocytes.* Journal of Biological Chemistry, 1996. **271**(44): p. 27360-27365.

340. Fleming, A., *On a remarkable bacteriolytic element found in tissues and secretions.* Proc. R Soc. Lond. B Biol. Sci., 1922(93): p. 306–317.

341. Elhussein Mohamed, O.Y., et al., *Local elevation of CCL22: A new trend in immunotherapy (skin model).* Journal of Cellular Immunotherapy, 2016. **2**(2): p. 79-84.

342. Layseca-Espinosa, E., et al., *CCL22-producing CD8alpha- myeloid dendritic cells mediate regulatory T cell recruitment in response to G-CSF treatment.* J Immunol, 2013. **191**(5): p. 2266-72.

343. Kusumoto, M., et al., *Expression of chemokine receptor CCR4 and its ligands (CCL17 and CCL22) in murine contact hypersensitivity.* J Interferon Cytokine Res, 2007. **27**(11): p. 901-10.

344. Katou, F., et al., *Macrophage-derived chemokine (MDC/CCL22) and CCR4 are involved in the formation of T lymphocyte-dendritic cell clusters in human inflamed skin and secondary lymphoid tissue.* Am J Pathol, 2001. **158**(4): p. 1263-70.

345. Trapnell, C., et al., *The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells.* Nat Biotechnol, 2014. **32**(4): p. 381-386.

346. Liu, S. and C. Trapnell, *Single-cell transcriptome sequencing: recent advances and remaining challenges.* F1000Res, 2016. **5**.

347. Seshadri, C., et al., *Human CD1a deficiency is common and genetically regulated.* J Immunol, 2013. **191**(4): p. 1586-93.

348. Aquino, A., et al., *Exogenous control of the expression of Group I CD1 molecules competent for presentation of microbial nonpeptide antigens to human T lymphocytes.* Clin Dev Immunol, 2011. **2011**: p. 790460.

349. Wirths, S., et al., *Activated CD8+ T lymphocytes induce differentiation of monocytes to dendritic cells and restore the stimulatory capacity of interleukin 10-treated antigen-presenting cells.* Cancer Res, 2002. **62**(17): p. 5065-8.

350. Nascimento, C.R., et al., *The short chain fatty acid sodium butyrate regulates the induction of CD1a in developing dendritic cells.* Immunobiology, 2011. **216**(3): p. 275-84.

351. Kolodziejczyk, A.A., et al., *The technology and biology of single-cell RNA sequencing.* Mol Cell, 2015. **58**(4): p. 610-20.

352. Wang, Y. and N.E. Navin, *Advances and applications of single-cell sequencing technologies.* Mol Cell, 2015. **58**(4): p. 598-609.

353. Potter, S.S., *Single-cell RNA sequencing for the study of development, physiology and disease.* Nat Rev Nephrol, 2018. **14**(8): p. 479-492.

354. Angerer, P., et al., *Single cells make big data: New challenges and opportunities in transcriptomics.* Current Opinion in Systems Biology, 2017. **4**: p. 85-91.

355. Yang, J., et al., *Establishment and function of tissue-resident innate lymphoid cells in the skin.* Protein Cell, 2017. **8**(7): p. 489-500.

356. Spits, H., et al., *Innate lymphoid cells--a proposal for uniform nomenclature.* Nat Rev Immunol, 2013. **13**(2): p. 145-9.

357. Walker, J.A., J.L. Barlow, and A.N. McKenzie, *Innate lymphoid cells--how did we miss them?* Nat Rev Immunol, 2013. **13**(2): p. 75-87.

358. Sonnenberg, G.F., et al., *SnapShot: innate lymphoid cells.* Immunity, 2013. **39**(3): p. 622-622 e1.

359. Sonnenberg, G.F. and D. Artis, *Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease.* Immunity, 2012. **37**(4): p. 601-10.

360. Monticelli, L.A., G.F. Sonnenberg, and D. Artis, *Innate lymphoid cells: critical regulators of allergic inflammation and tissue repair in the lung.* Curr Opin Immunol, 2012. **24**(3): p. 284-9.

361. Eberl, G., J.P. Di Santo, and E. Vivier, *The brave new world of innate lymphoid cells.* Nat Immunol, 2015. **16**(1): p. 1-5.

362. Robinette, M.L., et al., *Transcriptional programs define molecular characteristics of innate lymphoid cell classes and subsets.* Nat Immunol, 2015. **16**(3): p. 306-17.

363. Diefenbach, A., M. Colonna, and S. Koyasu, *Development, differentiation, and diversity of innate lymphoid cells.* Immunity, 2014. **41**(3): p. 354-365.

364. Constantinides, M.G., et al., *A committed precursor to innate lymphoid cells.* Nature, 2014. **508**(7496): p. 397-401.

365. Takatori, H., et al., *Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22.* J Exp Med, 2009. **206**(1): p. 35-41.

366. Vonarbourg, C., et al., *Regulated expression of nuclear receptor RORgammat confers distinct functional fates to NK cell receptor-expressing RORgammat(+) innate lymphocytes.* Immunity, 2010. **33**(5): p. 736-51.

367. Montaldo, E., K. Juelke, and C. Romagnani, *Group 3 innate lymphoid cells (ILC3s): Origin, differentiation, and plasticity in humans and mice.* Eur J Immunol, 2015. **45**(8): p. 2171-82.

368. Satoh-Takayama, N., et al., *IL-7 and IL-15 independently program the differentiation of intestinal CD3-NKp46+ cell subsets from Id2-dependent precursors.* J Exp Med, 2010. **207**(2): p. 273-80.

369. Bjorklund, A.K., et al., *The heterogeneity of human CD127(+) innate lymphoid cells revealed by single-cell RNA sequencing.* Nat Immunol, 2016. **17**(4): p. 451-60.

370. Koues, O.I., et al., *Distinct Gene Regulatory Pathways for Human Innate versus Adaptive Lymphoid Cells.* Cell, 2016. **165**(5): p. 1134-1146.

371. Melo-Gonzalez, F. and M.R. Hepworth, *Functional and phenotypic heterogeneity of group 3 innate lymphoid cells.* Immunology, 2017. **150**(3): p. 265-275.

372. Simoni, Y. and E.W. Newell, *Dissecting human ILC heterogeneity: more than just three subsets.* Immunology, 2018. **153**(3): p. 297-303.

373. Satoh-Takayama, N., *Heterogeneity and diversity of group 3 innate lymphoid cells: new cells on the block.* Int Immunol, 2016. **28**(1): p. 29-34.

374. Klose, C.S. and D. Artis, *Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis.* Nat Immunol, 2016. **17**(7): p. 765-74.

375. Withers, D.R. and M.R. Hepworth, *Group 3 Innate Lymphoid Cells: Communications Hubs of the Intestinal Immune System.* Front Immunol, 2017. **8**: p. 1298.

376. Hoorweg, K., et al., *Functional Differences between Human NKp44(-) and NKp44(+) RORC(+) Innate Lymphoid Cells.* Front Immunol, 2012. **3**: p. 72.

377. Cupedo, T., et al., *Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to RORC+ CD127+ natural killer-like cells.* Nat Immunol, 2009. **10**(1): p. 66-74.

378. Satoh-Takayama, N., et al., *Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense.* Immunity, 2008. **29**(6): p. 958-70.

379. Spits, H. and J.P. Di Santo, *The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling.* Nat Immunol, 2011. **12**(1): p. 21-7.

380. Cella, M., et al., *A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity.* Nature, 2009. **457**(7230): p. 722-5.

381. Bruder, D., et al., *Neuropilin-1: a surface marker of regulatory T cells.* Eur J Immunol, 2004. **34**(3): p. 623-630.

382. Weiss, J.M., et al., *Neuropilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3+ T reg cells.* J Exp Med, 2012. **209**(10): p. 1723-42, S1.

383. Wrzesinski, S.H., J.L. Fisher, and M.S. Ernstoff, *Genetic profiles of plasmacytoid (BDCA-4 expressing) DC subtypes-clues to DC subtype function in vivo.* Exp Hematol Oncol, 2013. **2**(1): p. 8.

384. Blomberg, S., et al., *Expression of the markers BDCA-2 and BDCA-4 and production of interferon-alpha by plasmacytoid dendritic cells in systemic lupus erythematosus.* Arthritis Rheum, 2003. **48**(9): p. 2524-32.

385. Rankin, L.C., et al., *The transcription factor T-bet is essential for the development of NKp46+ innate lymphocytes via the Notch pathway.* Nat Immunol, 2013. **14**(4): p. 389-95.

386. Klose, C.S., et al., *A T-bet gradient controls the fate and function of CCR6-RORgammat+ innate lymphoid cells.* Nature, 2013. **494**(7436): p. 261-5.

387. Vitenshtein, A., et al., *NK Cell Recognition of Candida glabrata through Binding of NKp46 and NCR1 to Fungal Ligands Epa1, Epa6, and Epa7.* Cell Host Microbe, 2016. **20**(4): p. 527-534.

388. Glatzer, T., et al., *RORgammat(+) innate lymphoid cells acquire a proinflammatory program upon engagement of the activating receptor NKp44.* Immunity, 2013. **38**(6): p. 1223-35.

389. Johnson, H., et al., *Cell death induced by granzyme C.* Blood, 2003. **101**(8): p. 3093-101.

390. Bernink, J.H., et al., *Interleukin-12 and -23 Control Plasticity of CD127(+) Group 1 and Group 3 Innate Lymphoid Cells in the Intestinal Lamina Propria.* Immunity, 2015. **43**(1): p. 146-60.

391. Gury-BenAri, M., et al., *The Spectrum and Regulatory Landscape of Intestinal Innate Lymphoid Cells Are Shaped by the Microbiome.* Cell, 2016. **166**(5): p. 1231-1246 e13.

392. Sanos, S.L., et al., *RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells.* Nat Immunol, 2009. **10**(1): p. 83-91.

393. Serafini, N., C.A. Vosshenrich, and J.P. Di Santo, *Transcriptional regulation of innate lymphoid cell fate.* Nat Rev Immunol, 2015. **15**(7): p. 415-28.

394. Cherrier, M., S. Sawa, and G. Eberl, *Notch, Id2, and RORgammat sequentially orchestrate the fetal development of lymphoid tissue inducer cells.* J Exp Med, 2012. **209**(4): p. 729-40.

395. Klose, C.S.N., et al., *Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages.* Cell, 2014. **157**(2): p. 340-356.

396. Moro, K., et al., *Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells.* Nature, 2010. **463**(7280): p. 540-4.

397. Possot, C., et al., *Notch signaling is necessary for adult, but not fetal, development of RORgammat(+) innate lymphoid cells.* Nat Immunol, 2011. **12**(10): p. 949-58.

398. Carotta, S., et al., *Identification of the earliest NK-cell precursor in the mouse BM.* Blood, 2011. **117**(20): p. 5449-52.

399. Hoyler, T., et al., *The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells.* Immunity, 2012. **37**(4): p. 634-48.

400. Yu, Y., et al., *Single-cell RNA-seq identifies a PD-1(hi) ILC progenitor and defines its development pathway.* Nature, 2016. **539**(7627): p. 102-106.

401. Eberl, G., et al., *An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells.* Nat Immunol, 2004. **5**(1): p. 64-73.

402. Ishizuka, I.E., et al., *Single-cell analysis defines the divergence between the innate lymphoid cell lineage and lymphoid tissue-inducer cell lineage.* Nat Immunol, 2016. **17**(3): p. 269-76.

403. Zook, E.C. and B.L. Kee, *Development of innate lymphoid cells.* Nat Immunol, 2016. **17**(7): p. 775-82.

404. Kim, S.H., et al., *Microbiota-derived butyrate suppresses group 3 innate lymphoid cells in terminal ileal Peyer's patches.* Sci Rep, 2017. **7**(1): p. 3980.

405. Kiss, E.A., et al., *Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles.* Science, 2011. **334**(6062): p. 1561-5.

406. Lee, J.S., et al., *AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch.* Nat Immunol, 2011. **13**(2): p. 144-51.

407. Qiu, J., et al., *The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells.* Immunity, 2012. **36**(1): p. 92-104.

408. Qiu, J., et al., *Group 3 innate lymphoid cells inhibit T-cell-mediated intestinal inflammation through aryl hydrocarbon receptor signaling and regulation of microflora.* Immunity, 2013. **39**(2): p. 386-99.

409. Gomez de Aguero, M., et al., *The maternal microbiota drives early postnatal innate immune development.* Science, 2016. **351**(6279): p. 1296-302.

410. van de Pavert, S.A., et al., *Maternal retinoids control type 3 innate lymphoid cells and set the offspring immunity.* Nature, 2014. **508**(7494): p. 123-7.

411. Spencer, S.P., et al., *Adaptation of innate lymphoid cells to a micronutrient deficiency promotes type 2 barrier immunity.* Science, 2014. **343**(6169): p. 432-7.

412. Goverse, G., et al., *Vitamin A Controls the Presence of RORgamma+ Innate Lymphoid Cells and Lymphoid Tissue in the Small Intestine.* J Immunol, 2016. **196**(12): p. 5148-55.

413. Konya, V., et al., *Vitamin D downregulates the IL-23 receptor pathway in human mucosal group 3 innate lymphoid cells.* J Allergy Clin Immunol, 2018. **141**(1): p. 279-292.

414. Chen, J., et al., *Dysbiosis caused by vitamin D receptor deficiency confers colonization resistance to Citrobacter rodentium through modulation of innate lymphoid cells.* Mucosal Immunol, 2015. **8**(3): p. 618-26.

415. Montaldo, E., et al., *Human RORgammat(+)CD34(+) cells are lineage-specified progenitors of group 3 RORgammat(+) innate lymphoid cells.* Immunity, 2014. **41**(6): p. 988-1000.

416. Scoville, S.D., et al., *A Progenitor Cell Expressing Transcription Factor RORgammat Generates All Human Innate Lymphoid Cell Subsets.* Immunity, 2016. **44**(5): p. 1140-50.

417. Verrier, T., et al., *Phenotypic and Functional Plasticity of Murine Intestinal NKp46+ Group 3 Innate Lymphoid Cells.* J Immunol, 2016. **196**(11): p. 4731-8.

418. Viant, C., et al., *Transforming growth factor-beta and Notch ligands act as opposing environmental cues in regulating the plasticity of type 3 innate lymphoid cells.* Sci Signal, 2016. **9**(426): p. ra46.

419. Zhong, C., et al., *Group 3 innate lymphoid cells continuously require the transcription factor GATA-3 after commitment.* Nat Immunol, 2016. **17**(2): p. 169-78.

420. Schmutz, S., et al., *Cutting edge: IL-7 regulates the peripheral pool of adult ROR gamma+ lymphoid tissue inducer cells.* J Immunol, 2009. **183**(4): p. 2217-21.

421. Chappaz, S., et al., *Kit ligand and Il7 differentially regulate Peyer's patch and lymph node development.* J Immunol, 2010. **185**(6): p. 3514-9.

422. Chappaz, S. and D. Finke, *The IL-7 signaling pathway regulates lymph node development independent of peripheral lymphocytes.* J Immunol, 2010. **184**(7): p. 3562-9.

423. Robinette, M.L., et al., *IL-15 sustains IL-7R-independent ILC2 and ILC3 development.* Nat Commun, 2017. **8**: p. 14601.

424. Tang, Q., et al., *Development of IL-22-producing NK lineage cells from umbilical cord blood hematopoietic stem cells in the absence of secondary lymphoid tissue.* Blood, 2011. **117**(15): p. 4052-5.

425. Montaldo, E., et al., *Development of human natural killer cells and other innate lymphoid cells.* Semin Immunol, 2014. **26**(2): p. 107-13.

426. Ambrosini, P., et al., *IL-1beta inhibits ILC3 while favoring NK-cell maturation of umbilical cord blood CD34(+) precursors.* Eur J Immunol, 2015. **45**(7): p. 2061-71.

427. Raykova, A., et al., *Interleukins 12 and 15 induce cytotoxicity and early NK-cell differentiation in type 3 innate lymphoid cells.* Blood Adv, 2017. **1**(27): p. 2679-2691.

428. Guo, L., I.S. Junttila, and W.E. Paul, *Cytokine-induced cytokine production by conventional and innate lymphoid cells.* Trends Immunol, 2012. **33**(12): p. 598-606.

429. Reynders, A., et al., *Identity, regulation and in vivo function of gut NKp46+RORgammat+ and NKp46+RORgammat- lymphoid cells.* EMBO J, 2011. **30**(14): p. 2934-47.

430. Cella, M., K. Otero, and M. Colonna, *Expansion of human NK-22 cells with IL-7, IL-2, and IL-1beta reveals intrinsic functional plasticity.* Proc Natl Acad Sci U S A, 2010. **107**(24): p. 10961-6.

431. Hepworth, M.R. and G.F. Sonnenberg, *Regulation of the adaptive immune system by innate lymphoid cells.* Curr Opin Immunol, 2014. **27**: p. 75-82.

432. Hepworth, M.R., et al., *Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria.* Nature, 2013. **498**(7452): p. 113-7.

433. Hepworth, M.R., et al., *Immune tolerance. Group 3 innate lymphoid cells mediate intestinal selection of commensal bacteria-specific CD4(+) T cells.* Science, 2015. **348**(6238): p. 1031-5.

434. von Burg, N., et al., *Activated group 3 innate lymphoid cells promote T-cell-mediated immune responses.* Proc Natl Acad Sci U S A, 2014. **111**(35): p. 12835-40.

435. Saez de Guinoa, J., et al., *CD1d-mediated activation of group 3 innate lymphoid cells drives IL-22 production.* EMBO Rep, 2017. **18**(1): p. 39-47.

436. Luci, C., et al., *Influence of the transcription factor RORgammat on the development of NKp46+ cell populations in gut and skin.* Nat Immunol, 2009. **10**(1): p. 75-82.

437. Villanova, F., et al., *Characterization of innate lymphoid cells in human skin and blood demonstrates increase of NKp44+ ILC3 in psoriasis.* J Invest Dermatol, 2014. **134**(4): p. 984-991.

438. Teunissen, M.B., et al., *Composition of innate lymphoid cell subsets in the human skin: enrichment of NCR(+) ILC3 in lesional skin and blood of psoriasis patients.* J Invest Dermatol, 2014. **134**(9): p. 2351-60.

439. Bruggen, M.C., et al., *In Situ Mapping of Innate Lymphoid Cells in Human Skin: Evidence for Remarkable Differences between Normal and Inflamed Skin.* J Invest Dermatol, 2016. **136**(12): p. 2396-2405.

440. Keren, A., et al., *Innate lymphoid cells 3 induce psoriasis in xenotransplanted healthy human skin.* J Allergy Clin Immunol, 2018. **142**(1): p. 305-308 e6.

441. Li, Z., et al., *Epidermal Notch1 recruits RORgamma(+) group 3 innate lymphoid cells to orchestrate normal skin repair.* Nat Commun, 2016. **7**: p. 11394.

442. Griffiths, C.E., et al., *A classification of psoriasis vulgaris according to phenotype.* Br J Dermatol, 2007. **156**(2): p. 258-62.

443. Di Meglio, P., F. Villanova, and F.O. Nestle, *Psoriasis.* Cold Spring Harb Perspect Med, 2014. **4**(8): p. 30.

444. Lowes, M.A., M. Suarez-Farinas, and J.G. Krueger, *Immunology of psoriasis.* Annu Rev Immunol, 2014. **32**: p. 227-55.

445. Bowcock, A.M. and J.G. Krueger, *Getting under the skin: the immunogenetics of psoriasis.* Nat Rev Immunol, 2005. **5**(9): p. 699-711.

446. Barisic-Drusko, V. and I. Rucevic, *Trigger factors in childhood psoriasis and vitiligo.* Coll Antropol, 2004. **28**(1): p. 277-85.

447. Quesada, J.R. and J.U. Gutterman, *Psoriasis and alpha-interferon.* Lancet, 1986. **1**(8496): p. 1466-8.

448. Balak, D.M. and E. Hajdarbegovic, *Drug-induced psoriasis: clinical perspectives.* Psoriasis (Auckl), 2017. **7**: p. 87-94.

449. Dhingra, N., et al., *Attenuated neutrophil axis in atopic dermatitis compared to psoriasis reflects TH17 pathway differences between these diseases.* J Allergy Clin Immunol, 2013. **132**(2): p. 498-501 e3.

450. Choy, D.F., et al., *Comparative transcriptomic analyses of atopic dermatitis and psoriasis reveal shared neutrophilic inflammation.* J Allergy Clin Immunol, 2012. **130**(6): p. 1335-43 e5.

451. Nestle, F.O., et al., *Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production.* Journal of Experimental Medicine, 2005. **202**(1): p. 135-143.

452. Valdimarsson, H., et al., *Psoriasis: a disease of abnormal Keratinocyte proliferation induced by T lymphocytes.* Immunol Today, 1986. **7**(9): p. 256-9.

453. Conrad, C., et al., *Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis.* Nat Med, 2007. **13**(7): p. 836-42.

454. Kryczek, I., et al., *Induction of IL-17+ T cell trafficking and development by IFN-gamma: mechanism and pathological relevance in psoriasis.* J Immunol, 2008. **181**(7): p. 4733-41.

455. Sonnenberg, G.F., L.A. Fouser, and D. Artis, *Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22.* Nat Immunol, 2011. **12**(5): p. 383-90.

456. Ward, N.L. and D.T. Umetsu, *A new player on the psoriasis block: IL-17A- and IL-22-producing innate lymphoid cells.* J Invest Dermatol, 2014. **134**(9): p. 2305-2307.

457. Piskin, G., et al., *In vitro and in situ expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin.* J Immunol, 2006. **176**(3): p. 1908-15.

458. Res, P.C., et al., *Overrepresentation of IL-17A and IL-22 producing CD8 T cells in lesional skin suggests their involvement in the pathogenesis of psoriasis.* PLoS One, 2010. **5**(11): p. e14108.

459. Boniface, K., et al., *A role for T cell-derived interleukin 22 in psoriatic skin inflammation.* Clin Exp Immunol, 2007. **150**(3): p. 407-15.

460. Nikoopour, E., S.M. Bellemore, and B. Singh, *IL-22, cell regeneration and autoimmunity.* Cytokine, 2015. **74**(1): p. 35-42.

461. Wolk, K., et al., *IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not.* J Mol Med (Berl), 2009. **87**(5): p. 523-36.

462. Arican, O., et al., *Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity.* Mediators Inflamm, 2005. **2005**(5): p. 273-9.

463. Martin, D.A., et al., *The emerging role of IL-17 in the pathogenesis of psoriasis: preclinical and clinical findings.* J Invest Dermatol, 2013. **133**(1): p. 17-26.

464. Harper, E.G., et al., *Th17 cytokines stimulate CCL20 expression in keratinocytes in vitro and in vivo: implications for psoriasis pathogenesis.* J Invest Dermatol, 2009. **129**(9): p. 2175-83.

465. Johansen, C., et al., *Characterization of the interleukin-17 isoforms and receptors in lesional psoriatic skin.* Br J Dermatol, 2009. **160**(2): p. 319-24.

466. Russell, C.B., et al., *Blockade of the IL-17R with AMG 827 leads to rapid reversal of gene expression and histopathologic abnormalities in psoriatic skin, including substantial pathway-specific effects within one week.* Journal of Investigative Dermatology, 2011. **131**: p. S11-S11.

467. Lowes, M.A., et al., *Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells.* J Invest Dermatol, 2008. **128**(5): p. 1207-11.

468. Liang, S.C., et al., *Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides.* J Exp Med, 2006. **203**(10): p. 2271-9.

469. Cargill, M., et al., *A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes.* Am J Hum Genet, 2007. **80**(2): p. 273-90.

470. Nair, R.P., et al., *Polymorphisms of the IL12B and IL23R genes are associated with psoriasis.* J Invest Dermatol, 2008. **128**(7): p. 1653-61.

471. Ellinghaus, E., et al., *Genome-wide association study identifies a psoriasis susceptibility locus at TRAF3IP2.* Nat Genet, 2010. **42**(11): p. 991-5.

472. Ellinghaus, E., et al., *Genome-wide meta-analysis of psoriatic arthritis identifies susceptibility locus at REL.* J Invest Dermatol, 2012. **132**(4): p. 1133-40.

473. Stuart, P.E., et al., *Genome-wide association analysis identifies three psoriasis susceptibility loci.* Nat Genet, 2010. **42**(11): p. 1000-4.

474. Huffmeier, U., et al., *Genetic variants of the IL-23R pathway: association with psoriatic arthritis and psoriasis vulgaris, but no specific risk factor for arthritis.* J Invest Dermatol, 2009. **129**(2): p. 355-8.

475. Marrakchi, S., et al., *Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis.* N Engl J Med, 2011. **365**(7): p. 620-8.

476. Onoufriadis, A., et al., *Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis.* Am J Hum Genet, 2011. **89**(3): p. 432-7.

477. Capon, F., *IL36RN mutations in generalized pustular psoriasis: just the tip of the iceberg?* J Invest Dermatol, 2013. **133**(11): p. 2503-2504.

478. Hussain, S., et al., *IL36RN mutations define a severe autoinflammatory phenotype of generalized pustular psoriasis.* J Allergy Clin Immunol, 2015. **135**(4): p. 1067-70 e9.

479. Setta-Kaffetzi, N., et al., *Rare pathogenic variants in IL36RN underlie a spectrum of psoriasis-associated pustular phenotypes.* J Invest Dermatol, 2013. **133**(5): p. 1366-9.

480. Lin, A.M., et al., *Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis.* J Immunol, 2011. **187**(1): p. 490-500.

481. Kim, H.Y., et al., *Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity.* Nat Med, 2014. **20**(1): p. 54-61.

482. Muir, R., et al., *Innate Lymphoid Cells Are the Predominant Source of IL-17A during the Early Pathogenesis of Acute Respiratory Distress Syndrome.* Am J Respir Crit Care Med, 2016. **193**(4): p. 407-16.

483. Amin, K., *The role of mast cells in allergic inflammation.* Respir Med, 2012. **106**(1): p. 9-14.

484. Leonardi, C.L., et al., *Etanercept as monotherapy in patients with psoriasis.* N Engl J Med, 2003. **349**(21): p. 2014-22.

485. Papp, K.A., et al., *Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2).* Lancet, 2008. **371**(9625): p. 1675-84.

486. Leonardi, C.L., et al., *Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1).* Lancet, 2008. **371**(9625): p. 1665-74.

487. Smith, S.H., et al., *Tapinarof Is a Natural AhR Agonist that Resolves Skin Inflammation in Mice and Humans.* J Invest Dermatol, 2017. **137**(10): p. 2110-2119.

488. Zaba, L.C., et al., *Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses.* J Exp Med, 2007. **204**(13): p. 3183-94.

489. Fantuzzi, F., et al., *Targeting tumor necrosis factor alpha in psoriasis and psoriatic arthritis.* Expert Opin Ther Targets, 2008. **12**(9): p. 1085-96.

490. Yost, J. and J.E. Gudjonsson, *The role of TNF inhibitors in psoriasis therapy: new implications for associated comorbidities.* F1000 Med Rep, 2009. **1**.

491. Leonardi, C., et al., *Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis.* N Engl J Med, 2012. **366**(13): p. 1190-9.

492. Papp, K.A., et al., *Brodalumab, an anti-interleukin-17-receptor antibody for psoriasis.* N Engl J Med, 2012. **366**(13): p. 1181-9.

493. Genovese, M.C., et al., *LY2439821, a humanized anti-interleukin-17 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: A phase I randomized, double-blind, placebo-controlled, proof-of-concept study.* Arthritis Rheum, 2010. **62**(4): p. 929-39.

494. Hueber, W., et al., *Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis.* Sci Transl Med, 2010. **2**(52): p. 52ra72.

495. Russell, C.B., et al., *Blockade of the IL-17R with AMG 827 leads to rapid reversal of gene expression and histopathologic abnormalities in human psoriatic skin.* Journal of Investigative Dermatology, 2010. **130**: p. S46-S46.

496. Wasilewska, A., et al., *Interleukin-17 inhibitors. A new era in treatment of psoriasis and other skin diseases.* Postepy Dermatol Alergol, 2016. **33**(4): p. 247-52.

497. Chiricozzi, A. and J.G. Krueger, *IL-17 targeted therapies for psoriasis.* Expert Opin Investig Drugs, 2013. **22**(8): p. 993-1005.

498. Dong, J. and G. Goldenberg, *New biologics in psoriasis: an update on IL-23 and IL-17 inhibitors.* Cutis, 2017. **99**(2): p. 123-127.

499. Levin, A.A. and J.M. Sobell, *Update on IL-17 Inhibitors for Psoriasis.* Current Dermatology Reports, 2017. **6**(2): p. 121-128.

500. Roman, M. and M.W. Chiu, *Spotlight on brodalumab in the treatment of moderate-to-severe plaque psoriasis: design, development, and potential place in therapy.* Drug Des Devel Ther, 2017. **11**: p. 2065-2075.

501. Papp, K., et al., *Tildrakizumab (MK-3222), an anti-interleukin-23p19 monoclonal antibody, improves psoriasis in a phase IIb randomized placebo-controlled trial.* Br J Dermatol, 2015. **173**(4): p. 930-9.

502. Papp, K.A., et al., *Risankizumab versus Ustekinumab for Moderate-to-Severe Plaque Psoriasis.* N Engl J Med, 2017. **376**(16): p. 1551-1560.

503. Gordon, K.B., et al., *A Phase 2 Trial of Guselkumab versus Adalimumab for Plaque Psoriasis.* N Engl J Med, 2015. **373**(2): p. 136-44.

504. Conrad, C., et al., *TNF blockade induces a dysregulated type I interferon response without autoimmunity in paradoxical psoriasis.* Nat Commun, 2018. **9**(1): p. 25.

505. Duerr, C.U., et al., *Type I interferon restricts type 2 immunopathology through the regulation of group 2 innate lymphoid cells.* Nat Immunol, 2016. **17**(1): p. 65-75.

506. Paoletti, L.C., et al., *Group B Streptococcus type II polysaccharide-tetanus toxoid conjugate vaccine.* Infect Immun, 1992. **60**(10): p. 4009-14.

507. Zavala, F., et al., *Rationale for development of a synthetic vaccine against Plasmodium falciparum malaria.* Science, 1985. **228**(4706): p. 1436-40.

508. Mukherjee, J., A. Casadevall, and M.D. Scharff, *Molecular characterization of the humoral responses to Cryptococcus neoformans infection and glucuronoxylomannan-tetanus toxoid conjugate immunization.* J Exp Med, 1993. **177**(4): p. 1105-16.

509. Palker, T.J., et al., *Mapping of Homologous, Amino-Terminal Neutralizing Regions of Human T-Cell Lymphotropic Virus Type-I and Type-Ii Gp46 Envelope Glycoproteins.* Journal of Virology, 1992. **66**(10): p. 5879-5889.

510. Demotz, S., et al., *Delineation of Several Dr-Restricted Tetanus Toxin T-Cell Epitopes.* Journal of Immunology, 1989. **142**(2): p. 394-402.

511. Panina-Bordignon, P., et al., *Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells.* Eur J Immunol, 1989. **19**(12): p. 2237-42.

512. Ho, P.C., et al., *Identification of two promiscuous T cell epitopes from tetanus toxin.* Eur J Immunol, 1990. **20**(3): p. 477-83.

513. Cellerai, C., et al., *Functional and phenotypic characterization of tetanus toxoid-specific human CD4+ T cells following re-immunization.* Eur J Immunol, 2007. **37**(4): p. 1129-38.

514. Kloverpris, H.N., et al., *Innate Lymphoid Cells Are Depleted Irreversibly during Acute HIV-1 Infection in the Absence of Viral Suppression.* Immunity, 2016. **44**(2): p. 391-405.

515. Zhang, Z., et al., *Plasmacytoid dendritic cells promote HIV-1-induced group 3 innate lymphoid cell depletion.* J Clin Invest, 2015. **125**(9): p. 3692-703.

516. Boyd, A., J.M. Ribeiro, and T.B. Nutman, *Human CD117 (cKit)+ innate lymphoid cells have a discrete transcriptional profile at homeostasis and are expanded during filarial infection.* PLoS One, 2014. **9**(9): p. e108649.

517. Vivier, E., et al., *Innate or adaptive immunity? The example of natural killer cells.* Science, 2011. **331**(6013): p. 44-9.

518. Spits, H. and L.L. Lanier, *Natural killer or dendritic: what's in a name?* Immunity, 2007. **26**(1): p. 11-6.

519. Blasius, A.L., et al., *Development and function of murine B220+CD11c+NK1.1+ cells identify them as a subset of NK cells.* J Exp Med, 2007. **204**(11): p. 2561-8.

520. Vosshenrich, C.A., et al., *CD11cloB220+ interferon-producing killer dendritic cells are activated natural killer cells.* J Exp Med, 2007. **204**(11): p. 2569-78.

521. Caminschi, I., et al., *Putative IKDCs are functionally and developmentally similar to natural killer cells, but not to dendritic cells.* J Exp Med, 2007. **204**(11): p. 2579-90.

522. Phillips, J.H., A.M. Le, and L.L. Lanier, *Natural killer cells activated in a human mixed lymphocyte response culture identified by expression of Leu-11 and class II histocompatibility antigens.* J Exp Med, 1984. **159**(4): p. 993-1008.

523. Hanna, J., et al., *Novel APC-like properties of human NK cells directly regulate T cell activation.* J Clin Invest, 2004. **114**(11): p. 1612-23.

524. Roncarolo, M.G., et al., *Natural-Killer-Cell Clones Can Efficiently Process and Present Protein Antigens.* Journal of Immunology, 1991. **147**(3): p. 781-787.

525. Feldmeyer, L., et al., *Interleukin-1, inflammasomes and the skin.* Eur J Cell Biol, 2010. **89**(9): p. 638-44.

526. Sims, J.E. and D.E. Smith, *The IL-1 family: regulators of immunity.* Nat Rev Immunol, 2010. **10**(2): p. 89-102.

527. Jensen, L.E., *Targeting the IL-1 family members in skin inflammation.* Curr Opin Investig Drugs, 2010. **11**(11): p. 1211-20.

528. Sanmiguel, J.C., et al., *Interleukin-1 regulates keratinocyte expression of T cell targeting chemokines through interleukin-1 receptor associated kinase-1 (IRAK1) dependent and independent pathways.* Cell Signal, 2009. **21**(5): p. 685-94.

529. Coccia, M., et al., *IL-1beta mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4(+) Th17 cells.* J Exp Med, 2012. **209**(9): p. 1595-609.

530. Mayer-Barber, K.D. and B. Yan, *Clash of the Cytokine Titans: counter-regulation of interleukin-1 and type I interferon-mediated inflammatory responses.* Cell Mol Immunol, 2017. **14**(1): p. 22-35.

531. Ludigs, K., et al., *Type I IFN-mediated regulation of IL-1 production in inflammatory disorders.* Cell Mol Life Sci, 2012. **69**(20): p. 3395-418.

532. Guarda, G., et al., *Type I interferon inhibits interleukin-1 production and inflammasome activation.* Immunity, 2011. **34**(2): p. 213-23.

533. Tian, Z., et al., *IL-1 beta attenuates IFN-alpha beta-induced antiviral activity and STAT1 activation in the liver: involvement of proteasome-dependent pathway.* J Immunol, 2000. **165**(7): p. 3959-65.

534. Kohase, M., et al., *Interleukin-1 can inhibit interferon-beta synthesis and its antiviral action: comparison with tumor necrosis factor.* J Interferon Res, 1988. **8**(4): p. 559-70.

535. Mayer-Barber, K.D., et al., *Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk.* Nature, 2014. **511**(7507): p. 99-103.

536. Henry, T., et al., *Type I IFN signaling constrains IL-17A/F secretion by gammadelta T cells during bacterial infections.* J Immunol, 2010. **184**(7): p. 3755-67.

537. Castiglia, V., et al., *Type I Interferon Signaling Prevents IL-1beta-Driven Lethal Systemic Hyperinflammation during Invasive Bacterial Infection of Soft Tissue.* Cell Host Microbe, 2016. **19**(3): p. 375-87.

538. Perkins, D.J., et al., *Salmonella Typhimurium Co-Opts the Host Type I IFN System To Restrict Macrophage Innate Immune Transcriptional Responses Selectively.* J Immunol, 2015. **195**(5): p. 2461-71.

539. Malefyt, R.D., et al., *Interleukin-10(Il-10) Inhibits Cytokine Synthesis by Human Monocytes - an Autoregulatory Role of Il-10 Produced by Monocytes.* Journal of Experimental Medicine, 1991. **174**(5): p. 1209-1220.

540. Huang, Y., L.M. Blatt, and M.W. Taylor, *Type 1 interferon as an antiinflammatory agent: inhibition of lipopolysaccharide-induced interleukin-1 beta and induction of interleukin-1 receptor antagonist.* J Interferon Cytokine Res, 1995. **15**(4): p. 317-21.

541. Tilg, H., et al., *Induction of circulating IL-1 receptor antagonist by IFN treatment.* J Immunol, 1993. **150**(10): p. 4687-92.

542. Coclet-Ninin, J., J.M. Dayer, and D. Burger, *Interferon-beta not only inhibits interleukin-1 beta and tumor necrosis factor-alpha but stimulates interleukin-1 receptor antagonist production in human peripheral blood mononuclear cells.* European Cytokine Network, 1997. **8**(4): p. 345-349.

543. Billiau, A., *Anti-inflammatory properties of Type I interferons.* Antiviral Res, 2006. **71**(2-3): p. 108-16.

544. Novikov, A., et al., *Mycobacterium tuberculosis triggers host type I IFN signaling to regulate IL-1beta production in human macrophages.* J Immunol, 2011. **187**(5): p. 2540-7.

545. Clark, R. and T. Kupper, *Old meets new: the interaction between innate and adaptive immunity.* J Invest Dermatol, 2005. **125**(4): p. 629-37.

546. Szegedi, K., et al., *Cytokine profiles in interstitial fluid from chronic atopic dermatitis skin.* J Eur Acad Dermatol Venereol, 2015. **29**(11): p. 2136-44.

547. Arias-Santiago, S., M.J. Espineira-Carmona, and J. Aneiros-Fernandez, *The Koebner phenomenon: psoriasis in tattoos.* CMAJ, 2013. **185**(7): p. 585.

548. Pedace, F.J., S.A. Muller, and R.K. Winkelmann, *The biology of psoriasis. An experimental study of the Koebner phenomenon.* Acta Derm Venereol, 1969. **49**(4): p. 390-400.

549. Sagi, L. and H. Trau, *The Koebner phenomenon.* Clin Dermatol, 2011. **29**(2): p. 231-6.

550. Powles, A.V., et al., *Epidermal Rupture Is the Initiating Factor for the Koebner Response in Psoriasis.* Acta Dermato-Venereologica, 1990. **70**(1): p. 35-38.

551. Dudakov, J.A., A.M. Hanash, and M.R.M. van den Brink, *Interleukin-22: Immunobiology and Pathology*, in *Annual Review of Immunology Vol 33*, D.R. Littman and W.M. Yokoyama, Editors. 2015. p. 747-785.

552. Fujita, H., *The role of IL-22 and Th22 cells in human skin diseases.* J Dermatol Sci, 2013. **72**(1): p. 3-8.

553. Sonnenberg, G.F., L.A. Fouser, and D. Artis, *Functional Biology of the IL-22-IL-22R Pathway in Regulating Immunity and Inflammation at Barrier Surfaces*, in *Mucosal Immunity*, S. Fagarasan and A. Cerutti, Editors. 2010. p. 1-+.

554. Ismail, A. and N. Yusuf, *Type I interferons: key players in normal skin and select cutaneous malignancies.* Dermatol Res Pract, 2014. **2014**: p. 847545.

555. Krasagakis, K., et al., *Effects of Interferons on Cultured Human Melanocytes Invitro - Interferon-Beta but Not Interferon-Alpha or Interferon-Gamma Inhibit Proliferation and All Interferons Significantly Modulate the Cell Phenotype.* Journal of Investigative Dermatology, 1991. **97**(2): p. 364-372.

556. Niederwieser, D., et al., *IFN-mediated induction of MHC antigen expression on human keratinocytes and its influence on in vitro alloimmune responses.* J Immunol, 1988. **140**(8): p. 2556-64.

557. Bielenberg, D.R., et al., *Expression of interferon-beta is associated with growth arrest of murine and human epidermal cells.* J Invest Dermatol, 1999. **112**(5): p. 802-9.

558. Yaar, M., et al., *Effects of alpha and beta interferons on cultured human keratinocytes.* J Invest Dermatol, 1985. **85**(1): p. 70-4.

559. Nickoloff, B.J., et al., *Antiproliferative effects of recombinant alpha- and gamma-interferons on cultured human keratinocytes.* Lab Invest, 1984. **51**(6): p. 697-701.

560. Lauwerys, B.R., J. Ducreux, and F.A. Houssiau, *Type I interferon blockade in systemic lupus erythematosus: where do we stand?* Rheumatology (Oxford), 2014. **53**(8): p. 1369-76.

561. Hao, J.Q., *Targeting interleukin-22 in psoriasis.* Inflammation, 2014. **37**(1): p. 94-9.

562. Wallace, D.J., M. A. Petri, and N. Olsen, *MEDI-545, an anti-interferon alpha monoclonal antibody, shows evidence of clinical activity in systemic lupus erythematosus*. Vol. 56. 2007. S526-S527.

563. Khamashta, M., et al., *Sifalimumab, an anti-interferon-alpha monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study.* Ann Rheum Dis, 2016. **75**(11): p. 1909-1916.

564. Petri, M., et al., *Sifalimumab, a human anti-interferon-alpha monoclonal antibody, in systemic lupus erythematosus: a phase I randomized, controlled, dose-escalation study.* Arthritis Rheum, 2013. **65**(4): p. 1011-21.

565. Merrill, J.T., et al., *Safety profile and clinical activity of sifalimumab, a fully human anti-interferon alpha monoclonal antibody, in systemic lupus erythematosus: a phase I, multicentre, double-blind randomised study.* Ann Rheum Dis, 2011. **70**(11): p. 1905-13.

566. Bissonnette, R., et al., *A randomized, double-blind, placebo-controlled, phase I study of MEDI-545, an anti-interferon-alfa monoclonal antibody, in subjects with chronic psoriasis.* J Am Acad Dermatol, 2010. **62**(3): p. 427-36.

567. Sabat, R., W. Ouyang, and K. Wolk, *Therapeutic opportunities of the IL-22-IL-22R1 system.* Nat Rev Drug Discov, 2014. **13**(1): p. 21-38.

568. Krueger, J.G., *Hiding under the skin: A welcome surprise in psoriasis.* Nat Med, 2012. **18**(12): p. 1750-1.

569. Lebwohl, M., et al., *Phase 3 Studies Comparing Brodalumab with Ustekinumab in Psoriasis.* N Engl J Med, 2015. **373**(14): p. 1318-28.

570. Guttman-Yassky, E., et al., *Efficacy and safety of fezakinumab (an IL-22 monoclonal antibody) in adults with moderate-to-severe atopic dermatitis*

inadequately controlled by conventional treatments: A randomized, double-blind, phase 2a trial. J Am Acad Dermatol, 2018. **78**(5): p. 872-881 e6.