

Immunity against *Candida albicans* skin infection

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

Sakeen Wali Kashem

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
Doctor of Philosophy

Daniel H. Kaplan (Advisor)

May 2016

© Sakeen Wali Kashem 2016
ALL RIGHTS RESERVED

Acknowledgements

I would like to thank my parents, my brother and my extended family for their support. You made personal sacrifices and invested in my education. I took this for granted growing up, but I now thank you for making me valuing academic curiosity.

I thank the members of the Kaplan lab, including, but not limited to, Botond, Chen, Brian C., Javed, Felix, Nathan, Aleh, Toshiro, Brian A., Beth and Krystal. Many mornings, I found myself waking up to wonder why I gave up the view of New York City for that of the unrelenting Minnesota winter. I would then arrive to find myself in the warmth of your company. Our discussions together were a key part of any and all experimental success, as well as my personal growth. I will also never forget all the croquet games we played, meetings we have attended and the list of lab mistakes we have made. I look forward to all of your successes.

I would like to acknowledge the support I have received from the students and staff of the Center for Immunology, MICaB, and the Medical Scientist Training Program. Thank you for being a part of my experiments and training and thank you for allowing me to be a small part of yours. I am exceptionally honored to be your colleague and am in gratitude for your continued friendship. I would also like to recognize the CFI faculty for providing me with advice, insight and direction. I would specifically like to thank Bryce Binstadt for his continued clinical mentorship. Your feedback is one of the best things to happen to me. I would also like to thank the Fife, Mueller and Jenkins labs for their collegiality, assistance and friendship. I thank Marc Jenkins for installing a spirit of approachability in the CFI and for opening his lab and lab meetings to the CFI.

Most importantly, I thank my mentor Daniel Kaplan for everything. In brief, you are the best mentor I could have asked for. Our scientific conversations were the highlight of my years in Minnesota. Thank you for teaching me how to ask questions. I look up to you and would be nothing without you.

Dedication

To the questions in science that are yet to be solved or asked...
and to all of the generous sacrifices that have allowed for my scientific pursuits.

Abstract

Candida albicans is a dimorphic commensal fungus that colonizes the healthy human skin, mucosa and reproductive tracts. *C. albicans* is also a predominant opportunistic fungal pathogen, leading to disease manifestations such as disseminated candidiasis and chronic mucocutaneous candidiasis (CMC). The differing host susceptibilities to the sites of *C. albicans* infection have revealed tissue compartmentalization with tailoring of immune responses based on site of infection. Furthermore, extensive studies of host genetics in rare cases of CMC have identified conserved genetic pathways involved in the immune recognition and response to the extracellular pathogens. In this dissertation, we focus on mouse skin as a site of *C. albicans* infection and define the mechanisms behind innate and adaptive resistance to *C. albicans* skin infection.

Contents

Acknowledgements	i
Dedication	ii
Abstract	iii
List of Tables	vii
List of Figures	viii
1 Introduction	1
1.1 Immunity against <i>Candida albicans</i> is compartmentalized to the route of infection	2
1.2 Innate immune response to <i>C. albicans</i> skin infections	6
1.2.1 Pattern recognition	6
1.2.2 Stromal cells	8
1.2.3 Cutaneous nerves	9
1.2.4 IL-23 and antigen presenting cells	9
1.2.5 IL-17 and $\gamma\delta$ T cells	10
1.2.6 Neutrophils	12
1.3 Adaptive Immunity against <i>C. albicans</i> skin infection	14
1.3.1 Th17 cell differentiation	14
1.3.2 Th1 cell differentiation and compartmentalization of the effector responses	15
1.4 Publication and contributions	17
2 Innate immunity against <i>Candida albicans</i> skin infection	19
2.1 Introduction	20

2.2	Results	21
2.2.1	IL-17A from $\gamma\delta$ T cells is required for resistance to cutaneous candidiasis	21
2.2.2	IL-17 producing $\gamma\delta$ T cells require IL-23	24
2.2.3	IL-23 from CD301b+ dermal DC is critical for IL-17 production by dermal $\gamma\delta$ T cells and pathogen resistance	26
2.2.4	Nociceptive neurons augment immunity to <i>C. albicans</i>	30
2.2.5	CGRP α drives IL-23 expression in CD301b+ dDC	33
2.3	Discussion	35
2.4	Methods	38
2.4.1	Mice	38
2.4.2	Generation and testing of bone marrow chimeric mice	38
2.4.3	Flow cytometry	38
2.4.4	Antibodies	38
2.4.5	qPCR	39
2.4.6	Infection models	39
2.4.7	Denervation	39
2.4.8	Behavioral assays	40
2.4.9	DRG cultures	40
2.4.10	Calcium imaging	41
2.4.11	CGRP release assay	41
2.4.12	Statistics and data representation	42
2.5	Publication and contributions	43
3	Adaptive immunity against <i>Candida albicans</i> skin infection	44
3.1	Introduction	45
3.2	Results	47
3.2.1	Distinct T helper cell responses mediated by <i>C. albicans</i> morphology	47
3.2.2	Th17 cell differentiation requires LC derived IL-6	50
3.2.3	LC-derived IL-6 requires Dectin-1 engagement	56
3.2.4	Absence of Dectin-1 ligands prevent Th17 cell induction by dermal DC	60
3.2.5	Compartmentalization of T helper responses	66
3.3	Discussion	68
3.4	Methods	71

3.4.1	Mice	71
3.4.2	Generation and testing of bone marrow chimeric mice	71
3.4.3	DC Depletion with diphtheria toxin	71
3.4.4	Tamoxifen treatment	71
3.4.5	Antibodies	71
3.4.6	Adoptive T cell transfer	72
3.4.7	Flow cytometry	72
3.4.8	DC sorting by flow cytometry and qPCR	73
3.4.9	<i>Candida albicans</i> strains	73
3.4.10	Infection models	75
3.4.11	LC Targeting with anti-huLangerin antibodies	75
3.4.12	Histology	75
3.5	Publication and contributions	76
4	Conclusion	77
	References	81

List of Tables

1.1	Immunodeficiencies leading to Chronic Mucocutaneous Candidiasis . . .	5
3.1	Primers for the generation of <i>Hwp1</i> -Ag	74

List of Figures

1.1	Steady-state skin leukocyte subsets.	4
1.2	Innate immunity against <i>C. albicans</i> skin infection.	13
1.3	Adaptive immunity against <i>C. albicans</i> skin infection.	18
2.1	Dermal $\gamma\delta$ T cells, but not $\alpha\beta$ T cells, are required for IL-17 mediated protection against <i>C. albicans</i>	23
2.2	IL-23 is required for IL-17 secretion and proliferation of dermal $\gamma\delta$ T cells. 25	
2.3	CD301b+ dermal DCs are required for IL-23 mediated protection against <i>C. albicans</i>	28
2.4	IL-23 from CD301b+ dermal DCs is necessary for IL-17 secretion and proliferation of dermal $\gamma\delta$ T cells.	29
2.5	CD301b+ dermal DCs reside by cutaneous nerves.	31
2.6	Nociception and sensory nerves are crucial mediators of anti-fungal immunity.	32
2.7	Neuropeptide CGRP α drives IL-23 response and cutaneous fungal resistance.	34
3.1	Langerhans cells are necessary and sufficient for Th17 responses to <i>C. albicans</i>	48
3.2	<i>Candida albicans</i> yeast drives Th17 cell differentiation in vivo.	49
3.3	Generation of <i>Hwp1</i> -Ag.	51
3.4	IL-1 β and IL-6 are required for Th17 cell differentiation during <i>C. albicans</i> skin infection.	52
3.5	LC derived IL-6, but not TGF- β or IL-1 β is required for Th17 cell differentiation.	54
3.6	Generation of bone marrow chimeras.	55
3.7	SC5314 and s20175.016 have similar virulence.	57

3.8	Dectin-1 engagement on LC leads to IL-6 production and Th17 cell differentiation.	58
3.9	Sort strategy for migratory Langerhans cells from skin draining lymph nodes.	59
3.10	CD11b+ Dermal DC are not required for Th17 cell differentiation. . . .	62
3.11	Characterization of Mgl2-DTR mice.	63
3.12	Dermal infection and <i>C. albicans</i> pseudohyphae do not induce Th17 cells without exogenous Dectin-1 ligands.	64
3.13	Th17 cells are generated only after epicutaneous infection with <i>Eno1</i> -Ag. . . .	65
3.14	Th1 and Th17 cells provide compartmentalized protection to <i>C. albicans</i>	67

Chapter 1

Introduction

1.1 Immunity against *Candida albicans* is compartmentalized to the route of infection

C. albicans is the most common of the disease-causing *Candida spp.* that naturally colonizes the skin, genital and/or intestinal mucosa in up to 70% of healthy individuals[1]. Under normal circumstances, the fungus does not cause disease but the absence of appropriate immune recognition and response mechanisms can lead to the inability to control *C. albicans* colonization and invasion. Chronic mucocutaneous candidiasis (CMC) is a rare non-life threatening condition that occurs in the setting of primary and acquired immunodeficiencies resulting in oropharyngeal candidiasis (OPC) or superficial mucosal and cutaneous lesions with thickening, hyperkeratosis and erythema of the skin or nailbeds. The genomic sequencing of HIV negative CMC patients has identified many genes that are critical for host defense against *Candida albicans*. Subsequent mechanistic studies have further defined the importance of specific pattern recognition receptors, dendritic cells, cytokines and T cell signaling events in immunity against *C. albicans* with the common theme of defects in innate and/or adaptive Interleukin-17 (IL-17) pathways (referred to as type 3 immunity)(**Table 1.1**).

In addition to infections at barrier surfaces, *Candida* species are also leading cause of fatal bloodstream infections. The increased rate is thought to result from increased use of immunosuppressive agents, anti-cancer treatments, increased anti-microbial resistance and the frequency of invasive surgeries[1]. The genetic susceptibilities associated with systemic candidiasis as well as infections of gastrointestinal and female reproductive organs differ significantly from those associated with CMC[2]. Unlike CMC patients that have defects with type 3 immunity, genetic defects upstream or downstream of IL-17 have not been implicated in patients with disseminated candidiasis[3]. Rather, type I Interferons have been demonstrated to play an important role in patients with systemic infections. In mice, while IL-17 plays a role in systemic candidiasis, IFN γ from Th1 and NK cells have been recently appreciated as key players in the host response[4, 5, 6, 7]. Susceptibility to gastrointestinal and vulvovaginal candidiasis is largely dictated by micro-environmental factors such as local nutrients, pH, bile acids and local commensal flora[8, 9]. The host response to *C. albicans* infections in tissues other than the skin has been recently reviewed in depth[10, 8, 9, 11].

Mouse models of OPC and cutaneous candidiasis demonstrate high fidelity to the human disease. Mice with genetic defects in the same pathways as those defective in patients with CMC have greatly increase susceptibility to skin and mucosal *C. albicans*

infections[10]. Unlike humans, *C. albicans* is not commensal fungus of mice[12]. Thus, mice allow for the examination of primary innate and adaptive immune responses against *C. albicans*. Cutaneous candidiasis models include either application of *C. albicans* onto stratum corneum-stripped epidermis or direct intradermal inoculation[13, 14]. The well characterized nature of the skin-resident and circulating leukocyte of the skin make the murine skin an ideal site of study of both innate and adaptive anti-fungal immune responses(**Figure 1.1**). Specifically, the IL-17 cytokine family has been identified to be essential against host defense, driving neutrophil recruitment and anti-microbial peptide production[10]. It has been appreciated that IL-17 can be produced by leukocytes from both the innate (e.g. ILCs, $\gamma\delta$ T cells) and adaptive immune system (e.g. CD4+ T cells)[15, 5]. This review focuses on recent advances in understanding the mechanisms by which the primary innate and secondary adaptive type 3 immune response develop during *C. albicans* infection. Better understanding these mechanisms can assist with the development of vaccines against *C. albicans* and other extracellular pathogens as well as provide insight into type 3 autoimmune diseases of the skin.

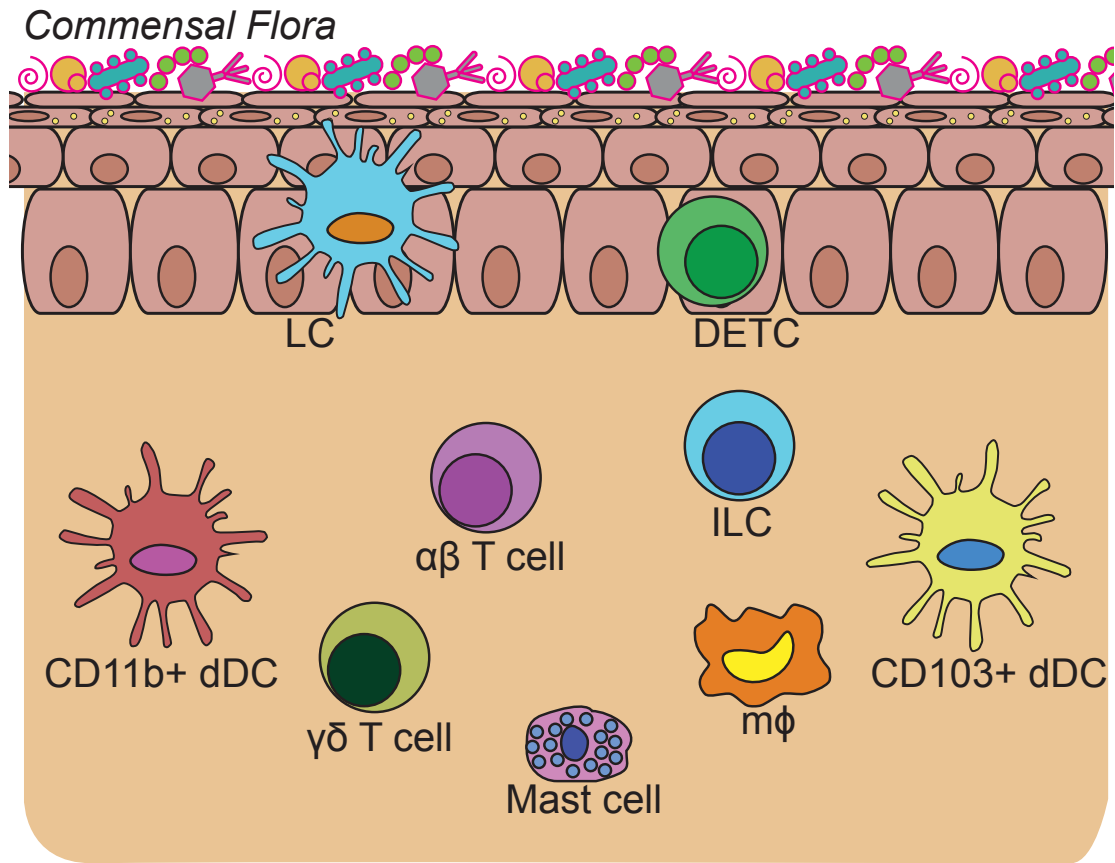


Figure 1.1: Steady-state skin leukocyte subsets.

The skin harbors a wide and diverse range of leukocyte subsets. The epidermis is resided by Langerhans cells (LCs) and dendritic epidermal T cells (DETCs). In the dermis, a much more diverse set of immune cells that include but are not limited to CD11b+ and CD103+ dermal dendritic cells (dDCs), innate lymphoid cells (ILCs), T cells, macrophages (m ϕ), and mast cells.

Table 1.1: Immunodeficiencies leading to Chronic Mucocutaneous Candidiasis

Gene	Phenotype	Reference
<i>Il2rg, Rag1, Rag2, Ada</i>	Loss of T and/or B cells	[16]
<i>Unc119, Magt1, Rag1</i>	Idiopathic CD4+ T cell lymphopenia	[17, 18]
<i>Stat3</i>	HIES due to defective IL-23R and IL-6R signaling and decreased Th17	[19, 20]
<i>Dock8</i>	HIES due to defective T cell synapse formation and dis-regulated DC migration	[21]
<i>Nfkbia</i>	Impaired TCR and NFkB signaling	[22]
<i>Il17f, Il17ra, Il17rc, Act1</i>	Defective IL-17A/F signaling	[23, 24, 25]
<i>Rorc</i>	Loss of IL-17A/F production, lymph nodes and impaired IFN γ response	[26]
<i>Aire</i>	Autoantibodies to IL-17A/F/IL-22	[27]
<i>Stat1</i>	Gain of function mutation leading to excessive type I and II IFN responses leading to decreased Th17	[28, 29, 23]
<i>Il12rb1</i>	Abolished response to IL-23 and IL-12	[30]
<i>Clec7a, Card9</i>	Defective phagocytosis, IL-1 β , IL-6 production by PBMC and IL-17 producing T cells	[31, 32]

1.2 Innate immune response to *C. albicans* skin infections

1.2.1 Pattern recognition

C-type lectins

C-type lectins encompass a large family of receptors that bind glycans through their extracellular carbohydrate recognition domain and mediate intracellular signaling via various cytoplasmic domains[33]. Dectin-1 is one of the most commonly studied and reviewed C-type lectins[34, 2, 33]. Dectin-1 recognizes β -glucan on the cell wall of most species of fungi including *C. albicans*[35]. Signaling of dectin-1 activates cells by a Syk dependent pathway, resulting in formation of the CARD9-BCL10-MALT1 trimer and activation of NF- κ B leading to transcription of pro-inflammatory cytokines such as IL-1 β , IL-6, and IL-12[34].

Humans with SNP variants of Dectin-1 and mutations of Card9 develop with CMC[32, 31]. These patients demonstrate impaired IL-1 β , IL-6, IL-22, and IL-17 production alongside impaired phagocytosis of *C. albicans* yeasts[31]. Work in mouse models has revealed that the role of Dectin-1 is more nuanced. Recognition by Dectin-1 varies by species, strain and life form (i.e. yeast vs. filamentous). Dectin-1 signaling has been shown to be required for protection from intravenous and oral candidiasis in vivo, but has also been shown to be redundant[36, 37]. These initially discordant results resulted from the use of different strains of *C. albicans*. Some strains of *C. albicans* have increased cell wall chitin resulting in lower availability of cell wall β -glucans for recognition by Dectin-1[38]. In addition, Dectin-1 specifically recognizes *C. albicans* yeast and not filamentous forms due to a difference in β -glucan availability (covered below)[39]. Recognition of fungi by other C-type lectin receptors, including the important concept of trained immunity, has been reviewed in depth elsewhere[2, 34, 33].

Toll like receptors

Several Toll-like Receptors (TLRs) recognize *C. albicans* cell wall polysaccharides including TLR-2, which recognizes phospholipomannans, and TLR-4, which recognizes O-linked mannans[40]. Activation of TLRs by their ligands leads to triggering of intracellular signaling pathways, such as MAPK (mitogen-activated protein kinase) and NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) pathways, leading to transcription and secretion of TNF α , IL-6 and/or type I interferons[41]. TLRs are expressed differentially on numerous cell types including keratinocytes, melanocytes,

dendritic cells and macrophages and can also cooperate with C-type lectins or the inflammasome to drive IL-1 β , TNF α and IL12 production[42, 43, 44, 45].

Deficiency of TLRs or adapter protein Myd88 significantly alters the survival of mice to intravenous *C. albicans* infection in vivo and Myd88 deficiency in Langerhans cells renders mice unable to mount Th17 response to *C. albicans* skin infection[46, 47]. However, deficiency of IRAK-4 or Myd88 lead to recurrent pyogenic bacterial infections and cold abscesses but do not lead to CMC in humans[22, 16]. Mutations of I κ Ba, which acts downstream of TLRs and of the T cell receptor (TCR) lead to CMC. Given the disparity of CMC susceptibility in I κ Ba variant patients compared to those with Myd88/IRAK4 deficiency, it is likely that the role of T cells trumps that of pattern recognition through TLRs in human resistance against CMC.

Nod like receptors

Nod-like receptors (NLRs) generally interact with apoptosis-associated speck-like protein containing a CARD (ASC) and procaspase-1 to form the inflammasome to convert procaspase-1 into active caspase-1, which in turn converts pro-IL-1 β and pro-IL-18 into mature IL-1 β and IL-18[48]. In humans, NLR mutations and polymorphisms have not been identified with CMC but a defective NLRP3 activation increases *C. albicans* colonization of the gut and NLRP3 polymorphism predisposes patients to recurrent vulvovaginal candidiasis (RVVC)[49, 50]. In mice, macrophage derived Nlrp3 recognizes filamentous forms of *C. albicans*, and deficiency of Asc and Nlrp3 and IL-1R1 leads to decreased *C. albicans* resistance in intravenous and oral routes of *C. albicans* infection[51, 52]. Nlrp10 has also been implicated in host defense against *C. albicans* by the induction of dendritic cell migration and differentiation of T helper cells[53, 54]. However, subsequent studies demonstrated that Nlrp10 deficient mice also possessed functional mutations of Dock8. Dock8, but not Nlrp10, was found to be responsible for DC migration[55, 56]. This supports the clinical association between Hyper IgE Syndrome (HIES) from Dock8 mutations and CMC[57]. Finally, stromal cell derived NLRC4 has also been demonstrated to be critical for mediating immunity against oral candidiasis[58]. While there has been some preliminary characterizations of NLRs in immunity against *C. albicans* disseminated and oral infections, the role of the inflammasome in mediating immunity against *C. albicans* skin infection is currently unknown.

1.2.2 Stromal cells

The uppermost layer of the avascular epidermis is the corneal layer that consists of dead keratinocytes, keratin and various hydrophobic lipids providing a physical barrier to the environment and to potential microbes. In addition, the corneal layer contains antimicrobial and anti-*Candida* peptides such as β -defensins and cathelicidins, which are produced by keratinocytes of subsequent layers in response to infection or colonization[59]. Notably, mice deficient of key components of the corneal layer are susceptible to *C. albicans* skin infections and, therefore, stratum corneum is a critical part of cutaneous host defense[60].

Beneath the corneal layer are the granular, spinous and basal layers that are composed of keratinocytes that express PRRs to initiate the early cutaneous immune responses. *C. albicans* has been shown to adhere to human keratinocytes and induce pro-inflammatory cytokine secretion in vitro but their role in directly activating KCs in vivo is unknown[61, 62]. Keratinocytes constitutively express the receptors for $\text{TNF}\alpha$, IL-17A and IL-22. $\text{TNF}\alpha$ and IL-17 acts on keratinocytes and epithelial cells to drive production of antimicrobial peptides such as β -defensins and S100 proteins and chemokines to drive the recruitment of neutrophils and inflammatory cells[63, 64]. IL-22 acts on keratinocyte to drive their proliferation by down-regulating genes involved in terminal differentiation[65]. Recent studies have also identified the role of keratinocytes in initiating immune responses through control of dendritic cell migration and recruitment of memory T cells into the skin[66, 67, 68, 69]. Thus, the interaction between *C. albicans* and epithelial cells of tissues is a critical topic of investigation going forward.

In addition to keratinocytes, melanocytes can also respond to *C. albicans*. Melanocytes are located in the basal layer of the epidermis and synthesize melanin to provide skin pigmentation. In invertebrates, melanocytes modulate melanin production during infection and inflammation can lead to hypo- or hyper-pigmentation in humans[70]. Melanin has also been demonstrated to have antimicrobial properties[71]. While early studies demonstrated that *C. albicans* negatively regulates transcription of melanogenesis genes, more recent study has shown that melanocytes may recognize *C. albicans* via Toll Like Receptor 4 to increase melanization and inhibit infection(**Figure 1.2**)[72, 73].

1.2.3 Cutaneous nerves

The skin is also a sensory organ that is highly innervated by terminal nerve fibers. Neuro-immune interactions have been appreciated in human and in mouse inflammatory and infection disease models[74]. Myeloid cells in the dermis and epidermis have been shown to localize closely with cutaneous nerve fibers, specifically fibers expressing CGRP[75, 76]. In the context of *C. albicans* vulvovaginal infection, *C. albicans* has been shown to stimulate pain, allodynia and development of CGRP+ nerve fibers[77]. In addition, zymosan has been used as a fundamental trigger of pain in rodent models[78]. Recently pathogens such as *C. albicans* and *Staphylococcus aureus* have been shown to directly activate sensory neurons taken from murine DRGs[15, 79]. Zymosan can activate sensory neurons directly while *S. aureus* can activate neurons directly or by causing neuronal lysis through its secreted toxins[79, 15]. Thus, it is possible that neurons can be activated by recognition of cell wall products by neuronal PRRs or through secreted microbial products. Other groups have suggested that *C. albicans* can metabolize host arachnoid acid to bi-products that can directly activate nociceptor TRPV1[80].

The activation of neurons by *C. albicans* lead to the increased secretion of the neuropeptide CGRP[15]. CGRP has been demonstrated to have antifungal properties in vitro, to act on KCs to drive proliferation in vitro, to skew LCs towards type 2 responses in vitro and to induce IL-23 secretion by dermal DCs in vivo(**Figure 1.2**)[81, 82, 83, 15]. In the mouse model of *C. albicans* epicutaneous infection, mechanical or chemical denervation by the TRPV1-desensitising resiniferatoxin (RTX) rendered mice less able to resist *C. albicans* skin infection due to diminished IL-23/IL-17 production. Addition of CGRP in RTX treated mice ameliorated the increased fungal burden of denervated mice[15]. Thus, pain sensation via TRPV1 channels and CGRP secretion by sensory nerves is critical for cutaneous host defense against *C. albicans*.

1.2.4 IL-23 and antigen presenting cells

IL-23 is a heterodimeric protein of IL-12p40 and IL-23p19 subunit that signal through heterodimeric IL-12Rb1 and IL-23R[84]. IL-23 has crucial roles in protection from pathogens and in the pathogenesis of autoimmunity by inducing a unique inflammatory gene signature that includes *Il17a*, *Il17f*, *Csf2*, *Tnfa* and others[85]. Mutation of IL-12Rb1 and the downstream STAT3 lead to CMC and reduced Th17 in humans[86, 30].

In mice, IL-23, but not IL-12, deficiency leads to increases susceptibility to intravenous, oral, epidermal and intradermal primary and secondary infection of *C. albicans* infections due to decreased IL-17, reduced neutrophilic infiltrates and epithelial hyperplasia[87, 88, 15, 14].

IL-23 has been demonstrated to be produced by dendritic cells after *C. albicans* infection in mice and humans[15, 89]. The skin harbors three major subtypes of dendritic cells. Langerhans cells are the only MHC-II positive cells in the epidermis while CD11b+ dermal DCs (dDCs) and CD103+ dDCs remake up for the majority and minority of the DC subsets in the dermis, respectively[90]. Mouse models of imiquimod induced psoriatic inflammation have implicated LCs and CD11b+ dDCs as the producers of IL-23 in vivo[91, 92]. In response to *C. albicans* skin infection, LCs are not responsible for IL-23 production or innate immune resistance against the fungus. Mice deficient of LCs show exaggerated NK cell mediated inflammation in response to heat killed *C. albicans* application to the footpad(**Figure 1.2**)[93, 15]. Additionally, ablation of LCs in mice induces exaggerated CHS and DTH responses, suggesting that LCs are immune-suppressive[94, 95, 96].

Furthermore, mice deficient in both LCs and CD103+ dDCs have intact immunity against *C. albicans*. Mgl2-DTR mice that can be depleted of CD11b+ dDCs and tissue resident macrophages have decreased IL-23 transcription, IL-17 production and increased fungal burden in skin after infection with *C. albicans*[15]. Mixed bone marrow chimeric mice in which CD11b+ dDCs lack IL-23 production have defective immune response to primary *C. albicans* skin infection, particularly due to decreased IL-17 production by dermal $\gamma\delta$ T cells (see below)[15]. Thus, CD11b+ dDCs are both necessary and sufficient for IL-23 driven anti-*C. albicans* response. Interestingly, CD11b+ dDC reside alongside dermal nerve fibers and also express the receptor for CGRP[76, 15]. CGRP released by sensory nerves after *C. albicans* infection acts on the CD11b+ dermal DCs to stimulate IL-23 production. Thus, the sensory nervous system and CD11b+ dermal DCs partake in a critical cutaneous circuit that drives host resistance to *C. albicans*.

1.2.5 IL-17 and $\gamma\delta$ T cells

The IL-17 family consists of six cytokines (IL-17A-IL-17F) that signal through five receptors (IL-17RA-IL-17RE). IL-17A and IL-17F form homo- and heterodimers and

signal through a dimer of IL-17RA and IL-17RC. Mutations in IL-17F, IL-17RA, IL-17RC and downstream signaling molecule Act1 all lead to CMC in humans[85, 10]. IL-17RA expression is found in both hematopoietic and non hematopoietic cells. In hematopoietic cells, IL-17 can signal on neutrophils and NK cells to drive anti-fungal immunity[97, 6]. In non-hematopoietic cells such as keratinocytes, IL-17 induces transcription of pro-inflammatory cytokines such as IL-6, G-CSF, chemokines such as Ccl20, and antimicrobial peptides S100A proteins and β -defensins[63, 98]. These keratinocyte signals are important for neutrophil trafficking and function and for host defense against *C. albicans* and other extracellular pathogens. Unlike IL-17A/F, IL-17C is produced by epithelial keratinocytes but not hematopoietic cells and signals through IL-17RA and IL-17RE[85]. IL-17C induces a gene profile strikingly similar to IL-17A and plays an important role in psoriasis pathogenesis but plays no detectable role in protection against oral, dermal, or disseminated candidiasis in vivo[99, 100]. Similarly, IL-17RE was dispensable for protection against candidiasis, further demonstrating the specific necessity of IL-17A/F signaling[100].

Th17 cells have been long considered the predominant source of IL-17 during *C. albicans* mucocutaneous infections (see below). Recently, tissue resident innate lymphoid cells (ILCs) and $\gamma\delta$ T cells have been appreciated as primary producers of effector cytokines in the site of *C. albicans* infection in mouse models[101, 102]. In humans, loss of function STAT3 mutations in humans lead to decreased number and function of IL-17 producing unconventional mucosal associated invariant T cells[103]. Unlike CD4+ T cells, which are primed by dendritic cell antigen presentation and signals in the secondary lymphoid organs to become IL-17 producers, innate lymphoid cells and $\gamma\delta$ T cells are pre-programmed into IL-17 secreting lineage in the bone marrow and thymus, respectively[104]. In humans, $\gamma\delta$ T cells have been shown to make IL-17 in response to IL-23 produced by DCs after *C. albicans* stimulation[89]. Thus, these cells may serve as primary responders in patients who have not yet developed *C. albicans* specific effector or memory T cells and may augment immunity early. In mouse models of OPC, some groups have found that $\gamma\delta$ T cells and innate like CD4+ T cells in tissues are both critical for resistance while other groups have demonstrated the need of ILCs for protection[102, 101]. In the skin, dermal $\gamma\delta$ T cells are the obligate source of IL-17 after epicutaneous *C. albicans*[15]. Most of the IL-17 secreting dermal $\gamma\delta$ T cells have a V γ 4 TCR[105]. These $\gamma\delta$ T cells are embryonically derived, functionally seeded into tissues with constitutive expression of IL-23R and can produce IL-17 and proliferate rapidly in response to IL-23 from CD301b+ dermal DC[106, 76, 15]. Thus, IL-17 production

is not restricted to CD4+ T cells after infection and tissue resident cells are important mediators of antifungal immunity.

1.2.6 Neutrophils

It has been long appreciated that IL-17 is a critical cytokine that drives recruitment and activation of neutrophils[107, 108]. Neutrophils have been demonstrated to be required for protection against mucosal and systemic *C. albicans* infections[109, 97]. Recently, neutrophils have also been shown to constitutively express Ror γ t, produce and respond to IL-17 in a mouse model of *Aspergillus fumigatus*[108]. Neutrophils are also critical for phagocytosis of *C. albicans* as they sense pathogen size via Dectin-1 and release neutrophil extracellular traps in response to *Candida albicans* filaments but not yeast[110]. In addition, adoptive transfer of neutrophilic myeloid derived suppressor cells have been shown to improve mice survival of invasive *C. albicans* infection[111]. Neutrophils in response to *C. albicans* infection have been further studied extensively and are covered elsewhere[40, 112, 2].

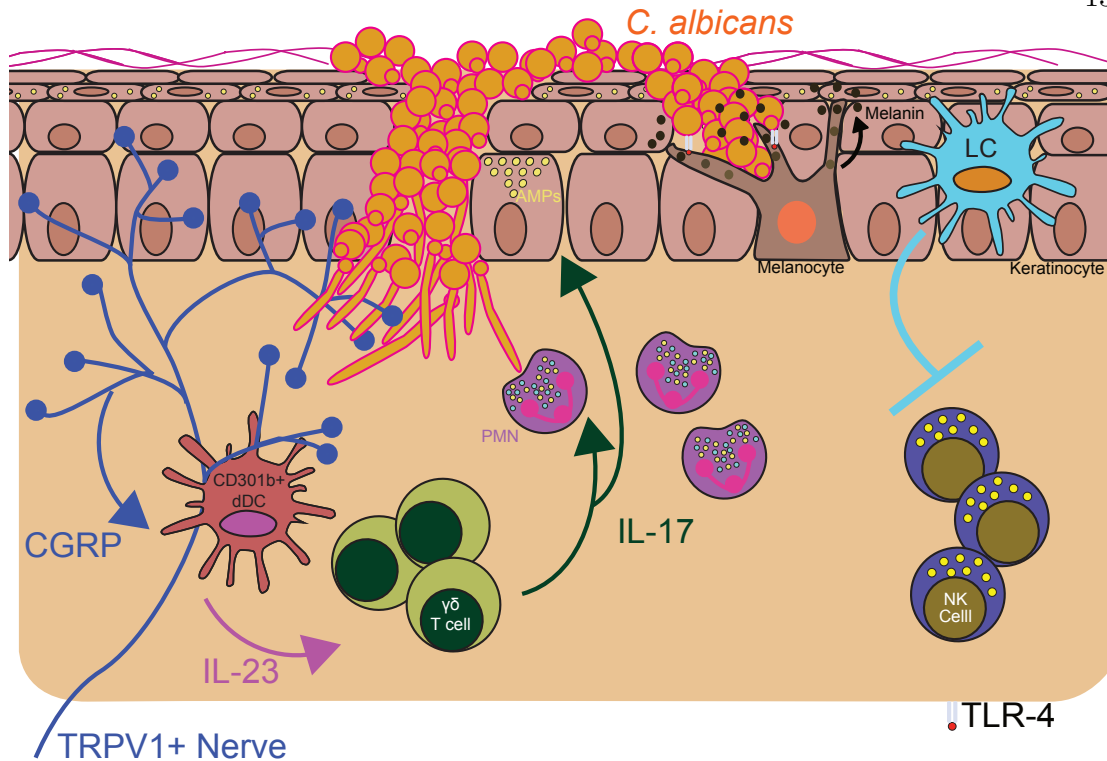


Figure 1.2: Innate immunity against *C. albicans* skin infection.

The skin has a layered innate immune system. *C. albicans* directly activates cutaneous sensory nerves to induce the release of calcitonin gene related peptide (CGRP). CGRP acts on CD301b+ dermal dendritic cells (dDC), which subsequently release IL-23.

IL-23 acts on dermal $\gamma\delta$ T cells to drive IL-17 production in the skin, leading to anti-*C. albicans* resistance through the presumed activation of neutrophils and antimicrobial peptides such as β -defensins. In addition, melanocytes in the basal epidermis can also recognize *C. albicans* via TLR-4 to drive production and release of melanin granules, which are antimicrobial in nature. Finally, Langerhans cells of the epidermis can suppress liver derived CD49a+ NK cells in response to *C. albicans* through unknown mechanisms.

1.3 Adaptive Immunity against *C. albicans* skin infection

The importance of adaptive IL-17 producing CD4+ T cells in protection against *C. albicans* has been demonstrated in mice and humans. In humans, Th17 cells are the critical mediators of anti-fungal barrier immunity. Patients with Th17 deficiencies in the context of STAT3 deficiency/HIES has increased susceptibility to mucocutaneous candidiasis[86]. Stimulation of nave human CD4+ T cells with *C. albicans* can induce the expansion of T cell clones that can induce the production of IL-17 and IFN γ that depended on IL-1 β [113]. Interestingly, memory T cell responses to *C. albicans* demonstrated functional heterogeneity with distinct Th1/Th2/Th17 cell subsets sharing the same T cell clone suggesting that polarized T cell responses might result from preferential expansion rather than T cell priming[114]. However, in mouse models of cutaneous candidiasis, both fungal morphology and dendritic cell subsets have been demonstrated to be important for differentiation of specific T helper subsets that lead to compartmentalized, tissue-specific response against secondary exposure of *C. albicans*(**Figure 1.3**)[5].

1.3.1 Th17 cell differentiation

During infection or inflammation, DCs migrate to the lymph node, upregulate co-stimulatory molecules and secrete cytokines that induces the proliferation and differentiation of effector and cytotoxic T cells[90]. Dock8 mutations in humans lead to defective T cell response and dendritic cell migration that causes HIES and CMC[115, 56, 57]. In mice, skin DCs prime distinct T helper responses that have differing functions in protective immunity against subsequent *C. albicans* infections[13]. Langerhans cells migrate to the lymph node 3 to 4 days after infection, where they express high amount of Th17 differentiating cytokines IL-1 β , IL-6 and TGF- β . Mice deficient in Langerhans cells have intact *C. albicans* specific T cell expansion but have significantly decreased Th17 cells[13]. In the epidermis, *C. albicans* colonizes as budding yeasts. *C. albicans* strains that are genetically locked into yeast, but not filaments, are capable of inducing Th17 differentiation through Langerhans cells[5]. The yeast morphology of *C. albicans* specifically provides accessibility ligands for the β -glucan receptor Dectin-1 in its bud scars[39]. In mice, Langerhans cells express the pattern recognition receptors Dectin-1 while the data in humans is more controversial[116, 117]. Humans with Dectin-1 polymorphisms and mice with Dectin-1 deficiency lead to decreased *C. albicans* specific

Th17 response[31]. Binding of Dectin-1 by *C. albicans* induces the secretion of the pro-inflammatory cytokine IL-6 by human PBMC and mouse Langerhans cells[5, 31]. IL-6, but not IL-1 β or TGF- β , from LC is necessary for Th17 cell differentiation and while IL-6 from other sources were dispensable. Finally, Langerhans cell deficiency of Myd88 have defective Th17 cell generation while LC migration is unaffected[47]. Thus, pattern recognition of *C. albicans* via Dectin-1 and TLRs by LCs is critical for driving Th17 cell generation.

As discussed previously, CD11b+ dermal DC are required for innate immunity against *C. albicans* and for Th17 generation against bacterial and fungal pathogens in other tissues[118, 119, 120]. CD11b+ dermal DCs also express Dectin-1 but are not required for Th17 generation after *C. albicans* infection due to the inaccessibility to Dectin-1 ligands of pseudohyphae in the dermis[13, 5]. Unlike Langerhans cells and CD11b+ dermal DCs, CD103+ dermal DCs do not express Dectin-1 and suppress Th17 cell differentiation presumably through anti-Th17 cytokines such as IL-27 and IL-12[5]. While CD103+ dDC and CD11b+ dDCs are not required for Th17 differentiation, they do play a role in activating and differentiating bystander IL-17 secreting CD8+ T cell responses that can protect against *C. albicans*[13, 121].

1.3.2 Th1 cell differentiation and compartmentalization of the effector responses

Unlike yeast form of *C. albicans*, filamentous *C. albicans* invade past the stratum corneum and the dermis and disseminate to systemic tissues. Epicutaneous and intravenous infection with a strain of *C. albicans* that expresses model antigens under a filament specific promoter induces Th1 response but not Th17 response[5]. Given that Batf3 deficient mice have defective Th1 cell differentiation to epicutaneous and disseminated *C. albicans* infection, CD103+ dermal DC likely induces Th1 cells through the secretion of IL-12 or IL-27[13]. In the oral mucosa, CD103+ and CD11b+ migratory DCs collaborate together to induce *C. albicans* specific T cell expansion and Th17 cell differentiation while oral LCs were dispensable[122]. Thus, there seems to be varied functions by different DCs in distinct tissues. Interestingly, a patient with IRF8 mutation lacking both conventional and plasmacytoid dendritic cells was recently found to be infected with oral candidiasis[123].

Like humans, mice infected with *C. albicans* demonstrate heterogeneous T helper profiles that includes both Th17 and Th1 cells. Mice lacking Th17 cells have impaired

protection against a secondary cutaneous, but not systemic, *C. albicans* infection while mice with exaggerated Th17 cells have greater protection against cutaneous, but not systemic, re-infection[5]. In addition, adoptive transfer of Th17 specific cells from *C. albicans* primed mice into nave animal afforded the host protection against oral and cutaneous, but not systemic, *C. albicans* infection[124, 5]. Conversely, mice lacking Th1 cells have impaired protection against secondary systemic, but not cutaneous, *C. albicans* infection while mice with exaggerated Th1 cells have increased resistance against systemic but not cutaneous re-infection. Finally, adoptive transfer of Th1 specific cells from *C. albicans* primed mice into nave animal afforded the host protection against systemic but not cutaneous *C. albicans* infection[5]. Thus, specific T helper subsets have compartmentalized immunity against distinct routes of *C. albicans* re-infection. While differential priming of T helper subsets has been appreciated in other models, the mechanism of compartmentalized protection is still unknown[125]. Whether distinct T helper subsets have different homing to tissues, or differing tissue specific maintenance or function in *C. albicans* infection is a necessary area of investigation to elucidate mechanism of T cell compartmentalization.

1.4 Publication and contributions

This chapter is modified from the article in press:

Sakeen W Kashem and Daniel H Kaplan. Skin immunity against *Candida albicans*.
Trends in Immunology. Featured Review. *In press*

Author contributions are as follows: SWK and DHK wrote and edited the manuscript.

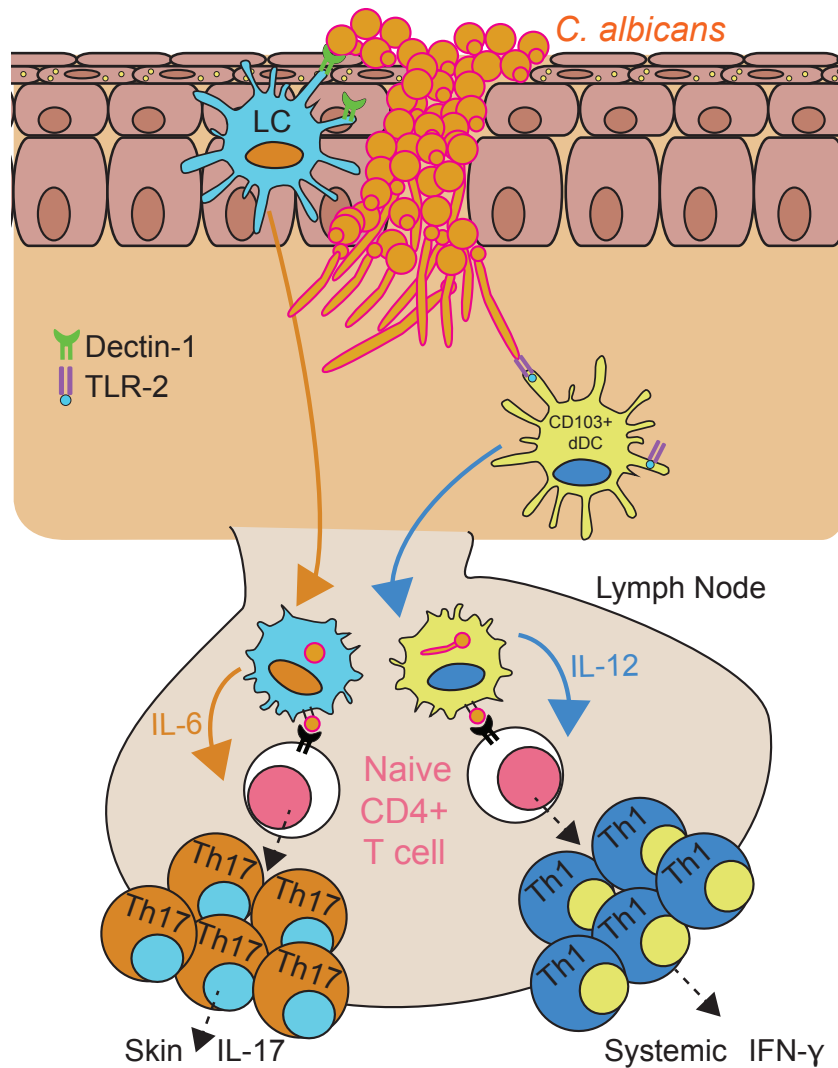


Figure 1.3: Adaptive immunity against *C. albicans* skin infection.

The skin generates heterogeneous T helper response that provides compartmentalized immunity against *C. albicans*. In the epidermis, *C. albicans* exists as yeasts. *C. albicans* yeasts are recognized by Dectin-1 on Langerhans cells (LCs). Dectin-1 engagement on LCs lead to production of IL-6 in the secondary lymphoid organs that differentiate nave CD4+ T cells to the Th17 cell lineage. These Th17 cells provide protection against secondary cutaneous infections but not against secondary systemic infections. Conversely, *C. albicans* invades as filamentous pseudohyphae in the dermis. Recognition of *C. albicans* filaments by CD103+ dDCs, presumably through TLR-2, lead to Th1 cell differentiation in the secondary lymphoid organs. These Th1 cells provide protection against secondary systemic infections but not secondary skin infection. Thus, recognition of distinct morphology of *C. albicans* via different dendritic cell subsets lead to tailored immune response that provide protect against specific subsequent routes of infections.

Chapter 2

Innate immunity against *Candida albicans* skin infection

2.1 Introduction

Candida albicans is a common, highly morbid pathogen at barrier sites such as the skin and oral cavity in the setting of immunosuppression. For example, patients with defective T helper 17 (Th17) cell immunity such as AIDS or hyper-IgE syndrome are prone to develop chronic mucocutaneous candidiasis (CMC)[126]. Mice with defective Th17 cell type responses are also more susceptible to both oral and cutaneous candidiasis[124, 5]. Patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) disorder have circulating neutralizing antibodies against IL-17 and develop CMC [127, 27]. Similarly, patients with IL-17 receptor A (IL-17RA) or IL-17F deficiencies also suffer from CMC[23, 128]. These patients have global defects in the IL-17 pathway that affects both IL-17 produced by Th17 cells as well as IL-17 derived from cells of the innate immune system. It is becoming increasingly clear that innate sources of IL-17 are crucial for host defense against *C. albicans* at barrier sites[102, 101]. In the skin, dendritic epidermal T cells (DETCs), dermal $\gamma\delta$ T cells, CD4+ and CD8+ $\alpha\beta$ T cells and innate lymphoid cells (ILCs) all can produce IL-17[129, 130, 131, 121]. IL-17 from dermal $\gamma\delta$ T cells is required for immunity against BCG, *Staphylococcus aureus*, Vaccinia virus and for autoimmune inflammation[132, 105, 130, 133, 134]. CD4+ and CD8+ T cells generate IL-17 responses to commensal organisms such as *Staphylococcus epidermitis* and protect against opportunistic pathogens[131, 121]. In mouse models of oropharyngeal candidiasis, IL-17 from $\alpha\beta$ and $\gamma\delta$ T cells as well as ILC3s mediate protection, though the contribution of each cell type remains controversial[102, 101].

Production of IL-17 by tissue resident lymphocytes is mediated by pro-inflammatory cytokines, notably IL-23[135, 136]. IL-23 signaling plays a critical role against mucocutaneous candidiasis as humans with IL-23R signaling defects are prone to CMC and mice with IL-23 deficiencies are susceptible to oral and cutaneous candidiasis[85, 128, 14]. There are at least three well defined subsets of cutaneous dendritic cells (DCs) - epidermal Langerhans cells (LCs), CD103+ dermal DCs (dDCs) and CD11b+ DCs[90]. LCs and CD11b+ dDCs both secrete IL-23 and conflicting reports suggest that each is an obligate source of IL-23 during imiquimod-induced dermatitis [92, 91]. Recently, the role of nociceptors has been appreciated in IL-23 mediated imiquimod-induced skin inflammation[76]. In addition, pathogens such as *S. aureus* can directly activate neurons isolated from the dorsal root ganglion (DRG) and induce pain hyper-responsiveness[79]. The relationship between nociception and innate immunity, however remains poorly characterized.

In our previous work, we developed an epicutaneous *C. albicans* infection model that does not require immunosuppression or dysbiosis for productive infection[13]. In this model, LCs are not required for pathogen clearance in nave mice. LCs were necessary and sufficient, however, for the development of antigen-specific Th17 cells that provided protection from a secondary infection in immune mice[5]. The cell types and cytokine networks responsible for resistance to *C. albicans* in the skin, however, have not been precisely defined. In this report, we examined the cellular sources of IL-17 and the inflammatory cascade that mediated resistance to cutaneous *C. albicans* infection in nave mice. We found that IL-23 from CD301b+ dDC that includes the CD11b+ dDC subset drove expansion and IL-17 production by dermal $\gamma\delta$ T cells, resulting in protection from *C. albicans*. In addition, transient receptor potential cation channel subfamily V member 1 (TRPV1) nociceptive neurons and the neuropeptide calcitonin gene-related peptide (CGRP) acted upstream of CD301b+ dDC to drive IL-23 expression. Thus, we have defined a sequence of coordinated events occurring early during *C. albicans* skin infection that link nociceptive neurons, dendritic cells and $\gamma\delta$ T cells in establishing host defense against a fungal infection in the skin.

2.2 Results

2.2.1 IL-17A from $\gamma\delta$ T cells is required for resistance to cutaneous candidiasis

In the oral mucosa, IL-17 from $\alpha\beta$ T cells, $\gamma\delta$ T cells and ILCs has been reported to be required for resistance to *C. albicans* infection[88, 102, 101, 137]. We have previously described a model of cutaneous candidiasis in which *C. albicans* yeast is applied onto the shaved back skin of immunocompetent mice. Infection, as measured by CFUs in the skin, peaks 2-3 days after inoculation but gradually declines over the course of 5 days[13].

To determine whether IL-17 was required to control infection, we skin-infected mice deficient in IL-17A and IL-17F production (*Il17af*^{-/-} mice). As expected, mice lacking IL-17A were highly susceptible to infection and had higher *C. albicans* burden three days after infection (**Figure 2.1A**). To identify the cellular source of IL-17A, we first analyzed the composition of the cellular infiltrate found in the skin of infected mice. Lymphoid cells in the skin can be identified using flow cytometric analysis of collagenase digested skin gated on CD90.2+ lin- (B220, CD11c, CD11b, F4/80) cells as: TCR $\gamma\delta$ hi

TCR β lo DETCs; TCR $\gamma\delta$ mid TCR β lo dermal $\gamma\delta$ T cells; TCR $\gamma\delta$ lo TCR β hi $\alpha\beta$ T cells (including CD4+ and CD8+ T cells); and a TCRlo population that includes NK and innate lymphoid cells (**Figure 2.1B**)[76]. During infection, the numbers of dermal $\gamma\delta$ T cells and CD4+ $\alpha\beta$ T cells were increased compared with nave mice (**Figure 2.1C**). Intracellular flow cytometry revealed that approximately 3% of the CD90.2+ cells in infected skin expressed IL-17A (**Figure 2.1D**). When we subsetted these cells, we observed that dermal $\gamma\delta$ T cells were the dominant source of IL-17A with a small amount contributed by CD4+ TCR β + cells (**Figure 2.1E**). Individual dermal $\gamma\delta$ T cells appeared to produce more IL-17A than CD4+ TCR β + cells based on MFI. DETC, CD8+ TCR β + cells and CD90.2+ TCR- innate lymphoid subsets did not produce appreciable amounts of IL-17.

To determine whether dermal $\gamma\delta$ T cells and $\alpha\beta$ T cells were required for resistance to *C. albicans*, we skin-infected control, *Rag1*^{-/-} (lacking both T cell subsets), and TCR α ^{-/-} (lacking $\alpha\beta$ T cells) mice (**Figure 2.1F**). Fungal burden was increased in *Rag1*^{-/-} but not TCR α ^{-/-} indicating a requirement for $\gamma\delta$ T cells. To confirm this finding and exclude any role for DETCs, we generated mixed bone marrow chimeras in which *Tcrd*^{-/-} bone marrow was transferred into irradiated B6.SJL-Ly5.2 (referred to as CD45.1 WT) hosts. As DETCs are radio-resistant, these mice have a selective absence of dermal $\gamma\delta$ T cells (unpublished observations)[138]. As expected, *Tcrd*^{-/-} →CD45.1 WT mice had a significantly higher fungal burden than the WT →CD45.1 WT controls (**Figure 2.1G**). These results indicate that resistance to primary *C. albicans* skin infection requires IL-17 produced by dermal $\gamma\delta$ T cells.

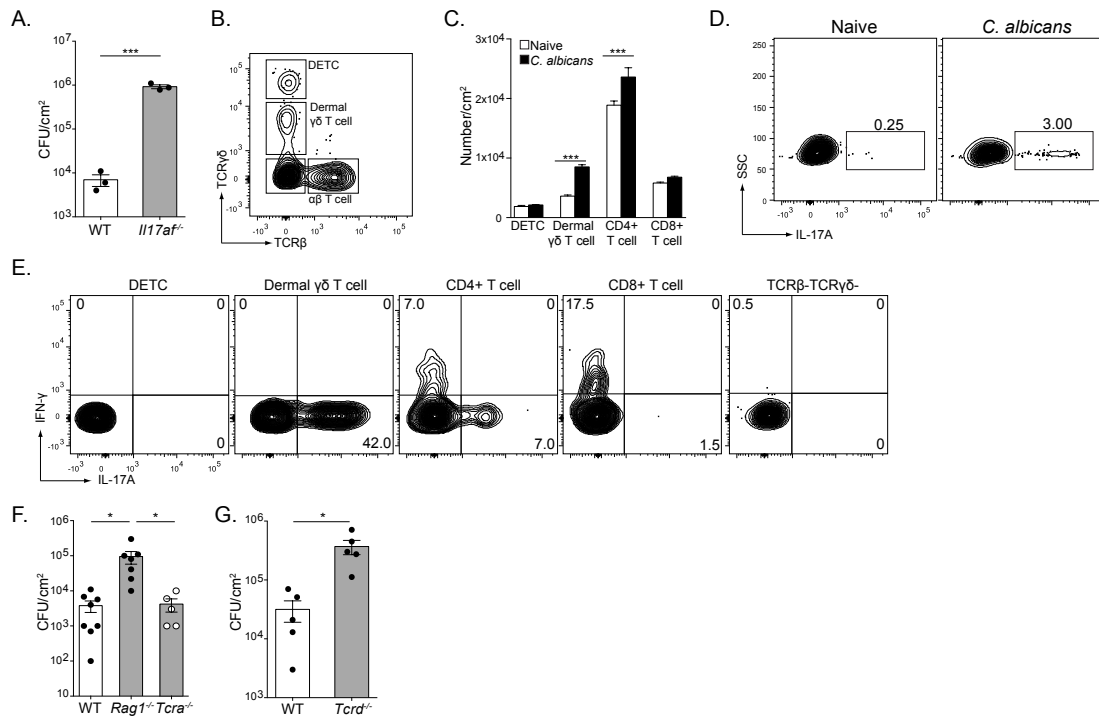


Figure 2.1: Dermal $\gamma\delta$ T cells, but not $\alpha\beta$ T cells, are required for IL-17 mediated protection against *C. albicans*.

Cohorts of mice were infected on their shaved dorsum with 2x10⁸ CFU *C. albicans*.

(A) Day 3 whole skin homogenates from wildtype or *Il17af*^{-/-} infected mice were plated onto YPAD agar plates and incubated at 30°C for 48 hours. The number colony forming units (CFU) per cm² of skin is shown. (B) Single cell suspensions from whole skin was obtained by enzymatic digestion and analyzed by flow cytometry gated as singlets, live/dead excluded, CD90.2+ and Lineage- (B220-CD11c-CD11b-F4/80-). Expression of TGR β and TCR $\gamma\delta$ is used to identify lymphoid subsets in the skin. (C)

The numbers in each lymphocyte subset as in (B) in nave (white) and *C. albicans* infected (black) mice is shown. (D) Cells isolated from skin were re-stimulated with PMA and Ionomycin in the presence of monensin and gated as in (B). Expression of IL-17A by CD90.2+ lymphocytes is shown. (E) Representative expression of IL-17A and interferon- γ (IFN- γ) by individual subsets from (D) are shown. (F) *C. albicans* CFU isolated from the skin of WT, *Rag1*^{-/-} and *Tcr α* ^{-/-} mice 3 days after infection is shown. (G) Lethally irradiated B6.SJL-Ly5.2 were re-constituted with bone marrow from WT C57BL/6 or *Tcrd*^{-/-} mice and 12 weeks later. *C. albicans* CFU 3 days after infection is shown. Scales represents mean $\pm$ SEM. Student's unpaired T-test representative of *P<0.05, **P<0.01, ***P<0.001. Data points in A, F, and G represent individual animals. Data is representative of at least three mice per group and at least three independent experiments.

2.2.2 IL-17 producing $\gamma\delta$ T cells require IL-23

We next sought to identify the factors required for production of IL-17 by dermal $\gamma\delta$ T cells. In vitro, $\gamma\delta$ T cell activation has been shown to require IL-1R and/or IL-23R signaling[139, 130]. As patients with deficiencies in IL-23 signaling but not IL-1 signaling suffer from chronic mucocutaneous candidiasis we focused on the role of IL-23 in response to *C. albicans* infection[128, 140]. Skin infection of IL-23p19 deficient mice (referred to as *Il23a*^{-/-}) resulted in a large reduction of dermal $\gamma\delta$ T cells compared with nave controls (**Figure 2.2A**). In addition to fewer cells, there was also a reduced frequency of cells expressing IL-17 resulting in a dramatic decrease in the number of dermal $\gamma\delta$ T cells producing IL-17 (**Figure 2.2B-C**). Skin infection with *C. albicans* was associated with a strong induction of dermal $\gamma\delta$ T cell proliferation as evidenced by the large number of Ki-67 positive cells after infection (**Figure 2.2D**). In the absence of IL-23, proliferation was greatly curtailed, consistent with observations that IL-23 drives proliferation of $\gamma\delta$ T cells in vitro [139]. Consistent with the importance of IL-17 from dermal $\gamma\delta$ T cells, we found that the fungal burden was greatly increased in *Il23a*^{-/-} mice (**Figure 2.2E**). Exogenous administration of IL-17A restored fungal burdens to the level observed in WT mice indicating that IL-23 acts upstream of IL-17 (**Figure 2.2E**). These results show that IL-23 is required for proliferation and production of IL-17A by dermal $\gamma\delta$ T cells during *C. albicans* infection.

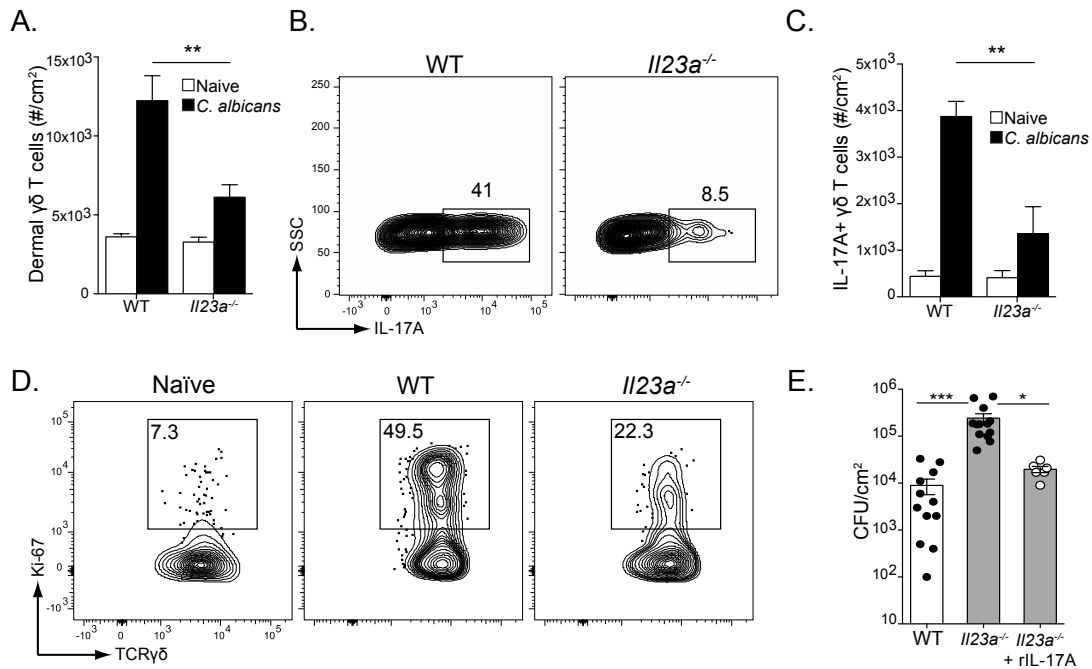


Figure 2.2: IL-23 is required for IL-17 secretion and proliferation of dermal $\gamma\delta$ T cells.

Mice were infected on their dorsum with 2×10^8 CFU *C. albicans*. Three days later, single cell suspensions from whole skin was obtained by enzymatic digestion and subjected to flow cytometry. (A) Total number of dermal $\gamma\delta$ T cells in WT or *Il23a*^{-/-} mice is shown in nave (white) or infected (black) mice. (B) As in (A) cells were re-stimulated with PMA and Ionomycin in the presence of monensin and analyzed for IL-17 expression. (C) The number of IL-17A-expressing dermal $\gamma\delta$ T cells in (B) is shown in nave (white) or infected (black) mice. (D) Dermal $\gamma\delta$ T cells in WT or *Il23a*^{-/-} mice were analyzed for proliferation by nuclear Ki-67 staining compared to nave mice. (E) *C. albicans* CFU from day 3 skin homogenates from WT, *Il23a*^{-/-} or *Il23a*^{-/-} mice administered $1 \mu\text{g}$ intradermal recombinant IL-17A (rIL-17A) 1 day prior and 1 day after infection. Scales represents mean $\pm$ SEM. Student's unpaired T-test representative of *P<0.05, **P<0.01, ***P<0.001. Data points in E represent individual animals. Data is representative of at least three independent experiments with cohorts of at least 6 mice.

2.2.3 IL-23 from CD301b+ dermal DC is critical for IL-17 production by dermal $\gamma\delta$ T cells and pathogen resistance

In other models, DCs have been shown to be an important source of IL-23[141, 92]. To determine whether IL-23 from DCs was required for cutaneous resistance to *C. albicans*, we skin-infected control and CD11c-DTR mice one day after treatment with diphtheria toxin (DT) to deplete all dendritic cells. DC-depleted mice expressed lower amounts of *Il23a* mRNA in the skin 16 hours after infection (**Figure 2.3A**). In addition, DC deficient mice were unable to clear *C. albicans* as efficiently as controls (**Figure 2.3B**). These findings indicate that dendritic cells are a non-redundant source of IL-23 that is required for clearance of *C. albicans* skin infection in vivo.

There are at least 3 major subsets of DCs in the skin. To evaluate whether LC or CD103+ dDCs are an obligate source of IL-23, we crossed huLangerin-DTA mice that lack LCs with *Batf3*^{-/-} mice that lack CD103+ dDCs to generate human Langerin-DTAx*Batf3*^{-/-} mice (referred to as LC^{-/-}x*Batf3*^{-/-} that lack both DC subsets[142, 5, 143]. Production of IL-23, numbers of dermal $\gamma\delta$ T cells, expression of IL-17 by dermal $\gamma\delta$ T cells, and *C. albicans* CFU were all unaffected by the absence of these DC subsets (**Figure 2.3C-F**). This indicates that CD103+ dDCs and LCs are not required for innate responses to *C. albicans*, and another source of IL-23 is sufficient.

The C-type lectin macrophage galactose lectin type 2 receptor (Mgl2; CD301b) is expressed by the bulk of CD103- dDCs in the dermis and can be ablated using Mgl2-DTR mice [144, 5]. CD301b+ dDCs are heterogeneously comprised of CD11b+CD64- conventional DCs and the recently defined CD11b+CD64+ cells that are now ontogenically defined as tissue macrophages[145]. *C. albicans* infection of Mgl2-DTR mice depleted of CD301b+ DC by injection of DT showed greatly decreased IL-23 expression in the skin compared to PBS treated mice (**Figure 2.3G**). In addition, the numbers of dermal $\gamma\delta$ T cells and IL-17 production by $\gamma\delta$ T cells was reduced (**Figure 2.3H-I**). Fungal burdens were also significantly increased in these mice (**Figure 2.3J**).

To determine whether IL-23 from the CD301b+ dDC was required for dermal $\gamma\delta$ T cell production of IL-17, we generated a series of mixed bone marrow chimeras. A 1:1 ratio of bone marrow isolated from Mgl2-DTR mice and bone marrow from either WT or *Il23a*^{-/-} mice was transferred into irradiated CD45.1 mice. After 12 weeks of reconstitution, mice were treated with either vehicle or DT to generate cohorts in which CD301b+ dDC are either IL-23 sufficient or deficient. Mgl2-DTR+*Il23a*^{-/-} → WT mice

in which CD301b+ dDCs are IL-23 deficient demonstrated reduced expression of IL-17 by dermal $\gamma\delta$ T cells compared with controls (**Figure 2.4A**). The numbers and proliferation of dermal $\gamma\delta$ T cells was also reduced (**Figure 2.4A-D**). Finally, the CFUs of *C. albicans* was increased in mice that lacked IL-23 production from CD301b+ dDC (**Figure 2.4E**). Thus, CD301b+ dDC are an obligate source of IL-23 that is required for activation and expansion of dermal $\gamma\delta$ T cells during *C. albicans* infection.

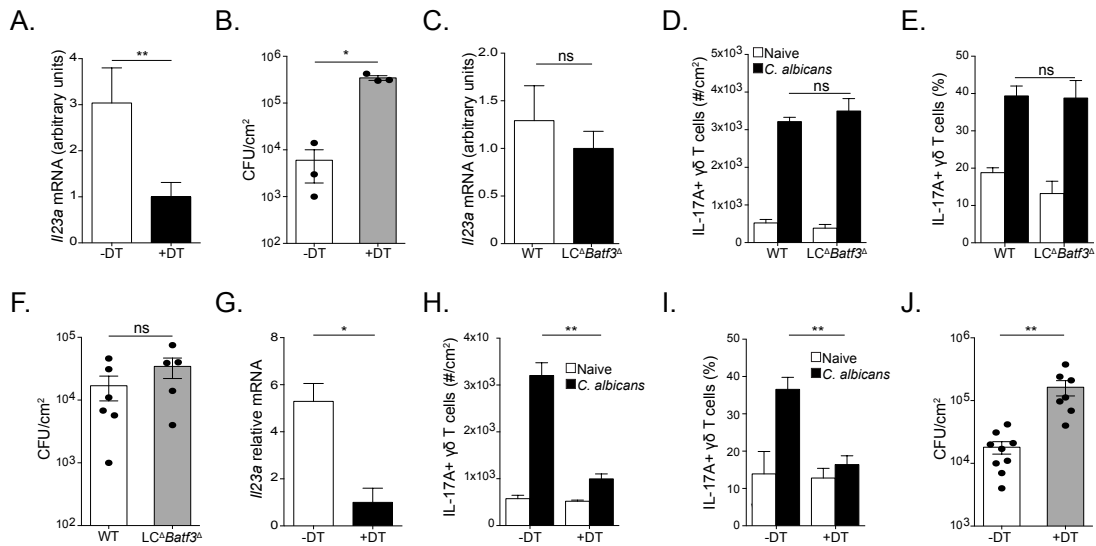


Figure 2.3: CD301b+ dermal DCs are required for IL-23 mediated protection against *C. albicans*.

CD11c-DTR mice were treated with 1 μ g of diphtheria toxin (DT) or vehicle day before infection with 2 $\times 10^8$ CFU *C. albicans*. (A) *Il23a* expression in whole skin tissue 16 hours after infection as analyzed by RTqPCR is shown. (B) *C. albicans* CFU in whole skin from control and DC-depleted mice 3 days after infection is shown. (C-F) Mice lacking LCs and CD103+ dDCs (LC^{-/-} x Batf3^{-/-}) were infected with *C. albicans*. (C) *Il23a* expression in skin was analyzed 16 hours after infection by RTqPCR. (D) Number and (E) percent of IL-17A expressing dermal $\gamma\delta$ T cells in mice kept naive (white) or infected with *C. albicans* (black) is shown. (F) Skin CFU 3 days after *C. albicans* infection in LC^{-/-} x Batf3^{-/-} depleted mice is shown. (G-J) Mgl2-DTR mice were treated with 1 μ g of DT or vehicle one day before infection with *C. albicans*. (G) *Il23a* expression in skin was assessed by RTqPCR 16 hours after infection. (H) Number and (I) percent of IL-17A expressing dermal $\gamma\delta$ T cells in mice kept naive (white) or infected with *C. albicans* (black) is shown. (J) CFU in skin mice 3 days after infection is shown. Scales represents mean $\pm$ SEM. Student's unpaired T-test representative of *P<0.05, **P<0.01, ***P<0.001. Data points in B, F, and J represent individual animals. Data is representative of at least three independent experiments with cohorts of at least 3 animals.

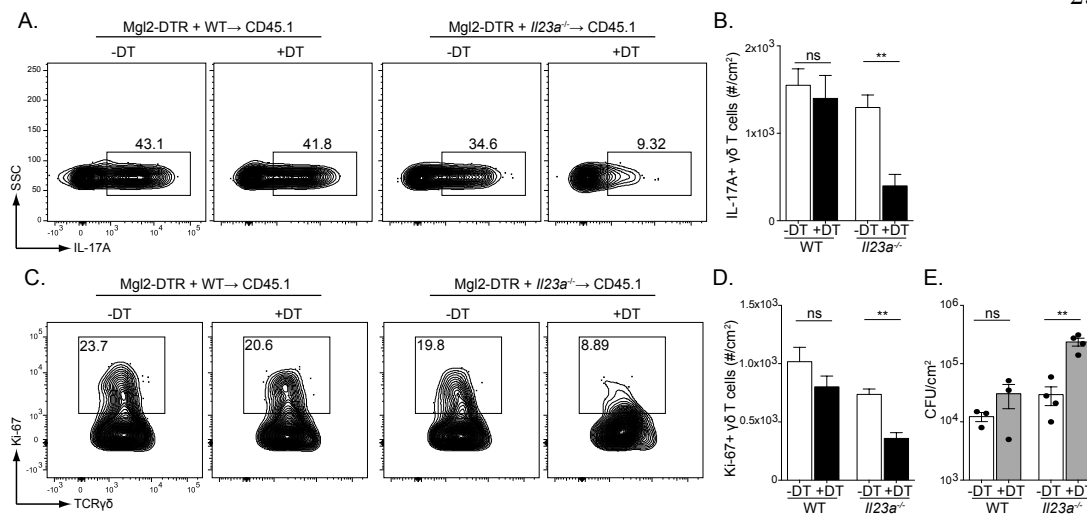


Figure 2.4: IL-23 from CD301b+ dermal DCs is necessary for IL-17 secretion and proliferation of dermal $\gamma\delta$ T cells.

Lethally irradiated B6.SJL-Ly5.2 (CD45.1) mice were re-constituted with 1:1 ratio of bone marrow from Mgl2-DTR mice and either WT or *Il23a*^{-/-} mice. After 12 weeks, mice were treated with 1 μ g of diphtheria toxin (DT) or vehicle 1 day before infection with 2×10^8 CFU *C. albicans*. (A) IL-17A expression of dermal $\gamma\delta$ T cells in mice 3 days after *C. albicans* infection is shown. (B) The number of IL-17-expressing dermal $\gamma\delta$ T cells in the skin from (A) is shown. (C) As in (A), Ki-67 staining of dermal $\gamma\delta$ T cells and (D) quantification of Ki-67+ dermal $\gamma\delta$ T cells 3 days after infection is shown. (E) *C. albicans* skin CFU from mixed bone marrow chimeric mice 3 days after infection. Student's unpaired T-test representative of * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Scales represents mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Data points in E represent individual animals. Data is representative of at least three independent experiments with cohorts of at least 3 mice.

2.2.4 Nociceptive neurons augment immunity to *C. albicans*

Cutaneous sensation of temperature and pain is transmitted by sensory fibers that express the cation channel TRPV1. CD301b+ dDCs reside near cutaneous these cutaneous nerve fibers and the role of nociceptors has been recently appreciated for IL-23 mediated imiquimod-induced inflammation(**Figure 2.5**) [76]. In addition, pathogens such as *S. aureus* can directly activate neurons isolated from the dorsal root ganglion (DRG) and induce pain hyper-responsiveness[79]. To determine whether *C. albicans* could directly affect sensory neurons, we measured changes in intracellular calcium via intracellular Fura2-AM fluorescence in primary cultures of mouse DRG neurons exposed to heat killed *C. albicans* (HKCA). Cells were stimulated with 30 mM potassium chloride (KCl) initially to determine viability before being exposed to HKCA (**Figure 2.6A**). After a 10 minute wash out period, cells were re-stimulated with 500mM of the TRPV1-specific agonist capsaicin (Cap) to assess neuronal specificity. Out of 53 total DRG neurons examined, Cap induced calcium transients in 24 neurons identifying them as TRPV1+ nociceptors (**Figure 2.6B**). Within the group of Cap-sensitive neurons, 17 also responded to HKCA. Furthermore, zymosan, a yeast cell wall derivative, also directly activated sensory neurons from the DRG (**Figure 2.6C**). Thus, the majority of TRPV1+ neurons are directly activated by *C. albicans* as measured by calcium mobilization.

We next examined whether sensory nociceptive fibers participated in anti-fungal responses. To ablate TRPV1+ sensory neurons, cohorts of mice were treated subcutaneously with escalating amounts of the capsaicin analog resiniferatoxin (RTX) and rested for 4 weeks[76]. Treated mice demonstrated increased tail flick latency to heat and absent nocifensive response to capsaicin administration confirming effective ablation of TRPV1+ nociceptors (**Figure 2.6D-E**). Mice were then skin infected with *C. albicans*. RTX treated mice demonstrated significantly decreased levels of IL-23 compared with vehicle treated controls (**Figure 2.6F**). In addition, the numbers IL-17+ dermal $\gamma\delta$ T cells were reduced and the fungal burden was increased (**Figure 2.6G-J**).

Recently, TRPV1 has been shown to be expressed and functional on T cells and keratinocytes[146, 147]. To insure that RTX treatment was not affecting these cells in addition to nociception, we mechanically disrupted the cutaneous nerves on one lateral half of the mice dorsum[148]. Infection on the denervated side demonstrated less *Il23a* and *Il17a* mRNA expression as well as higher fungal burden than the mock denervated

side (**Figure 2.6K-M**). These data indicate that sensory nerves and TRPV1+ nociceptors are crucial for the generation of cutaneous IL-23 and IL-17 responses and protection against *C. albicans*.

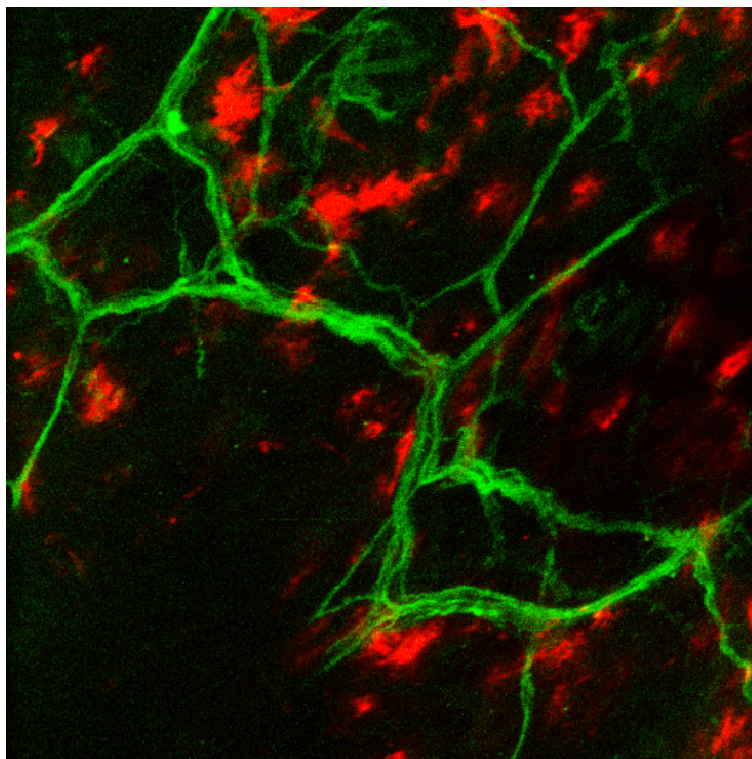


Figure 2.5: CD301b+ dermal DCs reside by cutaneous nerves.

Visualization of cutaneous nerve fibers (green; stained with anti- β III tubulin) and CD301b+ dDCs (red, stained with CD301b) in dermal skin sheets by confocal microscopy.

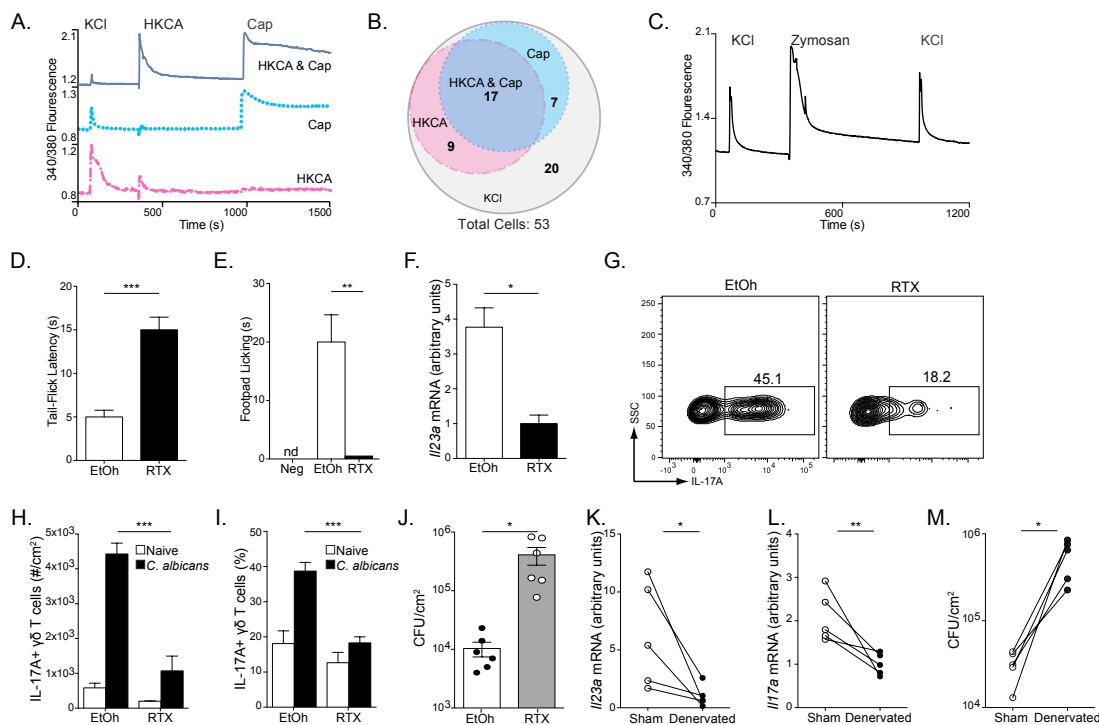


Figure 2.6: Nociception and sensory nerves are crucial mediators of anti-fungal immunity.

(A) Sensory neurons were dissociated from dorsal root ganglion of WT mice and cultured for 18-24 hr before being loaded with the ratiometric intracellular calcium indicator Fura2-AM. Cultured neurons were stimulated sequentially with 30mM KCl (10sec), heat killed 1×10^7 CFU *C. albicans* (HKCA; 15sec), and 500 mM Capsaicin (Cap; 10s). Traces showing calcium flux as indicated by the 340/380 ratio visualized by microscopy from 3 individual cells are shown. Black line represents cell that responded to HKCA and Cap, dotted blue line represents cell that responded to Cap but not HKCA and red dashed line represents cell that represents cell that responded to HKCA but not Cap. Data are representative of 53 cells tested. (B) Scaled Venn diagram representation of the 53 DRG neuron responsiveness to different stimuli. (C) Cultured neurons were stimulated sequentially with 30mM KCl (10sec), 1×10^7 CFU Zymosan (15sec), and 30mM KCl (10sec). Traces showing calcium flux as indicated by the 340/380 ratio visualized by microscopy from a single neuronal cell representative of 10 cells. (D-J) Cohorts of WT mice were injected s.q. with resiniferatoxin (RTX) or vehicle (EtOH). (D) Mice were checked for their latency to tail-flick after submersion in a 52°C waterbath. (E) Nociceptive behavior was assessed for 10 minutes after injection of vehicle or 5 μ g of capsaicin into the hindpaws of EtOH or RTX treated mice. Neg is defined as EtOH mice treated with vehicle. Mice were infected with *C. albicans* and (F) *Il23a* expression was quantified by RTqPCR in skin 16 hours later. (G) IL-17A expression by dermal $\gamma\delta$ T cells 3 days after infection is shown. (H) The number and (I) percentage of IL-17-expressing dermal $\gamma\delta$ T cells is shown in naive (white) and infected (black) mice. (J) *C. albicans* CFU in vehicle or RTX treated mice 3 days after infection is shown. (K-M) Mice were surgically denervated on one lateral side of their dorsum and infected with equal CFU of *C. albicans*. (K) *Il23a* and (L) *Il17a* expression in skin was quantified by RTqPCR 16 hours and 60 hours later, respectively. (M) CFU from mechanically denervated side of infected mice and mock denervated side are shown. Data from individual mice are connected by a line. Scales represent mean $\pm$ SEM. Student's unpaired (D-F; H-J) or paired (K-M) T-test representative of * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Data is representative of at least three independent experiments with cohorts of at least 5 mice.

2.2.5 CGRP α drives IL-23 expression in CD301b+ dDC

TRPV1+ neurons release numerous neuropeptides in the periphery [149, 150]. Given the importance of CD301b+ dDC in our system, we explored the expression pattern of neuropeptide receptors on cutaneous DC subsets from data generated by Immunological Genome Consortium[151]. Notably, CD11b+ DC express high levels of the CGRP receptors *Calcl* and *Ramp3* (**Figure 2.7A**). We confirmed the microarray findings by analyzing cell surface CALCRL expression on CD11b+ dDCs by flow cytometry (**Figure 2.7B**). This is consistent with other reports showing expression of CGRP receptors by CD11b+ DC in other tissues [152].

To determine whether sensory neurons released CGRP in response to *C. albicans*, we measured CGRP levels after stimulation of cultured DRG neurons with HKCA. HKCA stimulation resulted in increased concentrations of CGRP in the supernatant of DRG cultures (**Figure 2.7C**) relative to control treatment. Lysates of HKCA or control-treated cells had similar concentrations of CGRP, indicating that the CGRP increase in the supernatant of HKCA-stimulated cells was not due to HKCA-induced neuronal damage. Thus, *C. albicans* directly induce CGRP release from DRG neurons.

Since ablation of nociception by RTX has been shown to reduce the expression of CGRP in the skin and DRGs exposed to *S. aureus* supernatant or *C. albicans* have been shown to increase CGRP expression in vivo, we next tested whether CGRP participated in the anti-*C. albicans* immune response [79, 153]. We found that daily intradermal administration of CGRP α into the site of *C. albicans* infection increased the number of IL-17+ dermal $\gamma\delta$ T cells in the skin and reduced the fungal burden. Conversely, injection of the CGRP antagonist, CGRP32-37, reduced levels of IL-23, reduced the number of IL-17+ dermal $\gamma\delta$ T cells in the skin and increased the fungal burden (**Figure 2.7D-F**). Moreover, injection of CGRP α in RTX treated mice was able to restore IL-23 expression and fungal burden to levels seen in control mice (**Figure 2.7F-G**).

CGRP, as well as other neuropeptides, have been previously determined to be directly anti-microbial against *C. albicans*[81, 154]. To determine whether CGRP could suppress *C. albicans* infection independently of IL-23 mediated inflammation, we treated CD301b+ depleted Mgl2-DTR mice with CGRP α or vehicle. As shown previously, DT-treated Mgl2-DTR mice showed reduced IL-23 and an exaggerated fungal burden (**Figure 2.7H-I**). Unlike RTX treated mice, administration of CGRP α to Mgl2-DTR mice did not rescue the phenotype. Thus, these data indicate that CGRP α , produced by sensory nerves in response to *C. albicans*, acts upstream of CD301b+ dDCs to induce

IL-23 expression that induces IL-17 production from dermal $\gamma\delta$ T cells resulting in an effective innate immune response to cutaneous *C. albicans* infection.

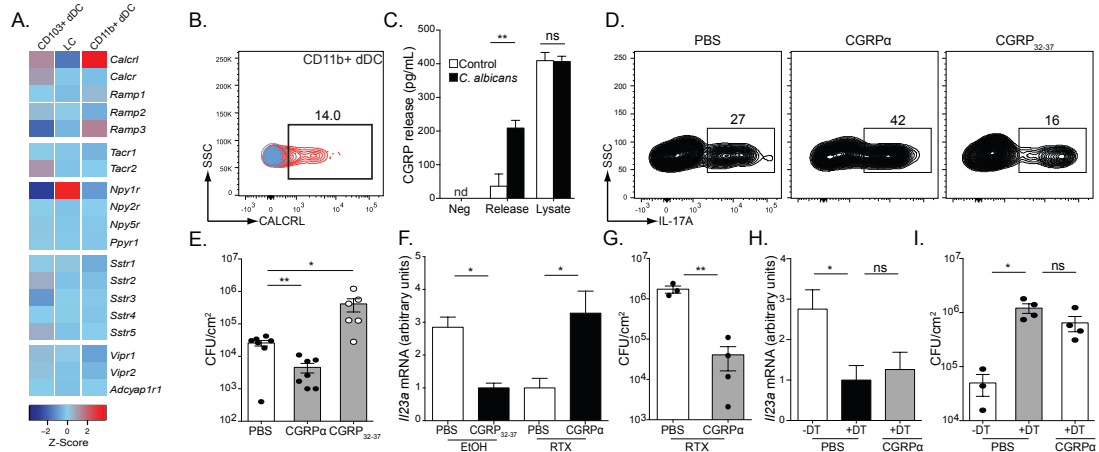


Figure 2.7: Neuropeptide CGRP α drives IL-23 response and cutaneous fungal resistance.

(A) Gene expression patterns of CGRP, substance P, neuropeptide Y, somatostatin and vaso-active intestinal peptide receptors on the indicated skin dendritic cell subsets. Raw data was obtained from the Immunological Genome Consortium. (B) Expression of CGRP receptor CALCRL (red) with isotype control (blue) on skin CD11b+ DC subsets. (D-I) Cohorts of WT mice were treated intradermally with either PBS or 0.5 μ g of peptide CGRP α or inhibitor CGRP32-37 on day -1, 0, 1 and 2 of *C. albicans* infection. (C) Cultured DRG neurons were incubated for 10 min in HEPES buffer to measure basal release (Neg) and then incubated for 10 min in HEPES buffer (release, open bars) or HKCA (release, black bars). CGRP levels in supernatants and cell lysates (lysate) were determined by ELISA. (D) IL-17A expression of PMA and Ionomycin stimulated dermal $\gamma\delta$ T cells in mice 3 days after infection is shown. (E) Skin CFU three days after infection from mice treated with PBS, CGRP α or inhibitor CGRP32-37 is shown. (F-G) Four-week-old WT mice were injected with RTX or vehicle (EtOH) subcutaneously in flank. Four to six weeks later, mice were either treated with PBS, CGRP α or inhibitor CGRP32-37. (F) Expression of *Il23a* in skin 16 hours after infection was determined by RTqPCR. (G) Skin CFU three days after infection is shown. (H-I) Mgl2-DTR mice were treated with 1 μ g of diphtheria toxin (DT) or vehicle 1 day before infection with *C. albicans*. Mice were given intradermal PBS or CGRP α on days 0-2 after infection. (H) Expression of *Il23a* in skin 16 hours after infection was assessed by RTqPCR. (I) Skin CFU three days after infection is shown. Student's unpaired T-test representative of *P<0.05, **P<0.01, ***P<0.001. Data is representative of at least three independent experiments with cohorts of at least 3 mice.

2.3 Discussion

Our work shows that resistance to *C. albicans* skin infection requires IL-17A and identifies dermal $\gamma\delta$ T cells as the dominant and primary source of this cytokine. IL-23 derived from CD301+ dDCs was required for proliferation and IL-17A production by dermal $\gamma\delta$ T cells. Ablation of dermal $\gamma\delta$ T cells, CD301b+ dDCs, or selective deletion of IL-23 in CD301b+ dDCs greatly increased susceptibility to *C. albicans* skin infection. In addition, we found that sensory neurons were capable of directly responding to *C. albicans*. Ablation of nociceptors or cutaneous nerves reduced production of IL-23 from CD301b+ dDCs and resulted in inefficient clearance of *C. albicans* through a CGRP-dependent mechanism. Thus, we have defined a series of coordinated events linking nociceptive sensory neurons, dendritic cells, and $\gamma\delta$ T cells that together provide critical host defense to a fungal pathogen early during skin infection.

Our observation that *Il17af*^{-/-} mice were highly susceptible to skin infection with *C. albicans* is consistent with the well documented requirement for this cytokine in protection against *C. albicans* at barrier surfaces in both humans and mice[155, 156, 88, 102, 101, 98, 137, 6, 157]. In mice, Th17 cells provide protection from secondary infection with *C. albicans*[124, 5]. *C. albicans* is a commensal microorganism in humans, but not in mice. Thus, protection in nave mice relies on IL-17 from cells of the innate immune system that can provide immediate protection rather than Th17 cells that arise only several days after infection. In the well-studied oropharyngeal model of mucosal candidiasis, $\gamma\delta$ T cells, natural IL-17+ $\alpha\beta$ T cells and ILC3 have been demonstrated to be important sources of IL-17[88, 102, 101]. In the skin, *C. albicans* infection induced production of IL-17 primarily from dermal $\gamma\delta$ T cells. These cells are also the dominant source of IL-17 in the response to *S. aureus* skin infection or imiquimod application[130, 105, 134]. Ablation of dermal $\gamma\delta$ T cells increased fungal burden confirming the importance of this cell type. We also observed a small subset of IL-17 producing CD4+ T cells that may represent natural Th17 cells, but they were not an obligate source of IL-17 in the skin. Notably, DETCs, ILC3s and CD8+ T cells that produce IL-17 in other models were not important sources during *C. albicans* infection.

Dermal $\gamma\delta$ T cells constitutively express the both subunits of the receptor for IL-23 and proliferate in response to IL-23 in vitro[139, 130]. In vivo, both *C. albicans* and imiquimod application induce IL-17 production by dermal $\gamma\delta$ T cells [105, 158]. We found that dermal $\gamma\delta$ T cells required IL-23 for both induction of IL-17 and proliferation. Notably, although proliferation and IL-17 production was greatly diminished in *Il23a*^{-/-}

mice, it was not completely abolished. This may represent a varying requirement for IL-23 within heterogeneous populations of dermal $\gamma\delta$ T cells. In addition, IL-1 β is known to be induced during *C. albicans* infection and may be partially sufficient to activate dermal $\gamma\delta$ T cells in the absence of IL-23[139, 130, 52]. In both the skin and the small intestines, DCs are the primary source of IL-23 during inflammation[92, 159, 13, 91]. We have previously observed that LCs and CD11b+ dDCs produce much higher levels of IL-23 mRNA than CD103+ dDCs during *C. albicans* skin infection [13]. Our current observation that mice lacking CD301b+ dDC (Mgl2-DTR mice) had reduced levels of IL-23 and reduced resistance to *C. albicans* infection coupled with the absence of a phenotype in mice lacking both LCs and CD103+ dDCs (LC^{-/-} Batf3^{-/-} mice) argues that CD301b+ dDC are the source of IL-23. This was confirmed using mixed bone marrow chimeras in which CD301b+ dDC were rendered IL-23 deficient. It is important to note that CD301b+ dDCs are heterogeneous and include all CD11b+ conventional DCs in the dermis but also include populations of CD64+ monocyte derived DCs and resident macrophages[5]. The depletion of CD301b using DTR-depleter mice precluded a definitive identification of which subset within the CD301b+ population was required. In support of CD11b+ dDCs, others have found using Itgax^{Cre} x Irf4^{fl/fl} mice that CD11b+ DCs induce IL-17 expression in skin CD8+ T cells after *S. epidermidis* colonization and in lung CD4+ T cells after *Aspergillus fumigatus* infection[121, 120]. Human homologues of all these subsets, however, make IL-23 in similar amounts raising the possibility that CD301b+ cells other than CD11b+ dDC may also contribute IL-23 during *C. albicans* infection[120, 160].

The interaction of nociceptive neurons and dermal dendritic cells has recently been appreciated. TRPV1+ nociceptive nerve fibers in the skin are in close contact with dermal DCs and are required for their production of IL-23 in the context of imiquimod induced skin inflammation[76]. Denervation also reduces disease severity in patients with the IL-17 and IL-23 mediated autoimmune diseases psoriasis and rheumatoid arthritis, as well as in mouse models of these diseases[76, 148, 161, 162, 163]. Patients with spinal cord injuries have decreased levels of IL-23 and IL-17 as well as frequent fungal skin infections below the site of neurological lesion[164, 165]. In addition, the skin pathogen *S. aureus* directly activates sensory nerves resulting in hyper-responsiveness to pain and the yeast cell wall product, zymosan, has been used extensively to experimentally induce pain[79, 78],

Our data connects these observations and demonstrates that sensory neurons can directly sense a pathogen and then enhance host resistance via secretion of neuropeptides.

We found that CD301b+ dDCs have reduced expression of IL-23 after RTX ablation of TRPV1+ neurons or physical denervation resulting in reduced $\gamma\delta$ T cell activation and reduced resistance to *C. albicans* infection. Expression of IL-23 and host defense could be inhibited by administering the CGRP peptide antagonist, CGRP32-37, and augmented by the addition of the CGR agonist, CGRP α . Moreover, CGRP α rescued IL-23 responses in RTX-treated WT mice but did not rescue mice lacking CD301b+ dDCs, indicating that CGRP α does not directly inhibit *C. albicans* proliferation or function indirectly through other cell types such as keratinocytes or fibroblasts[82]. Thus, CGRP α acts downstream of TRPV1+ nociceptors but acts upstream of CD301b+ dDCs. Notably, cultured DRGs were able to flux calcium and release CRGP in response to exposure to *C. albicans*. Many of these DRG neurons were also capsaicin responsive, which is consistent with the observed reduction of anti-*C. albicans* responses in RTX treated mice. Capsaicin insensitive DRGs also responded to *C. albicans* suggesting that other sensory neurons may also participate in the anti-*C. albicans* response. Although our data indicates that *C. albicans* can directly activate sensory neurons in DRG cultures, it does not negate the likely role of inflammation induced pain response or indirect activation of nociceptors in vivo.

We focused on CGRP rather than other neuropeptides based on the high expression levels of CGRP receptors on CD11b+ dDC and the ability of *C. albicans* to directly induce CGRP release by DRG neurons. In the lung, these DCs also express CGRP receptors and promote airway hyperresponsiveness[152]. In murine vulvovaginal candidiasis, pain was accompanied by increased expression of CGRP in peripheral nerves[77]. In addition, CGRP has also been implicated in the pathogenesis of psoriasis[166, 167]. It is important to note that although our data highlights the importance of CGRP α , it does not exclude a role for other neuropeptides in host defense against *C. albicans*.

In summary, we have elucidated a mechanism of innate host defense against *C. albicans* infection in the skin that involves pain sensing neurons, dermal dendritic cells and dermal $\gamma\delta$ T cells as well as the soluble factors, CGRP α , IL-23 and IL-17. This work provides insight into the complex interplay of cells of the innate immune system at a barrier tissue and offers potential avenues of therapeutic interventions to suppress IL-23 and/or IL-17 mediated autoimmune skin diseases and augment antimicrobial therapy.

2.4 Methods

2.4.1 Mice

Mgl2^{eGFP-DTR/wt} (referred to as Mgl2-DTR), human Langerin-DTAxBatf3^{-/-} (referred to as LC^{-/-} xBatf3^{-/-}), CD11c-DTR, *Rag1*^{-/-}, *Tcra*^{-/-}, *Tcrd*^{-/-}, *Il23a*^{-/-} and *Il17af*^{-/-} mice have been previously described[144, 94, 143, 142, 168, 169, 170, 171, 172, 106]. C57BL/6 and B6.SJL-Ly5.2 mice were purchased from National Cancer Institute (Frederick, MD). All experiments were performed with 6-to 8-week-old female mice. Mice were housed in microisolator cages and fed irradiated food and acidified water. The University of Minnesota institutional care and use committee approved all mouse protocols.

2.4.2 Generation and testing of bone marrow chimeric mice

Given that re-constitution of dermal $\gamma\delta$ cells vary by the recipient, we used six weeks old B6.SJL-Ly5.2 mice that were irradiated using X-ray irradiator as previously described[139, 105]. The mice received two split doses at 500 cGy each. The following day, 5×10^6 bone marrow cells isolated from specified mice were injected intravenously. Mice were rested for at least 12 weeks prior to experiments. The efficiency of chimerism was determined by flow cytometry of congenic markers on PBMC, lymph node, dermis and epidermis. Re-constitution of dermal $\gamma\delta$ cells were determined to be of donor origin at >90 %, while DETCs were of host origin (data not shown).

2.4.3 Flow cytometry

Single-cell skin suspensions were obtained and stained as previously described[5]. For evaluating cytokine expression, cells were incubated for 2hr in complete IDMEM supplemented with PMA (50 ng/ml) and ionomycin (1.5 μ M; Sigma-Aldrich, St. Louis, MO), with GolgiStop (BD PharMingen, San Jose, CA). The intracellular cytokine staining was performed with BD Bioscience Cytofix/Cytoperm kit (BD Biosciences, San Jose, CA) in accordance with the manufacturer's instructions. Samples were analyzed on LSR-II flow cytometers (BD Biosciences). Data were analyzed with FlowJo software (TreeStar, Ashland, OR).

2.4.4 Antibodies

Fluorochrome-conjugated antibodies to CD4 (GK1.5), CD8a (53-6.7), B220 (RA3-6B2), F4/80 (BM8), CD11b (M1/70), CD103 (2E7), I-A/I-E (M5/114.15.2), CD11c (N418),

CD90.2 (30-H12), IFN- γ (XMG1.2), TCR $\gamma\delta$ (UC7-13D5), TCR β (H57-597), PE Donkey anti-rabbit IgG (Poly4064) anti- β tubulin III (TUJ1) and CD301b (URA-1) and IL-17A (TC11-18H10.1) were purchased from Biolegend (San Diego, CA). CD207 (929F3.01) antibody was purchased from Dendritics (Lyon, France). Rabbit anti-calcitonin receptor-like receptor (CRLR - referred to as CALCRL; H-42) antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Live/dead fixable viability dye was purchased from Invitrogen (Carlsbad, CA).

2.4.5 qPCR

RNA was isolated using an RNeasy Mini Kit (Qiagen, Valencia, CA) and quantified from Nanodrop readings (NanoDrop, Wilmington, DE). cDNA was generated using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). TaqMan Gene Expression Master Mix, TaqMan Gene Expression Assays for IL-23p19 and IL-17A was used. ABI Prism 7900HT (Applied Biosystems) were used to complete the qPCR. All kits were completed according the manufacturer's instructions. All Ct values were normalized to HPRT expression and are shown as $2^{-\Delta Ct}$ to nave.

2.4.6 Infection models

The skin infection was performed as described [47, 13]. Mice were first anesthetized with a mixture of ketamine and xylazine (100/10 mg/kg body weight), shaved on the back with electric clipper, and chemically depilated with Nair hair remover (Church & Dwight, Princeton, NJ) per the manufacturer's instructions. The stratum corneum was removed with 10 strokes with 220 grit sandpaper (3M, St Paul, MN). After washing with sterile PBS, 2×10^8 C. albicans in 50 μ l of sterile PBS was applied on to the skin. Skin was harvest from mice 3 days later and homogenized and serially diluted onto YPAD plates and incubated at 30°C for 24-48 hours to assess the number of colony forming units. In some instances, CGRP α or CGRP32-37 (Genscript, Piscataway, NJ) was administered intradermally at day -1, 0, 1 and 2 during infection at total 0.5 μ g per dose spread over 10 sites in the back. When specified, 1.0 μ g recombinant IL-17A (R&D Biosystems, Minneapolis, MN) was administered day -1 and 1 during infection.

2.4.7 Denervation

Resineferatoxin (RTX; LC Laboratories, Woburn, MA) was injected subcutaneously into the flank of 4 week old female mice in at 30 μ g/kg, 70 μ g/kg and 100 μ g/kg doses

on consecutive days[76]. Control mice were treated with vehicle EtOh (ethanol diluted in PBS). Mice were rested for 4-6 weeks before confirmation by tail-flick assay. For mechanical deafferentation, mice were anesthetized and an incision 2 to 3 cm at the dorsal midline of the back skin was made[148, 173]. The dorsal cutaneous nerves from T3-T12 were exposed under a dissection microscope at both sides. On the right side, nerves were dissected close to the skin and the left side was kept as sham control. The skin was then closed by steel wound clips. Denervation was confirmed by touch sensitivity. Mice were rested for 7 days to avoid effects of immediate crush injury and inflammation and then infected.

2.4.8 Behavioral assays

Ethanol or resiniferatoxin treated mice were rested 24 hours prior to experiment to allow for adjustment to the experimenter before behavioral assessment assays. Responsiveness to noxious heat stimuli was performed as previously described[174]. The distal half of the tail of a lightly restrained EtOh or RTX treated mice is dipped into thermostatically controlled circulating water bath. Latency to heat stimulus response by a vigorous flexion of the tail is measured using a stop-watch. Responsiveness to capsaicin was evaluated as previously described[174, 175]. Briefly, mice were placed in clear plastic containers with four surrounding mirrors and allowed to acclimate for 30 minutes before pain assessment. At the time of the experiment, either vehicle (DMSO diluted in saline) or 5 μ g capsaicin (diluted in DMSO in saline) was injected intradermally into the footpad of EtOh or RTX treated mice. The response was videotaped for 10 minutes and quantified in amount of seconds spent licking injected footpad during the observational period.

2.4.9 DRG cultures

Primary cultures of mouse DRG neurons were prepared using methods similar to those previously described[176]. Briefly, DRG were collected from cervical, thoracic, and lumbar spinal levels of two adult female mice C57BL/6 mice and placed in PBS. Cells were enzymatically dissociated in Collagenase-D (1.5mg/ml, two 60 minute incubations; Roche Diagnostics) and triturated through fire-constricted Pasteur pipettes. This cell suspension was passed over a 100 μ m filter before plating onto the center of coverslips 25mm in diameter that had been coated with poly-L-lysine (10 μ g/ml; Sigma-Aldrich, St. Louis, MO) and laminin (10 μ g/ml; Sigma-Aldrich, St. Louis, MO) in a 6 well culture

dish. After 1.5 hours, F12/Ham's media containing 10% fetal bovine serum was added to each well.

2.4.10 Calcium imaging

18 - 24 hours after plating onto coverslips, cells were incubated in the ratiometric intracellular calcium indicator, Fura2-AM (Invitrogen Life Technologies; 1.5 μ M diluted in HEPES buffer containing 1% bovine serum albumin) at 37°C for 1h. Coverslips were then transferred to a recording chamber and perfused at a rate of 1.8ml/minute with HEPES-Hank's buffer (25mM HEPES, 135mM NaCl, 2.5mM CaCl₂, 3.5mM KCl, 1mM MgCl₂, and 3.3mM glucose, pH 7.4 and 335-340 mOsm adjusted with sucrose). Images were acquired using a Cool One SensiCam CCD camera (COOKE, Auburn Hills, MI) at approximately 1 second intervals using an Olympus IX71 inverted microscope equipped with a 40x oil immersion objective. Excitation wavelengths (340- and 380-nm) and shutter speed were controlled by a DeltaRam X controller and LPS-220B lamp (PTI). ImageMaster 5.0 software (PTI) was used to collect and analyze all images. Cells were stimulated with: 30mM KCl (10s), followed by 5 min perfusion of HEPES buffer, heat-killed 1x10⁷ *Candida albicans* (resuspended in HEPES buffer and applied directly to the coverslip;15s), followed by a 10 min washout, and 500mM Capsaicin (10s) or 30mM KCl (10s), followed by a 5 min washout.

2.4.11 CGRP release assay

After 48 h in culture, DRG neurons (approx. 20,000 cells/well) were washed once with HEPES buffer and then incubated in HEPES buffer for 10 min (for measurement of basal release) followed by a 10-min incubation with HEPES or HKCA resuspended in HEPES buffer (for measurement of stimulated release). Supernatants from the basal and stimulated release were collected and centrifuged for 5 min at 14,000 g and the pellet was discarded. Cells were lysed in 100 μ l of lysis buffer. CGRP levels in the basal and stimulated release supernatants and in cell lysates were measured by ELISA (Cayman Chemical, Ann Arbor, MI). To control for HKCA-derived crossreactivity in the stimulated supernatant, a separate aliquot of HKCA was resuspended in HEPES buffer and centrifuged and assayed along with the treatment samples. Absorbance in assay wells incubated with control HKCA supernatant was indistinguishable from background.

2.4.12 Statistics and data representation

Results are presented as mean $\pm$ standard error unless noted and groups were compared by unpaired Student's t test using GraphPad Prism software (San Diego, CA). Heatmap representation of microarray gene expression data was performed with the normalized data in `heatmaps.2` function of package `Gplots` in the statistical programming language R. Venn diagram proportional and scaled to their representative size of DRG neuron responsiveness was created with package `Venn` in statistical programming language R.

2.5 Publication and contributions

This chapter is modified from the published article:

Sakeen W Kashem, Maureen S Riedl, Chen Yao, Christopher N Honda, Lucy Vulchanova, and Daniel H Kaplan. Nociceptive Sensory Fibers Drive Interleukin-23 Production from CD301b+ Dermal Dendritic Cells and Drive Protective Cutaneous Immunity. *Immunity*, 43(3):515-526, September 2015.

Author contributions are as follows: SWK performed and analyzed all experiments. MSR, CNH and LV performed experiments related to DRG neuron stimulations. CY assisted on experiments pertinent to manuscript revision. SWK and DHK designed all experiments and wrote the manuscript. All authors were involved in editing the original published manuscript.

Chapter 3

Adaptive immunity against *Candida albicans* skin infection

3.1 Introduction

Candida albicans is a human commensal and a common mucosal and systemic pathogen in the setting of immunosuppression. Chronic mucocutaneous candidiasis (CMC) occurs in the absence of innate sources of interleukin-17 (IL-17) and IL-22 as well as in the setting of ineffective T helper-17 (Th17) cell-type immunity in both mice and in patients (e.g. HIV/AIDS or hyper IgE syndrome)[124, 126]. CMC is also associated with mutations in the fungal recognition C-type lectin receptor Dectin-1 or its down stream signaling molecules[31, 32]. In contrast, systemic candidiasis predominantly occurs in the clinical setting of neutropenia and in mice with innate immune defects (e.g. *Il6*^{-/-} mice) resulting in ineffective neutrophil activation but does not occur in the absence of Th17 cell-associated or adaptive immunity[6, 11].

C. albicans is dimorphic and grows as yeast at 30°C and as a filamentous or hyphal form at 37°C. On the stratum corneum of the skin, *C. albicans* exist as budding yeasts. Pathogenic *C. albicans* in the dermis and systemic organs exists predominantly as pseudohyphae[40]. *C. albicans* mutants that are unable to form filaments, fail to establish robust infections suggesting that the yeast to hyphal transition is required for virulence[177]. This transition has also been suggested to be required for the development of anti-Candida Th17 cell responses thereby allowing for discrimination between commensal and invasive *C. albicans*[40]. In vitro, *C. albicans* pseudohyphae induce Dectin-1 mediated Th17 cell differentiation[178]. *C. albicans* pseudohyphae, however, have also been reported to promote Th1 and Th2 cell differentiation in vitro[179, 180]. Importantly, T helper (Th) cell responses in vivo to yeast and filamentous forms of *C. albicans* and the ability of these adaptive responses to provide protection remain unclear.

In the skin, there are at least three well defined subsets of dendritic cells (DC) - epidermal Langerhans cells (LC), CD103+ dermal DC (dDC) and CD11b+ dDC[90]. These DC subsets migrate from the skin into regional lymph nodes during infection where they present antigen to nave T cells and secrete cytokines that determine Th cell differentiation[181]. During epicutaneous infection with *C. albicans*, CD103+ dDC generate IL-12 and are required for differentiation of Th1 cells[13]. LC are required for Th17 cell differentiation and express high amounts of Th17 cell inducing cytokines IL-1 β , transforming growth factor- β (TGF- β) and IL-6. The importance of individual cytokines in generating Th17 cells in vivo is controversial and varies by tissue. It

has been demonstrated that IL-1 β and IL-6 are both necessary for Th17 cell differentiation in peripheral tissues including skin[182]. Th17 cell induction in the spleen is independent of IL-6 but not IL-1 β . In the intestines, several studies have confirmed an IL-1 β dependence for Th17 cell development while the requirement for IL-6 remains controversial[182, 119].

CD11b+ dDC make up the majority of the skin migratory DC and drive Th2 cell differentiation in the setting of dermal papain injection or parasitic infection[144]. In the setting of *C. albicans* infection, this DC subset generates high amounts of IL-1 β and to a lesser extent IL-6 and IL-12[13]. Their ability, however, to promote Th cell differentiation other than Th2 cell differentiation remains unexplored[144]. Based on the functional differences between skin DC subsets and the transition of *C. albicans* from yeast to filamentous forms during epidermal invasion, we hypothesized that DC subsets and *C. albicans* morphology together determine Th cell differentiation.

Because of the requirement for immunosuppression to establish robust and consistent oropharyngeal *C. albicans* infection, we have focused on epicutaneous *C. albicans* infection. We found that infection with *C. albicans* yeast but not pseudohyphae was capable of inducing Th17 cell responses through a mechanism that required interaction with LC, engagement of Dectin-1 and LC-derived IL-6. *C. albicans* in the dermis failed to induce Th17 cell differentiation despite the expression of Dectin-1 on CD11b+ dDC due to the absence of Dectin-1 ligation by pseudohyphae that are the dominant morphology at that site. Finally, we demonstrated that Th17 but not Th1 cells were protective against secondary cutaneous infections while Th1 but not Th17 cells were protective against secondary systemic infections. Thus, *C. albicans* morphology and skin DC subsets drive distinct Th cell responses that provide protection from either cutaneous or systemic infections.

3.2 Results

3.2.1 Distinct T helper cell responses mediated by *C. albicans* morphology

We have previously generated recombinant *C. albicans* derived from the standard SC5314 strain that expresses the peptides 2W1S and E α , under the ubiquitous *Eno1* promoter (*Eno1*-Ag). Using a TE α CD4+ T cell adoptive transfer system, we showed that mice that express Diphtheria toxin fragment A (DTA) under a human Langerin (huLangerin) promoter with a constitutive absence of LC (henceforth referred to as LC-) failed to generate antigen-specific Th17 cells[13]. To exclude the possibility that this phenotype resulted from a chronic absence of LC, we repeated these experiments using mice in which express the Diphtheria toxin receptor (DTR) within huLangerin (referred to as huLangerin-DTR mice) that allow for inducible ablation of LC. As expected, ablation of LC just prior to *Eno1*-Ag infection also prevented efficient Th17 cell differentiation (**Figure 3.1A**). We next examined the responses of endogenous antigen specific CD4+ T cells to *C. albicans* using I-Ab:p2W1S tetramer staining 8 days post infection. Expansion of p2W1S-specific cells was equivalent in control and LC deficient mice (**Figure 3.2A**). Expansion of cells producing IL-17A but not interferon- γ (IFN- γ), however, was significantly reduced in LC deficient mice (**Figure 3.2B**). Thus, consistent with our earlier observations using adoptive transfer of T cell receptor (TCR) transgenic T cells specific for E α (TE α), LC are required for efficient differentiation of endogenous nave CD4+ T cells into Th17 cells.

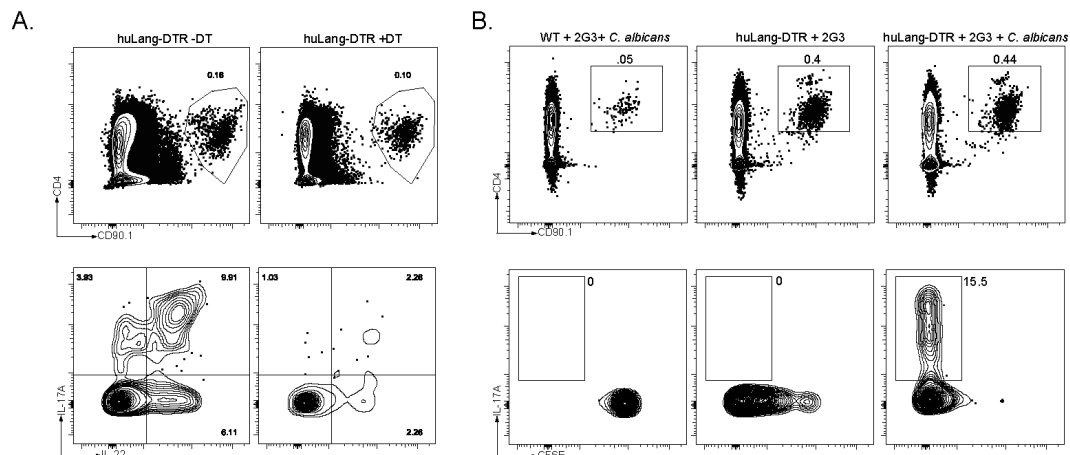


Figure 3.1: Langerhans cells are necessary and sufficient for Th17 responses to *C. albicans*.

(A) Human Langerin-DTR mice were adoptively transferred with 3×10^5 TE α cells and treated the next day either with PBS or $1 \mu\text{g}$ diphtheria toxin i.p. to selectively deplete Langerhans cells. Four days later mice were epicutaneously infected with 2×10^8 CFU of *Eno1*-Ag. T cells were isolated from skin draining lymph nodes and stimulated with PMA/Ionomycin. Expansion (top row) and cytokine production as determined by intracellular flow cytometry is shown (bottom row). (B) WT or Human Langerin-DTR mice received 3×10^5 TE α cells. One day later mice were immunized with $1 \mu\text{g}$ 2G3-E α ip. Six hours later, they were either mock or SC5314 epicutaneously infected. Four days later, T cells were isolated from skin draining lymph nodes and stimulated with PMA/Ionomycin. Expansion (top row) and cytokine production as determined by intracellular flow cytometry (bottom row) is shown.

Representative data from 3 experiments is shown.

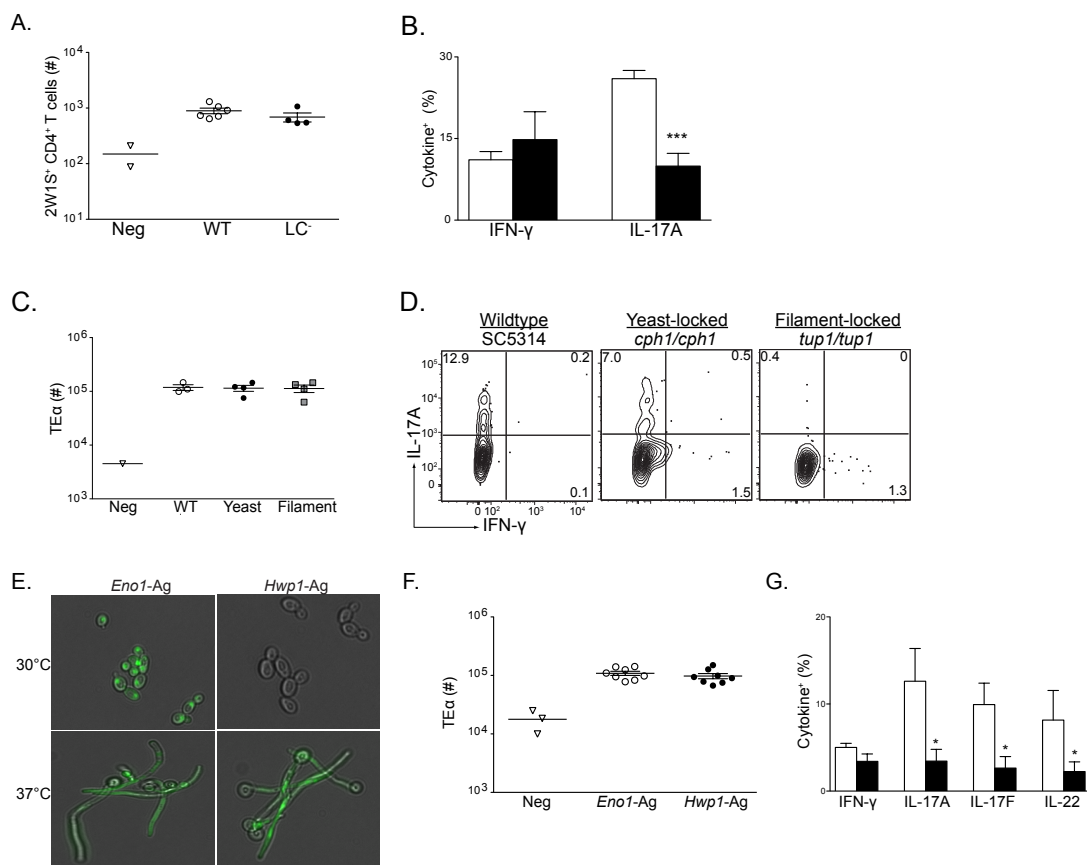


Figure 3.2: *Candida albicans* yeast drives Th17 cell differentiation in vivo.

(A) The number of 2W1S-specific CD4⁺ T cells in six skin draining lymph nodes (axillary, brachial, inguinal) and spleen 8 days after mock (Neg) or skin infection with *Eno1-Ag* in wildtype (WT) or LC deficient mice (LC^{-/-} mice is shown). (B) Percentage of PMA and Ionomycin stimulated 2W1S-specific cells in WT (white) or LC^{-/-} (black) mice expressing the indicated cytokines. (C) Antigen was targeted to huLangerin transgenic mice by i.p injection of 1.0 μ g 2G3-E α . Adjuvant was provided by skin infection with wild-type, *cph1/cph1*, or *tup1/tup1* strains of *C. albicans*. TE α numbers were assessed in the skin draining lymph nodes of mice 4 days after infection. Neg refers to unimmunized mice. (D) The expression of IFN- γ and IL-17A in PMA + Ionomycin stimulated E α -specific CD4⁺ TE α cells from (C) is shown. (E) GFP expression in *Eno1-Ag* or *Hwp1-Ag* *C. albicans* under yeast or filamentous growth conditions was determined by immunofluorescence. (F) TE α expansion and (G) cytokines production 4 days after infection of WT mice with *Eno1-Ag* (white) or *Hwp1-Ag* (black) or nave (Neg). Each symbol represents data from an individual animal. Scales represents mean $\pm$ SEM. *p<0.05, **p<0.01, ***p<0.001

To determine whether recognition by LC of yeast or filamentous forms of *C. albicans* affects Th cell differentiation, we selectively targeted antigen to LC by i.p. injection of 1.0 μ g anti-huLangerin mAb-E α conjugates (2G3-E α) in human Langerin transgenic mice (huLangerin) (**Figure 3.1B**)[95, 183, 184, 13]. HuLangerin is expressed exclusively by LC in these mice resulting in efficient and LC-selective acquisition of 2G3-E α . As a source of adjuvant, we epicutaneously infected 2G3-E α targeted huLangerin mice with wild-type SC5314 as well as *cph/cph* and *tup1/tup1* mutants derived from SC5314 that are locked into yeast or hyphal forms, respectively[185, 186]. Expansion of adoptively transferred TE α cells was equivalent in mice infected with all 3 strains (**Figure 3.2C**). Notably, Th17 cell differentiation was intact in the mice infected with the yeast-locked *cph1* mutant but was absent in those infected with the filament locked *tup1* mutant suggesting that LC-mediated Th17 cell differentiation requires the presence of *C. albicans* yeast forms (**Figure 3.2D**).

To determine the effect of *C. albicans* morphology under conditions in which antigen presentation is not limited to LC, we next generated recombinant *C. albicans* in which expression of a fusion protein of GFP with the antigens E α and 2W1S was driven by the promoter for the pseudohyphae specific gene *Hwp1* (*Hwp1*-Ag)[187]. Unlike *Eno1*-Ag where GFP expression was present in all *C. albicans* morphologies, expression of GFP by *Hwp1*-Ag was absent in yeast and observed only for filamentous forms (**Figure 3.2B and 3.3A-E**). Both *Eno1*-Ag and *Hwp1*-Ag strains demonstrated similar virulence in vivo after epicutaneous or intravenous infection (**Figure 3.3F-G**). Epicutaneous infection of WT mice with *Eno1*-Ag and *Hwp1*-Ag induced similar antigen specific CD4+ T cells expansion and Th1 cell differentiation (**Figure 3.2F**). Notably, mice infected with *Hwp1*-Ag failed to efficiently induce Th17 cell-associated responses (**Figure 3.2G**). Thus, LC and *C. albicans* yeast but not pseudohyphae are required for optimal Th17 cell differentiation.

3.2.2 Th17 cell differentiation requires LC derived IL-6

In vitro, Th17 cell differentiation of nave cells requires combinations of IL-1 β , IL-6, IL-23 and TGF- β [188, 189]. The requirement for IL-1 β and IL-6 in vivo differs based on the site of immunization[182]. To determine which cytokines are responsible for LC-mediated Th17 cell induction, we first compared responses in IL-1 β and IL-6 deficient mice. We found that both IL-1 β and IL-6 deficiency resulted in diminished Th17 cell responses (**Figure 3.4A-B**). Notably, Th17 cells were entirely absent in *Il6*^{-/-} mice and

was associated with a reciprocal increase in Th1 cells (Figure 3.4B-C).

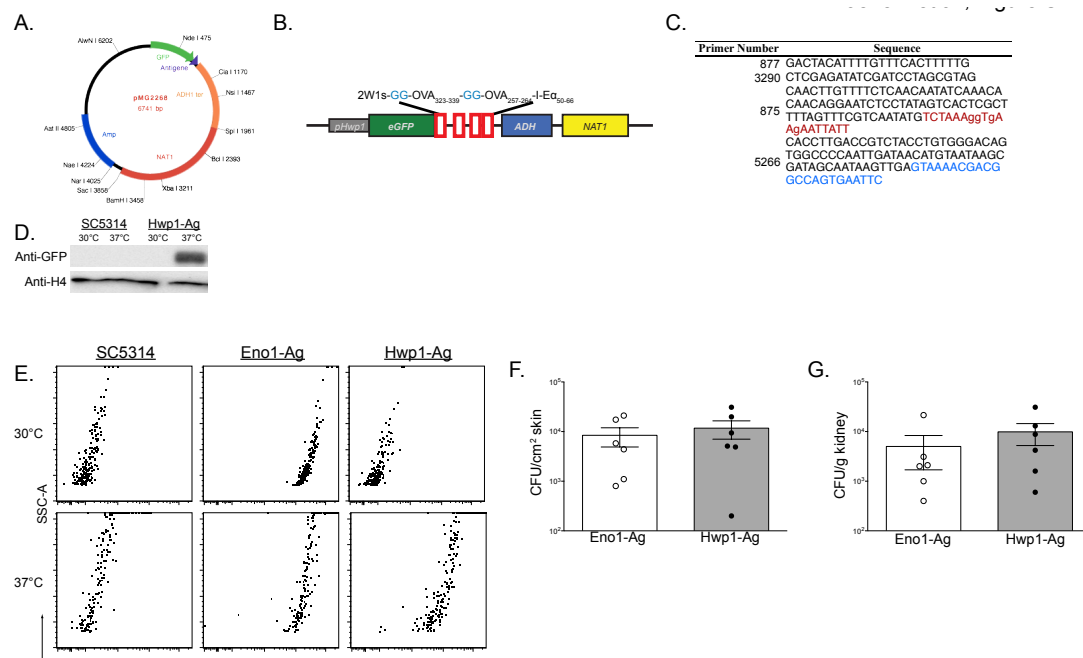


Figure 3.3: Generation of *Hwp1*-Ag.

(A) Design of vector inserting GFP-Ag cassette behind the hyphal wall protein 1 promoter. (B) Schematic drawing of Hwp1-Ag. (C) Primers used in design of *Hwp1*-Ag. (D) Western blot showing GFP and control H4 expression in *Eno1*-Ag compared to *Hwp1*-Ag under different morphological growth conditions. (E) GFP expression as demonstrated by flow cytometry of SC5314, *Eno1*-Ag and *Hwp1*-Ag under yeast (top) and filamentous growth conditions (bottom). (F) WT mice were infected epicutaneously with 2×10^8 *Eno1*-Ag or *Hwp1*-Ag. CFU from 1 cm² skin sections harvested 3 days after infection from the central back are shown. (G) WT mice were i.v. infected with 1×10^5 *Eno1*-Ag or *Hwp1*-Ag. CFU of kidneys harvested 3 days after infection are shown. Symbols represent data from individual mice.

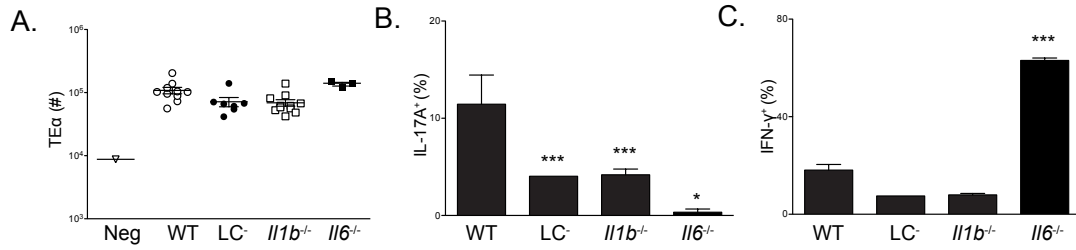


Figure 3.4: IL-1 β and IL-6 are required for Th17 cell differentiation during *C. albicans* skin infection.

(A) Expansion of TE α cells isolated from skin draining lymph nodes 4 days after skin infection of the indicated mouse strains with *Eno1*-Ag *C. albicans*. Each symbol represents results from an individual animal. (B) The percent of ex vivo PMA and Ionomycin stimulated TE α cells in cutaneous lymph nodes that express IL-17A or (C) IFN- γ as determined intracellular flow cytometry is shown. Neg refers to mock infected WT mice. Scales represents mean $\pm$ SEM. * p <0.05, ** p <0.01, *** p <0.001. Data is representative of at least three independent experiments.

Since expression of IL-1 β , TGF- β , and IL-6 was increased in LC during *C. albicans* infection[13], we next sought to determine which cytokines were required for Th17 cell differentiation. To generate LC-specific ablation of TGF- β 1, we used huLangerin^{creERT2} x *Tgfb1*^{fl/fl} mice that efficiently and selectively excise TGF- β 1 in LC after administration of tamoxifen[190]. We have previously reported that ablation of TGF- β 1 in LC results in a gradual spontaneous migration of LC out of the epidermis over the course of weeks. To avoid this potential confounding factor, we infected mice on day 5 after tamoxifen administration, a time point when TGF- β has been efficiently excised but LCs have not begun to migrate in large numbers. As controls, we also infected tamoxifen treated huLangerin^{creERT2} x YFP and huLangerin^{creERT2} x *Tgfb1*^{fl/fl} mice which maintain TGF- β expression but also have increased homeostatic migration[190]. Antigen specific T cell expansion and Th cell phenotype differentiation was equivalent in all 3 strains demonstrating that LC-derived TGF- β is not required for Th17 cell differentiation (**Figure 3.5A-B**).

To generate LC-specific ablation of IL-1 β and IL-6, we generated WT $\rightarrow$ *Il1b*^{-/-} and WT $\rightarrow$ *Il6*^{-/-} bone marrow chimeras. LC are the only radio-resistant antigen presenting cell (APC) in the skin and remained of cytokine deficient host origin while the hematopoietic system achieved 90-96% chimerism (**Figure 3.6A-C**). Infected WT $\rightarrow$ *Il1b*^{-/-} chimeras developed cell proliferation and Th differentiation similar to control WT $\rightarrow$ WT mice indicating that LC-derived IL-1 β is not required for Th17 induction (**Figure 3.5C-D**). In contrast, Th17 cell differentiation was reduced in WT $\rightarrow$ *Il6*^{-/-} chimeras indicating that LC-derived IL-6 is required for Th17 (**Figure 3.5E-F**). Moreover, Th17 cell differentiation was intact in *Il6*^{-/-} WT mice in which LC were the only IL-6 sufficient hematopoietic cell type (**Figure 3.5H**). Thus, IL-6 derived from LC is necessary for Th17 cell induction during skin infection.

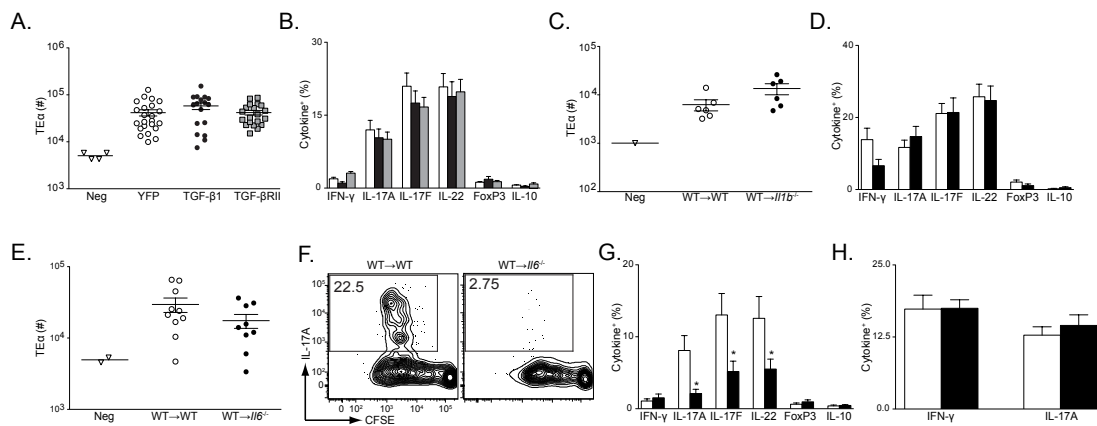


Figure 3.5: LC derived IL-6, but not TGF- β or IL-1 β is required for Th17 cell differentiation.

(A) huLangerin^{creERT2} *Tgfb1*^{fl/fl} mice were treated with tamoxifen to generate LC-specific ablation of TGF- β 1. These mice were skin infected with *Eno1*-Ag *C. albicans* along with tamoxifen treated huLangerin^{creERT2} *Tgfb2*^{fl/fl} and YFP controls.

Expansion of TE α at day 4 along with (B) cytokines expression is shown. (C) Expansion and (D) cytokine production by TE α cells in *Eno1*-Ag infected WT→WT (white) or WT→*Il1b*^{-/-} (black) bone marrow chimeric mice is shown. (E) The expansion, (F) expression of IL-17A by TE α cells and (G) percent of TE α cells producing the indicated cytokines in WT→WT (white) or WT→*Il6*^{-/-} (black) mice is shown. (H) Cytokine production by TE α cells in WT→WT (white) or *Il6*^{-/-} WT (black) mice is shown. Neg refers to mock infected WT mice. Scales represents mean $\pm$ SEM. *P<0.05, **P<0.01, ***P<0.001. Data is representative of at least three independent experiments.

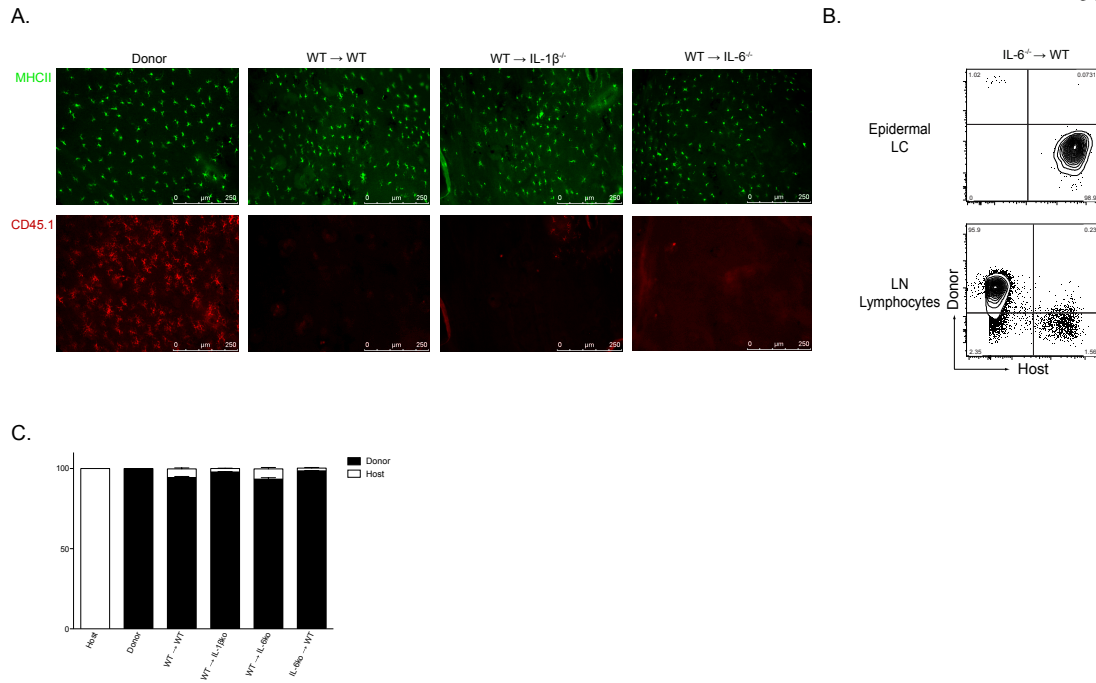


Figure 3.6: Generation of bone marrow chimeras.

Six weeks old knock-out, C57BL/6 or Ly5.2 congenically marked mice were irradiated with split doses of 500 cGy. The following day, 5×10^6 bone marrow cells isolated from the specified mice were injected intravenously. Mice were rested for at least 6 weeks prior to experiments. Chimerism was assessed by expression of CD45.1 and CD45.2 by

LC in epidermis as well as CD45+ lymphocytes in blood and lymph nodes. (A) Demonstration of the persistence of host radio-resistant CD45.2 LC in recipient skin.

(B) The percentage of host (CD45.2) and donor (CD45.1) skin LC (top panel) and total LN lymphocytes (bottom) in IL-6^{-/-} → WT bone marrow chimeric mice is shown.

(C) The relative degree of chimerism in the lymph nodes of the indicated chimeras based on expression of CD45.1 and CD45.2 is shown.

3.2.3 LC-derived IL-6 requires Dectin-1 engagement

A subset of patients with chronic mucocutaneous candidiasis (CMC) have mutations in the transcription factor STAT3, that acts downstream of the IL-6 receptor[126]. Patients with Dectin-1 polymorphisms also suffer from CMC and have decreased IL-6 production by their peripheral blood mononuclear cells (PBMCs)[31]. In addition, budding yeast engages Dectin-1 while yeast but not pseudohyphae trigger IL-6 secretion in human PBMCs[191]. Since Th17 cell differentiation depends on LC-derived IL-6, we next sought to determine whether Th17 cell differentiation required engagement of Dectin-1 on LC. We took advantage of a clinical isolate, s20175.016, that has similar virulence as SC5314 but its immune recognition is independent of Dectin-1 (**Figure 3.7A-B**)[38]. Antigen was targeted to LC in huLangerin mice using 2G3-E α followed by infection with SC5314 or s20175.016. As expected, CD4⁺ T cell expansion and Th1 cell differentiation were comparable between the two experimental groups but Th17 cell differentiation was reduced in mice infected with s20175.016 (**Figure 3.8A-B**). Analysis of LC isolated from the skin draining lymph nodes 3 days after infection revealed a large reduction in IL-6 production in mice infected with s20175.016 compared with SC5314 (**Figure 3.8C and 3.9**). Moreover, intradermal injection of 1 μ g recombinant IL-6 at the time of infection restored Th17 cell differentiation (**Figure 3.8D**). Finally, we directly explored the necessity of Dectin-1 signaling by comparing the host response to *C. albicans* infection in Dectin-1 deficient (*Clec7a*^{-/-}) mice. While WT and *Clec7a*^{-/-} mice strains exhibited similar T cell expansion, Dectin-1 deficient host demonstrated diminished Th17 cell responses (**Figure 3.8E-F**). Thus Th17 cell differentiation requires engagement of Dectin-1 on LC that results in increased expression of IL-6.

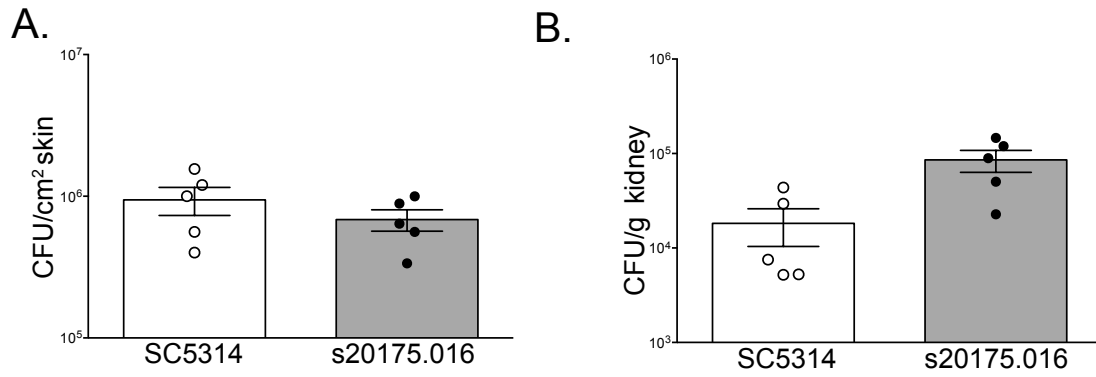


Figure 3.7: SC5314 and s20175.016 have similar virulence.

(A) WT mice were intradermally infected with 2×10^8 SC5314 or s20175.016. Mice were euthanized 3 days later and 1.0 cm^2 skin sections harvested from central back of mice. The total CFU is shown. B) WT mice were i.v. infected with 1×10^5 SC5314 or s20175.016. The CFU in kidneys isolated 2 days after infection is shown. Each symbol represents an individual animal.

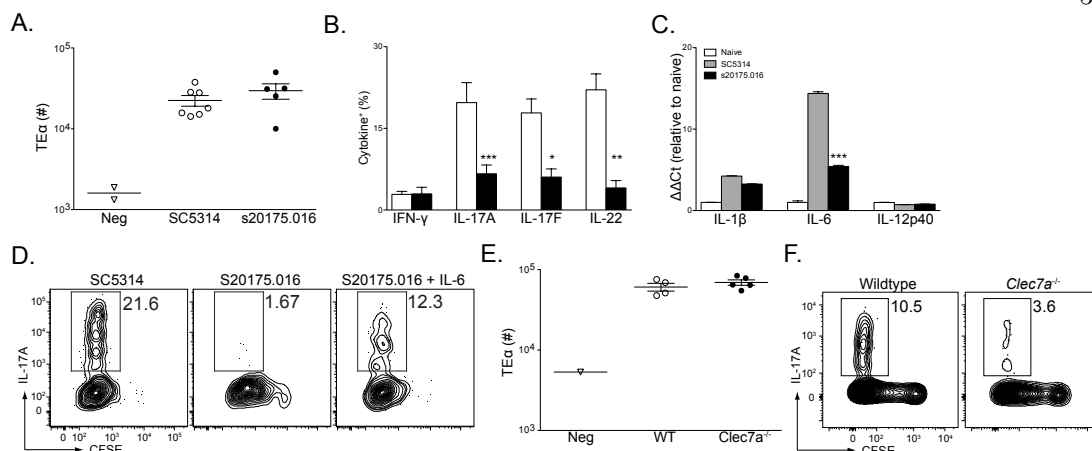


Figure 3.8: Dectin-1 engagement on LC leads to IL-6 production and Th17 cell differentiation.

Antigen was directed to LC in huLangerin transgenic by i.p. injection of 1.0 μ g 2G3-E α followed by skin infection with SC5314 (white) or s20175.016 (black) strains of *C. albicans*. (A) TE α expansion and (B) cytokine production by TE α cells is shown. (C) LC were isolated from the skin draining lymph nodes by flow cytometry sorting on day 3 after skin infection with vehicle (white), SC5314 (gray) or s20175.016 (black). Expression of the indicated cytokines was determined by qPCR and is shown normalized to uninfected mice. (D) Antigen was targeted to LC (as in (A)) followed by infection with SC5314 or S20175.016. At the time of infection, PBS or 1.0 μ g of rIL-6 was injected at the site of infection. CSFE dilution of TE α cells as well as expression of IL-17A is shown. (E) Expansion of TE α cells in the skin draining lymph nodes of WT and Dectin-1 deficient mice 4 days after infection with *Eno1*-Ag. (F) CSFE dilution and expression of IL-17A in PMA and Ionomycin stimulated TE α cells isolated from WT and Dectin-1 deficient mice on day 4 after infection. Neg refers to mock infected WT mice. Scales represents mean $\pm$ SEM. *P<0.05, **P<0.01, ***P<0.001. Data is representative of at least two independent experiments.

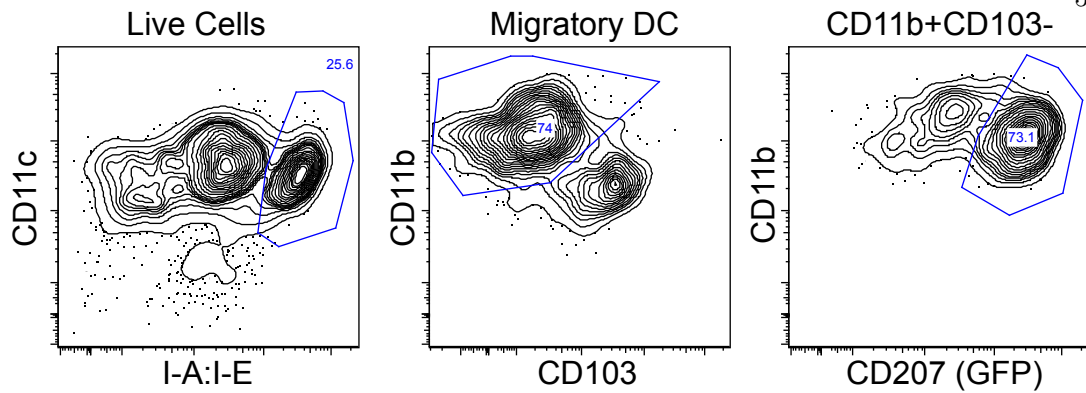


Figure 3.9: Sort strategy for migratory Langerhans cells from skin draining lymph nodes.

(A) Groups of Langerin-eGFP mice were either mock, SC5314 or s20175.016 epicutaneously infected. 3 days later DC were purified from skin draining lymph nodes by CD11c+ magnetic bead selection and sorted on live, singlets. LC were identified as CD11c hi, I-A/I-E hi, CD11b hi, CD103 negative, GFP positive cells.

3.2.4 Absence of Dectin-1 ligands prevent Th17 cell induction by dermal DC

To examine the function of dermal DC subsets in the induction of Th17 cell responses, we first compared expression of Dectin-1 by each subset. Dectin-1 is expressed by LC and CD11b+ dDC but not by CD103+ dDC (**Figure 3.10A**). The absence of Dectin-1 on CD103+ dDC is consistent with our previous observation that this DC subset was required for Th1 but not Th17 cell induction[13].

Since Th17 cell differentiation is reduced but not ablated in the absence of LC and since CD11b+ DC in the lung are required for Th17 cell responses during *Aspergillus fumigatus* infection[120], we hypothesized that CD11b+ dDC could also contribute to Th17 cell during skin infection. Macrophage galactose C-type lectin type 2 (Mgl2) is expressed by most CD11b+ dDC. Administration of diphtheria toxin (DT) to the recently reported Mgl2-DTR mice results in ablation of CD11b+ dDC and LC (**Figure 3.11A-C**)[144]. DT treated Mgl2-DTR mice have reduced Th17 cell differentiation that could result from deletion of either subset (**Figure 3.11D**). To selectively ablate CD11b+ dDC, we generated Mgl2-DTR→WT chimeric mice (**Figure 3.11C**). Notably, antigen-specific T cells expansion and Th phenotype was unaltered in DT treated Mgl2-DTR→WT mice infected with *Eno1*-Ag indicating that CD11b+ dDC are not required for Th17 cell differentiation (**Figure 3.10B-C**). To determine whether this DC subset could be sufficient for Th17 cell induction, we generated LC^{-/-}x Batf3^{-/-} mice that have a genetic absence of both LC and CD103+ dDC. *Eno1*-Ag infection of these mice in which CD11b+ dDC are the dominant skin-resident DC subset demonstrated defective Th1 and Th17 cell differentiation (**Figure 3.10D-E**) that is consistent with the absence of Th17 cells generated by LC and Th1 cells induced by CD103+ dDC. Thus, CD11b+ dDC are neither necessary nor sufficient for Th17 cell generation during epicutaneous infection with *C. albicans*.

Despite the equivalent degree of T cells expansion in LC^{-/-}xBatf3^{-/-} mice, we considered the possibility that CD11b+ dDC have limited access to antigen during an epicutaneous infection. In wild-type mice, we compared epicutaneous with dermal infection using the same inoculum of *Eno1*-Ag. Though T cell expansion and Th1 cell differentiation was similar, Th17 cell differentiation was greatly reduced in response to intradermal infection (**Figure 3.12A-B**). Induction of Th17 cell was also absent in response to an i.v. systemic infection (**Figure 3.13A-B**). Notably, addition of the Dectin-1 ligand, curdlan, with the intradermal inoculation rescued the Th17 cell response suggesting

that Dectin-1 ligation does not occur in the dermis during *C. albicans* infection but that CD11b+ dDC do have the capacity to induce Th17 cells(**Figure 3.12C**). During epicutaneous *C. albicans* infection, yeast are found on the epidermis and invasive filamentous forms in the dermis (**Figure 3.12D**)[40]. To test whether hyphal forms of *C. albicans* are unable to induce Th17 cell due to the absence of Dectin-1 ligands, we infected mice both epicutaneously with *Hwp1*-Ag with the addition of exogenous curdlan or vehicle (**Figure 3.12E**). The addition of Dectin-1 ligands rescued the ability of antigen expressed by hyphal forms of *C. albicans* to generate Th17 cell responses.

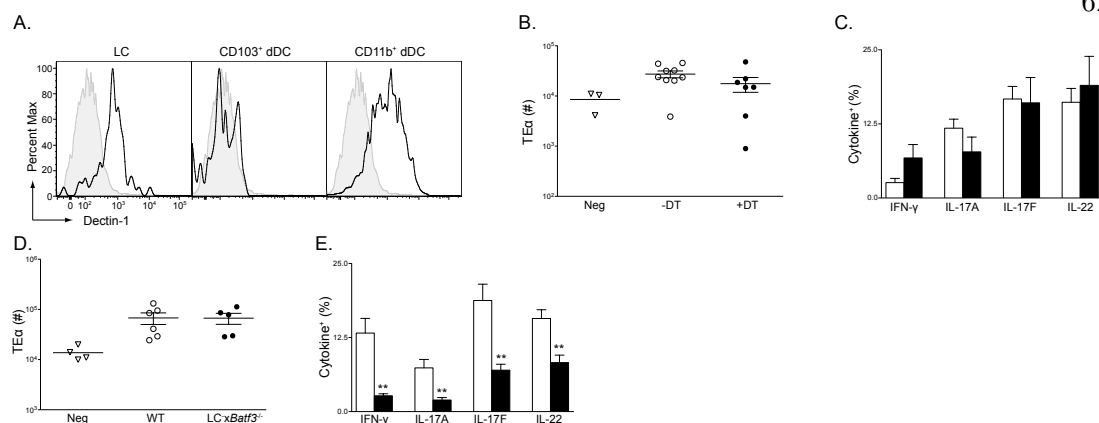


Figure 3.10: CD11b⁺ Dermal DC are not required for Th17 cell differentiation.

(A) Surface expression of Dectin-1 by the indicated skin DC subset isolated from the skin under steady-state conditions is shown. (B) Mgl2-DTR→WT chimeric mice were treated with vehicle (white circles) or diphtheria toxin (black circles) and skin infected with *Eno1*-Ag 1 day later. The expansion of TEα cells isolated from skin draining lymph nodes 4 days after infection is shown. Neg represents mock infected WT mice (triangles). (C) Expression of the indicated cytokines from PMA and Ionomycin TEα cells in (B) is shown. (D) Expansion and (E) cytokine production by TEα cells in WT (white) or LC-xBatf3^{-/-} (black) mice 4 days after infection with *Eno1*-Ag is shown.

Scales represents mean $\pm$ SEM. *P<0.05, **P<0.01, ***P<0.001. Data is representative of at least three independent experiments.

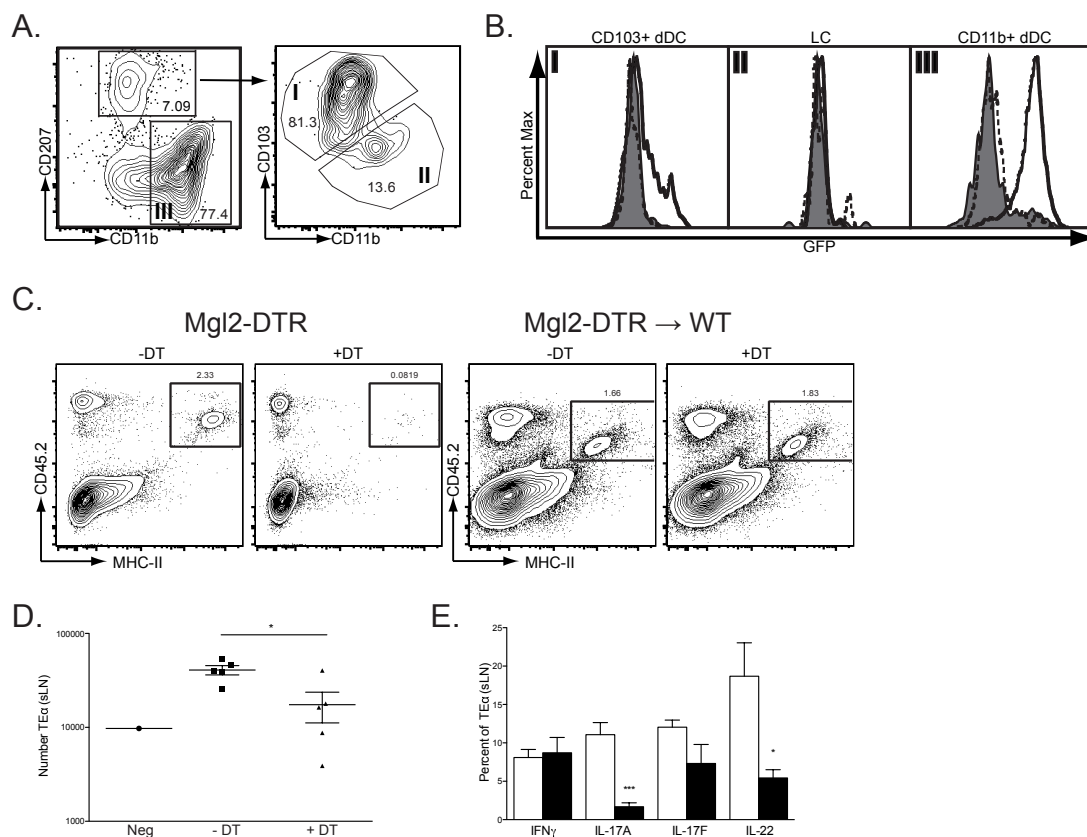


Figure 3.11: Characterization of Mgl2-DTR mice.

Dendritic cells were isolated from skin draining lymph nodes and gated as singlet, live, MHC-II^{hi}, CD11c^{hi} cells. (A) DC were further gating using CD103, CD11b and CD207 to identify 3 groups (I-CD103+ dDC, II-LC, III-CD11b+ dDC). (B) Expression of Mgl2 in the indicated DC populations as indicated by expression GFP in WT (gray filled), or Mgl2-DTR → WT mice 1 day after injection of vehicle (black line) or DT (dotted line). (C) Single cell epidermal suspensions were stained with MHC-II and CD45.2 to identify the percentage of LC in the epidermis of Mgl2-DTR (left) or Mgl2-DTR → WT (right) mice one day after treatment with vehicle or DT. (D) Mgl2-DTR mice were transplanted with 3×10^5 TE α cells and treated with PBS (white) or DT (black). On day +1, mice were either mock infected or epicutaneously infected with *Eno1*-Ag. Expansion and (E) cytokine production of PMA/Ionomycin stimulated CD90.1+ TE α cells in Mgl2-DTR mice treated with PBS (white) or DT (black) is shown.

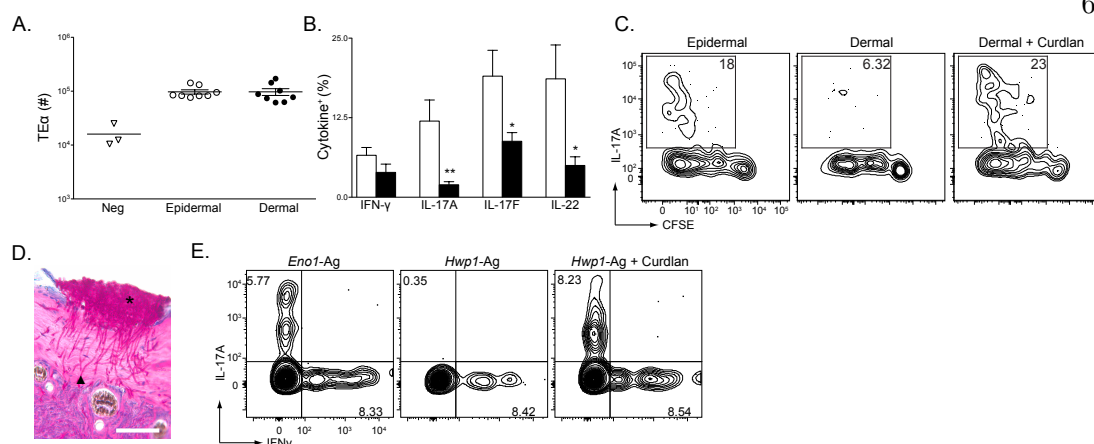


Figure 3.12: Dermal infection and *C. albicans* pseudohyphae do not induce Th17 cells without exogenous Dectin-1 ligands.

(A and B) WT mice were infected with an equivalent inoculum of *Eno1*-Ag *C. albicans* on the epidermis (white) or by intradermal injection (black). Expansion and cytokine production by PMA and Ionomycin stimulated TE α cells isolated from skin draining lymph nodes is shown. (C) As in (A), WT mice were infected epicutaneously or by dermal injection into which purified curdlan or vehicle has been added to the inoculum. CFSE dilution and expression of IL-17A by PMA and Ionomycin stimulated TE α cells is shown. (D) PAS stain of skin 2 days after *C. albicans* infection showing budding yeast (asterisk) restricted to the epidermis and numerous penetrating pseudohyphae in the dermis (arrowhead) (bar=150 μ m). (E) The expression of IL-17A and IFN γ by PMA and Ionomycin stimulated TE α cells 4 days after epicutaneous infection of WT mice with *Eno1*-Ag or *Hwp1*-Ag is shown. Curdlan or vehicle alone was provided by dermal injection at the time of infection. Neg represents mock infected WT mice. Scales represents mean $\pm$ SEM. *P<0.05, **P<0.01, ***P<0.001

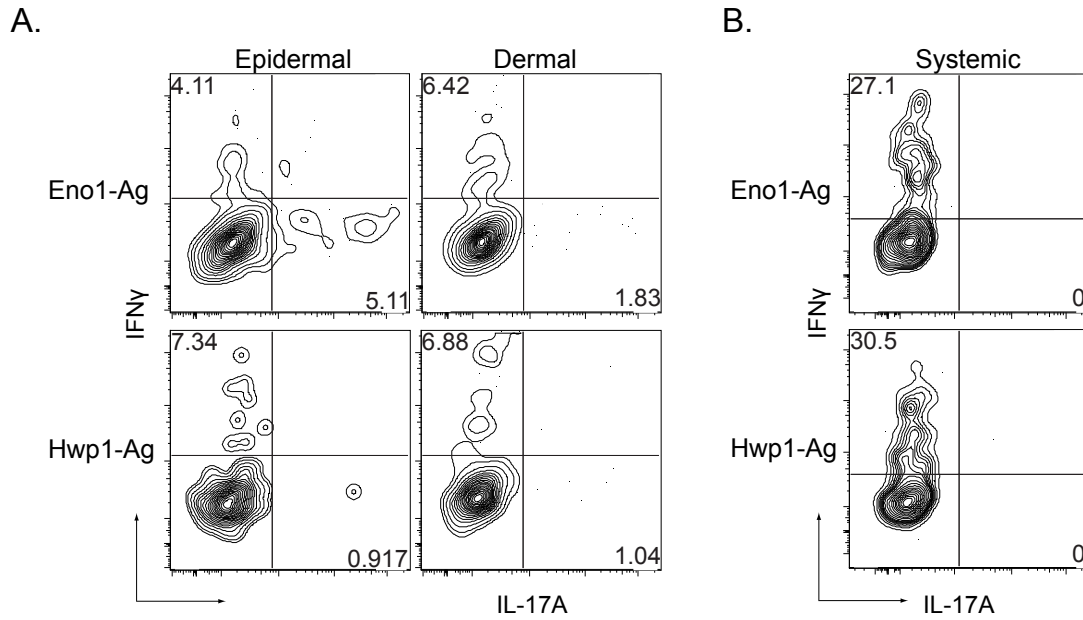


Figure 3.13: Th17 cells are generated only after epicutaneous infection with *Eno1*-Ag.

(A) Mice adoptively transferred with TE α cells were infected epicutaneously (left) or dermally (right) with either 2×10^8 Eno-1Ag (top panels) or Hwp-1Ag (bottom panels).

Four days later, T cells were isolated from lymph nodes and stimulated with PMA/Ionomycin. The expression of IL-17 and IFN- γ production by CD90.1 TE α cells is shown. (B) as in (A) except mice were i.v. infected with 10^5 Eno1-Ag or Hwp1-Ag.

Data are representative of 3 experiments.

3.2.5 Compartmentalization of T helper responses

The ability of *C. albicans* to induce Th17 cell in response to an epidermal infection and Th1 cells in response to an invasive infection raises the possibility that specific Th cell phenotypes could provide protection at distinct anatomical sites. To test this hypothesis, we epicutaneously infected WT, *Il6*^{-/-} and Batf3^{-/-} mice with *Eno1*-Ag. As expected, *Il6*^{-/-} mice generated a robust Th1 cell response in the absence of Th17 cell and Batf3^{-/-} mice developed a strong Th17 cell response in the absence of Th1 cells (**Figure 3.14A**). Separate cohorts of mice were re-challenged by skin infection 9 days after spontaneous clearance of the primary infection. The number of colony forming units (CFU) found in nave mice challenged with SC5314 was similar in all 3 groups (open symbol) (**Figure 3.14B**). Notably, though IL-6 is an innate cytokine important for resistance to systemic *C. albicans* infection, naive *Il6*^{-/-} mice did not develop an exaggerated cutaneous infection[192]. Secondarily infected (closed symbols) WT mice had an approximate 10 fold increase in fungal resistance. In contrast, Batf3^{-/-} mice had approximately 100 fold increase in pathogen resistance and *Il6*^{-/-} mice showed no increased resistance to re-infection compared to nave mice (**Figure 3.14B**).

To test whether this was a CD4⁺ T cell dependent process, we purified CD4⁺ T cells from *Il6*^{-/-} and Batf3^{-/-} mice infected 7 days earlier and adoptively transferred them into nave hosts that were then skin infected. Nave recipients of CD4⁺ T cells from Batf3^{-/-} but not *Il6*^{-/-} mice were protected against a cutaneous infection. Thus, the Th17 cells that arise in response to a cutaneous *C. albicans* infection provide protection against subsequent skin challenges (**Figure 3.14C**). In contrast, systemic challenge of previously skin infected mice by i.v. inoculation revealed protection in WT and *Il6*^{-/-} mice but not in Batf3^{-/-} mice (**Figure 3.14D**). Similarly, adoptive transfer of CD4⁺ T cells from skin infected *Il6*^{-/-} mice but not Batf3^{-/-} mice into nave recipients conferred protection against systemic candidiasis (**Figure 3.14E**). Thus, mice with expanded numbers of Th17 cell effectors were more resistant to cutaneous *C. albicans* challenge while mice with expanded numbers of Th1 cells were more resistant to systemic challenge. We conclude that Th cell effector cells appear to be functionally compartmentalized with Th17 cells providing skin protection and Th1 cells providing systemic protection.

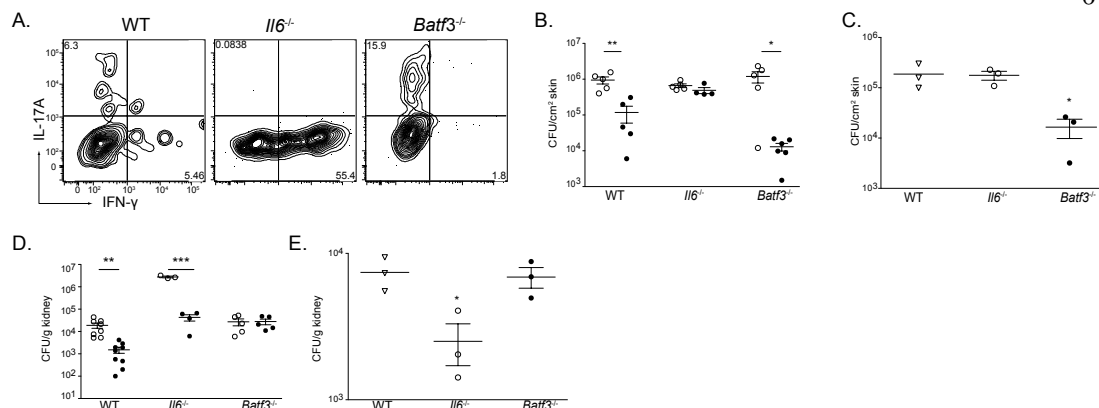


Figure 3.14: Th1 and Th17 cells provide compartmentalized protection to *C. albicans*.

WT, *Il6*^{-/-} and *Batf3*^{-/-} mice were epicutaneously infected with *Eno1*-Ag. (A) The expression of IL-17A and IFN- γ by PMA and Ionomycin stimulated CD4⁺ TE α cells isolated from skin draining lymph nodes 4 days after infection is shown. (B) The indicated strains of mice were mock infected (white) or skin infected with SC5314. Mice were re-challenged in the skin with SC5314 9 days after spontaneous clearance of the initial infection. Fungal skin burdens of individual mice is shown as CFU/cm².

(C) CD4⁺ T cells were purified from skin draining LN and spleen of nave WT, SC5314 infected *Il6*^{-/-} or SC5314 infected *Batf3*^{-/-} mice 7 days after infection and adoptively transferred into nave WT recipients. Recipients were skin infected with SC5314 1 day after transfer and CFU was assessed at the infected area 2 days later.

(D) As in (B) except that mice were challenged by i.v. infection with 10⁵ SC5314. (E) As in (C) except that mice were challenged by i.v. infection with 10⁵ SC5314. Fungal burdens in the kidneys expressed as CFU/g is shown. Scales

represents mean $\pm$ SEM. *P<0.05, **P<0.01, ***P<0.001.

3.3 Discussion

Herein we have demonstrated that the differentiation of Th17 cells in response to *C. albicans* skin infection occurs during infection with *C. albicans* yeast but not pseudohyphae. Th17 cell induction required the engagement of Dectin-1 and subsequent expression of IL-6 by Langerhans cells. CD11b+ dDC expressed Dectin-1 but were neither necessary nor sufficient for Th17 cell responses. The inability of *C. albicans* in the dermis to induce Th17 cells resulted from a morphologic change from yeast to pseudohyphae that occurred during invasion and could be rescued by the addition of exogenous Dectin-1 ligand. Finally, we found that Th17 cell expansion provided protection against cutaneous but not systemic challenge while the Th1 cell expansion provided protection against systemic but not cutaneous challenge.

The ability of *C. albicans* morphology to dictate immune responses has been an area of considerable controversy. Our data using mutants that are locked into yeast (*cph1/cph1*) or pseudohyphae (*tup1/tup1*) as well as a recombinant *C. albicans* strain in which model antigens are expressed exclusively by pseudohyphae (*Hwp1*-Ag) demonstrate that, in vivo, *C. albicans* yeast but not pseudohyphae induce Th17 cell differentiation. This is consistent with the finding that the ligand for Dectin-1 is largely accessible in the scars or patches of budding yeasts[39, 40]. Using in vitro methods, others have found that *C. albicans* pseudohyphae can induce Dectin-1 mediated Th17 cell differentiation through an IL-1 β -dependent mechanism as well as induce Th2 or Tr1 cell responses[178, 180]. These data can be reconciled by the differing availability in vitro vs. in vivo of Dectin-1 ligands on morphologic forms of *C. albicans*[38].

Differentiation of Th17 requires TGF- β as well as IL-1 β and/or IL-6 depending on the site of immunization[182]. Using total cytokine deficient mice, we found that Th17 cell development during skin infection is dependent on both IL-1 β and IL-6. By generating mice with LC selective defects of TGF- β 1, IL-1 β , and IL-6 we found that LC were a non-redundant source of only IL-6. Though LC-derived IL-1 β and TGF- β were not required, there are many other cellular sources of these cytokines that could be either be the primary source during infection or provide functionally sufficient quantities in the absence of LC. Notably, specific ablation of MyD88 in LC resulted in reduced expression of IL-6 and defective Th17 differentiation during *C. albicans* infection suggesting that IL-1 β could potentiate IL-6 expression by LC[47]. It is important to note that Dectin-1 also cooperates with toll-like receptor-2 (TLR-2) and TLR-4 to mount pro-inflammatory responses[43, 42]. In addition to any possible role for IL-1 β , we found that engagement

of Dectin-1 on LC was required for optimal IL-6 expression. Infection with a Dectin-1 independent strain induced only low expression of IL-6 by LC that failed to induce Th17 cell differentiation. Addition of exogenous IL-6 was able to rescue the Th17 cell phenotype. Similarly, patients with Dectin-1 deficiency suffer from CMC and have defective IL-6 production in response to *C. albicans*[31]. Thus, we have defined a model in which interaction of LC with *C. albicans* yeast results in a Dectin-1 dependent expression of IL-6 that is required for Th17 cell differentiation during skin infection.

In the dermis, Dectin-1 is expressed by CD11b+ dDC but not CD103+ dDC. The absence of Dectin-1 on CD103+ dDC is consistent with our prior observations that these cells were required for induction of Th1 cell differentiation. Despite expressing Dectin-1, CD11b+ dDC were neither necessary nor sufficient for optimal induction of Th17 cell responses to either an epicutaneous or dermal infection with *C. albicans*. Importantly, during a dermal infection or an epicutaneous infection with *Hwp1*-Ag, situations in which antigen bypasses LC in the epidermis, efficient Th17 cell differentiation was restored by addition of the exogenous Dectin-1 ligand, curdlan. CD11b+ dDC have been reported to drive Th2 responses to papain immunization and *nippostrongylus* infection[144]. Our data suggests that in addition to Th2 cells, these cells may also have the capacity to induce Th17 cell differentiation given the appropriate adjuvant. Indeed, similar IRF4+CD11b+ DCs can produce Th17 cell responses in other tissues[120, 119]. Thus, the inability of CD11b+ dDC to induce Th17 cells is not intrinsic to CD11b+ dDC but results from the morphologic change of *C. albicans* into pseudohyphae as it invades into the dermis and likely represents an adaption of *C. albicans* to evade immune responses.

Humans are chronically exposed to *C. albicans* and circulating anti-Candida CD4+ Th1 and Th17 cells have been identified[113]. The importance of adaptive responses to *C. albicans* has been demonstrated by the high prevalence of mucocutaneous candidiasis in patients with Th17 cell defects (e.g. hyper IgE syndrome) and some immunodeficiencies (e.g. HIV)[126]. Our results as well as the work of others has found that adaptive responses to *C. albicans* infection in mice are also dominated by Th1 and Th17 cells and provide protection from secondary infection thus mimicking the response seen in humans[113, 23, 140, 155]. We found that *Batf3*^{-/-} mice lacking CD103+ dDC and *Il6*^{-/-} mice developed either greatly exaggerated or absent Th17 cell development, respectively, without any alteration in skin innate responses to *C. albicans*. Secondary skin infection of mice with exaggerated Th17 cell expansion resulted in significant increase in protection compared with mice lacking Th17 cells that showed no evidence of protection.

Mice receiving exogenous *C. albicans* primed Th17 but not Th1 cells also were protected from skin infection. Thus, consistent with the well-documented role of Th17 cells in protection from CMC, Th17 cell expansion provided protection from secondary skin infection[126]. In addition, the ability of *C. albicans* yeast to drive Th17 cell responses and provide protection argues against the concept that the failure of yeast to generate Th17 cell is a mechanism that maintains *C. albicans* commensalism[193, 194].

Notably, we found that Th17 cell expansion failed to provide protection from systemic infection. Rather, enhanced protection from systemic challenge occurred in *Il6*^{-/-} mice that developed exaggerated Th1 cells as well as nave recipients of primed CD4⁺ T cells isolated from infected *Il6*^{-/-} mice. Notably, this protection developed despite an enhanced susceptible to primary disseminated *C. albicans* that occurs in *Il6*^{-/-} mice[192]. It was also independent of the phenomena of reprogrammed monocytes that can provide protection from systemic infection through an IL-6 dependent mechanism[195]. Notably, patients with mutations in STAT1 that reduce signaling of IFN- γ develop increased rates of systemic infection with *C. albicans* and other fungi[23]. We speculate that IFN- γ or granulocyte-macrophage colony stimulating factor (GM-CSF) derived from Th1 cell effectors may enhance the anti-candida responses by promoting increased NK and/or macrophage activity[6]. In summary, these data reveal a role for *C. albicans* morphology and interaction with a single DC subset for the generation of Th17 cell responses. In addition, the tissue selective protection afforded by Th17 and Th1 cell effectors reveals a compartmentalization of Th function that suggests a rational basis for the development of Th phenotype-specific *C. albicans* vaccines.

3.4 Methods

3.4.1 Mice

Mgl2^{eGFP-DTR/wt} (referred to as Mgl2-DTR), human Langerin-DTA (LC-), human Langerin-DTAxBatf3^{-/-} (referred to as LC-xBatf3^{-/-}), huLangerin-DTR, muLangerin-eGFP, human Langerin^{creERT2}, YFP, *Tgfb1*^{fl/fl}, *Tgfb2*^{fl/fl}, *Clec7a*^{-/-} mice have been previously described [144, 142, 94, 190, 36]. *Il1b*^{-/-} and *Il6*^{-/-} mice were purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/6 and B6-Ly5.2/Cr were purchased from the National Cancer Institute or Charles River laboratories (Frederick, MD). CD90.1 Rag1^{-/-} CD4⁺ TE α TCR-transgenic mice that recognize E α 50-66 in I-Ab on C57BL/6 backgrounds were also used [196]. All experiments were performed with 6- to 12-week-old and sex-matched mice. Mice were housed in microisolator cages and fed irradiated food and acidified water. The University of Minnesota institutional care and use committee approved all mouse protocols.

3.4.2 Generation and testing of bone marrow chimeric mice

Six weeks old cytokine deficient, wild type C57BL/6 or Ly5.2 congenically marked mice were irradiated using X-ray irradiator as previously described. The mice received two split dose at 500 cGy each. The following day, 5x10⁶ bone marrow cells isolated from specified mice were injected intravenously. Mice were rested for at least 6 weeks prior to experiments. The efficiency of chimerism was determined flow cytometry of congenic markers on PBMC, lymph node and epidermis.

3.4.3 DC Depletion with diphtheria toxin

Mgl2-DTRmice were i.p. injected with 1 μ g of diphtheria toxin (List Biological Laboratories, Campbell, CA) 2 days before infection, as previously described [95, 13].

3.4.4 Tamoxifen treatment

Tamoxifen (Sigma Aldrich) was dissolved in corn oil (Sigma Aldrich) and 10% ethanol and was administered 5 consecutive days by i.p. injection at 0.05 mg/g of mouse weight.

3.4.5 Antibodies

Fluorochrome-conjugated antibodies to CD4 (GK1.5), CD11b (M1/70), CD11c (N418), CD45.1(A20), CD45.2 (104), CD90.1 (OX-7), CD103 (2E7), I-A/I-E (M5/114.15.2),

Dectin-1 (RH1), IFN- γ (XMG1.2), IL-10 (JES5-16E3) and IL-17A (TC11-18H10.1) were purchased from BioLegend (San Diego, CA). Anti-mouse Langerin (L31), IL-17F (18F10), IL-22 (1H8PWSR), Foxp3 (FJK-16S) were acquired from eBioscience (San Diego, CA).

3.4.6 Adoptive T cell transfer

T cells were adoptively transferred as previously described [13]. Briefly, skin-draining lymph nodes, spleen and mesenteric lymph node of TE α mice were disrupted through a cell strainer and washed with sterile HBSS and labeled with CFSE (Invitrogen, Carlsbad, CA) in accordance with the manufacturer instructions. The cells after 2 washes were resuspended in sterile PBS at a concentration of 1×10^6 cell/ml and 300 μ l (3×10^5 cells) were injected intravenously into recipients. In certain experiments, single cell suspension from secondary lymphoid organs from either nave wildtype or skin infected IL-6 or Batf3 deficient mice were obtained. CD4+ T cells were magnetically isolated at >95% purity (STEMCELL Technologies, British Columbia, Canada) according to manufacturers directions. A total of 5×10^6 million purified CD4+ T cells were transferred into nave hosts in 300 μ l PBS.

3.4.7 Flow cytometry

Single-cell suspensions of epidermal cells were obtained as previously defined [94]. Briefly skin from ear or trunk were obtained and incubated for 2 hours at 37°C in 0.3% trypsin (Sigma-Aldridge, St. Louis, MO) in 150 mM NaCl, 0.5 mM KCl, 0.5 mM glucose. The epidermis was physically separated from the dermis. Dermal cells were obtained from whole flank skin. Samples were minced and incubated for 2 hr at 37°C in collagenase XI (4830 U/ml, Sigma), hyaluronidase (260 U/ml, Sigma), DNase (0.1 mg/ml, ICN), 10 mM HEPES (Sigma) in RPMI (Invitrogen). The resulting cells were filtered through a 40 μ m filter. Lymph node (axillary, brachial, and inguinal) and spleen cells were incubated in 400 and 150 U/ml Collagenase D for preparation of dendritic cells or smashed through a filter for lymphocytes (Roche Applied Science, Indianapolis, IN), respectively, for 90 min prior to erythroid cell lysis in ACK buffer (Biowhittaker, Walkersville, MD). Single-cell suspensions were pretreated for 10 min at 4°C with 2.4G2 except when anti-Rat secondaries were used, in which case cells were blocked with mouse Ig (Sigma). For evaluating cytokine expression, cells were incubated for 5 hours in complete IDMEM supplemented with PMA (50 ng/ml) and Ionomycin (1.5 μ M; Sigma-Aldrich, St. Louis,

MO), with GolgiStop (BD PharMingen, San Jose, CA) added for the final 4 hr. The intracellular cytokine staining was performed with BD Bioscience Cytofix/Cytoperm kit (BD Biosciences, San Jose, CA) in accordance with the manufacturer instructions. Samples were analyzed on LSR-II flow cytometers (BD Biosciences). Data were analyzed with FlowJo software (TreeStar, Ashland, OR). Detection of 2W1S specific CD4+ T cells was performed as published with an I-Ab-2W1S tetramer (a kind gift of M. Jenkins) after ex vivo stimulation of secondary organs as previously described [197].

3.4.8 DC sorting by flow cytometry and qPCR

Single cell suspension of LN cells were enriched by CD11c MACS positive selection (Miltenyi Biotec, Auburn, CA). LCs were sorted using a FACSaria cell sorter based as GFP+, MHCII+, CD11c+, CD11b+, CD103-CD8-. RNA was isolated using an RNeasy Mini Kit (Qiagen, Valencia, CA) and quantified from Nanodrop readings (Nanodrop, Wilmington, DE). cDNA was generated using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). TaqMan Gene Expression Master Mix, TaqMan Gene Expression Assays for IL-1, IL-6 and IL-12p40 was used. ABI Prism 7900HT(Applied Biosystems) were used to complete the qPCR. All kits were completed according the manufacturer instructions. All Ct values were normalized to HPRT expression and are shown as $2^{-\Delta Ct}$ to nave.

3.4.9 *Candida albicans* strains

Recombinant *C. albicans* used in this study was derived from SC5314 and was designed as previously described [13]. They were grown at in rich medium at 30°C (YPAD), or synthetic complete medium. Escherichia coli strain XLI-blue (Stratagene, La Jolla, CA, USA), growth conditions, DNA manipulations and primer design and synthesis were essentially as described previously. Transformants were selected on YPAD medium containing 400 μ g/ml nourseothricin and were screened by PCR using oligonucleotide primers listed (Figure S2C). Briefly, strain *Hwp1*-eGFP-Ag was constructed as follows: plasmid pMG2268 was PCR with Primers 875 and 5266. PCR product was transformed into wild-type strain SC5314 using standard methods[13]. Transformants were selected on YPAD medium containing 400 μ g/ml nourseothricin. Integration at the *Hwp1* locus was confirmed using 2 primers (877/3290) confirming integration of GFP-antigen at *Hwp1*. *C. albicans* strains s20175.016, yeast locked strain (JKC19) and filament locked strain (BCa2-9) were kindly provided by Dr. Neal Gow (University of Aberdeen) [38].

Growth of fungi occurred after inoculation of a colony at 30°C in YPAD overnight and, the next day, diluted 1:10 and cultured in either 30°C in YPAD until OD600 reached 1.5 for yeast morphology or at 37°C in RPMI with 10% FBS for 3 hours for filamentous forms.

Table 3.1: Primers for the generation of *Hwp1*-Ag

Primer Number	Primer Sequence
877	GACTACATTTTGTTTCACTTTTTG
3290	CTCGAGATATCGATCCTAGCGTAGCAACT TGTTTTCTCAACAATATCAAACACAACA GGAATCTC CTATAGTCACTCGCT
875	TTTAGTTTCGTCAATATGTCTAAAaggTgAAg AATTATTCACCTTGACCGTCTACCTGTG GGACAG
5266	TGGCCCCAATTGATAACATGTAATAAGCGA TAGCAATAAGTTGAGTAAAACGACGGCC AGTGAATTC

3.4.10 Infection models

The skin infection was performed as described [13]. Briefly, Mice were first anesthetized with a mixture of ketamine and xylazine (100/10 mg/kg body weight), shaved on the back with electric clipper, and chemically depilated with Nair hair remover (Church & Dwight, Princeton, NJ) per the manufacturer instructions. The stratum corneum was removed with 15 strokes with 220 grit sandpaper (3M, St Paul, MN). After washing with sterile PBS, 2×10^8 *C. albicans* in 50 μ l of sterile PBS was applied on to the skin. In some experiments, same dose of *C. albicans* or 50ug curdlan was injected intradermally spaced over 10 sites on the back. Where indicated, recombinant murine IL-6 (BioLegend) was injected into 4 different sites in the dorsum of the mice intradermally at a total dose of 1 μ g per mice. IV infections with *Eno1*-Ag and *Hwp1*-Ag for assessment of T cell expansion were performed at 3×10^5 CFU in PBS. In re-challenge experiments, previously mock or epicutaneously infected mice were challenged with 1×10^6 CFU *C. albicans* by intradermal injected into a single site or by 1×10^5 CFU intravenously. Skin and kidneys were harvest from mice 3 days later and homogenized and serially diluted onto YPAD plates and incubated at 30°C for 24-48 hours to assess the number of colony forming units.

3.4.11 LC Targeting with anti-huLangerin antibodies

Anti human-Langerin antibodies (2G3) conjugated to antigen were generated and administered as previously described[13]. Briefly, WT and huLangerin-DTR mice with were immunized by intraperitoneal injection of 1.0 μ g of 2G3-E α in 100 μ l of sterile PBS. Six hours later, the mice were infected with the noted pathogen strains as above.

3.4.12 Histology

Immunofluorescence on ear epidermis was performed as previously described [94]. Images were captured with a microscope (DM5500; Leica) with digital system and LAS AF software (version 1.5.1). Periodic Acid-Schiff (PAS) staining for fungal invasion was performed 2 days after infection with SC5314 on formaldehyde fixed frozen sections with commercial PAS staining kit (Sigma-Aldrich).

3.5 Publication and contributions

This chapter is modified from the published article:

Sakeen W Kashem, Botond Z Igyarto, Maryam Gerami-Nejad, Yosuke Kumamoto, Javed Mohammed, Elizabeth Jarrett, Rebecca A Drummond, Sandra M Zurawski, Gerard Zurawski, Judith Berman, Akiko Iwasaki, Gordon D Brown, and Daniel H Kaplan. *Candida albicans* Morphology and Dendritic Cell Subsets Determine T Helper Cell Differentiation. *Immunity*, 42(2):356-366, February 2015.

Author contributions are as follows: SWK and BZI contributed equally. SWK performed most and analyzed all experiments. BZI performed experiments relating to **(Figure 3.4)** and **(Figure 3.5A-G)**. MGN, YK, JM, EJ, RAD, SMZ, GZ, JB, AI, GDB assisted with creation of reagents or experiments. SWK and DHK designed all experiments and wrote the manuscript with the help of BZI. All authors were involved in editing the original published manuscript.

Chapter 4

Conclusion

In my thesis research, I aimed to investigate the mechanisms by which innate and adaptive immunity is generated in response to *Candida albicans* infection. In the context of a primary *C. albicans* infection, innate resistance to *C. albicans* in mucosal tissues requires production of IL-17A by tissue resident cells early during infection. In the skin, we found that dermal $\gamma\delta$ T cells were the dominant source of IL-17A during *C. albicans* infection and were required for pathogen resistance. Induction of IL-17A from dermal $\gamma\delta$ T cells and resistance to *C. albicans* required IL-23 from CD301b+ dermal dendritic cell (dDC) and was independent of CD103+ dDC and Langerhans cells. In addition, we found that sensory neurons are directly activated by *C. albicans*. Chemical ablation of transient receptor potential cation channel subfamily V member 1 (TRPV1+) neurons and cutaneous denervation resulted in reduced expression of IL-23 by CD301b+ dDC and increased susceptibility to *C. albicans* infection that could be rescued by exogenous calcitonin gene related peptide (CGRP). These data define a model in which nociceptive pathways in the skin drive production of IL-23 by CD301b+ dDC resulting in IL-17A from $\gamma\delta$ T cells and resistance to cutaneous candidiasis.

These findings suggest that sensory neurons are able to sense pathogens directly. Innate immune cells can directly detect infection through pattern recognition receptors that include Toll-like receptors, RIG- like receptors Nod-like receptors, C-type lectin receptors, formylated peptide receptors (FPRs) and scavenger receptors[198, 79]. Nociceptive neurons can express FPRs, several TLRs including TLRs 3, 4, 7, and 9, as well as have an active inflammasome to stimuli[199]. For example, imiquimod can directly activate wildtype, but not TLR7^{-/-} sensory neurons[200]. Ablation of TRPV1+ nociceptors in mice makes the host unable to mount a proper response to imiquimod induced IL-23/IL-17 mediated inflammation in the skin[76]. Nociceptive neurons can also be activated by N-formylated peptides from *Staphylococcus aureus* through FPRs as well as via pore formation and ionic changes induced by secreted bacterial hemolysins [79]. Notably, commensal microbes have been also implicated in mediating pain hypersensitivity, as germ free mice have delayed pain responsiveness [201]. While we find that *C. albicans* and zymosan can directly active DRG harvested sensory neurons, it is unclear the mechanism by which this activation happens. In addition, whether *C. albicans* can activate pattern recognition receptor signaling to sufficiently induce CGRP release or induce singlas to the central nervous system will be an important area of investigation going forward. Finally, the role of other neuropeptides, such as substance P, must be considered in the context of *C. albicans* skin infection.

Our work on innate immune resistance also demonstrate a subset specific effector

specialization of antigen presenting cells in tissue. CD301b+ dDCs account for the majority of the dermal DCs. While the role of distinct DC subsets has been appreciated in the context of adaptive immunity against skin infections, little is known about effector functions of specific DC subsets. In the model of imiquimod induced IL-23/IL-17 inflammation, Langerhans cells had been implicated in both initiating and suppressing inflammation[91, 202]. In the context of heat-killed *C. albicans* injection, Langerhans cells suppress CD49a+ liver NK cells to suppress footpad swelling [93]. Therefore, the current findings on the role of skin myeloid cells in innate immunity is relative to the context and the timing of the model and needs further evaluation. A simplified approach going forward may be to analyze skin DC subsets and their unique transcriptional profile to clue functional specificity. For example, CD301b+ dermal DCs are characterized by their unique expression of interferon regulatory factor 4 (IRF4)[203, 144]. The transcriptional factor IRF4 in BMDC binds to promoters of the genes for IL-12p40, IL-23p19 and CXCL16, all of which act of dermal IL-17 secreting $\gamma\delta$ T cells (unpublished work). Thus, careful analyses of myeloid subsets and their transcriptional profile changes during infection will be critical for understanding their function in host defense.

In the context of adaptive immunity, mucocutaneous immunity to *C. albicans* requires Th17 differentiation that is thought to depend on recognition of filamentous *C. albicans* while systemic resistance is considered T cell independent. Using a murine skin infection model, we compared in vivo T helper responses to yeast and filamentous *C. albicans*. Unexpectedly, we found that only yeast induced Th17 responses through a mechanism that required Dectin-1 mediated expression of IL-6 by Langerhans cells. Filamentous forms induced Th1 without Th17 due to the absence of Dectin-1 ligation. Notably, Th17 responses provided selective protection against secondary cutaneous infection while Th1 provided protection against systemic infection. Thus, *C. albicans* morphology drives distinct Th responses that provide tissue specific protection. It will be important to explore whether distinct morphologies of *C. albicans* activate sensory neurons and trigger innate immune pathways. In addition, the role of IL-23 in initiating and maintaining adaptive immunity still needs to be explored.

These findings provide novel insight into compartmentalization of Th responses, *C. albicans* pathogenesis and have critical implications for vaccine strategies. Current vaccination strategies being explored for *C. albicans* include immunizing for the immunodominant epitope of *C. albicans*, pALS3[204, 205]. The mouse models of such vaccination strategies have been promising, but the result from human trials is currently

unknown. Interestingly, these vaccination strategies provide cross-protection to *S. aureus* infections even though the bacterial pathogen does not express a cross-reactive epitope. Consideration for how *C. albicans* vaccination strategies impart memory T cell response in the skin compared to the systemic organs and how vaccines affect the commensal composition of mucosal surfaces post-vaccination must be given for immunization approaches.

Conversely, biologic therapy of IL-17 is currently being evaluated for the treatment of psoriasis and psoriatic arthritis[206, 207, 208, 209, 210]. Initial results from 12-week trials demonstrate that patients treated with anti-IL-17RA blockade, but not with anti-IL-17A monoclonal antibodies, are at a significantly higher risk for developing mucocutaneous candidiasis. However, the number of patients who experience barrier *C. albicans* infections with anti-IL-17RA therapy is still extremely small. It is unknown why certain biologics trigger *C. albicans* infections and only certain patients experience *C. albicans* infections. Detailed examination of patients treated with anti-IL-23/IL-17 biologics in regards to their therapeutic efficacy and occurrence of candidiasis will reveal important clues about the distinctions of immunity and inflammation and highlight the potential avenues of infection-free anti-inflammatory therapy.

In conclusion, *C. albicans* infection skin and mucosal infections have been a fruitful model of investigation of how IL-17 responses are developed. Findings addressed in this this dissertation provide a mechanistic insight into skin immunity that may have implications for both vaccination strategies and treatment autoimmunity.

References

- [1] Joshua Perlroth, Bryan Choi, and Brad Spellberg. Nosocomial fungal infections: epidemiology, diagnosis, and treatment. *Medical mycology*, 45(4):321–346, June 2007.
- [2] David M Underhill and Eric Pearlman. Immune Interactions with Pathogenic and Commensal Fungi: A Two-Way Street. *Immunity*, 43(5):845–858, November 2015.
- [3] Joshua D Milner and Steven M Holland. The cup runneth over: lessons from the ever-expanding pool of primary immunodeficiency diseases. *Nature reviews. Immunology*, 13(9):635–648, September 2013.
- [4] Sanne P Smeekens, Aylwin Ng, Vinod Kumar, Melissa D Johnson, Theo S Plantinga, Cleo van Diemen, Peer Arts, Eugène T P Verwiel, Mark S Gresnigt, Karin Fransen, Suzanne van Sommeren, Marije Oosting, Shih-Chin Cheng, Leo A B Joosten, Alexander Hoischen, Bart-Jan Kullberg, William K Scott, John R Perfect, Jos W M van der Meer, Cisca Wijmenga, Mihai G Netea, and Ramnik J Xavier. Functional genomics identifies type I interferon pathway as central for host defense against *Candida albicans*. *Nature communications*, 4:1342, 2013.
- [5] Sakeen W Kashem, Botond Z Igyártó, Maryam Gerami-Nejad, Yosuke Kumamoto, Javed Mohammed, Elizabeth Jarrett, Rebecca A Drummond, Sandra M Zurawski, Gerard Zurawski, Judith Berman, Akiko Iwasaki, Gordon D Brown, and Daniel H Kaplan. *Candida albicans* Morphology and Dendritic Cell Subsets Determine T Helper Cell Differentiation. *Immunity*, 42(2):356–366, February 2015.

- [6] Eva Bar, Paul G Whitney, Kathrin Moor, Caetano Reis e Sousa, and Salome LeibundGut-Landmann. IL-17 regulates systemic fungal immunity by controlling the functional competence of NK cells. *Immunity*, 40(1):117–127, January 2014.
- [7] Salome LeibundGut-Landmann, Olaf Gross, Matthew J Robinson, Fabiola Osorio, Emma C Slack, S Vicky Tsoni, Edina Schweighoffer, Victor Tybulewicz, Gordon D Brown, Jurgen Ruland, and Caetano Reis e Sousa. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nature immunology*, 8(6):630–638, June 2007.
- [8] Carol A Kumamoto. Inflammation and gastrointestinal Candida colonization. *Current opinion in microbiology*, 14(4):386–391, August 2011.
- [9] A Cassone. Vulvovaginal Candida albicans infections: pathogenesis, immunity and vaccine prospects. *BJOG : an international journal of obstetrics and gynaecology*, 122(6):785–794, May 2015.
- [10] Heather R Conti and Sarah L Gaffen. IL-17-Mediated Immunity to the Opportunistic Fungal Pathogen Candida albicans. *Journal of immunology (Baltimore, Md. : 1950)*, 195(3):780–788, August 2015.
- [11] Frank L van de Veerdonk, Bart-Jan Kullberg, and Mihai G Netea. Pathogenesis of invasive candidiasis. *Current opinion in critical care*, 16(5):453–459, October 2010.
- [12] Iliyan D Iliev, Vincent A Funari, Kent D Taylor, Quoclinh Nguyen, Christopher N Reyes, Samuel P Strom, Jordan Brown, Courtney A Becker, Phillip R Fleshner, Marla Dubinsky, Jerome I Rotter, Hanlin L Wang, Dermot P B McGovern, Gordon D Brown, and David M Underhill. Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. *Science (New York, N.Y.)*, 336(6086):1314–1317, June 2012.
- [13] Botond Z Igyártó, Krystal Haley, Daniela Ortner, Aleh Bobr, Maryam Gerami-Nejad, Brian T Edelson, Sandra M Zurawski, Bernard Malissen, Gerard Zurawski, Judith Berman, and Daniel H Kaplan. Skin-resident murine dendritic cell subsets promote distinct and opposing antigen-specific T helper cell responses. *Immunity*, 35(2):260–272, August 2011.

- [14] Shinji Kagami, Heather L Rizzo, Stephen E Kurtz, Lloyd S Miller, and Andrew Blauvelt. IL-23 and IL-17A, but not IL-12 and IL-22, are required for optimal skin host defense against *Candida albicans*. *Journal of immunology (Baltimore, Md. : 1950)*, 185(9):5453–5462, November 2010.
- [15] Sakeen W Kashem, Maureen S Riedl, Chen Yao, Christopher N Honda, Lucy Vulchanova, and Daniel H Kaplan. Nociceptive Sensory Fibers Drive Interleukin-23 Production from CD301b+ Dermal Dendritic Cells and Drive Protective Cutaneous Immunity. *Immunity*, 43(3):515–526, September 2015.
- [16] Fanny Lanternier, Sophie Cypowyj, Capucine Picard, Jacinta Bustamante, Olivier Lortholary, Jean-Laurent Casanova, and Anne Puel. Primary immunodeficiencies underlying fungal infections. *Current opinion in pediatrics*, 25(6):736–747, December 2013.
- [17] Dina S Ahmad, Mohammad Esmadi, and William C Steinmann. Idiopathic CD4 Lymphocytopenia: Spectrum of opportunistic infections, malignancies, and autoimmune diseases. *Avicenna journal of medicine*, 3(2):37–47, April 2013.
- [18] P Manchado Lopez, J M Ruiz de Morales, I Ruiz González, and M A Rodriguez Prieto. Cutaneous infections by papillomavirus, herpes zoster and *Candida albicans* as the only manifestation of idiopathic CD4+ T lymphocytopenia. *International journal of dermatology*, 38(2):119–121, February 1999.
- [19] Steven M Holland, Frank R DeLeo, Houda Z Elloumi, Amy P Hsu, Gulbu Uzel, Nina Brodsky, Alexandra F Freeman, Andrew Demidowich, Joie Davis, Maria L Turner, Victoria L Anderson, Dirk N Darnell, Pamela A Welch, Douglas B Kuhns, David M Frucht, Harry L Malech, John I Gallin, Scott D Kobayashi, Adeline R Whitney, Jovanka M Voyich, James M Musser, Cristina Woellner, Alejandro A Schäffer, Jennifer M Puck, and Bodo Grimbacher. STAT3 mutations in the hyper-IgE syndrome. *The New England journal of medicine*, 357(16):1608–1619, October 2007.
- [20] Joshua D Milner, Jason M Brenchley, Arian Laurence, Alexandra F Freeman, Brenna J Hill, Kevin M Elias, Yuka Kanno, Christine Spalding, Houda Z Elloumi, Michelle L Paulson, Joie Davis, Amy Hsu, Ava I Asher, John O’Shea, Steven M Holland, William E Paul, and Daniel C Douek. Impaired T(H)17 cell

differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature*, 452(7188):773–776, April 2008.

- [21] Qian Zhang, Jeremiah C Davis, Ian T Lamborn, Alexandra F Freeman, Huie Jing, Amanda J Favreau, Helen F Matthews, Joie Davis, Maria L Turner, Gulbu Uzel, Steven M Holland, and Helen C Su. Combined immunodeficiency associated with DOCK8 mutations. *The New England journal of medicine*, 361(21):2046–2055, November 2009.
- [22] Capucine Picard, Jean-Laurent Casanova, and Anne Puel. Infectious diseases in patients with IRAK-4, MyD88, NEMO, or $\text{I}\kappa\text{B}\alpha$ deficiency. *Clinical microbiology reviews*, 24(3):490–497, July 2011.
- [23] Anne Puel, Sophie Cypowyj, Jacinta Bustamante, Jill F Wright, Luyan Liu, Hye Kyung Lim, Mélanie Migaud, Laura Israel, Maya Chrabieh, Magali Audry, Matthew Gumbleton, Antoine Toulon, Christine Bodemer, Jamila El-Baghdadi, Matthew Whitters, Theresa Paradis, Jonathan Brooks, Mary Collins, Neil M Wolfman, Saleh Al-Muhsen, Miguel Galicchio, Laurent Abel, Capucine Picard, and Jean-Laurent Casanova. Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. *Science (New York, N.Y.)*, 332(6025):65–68, April 2011.
- [24] Yun Ling, Sophie Cypowyj, Caner Aytakin, Miguel Galicchio, Yildiz Camcioglu, Serdar Nepesov, Aydan Ikinciogullari, Figen Dogu, Aziz Belkadi, Romain Levy, Mélanie Migaud, Bertrand Boisson, Alexandre Bolze, Yuval Itan, Nicolas Goudin, Julien Cottineau, Capucine Picard, Laurent Abel, Jacinta Bustamante, Jean-Laurent Casanova, and Anne Puel. Inherited IL-17RC deficiency in patients with chronic mucocutaneous candidiasis. *The Journal of Experimental Medicine*, 212(5):619–631, May 2015.
- [25] Bertrand Boisson, Chenhui Wang, Vincent Pedergnana, Ling Wu, Sophie Cypowyj, Michel Rybojad, Aziz Belkadi, Capucine Picard, Laurent Abel, Claire Fieschi, Anne Puel, Xiaoxia Li, and Jean-Laurent Casanova. An ACT1 mutation selectively abolishes interleukin-17 responses in humans with chronic mucocutaneous candidiasis. *Immunity*, 39(4):676–686, October 2013.
- [26] Satoshi Okada, Janet G Markle, Elissa K Deenick, Federico Mele, Dina Averbuch, Macarena Lagos, Mohammed Alzahrani, Saleh Al-Muhsen, Rabih Halwani,

- Cindy S Ma, Natalie Wong, Claire Soudais, Lauren A Henderson, Hiyam Marzouqa, Jamal Shamma, Marcela Gonzalez, Rubén Martinez-Barricarte, Chizuru Okada, Danielle T Avery, Daniela Latorre, Caroline Deswarte, Fabienne Jabot-Hanin, Egidio Torrado, Jeffrey Fountain, Aziz Belkadi, Yuval Itan, Bertrand Boisson, Mélanie Migaud, Cecilia S Lindestam Arlehamn, Alessandro Sette, Sylvain Breton, James McCluskey, Jamie Rossjohn, Jean-Pierre de Villartay, Despina Moshous, Sophie Hambleton, Sylvain Latour, Peter D Arkwright, Capucine Picard, Olivier Lantz, Dan Engelhard, Masao Kobayashi, Laurent Abel, Andrea M Cooper, Luigi D Notarangelo, Stephanie Boisson-Dupuis, Anne Puel, Federica Sallusto, Jacinta Bustamante, Stuart G Tangye, and Jean-Laurent Casanova. IMMUNODEFICIENCIES. Impairment of immunity to *Candida* and *Mycobacterium* in humans with bi-allelic RORC mutations. *Science (New York, N.Y.)*, 349(6248):606–613, August 2015.
- [27] Kai Kisand, Anette S Bøe Wolff, Katarina Trebusak Podkrajsek, Liina Tserel, Maire Link, Kalle V Kisand, Elisabeth Ersvaer, Jaakko Perheentupa, Martina Moter Erichsen, Nina Bratanic, Antonella Meloni, Filomena Cetani, Roberto Perniola, Berrin Ergun-Longmire, Noel Maclaren, Kai J E Krohn, Mikulás Pura, Berthold Schalke, Philipp Ströbel, Maria Isabel Leite, Tadej Battelino, Eystein S Husebye, Pärt Peterson, Nick Willcox, and Anthony Meager. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. *The Journal of Experimental Medicine*, 207(2):299–308, February 2010.
- [28] Luyan Liu, Satoshi Okada, Xiao-Fei Kong, Alexandra Y Kreins, Sophie Cypowyj, Avinash Abhyankar, Julie Toubiana, Yuval Itan, Magali Audry, Patrick Nitschke, Cécile Masson, Beata Toth, Jérôme Flatot, Mélanie Migaud, Maya Chrabieh, Tatiana Kochetkov, Alexandre Bolze, Alessandro Borghesi, Antoine Toulon, Julia Hiller, Stefanie Eyerich, Kilian Eyerich, Vera Gulácsy, Ludmyla Chernyshova, Viktor Chernyshov, Anastasia Bondarenko, Rosa María Cortés Grimaldo, Lizbeth Blancas-Galicia, Ileana Maria Madrigal Beas, Joachim Roesler, Klaus Magdorf, Dan Engelhard, Caroline Thumerelle, Pierre-Régis Burgel, Miriam Hoernes, Barbara Drexel, Reinhard Seger, Theresia Kusuma, Annette F Jansson, Julie Sawalle-Belohradsky, Bernd Belohradsky, Emmanuelle Jouanguy, Jacinta Bustamante, Mélanie Bué, Nathan Karin, Gizi Wildbaum, Christine Bodemer, Olivier Lortholary, Alain Fischer, Stéphane Blanche, Saleh Al-Muhsen, Janine Reichenbach,

- Masao Kobayashi, Francisco Espinosa Rosales, Carlos Torres Lozano, Sara Sebnem Kilic, Matias Oleastro, Amos Etzioni, Claudia Traidl-Hoffmann, Ellen D Renner, Laurent Abel, Capucine Picard, László Maródi, Stephanie Boisson-Dupuis, Anne Puel, and Jean-Laurent Casanova. Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. *The Journal of Experimental Medicine*, 208(8):1635–1648, August 2011.
- [29] Frank L van de Veerdonk, Theo S Plantinga, Alexander Hoischen, Sanne P Smeekens, Leo A B Joosten, Christian Gilissen, Peer Arts, Diana C Rosentul, Andrew J Carmichael, Chantal A A Smits-van der Graaf, Bart-Jan Kullberg, Jos W M van der Meer, Desa Lilic, Joris A Veltman, and Mihai G Netea. STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. *The New England journal of medicine*, 365(1):54–61, July 2011.
- [30] Monia Ouederni, Ozden Sanal, Aydan Ikinogullari, Ilhan Tezcan, Figen Dogu, Ithaisa Sologuren, Sigifredo Pedraza-Sánchez, Melike Keser, Gonul Tanir, Chris Nieuwhof, Elena Colino, Dinakantha Kumararatne, Jacov Levy, Necil Kutukculer, Caner Aytekin, Estefanía Herrera-Ramos, Micah Bhatti, Neslihan Karaca, Ridha Barbouche, Arnon Broides, Ekaterini Goudouris, José Luis Franco, Nima Parvaneh, Ismail Reisli, Alexis Strickler, Anna Shcherbina, Ayper Somer, Anthony Segal, Alfonso Angel-Moreno, José Luis Lezana-Fernandez, Mohamed Bejaoui, Miriam Bobadilla-Del Valle, Salem Kachboura, Timothy Sentongo, Imen Ben-Mustapha, Jacinta Bustamante, Capucine Picard, Anne Puel, Stephanie Boisson-Dupuis, Laurent Abel, Jean-Laurent Casanova, and Carlos Rodríguez-Gallego. Clinical features of Candidiasis in patients with inherited interleukin 12 receptor $\beta 1$ deficiency. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 58(2):204–213, January 2014.
- [31] Bart Ferwerda, Gerben Ferwerda, Theo S Plantinga, Janet A Willment, Annemiek B van Spruiel, Hanka Venselaar, Clara C Elbers, Melissa D Johnson, Alessandra Cambi, Cristal Huysamen, Liesbeth Jacobs, Trees Jansen, Karlijn Verheijen, Laury Masthoff, Servaas A Morré, Gert Vriend, David L Williams, John R Perfect, Leo A B Joosten, Cisca Wijmenga, Jos W M van der Meer, Gosse J Adema, Bart-Jan Kullberg, Gordon D Brown, and Mihai G Netea. Human dectin-1 deficiency and mucocutaneous fungal infections. *The New England journal of medicine*, 361(18):1760–1767, October 2009.

- [32] Erik-Oliver Glocker, Andre Hennigs, Mohammad Nabavi, Alejandro A Schäffer, Cristina Woellner, Ulrich Salzer, Dietmar Pfeifer, Hendrik Veelken, Klaus Warnatz, Fariba Tahami, Sarah Jamal, Annabelle Manguiat, Nima Rezaei, Ali Akbar Amirzargar, Alessandro Plebani, Nicole Hanneschläger, Olaf Gross, Jurgen Ruland, and Bodo Grimbacher. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. *The New England journal of medicine*, 361(18):1727–1735, October 2009.
- [33] David Sancho and Caetano Reis e Sousa. Signaling by myeloid C-type lectin receptors in immunity and homeostasis. *Annual review of immunology*, 30(1):491–529, 2012.
- [34] Ivy M Dambuza and Gordon D Brown. C-type lectins in immunity: recent developments. *Current opinion in immunology*, 32:21–27, February 2015.
- [35] G D Brown and S Gordon. Immune recognition. A new receptor for beta-glucans. *Nature*, 413(6851):36–37, September 2001.
- [36] Philip R Taylor, S Vicky Tsoni, Janet A Willment, Kevin M Dennehy, Marcela Rosas, Helen Findon, Ken Haynes, Chad Steele, Marina Botto, Siamon Gordon, and Gordon D Brown. Dectin-1 is required for beta-glucan recognition and control of fungal infection. *Nature immunology*, 8(1):31–38, January 2007.
- [37] Shinobu Saijo, Noriyuki Fujikado, Takahisa Furuta, Soo-hyun Chung, Hayato Kotaki, Keisuke Seki, Katsuko Sudo, Shizuo Akira, Yoshiyuki Adachi, Naohito Ohno, Takeshi Kinjo, Kiwamu Nakamura, Kazuyoshi Kawakami, and Yoichiro Iwakura. Dectin-1 is required for host defense against *Pneumocystis carinii* but not against *Candida albicans*. *Nature immunology*, 8(1):39–46, January 2007.
- [38] Mohlopheni J Marakalala, Simon Vautier, Joanna Potrykus, Louise A Walker, Kelly M Shepardson, Alex Hopke, Hector M Mora-Montes, Ann Kerrigan, Mihai G Netea, Graeme I Murray, Donna M Maccallum, Robert Wheeler, Carol A Munro, Neil A R Gow, Robert A Cramer, Alistair J P Brown, and Gordon D Brown. Differential adaptation of *Candida albicans* in vivo modulates immune recognition by dectin-1. *PLoS pathogens*, 9(4):e1003315, 2013.
- [39] Benjamin N Gantner, Randi M Simmons, and David M Underhill. Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. *The EMBO journal*, 24(6):1277–1286, March 2005.

- [40] Neil A R Gow, Frank L van de Veerdonk, Alistair J P Brown, and Mihai G Netea. Candida albicans morphogenesis and host defence: discriminating invasion from colonization. *Nature reviews. Microbiology*, 10(2):112–122, February 2012.
- [41] Kiyoshi Takeda, Tsuneyasu Kaisho, and Shizuo Akira. Toll-like receptors. *Annual review of immunology*, 21(1):335–376, 2003.
- [42] Benjamin N Gantner, Randi M Simmons, Scott J Canavera, Shizuo Akira, and David M Underhill. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *The Journal of Experimental Medicine*, 197(9):1107–1117, May 2003.
- [43] Gerben Ferwerda, Friederike Meyer-Wentrup, Bart-Jan Kullberg, Mihai G Netea, and Gosse J Adema. Dectin-1 synergizes with TLR2 and TLR4 for cytokine production in human primary monocytes and macrophages. *Cellular microbiology*, 10(10):2058–2066, October 2008.
- [44] Mariel E Eberle and Alexander H Dalpke. Dectin-1 stimulation induces suppressor of cytokine signaling 1, thereby modulating TLR signaling and T cell responses. *Journal of immunology (Baltimore, Md. : 1950)*, 188(11):5644–5654, June 2012.
- [45] Giorgio Trinchieri and Alan Sher. Cooperation of Toll-like receptor signals in innate immune defence. *Nature reviews. Immunology*, 7(3):179–190, March 2007.
- [46] Silvia Bellocchio, Claudia Montagnoli, Silvia Bozza, Roberta Gaziano, Giordano Rossi, Salamatu S Mambula, Annunciata Vecchi, Alberto Mantovani, Stuart M Levitz, and Luigina Romani. The contribution of the Toll-like/IL-1 receptor superfamily to innate and adaptive immunity to fungal pathogens in vivo. *Journal of immunology (Baltimore, Md. : 1950)*, 172(5):3059–3069, March 2004.
- [47] Krystal Haley, Botond Z Igyártó, Daniela Ortner, Aleh Bobr, Sakeen Kashem, Dominik Schenten, and Daniel H Kaplan. Langerhans cells require MyD88-dependent signals for Candida albicans response but not for contact hypersensitivity or migration. *Journal of immunology (Baltimore, Md. : 1950)*, 188(9):4334–4339, May 2012.
- [48] Fabio Martinon, Annick Mayor, and Jürg Tschopp. The inflammasomes: guardians of the body. *Annual review of immunology*, 27(1):229–265, 2009.

- [49] Linda M Rehaume, Thierry Jouault, and Mathias Chamaillard. Lessons from the inflammasome: a molecular sentry linking Candida and Crohn's disease. *Trends in immunology*, 31(5):171–175, May 2010.
- [50] Ahinoam Lev-Sagie, Diana Prus, Iara M Linhares, Yuval Lavy, William J Ledger, and Steven S Witkin. Polymorphism in a gene coding for the inflammasome component NALP3 and recurrent vulvovaginal candidiasis in women with vulvar vestibulitis syndrome. *American journal of obstetrics and gynecology*, 200(3):303.e1–6, March 2009.
- [51] Amy G Hise, Jeffrey Tomalka, Sandhya Ganesan, Krupen Patel, Brian A Hall, Gordon D Brown, and Katherine A Fitzgerald. An essential role for the NLRP3 inflammasome in host defense against the human fungal pathogen *Candida albicans*. *Cell host & microbe*, 5(5):487–497, May 2009.
- [52] Sophie Joly, Ning Ma, Jeffrey J Sadler, David R Soll, Suzanne L Cassel, and Fayyaz S Sutterwala. Cutting edge: *Candida albicans* hyphae formation triggers activation of the Nlrp3 inflammasome. *Journal of immunology (Baltimore, Md. : 1950)*, 183(6):3578–3581, September 2009.
- [53] Sophie Joly, Stephanie C Eisenbarth, Alicia K Olivier, Adam Williams, Daniel H Kaplan, Suzanne L Cassel, Richard A Flavell, and Fayyaz S Sutterwala. Cutting edge: Nlrp10 is essential for protective antifungal adaptive immunity against *Candida albicans*. *Journal of immunology (Baltimore, Md. : 1950)*, 189(10):4713–4717, November 2012.
- [54] Stephanie C Eisenbarth, Adam Williams, Oscar R Colegio, Hailong Meng, Till Strowig, Anthony Rongvaux, Jorge Henao-Mejia, Christoph A Thaiss, Sophie Joly, David G Gonzalez, Lan Xu, Lauren A Zenewicz, Ann M Haberman, Eran Elinav, Steven H Kleinstein, Fayyaz S Sutterwala, and Richard A Flavell. NLRP10 is a NOD-like receptor essential to initiate adaptive immunity by dendritic cells. *Nature*, 484(7395):510–513, April 2012.
- [55] Jayendra Kumar Krishnaswamy, Arpita Singh, Uthaman Gowthaman, Renee Wu, Pavane Gorrepati, Manuela Sales Nascimento, Antonia Gallman, Dong Liu, Anne Marie Rhebergen, Samuele Calabro, Lan Xu, Patricia Ranney, Anuj Srivastava, Matthew Ranson, James D Gorham, Zachary McCaw, Steven R Kleeberger, Leonhard X Heinz, André C Müller, Keiryn L Bennett, Giulio Superti-Furga,

- Jorge Henao-Mejia, Fayyaz S Sutterwala, Adam Williams, Richard A Flavell, and Stephanie C Eisenbarth. Coincidental loss of DOCK8 function in NLRP10-deficient and C3H/HeJ mice results in defective dendritic cell migration. *Proceedings of the National Academy of Sciences of the United States of America*, 112(10):3056–3061, March 2015.
- [56] Yosuke Harada, Yoshihiko Tanaka, Masao Terasawa, Markus Pieczyk, Katsuyoshi Habiro, Tomoya Katakai, Kyoko Hanawa-Suetsugu, Mutsuko Kukimoto-Niino, Tomoko Nishizaki, Mikako Shirouzu, Xuefeng Duan, Takehito Uruno, Akihiko Nishikimi, Fumiyuki Sanematsu, Shigeyuki Yokoyama, Jens V Stein, Tatsuo Kinashi, and Yoshinori Fukui. DOCK8 is a Cdc42 activator critical for interstitial dendritic cell migration during immune responses. *Blood*, 119(19):4451–4461, May 2012.
- [57] Susan Farmand and Mikael Sundin. Hyper-IgE syndromes: recent advances in pathogenesis, diagnostics and clinical care. *Current opinion in hematology*, 22(1):12–22, January 2015.
- [58] Jeffrey Tomalka, Sandhya Ganesan, Elaheh Azodi, Krupen Patel, Parth Majumdar, Brian A Hall, Katherine A Fitzgerald, and Amy G Hise. A novel role for the NLRC4 inflammasome in mucosal defenses against the fungal pathogen *Candida albicans*. *PLoS pathogens*, 7(12):e1002379, December 2011.
- [59] R S Ali, A Falconer, M Ikram, C E Bissett, R Cerio, and A G Quinn. Expression of the peptide antibiotics human beta defensin-1 and human beta defensin-2 in normal human skin. *The Journal of investigative dermatology*, 117(1):106–111, July 2001.
- [60] Richard Jennemann, Mariona Rabionet, Karin Gorgas, Sharon Epstein, Alexander Dalpke, Ulrike Rothermel, Aline Bayerle, Franciscus van der Hoeven, Silke Imgrund, Joachim Kirsch, Walter Nickel, Klaus Willecke, Howard Riezman, Hermann-Josef Gröne, and Roger Sandhoff. Loss of ceramide synthase 3 causes lethal skin barrier disruption. *Human molecular genetics*, 21(3):586–608, February 2012.
- [61] Crisanto M Lopez, Reinhard Wallich, Kristian Riesbeck, Christine Skerka, and Peter F Zipfel. *Candida albicans* uses the surface protein Gpm1 to attach to

human endothelial cells and to keratinocytes via the adhesive protein vitronectin. *PloS one*, 9(3):e90796, 2014.

- [62] Yuping Ran, Masashi Yamazaki, Ryoji Tsuboi, and Hideoki Ogawa. Adherence and proliferation of keratinocytes cultured with *Candida albicans*. *The Journal of dermatology*, 41(6):554–556, June 2014.
- [63] Spencer C Liang, Xiang-Yang Tan, Deborah P Luxenberg, Riyez Karim, Kyriaki Dunussi-Joannopoulos, Mary Collins, and Lynette A Fouser. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *The Journal of Experimental Medicine*, 203(10):2271–2279, October 2006.
- [64] Mark Peric, Sarah Koglin, Song-Min Kim, Shin Morizane, Robert Besch, Jörg C Prinz, Thomas Ruzicka, Richard L Gallo, and Jürgen Schaubert. IL-17A enhances vitamin D3-induced expression of cathelicidin antimicrobial peptide in human keratinocytes. *Journal of immunology (Baltimore, Md. : 1950)*, 181(12):8504–8512, December 2008.
- [65] K E Nograles, L C Zaba, E Guttman-Yassky, J Fuentes-Duculan, M Suárez-Fariñas, A Cardinale, A Khatcherian, J Gonzalez, K C Pierson, T R White, C Pensabene, I Coats, I Novitskaya, M A Lowes, and J G Krueger. Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. *The British journal of dermatology*, 159(5):1092–1102, November 2008.
- [66] Sonja Didovic, Friederike V Opitz, Bernhard Holzmann, Irmgard Förster, and Heike Weighardt. Requirement of MyD88 signaling in keratinocytes for Langerhans cell migration and initiation of atopic dermatitis-like symptoms in mice. *European journal of immunology*, pages n/a–n/a, December 2015.
- [67] Yaming Wang, Mattia Bugatti, Tyler K Ulland, William Vermi, Susan Gilfillan, and Marco Colonna. Non-redundant roles of keratinocyte-derived IL-34 and neutrophil-derived CSF1 in Langerhans cells renewal in the steady-state and skin inflammation. *European journal of immunology*, pages n/a–n/a, December 2015.
- [68] Keisuke Nagao, Tetsuro Kobayashi, Kazuyo Moro, Manabu Ohyama, Takeya Adachi, Daniela Y Kitashima, Satoshi Ueha, Keisuke Horiuchi, Hideaki Tanizaki,

- Kenji Kabashima, Akiharu Kubo, Young-hun Cho, Björn E Clausen, Kouji Matsushima, Makoto Suematsu, Glaucia C Furtado, Sergio A Lira, Joshua M Farber, Mark C Udey, and Masayuki Amagai. Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin. *Nature immunology*, 13(8):744–752, August 2012.
- [69] Takeya Adachi, Tetsuro Kobayashi, Eiji Sugihara, Taketo Yamada, Koichi Ikuta, Stefania Pittaluga, Hideyuki Saya, Masayuki Amagai, and Keisuke Nagao. Hair follicle-derived IL-7 and IL-15 mediate skin-resident memory T cell homeostasis and lymphoma. *Nature Medicine*, 21(11):1272–1279, November 2015.
- [70] Philippe Gasque and Marie Christine Jaffar-Bandjee. The immunology and inflammatory responses of human melanocytes in infectious diseases. *The Journal of infection*, 71(4):413–421, October 2015.
- [71] J A Mackintosh. The antimicrobial properties of melanocytes, melanosomes and melanin and the evolution of black skin. *Journal of theoretical biology*, 211(2):101–113, July 2001.
- [72] S Kippenberger, A Bernd, I Menzel, S Loitsch, A Ramirez-Bosca, and R Kaufmann. *Candida albicans* suppresses transcription of melanogenesis enzymes in cultured melanocytes. *Mycoses*, 40(9-10):373–375, December 1997.
- [73] Cecilia V Tapia, Maryanne Falconer, Fabián Tempio, Felipe Falcón, Mercedes López, Marisol Fuentes, Claudio Alburquenque, José Amaro, Sergio A Bucarey, and Anna Di Nardo. Melanocytes and melanin represent a first line of innate immunity against *Candida albicans*. *Medical mycology*, 52(5):445–454, July 2014.
- [74] Jose Ordovas-Montanes, Seth Rakoff-Nahoum, Siyi Huang, Lorena Riol-Blanco, Olga Barreiro, and Ulrich H von Andrian. The Regulation of Immunological Processes by Peripheral Neurons in Homeostasis and Disease. *Trends in immunology*, 36(10):578–604, October 2015.
- [75] J Hosoi, G F Murphy, C L Egan, E A Lerner, S Grabbe, A Asahina, and R D Granstein. Regulation of Langerhans cell function by nerves containing calcitonin gene-related peptide. *Nature*, 363(6425):159–163, May 1993.
- [76] Lorena Riol-Blanco, Jose Ordovas-Montanes, Mario Perro, Elena Naval, Aude Thiriot, David Alvarez, Silke Paust, John N Wood, and Ulrich H von Andrian.

- Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. *Nature*, 510(7503):157–161, June 2014.
- [77] Melissa A Farmer, Anna M Taylor, Andrea L Bailey, Alexander H Tuttle, Leigh C MacIntyre, Zarah E Milagrosa, Halley P Crissman, Gary J Bennett, Alfredo Ribeiro-da Silva, Yitzchak M Binik, and Jeffrey S Mogil. Repeated vulvovaginal fungal infections cause persistent pain in a mouse model of vulvodynia. *Science translational medicine*, 3(101):101ra91–101ra91, September 2011.
- [78] Ke Ren and Ronald Dubner. Inflammatory Models of Pain and Hyperalgesia. *ILAR journal / National Research Council, Institute of Laboratory Animal Resources*, 40(3):111–118, June 1999.
- [79] Isaac M Chiu, Balthasar A Heesters, Nader Ghasemlou, Christian A Von Hehn, Fan Zhao, Johnathan Tran, Brian Wainger, Amanda Strominger, Sriya Muralidharan, Alexander R Horswill, Juliane Bubeck Wardenburg, Sun Wook Hwang, Michael C Carroll, and Clifford J Woolf. Bacteria activate sensory neurons that modulate pain and inflammation. *Nature*, 501(7465):52–57, September 2013.
- [80] L De Petrocellis, R Deva, F Mainieri, M Schaefer, T Bisogno, R Ciccoli, A Ligresti, K Hill, S Nigam, G Appendino, and V Di Marzo. Chemical synthesis, pharmacological characterization, and possible formation in unicellular fungi of 3-hydroxy-anandamide. *Journal of lipid research*, 50(4):658–666, April 2009.
- [81] Ikhlas A El Karim, Gerard J Linden, David F Orr, and Fionnuala T Lundy. Antimicrobial activity of neuropeptides against a range of micro-organisms from skin, oral, respiratory and gastrointestinal tract sites. *Journal of neuroimmunology*, 200(1-2):11–16, August 2008.
- [82] Dennis Roggenkamp, Sarah Köpnick, Franz Stäb, Horst Wenck, Martin Schmelz, and Gitta Neufang. Epidermal nerve fibers modulate keratinocyte growth via neuropeptide signaling in an innervated skin model. *The Journal of investigative dermatology*, 133(6):1620–1628, June 2013.
- [83] Wanhong Ding, Lori L Stohl, John A Wagner, and Richard D Granstein. Calcitonin gene-related peptide biases Langerhans cells toward Th2-type immunity. *Journal of immunology (Baltimore, Md. : 1950)*, 181(9):6020–6026, November 2008.

- [84] Christi Parham, Madaline Chirica, Jacqueline Timans, Elena Vaisberg, Marilyn Travis, Jeanne Cheung, Stefan Pflanz, Rebecca Zhang, Komal P Singh, Felix Vega, Wayne To, Janet Wagner, Anne-Marie O'Farrell, Terrill McClanahan, Sandra Zurawski, Charles Hannum, Daniel Gorman, Donna M Rennick, Robert A Kastelein, Rene de Waal Malefyt, and Kevin W Moore. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *Journal of immunology (Baltimore, Md. : 1950)*, 168(11):5699–5708, June 2002.
- [85] Sarah L Gaffen, Renu Jain, Abhishek V Garg, and Daniel J Cua. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nature reviews. Immunology*, 14(9):585–600, September 2014.
- [86] Ludovic de Beaucoudrey, Anne Puel, Orchidée Filipe-Santos, Aurélie Cobat, Pegah Ghandil, Maya Chrabieh, Jacqueline Feinberg, Horst von Bernuth, Arina Samarina, Lucile Jannière, Claire Fieschi, Jean-Louis Stéphan, Catherine Boileau, Stanislas Lyonnet, Guillaume Jondeau, Valérie Cormier-Daire, Martine Le Merrier, Cyrille Hoarau, Yvon Lebranchu, Olivier Lortholary, Marie-Olivia Chandesris, François Tron, Eleonora Gambineri, Lucia Bianchi, Carlos Rodríguez-Gallego, Simona E Zitnik, Julia Vasconcelos, Margarida Guedes, Artur Bonito Vitor, László Maródi, Helen Chapel, Brenda Reid, Chaim Roifman, David Nadal, Janine Reichenbach, Isabel Caragol, Ben-Zion Garty, Figen Dogu, Yildiz Camcioglu, Sanyie Gülle, Ozden Sanal, Alain Fischer, Laurent Abel, Birgitta Stockinger, Capucine Picard, and Jean-Laurent Casanova. Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. *The Journal of Experimental Medicine*, 205(7):1543–1550, July 2008.
- [87] Paul G Whitney, Eva Bar, Fabiola Osorio, Neil C Rogers, Barbara U Schraml, Safia Deddouche, Salome LeibundGut-Landmann, and Caetano Reis e Sousa. Syk signaling in dendritic cells orchestrates innate resistance to systemic fungal infection. *PLoS pathogens*, 10(7):e1004276, July 2014.
- [88] Heather R Conti, Fang Shen, Namrata Nayyar, Eileen Stocum, Jianing N Sun, Matthew J Lindemann, Allen W Ho, Justine Hoda Hai, Jeffrey J Yu, Ji Won Jung, Scott G Filler, Patricia Masso-Welch, Mira Edgerton, and Sarah L Gaffen. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against

oral candidiasis. *The Journal of Experimental Medicine*, 206(2):299–311, February 2009.

- [89] Christina O Maher, Katie Dunne, Ross Comerford, Siobhán O’Dea, Aisling Loy, James Woo, Thomas R Rogers, Fiona Mulcahy, Pádraic J Dunne, and Derek G Doherty. Candida albicans stimulates IL-23 release by human dendritic cells and downstream IL-17 secretion by V δ 1 T cells. *Journal of immunology (Baltimore, Md. : 1950)*, 194(12):5953–5960, June 2015.
- [90] Daniel H Kaplan. In vivo function of Langerhans cells and dermal dendritic cells. *Trends in immunology*, 31(12):446–451, December 2010.
- [91] Ryutaro Yoshiki, Kenji Kabashima, Tetsuya Honda, Satoshi Nakamizo, Yu Sawada, Kazunari Sugita, Haruna Yoshioka, Shun Ohmori, Bernard Malissen, Yoshiki Tokura, and Motonobu Nakamura. IL-23 from Langerhans cells is required for the development of imiquimod-induced psoriasis-like dermatitis by induction of IL-17A-producing $\gamma\delta$ T cells. *The Journal of investigative dermatology*, 134(7):1912–1921, July 2014.
- [92] Christian Wohn, Julia L Ober-Blöbaum, Stefan Haak, Stanislav Pantelyushin, Cheolho Cheong, Sonja P Zahner, Sabina Onderwater, Marius Kant, Heike Weighardt, Bernhard Holzmann, Boris Reizis, Burkhard Becher, Errol P Prens, and Björn E Clausen. Langerin(neg) conventional dendritic cells produce IL-23 to drive psoriatic plaque formation in mice. *Proceedings of the National Academy of Sciences of the United States of America*, 110(26):10723–10728, June 2013.
- [93] Felix Scholz, Shruti Naik, Fayyaz S Sutterwala, and Daniel H Kaplan. Langerhans Cells Suppress CD49a+ NK Cell-Mediated Skin Inflammation. *Journal of immunology (Baltimore, Md. : 1950)*, 195(5):2335–2342, September 2015.
- [94] Daniel H Kaplan, Mathew C Jenison, Sem Saeland, Warren D Shlomchik, and Mark J Shlomchik. Epidermal langerhans cell-deficient mice develop enhanced contact hypersensitivity. *Immunity*, 23(6):611–620, December 2005.
- [95] Aleh Bobr, Irlanda Olvera-Gomez, Botond Z Igyártó, Krystal M Haley, Kristin A Hogquist, and Daniel H Kaplan. Acute ablation of Langerhans cells enhances skin immune responses. *Journal of immunology (Baltimore, Md. : 1950)*, 185(8):4724–4728, October 2010.

- [96] Botond Z Igyártó, Matthew C Jenison, Jan C Dudda, Axel Roers, Werner Müller, Pandelakis A Koni, Daniel J Campbell, Mark J Shlomchik, and Daniel H Kaplan. Langerhans cells suppress contact hypersensitivity responses via cognate CD4 interaction and langerhans cell-derived IL-10. *Journal of immunology (Baltimore, Md. : 1950)*, 183(8):5085–5093, October 2009.
- [97] Anna R Huppler, Heather R Conti, Nydiaris Hernández-Santos, Toni Darville, Partha S Biswas, and Sarah L Gaffen. Role of neutrophils in IL-17-dependent immunity to mucosal candidiasis. *Journal of immunology (Baltimore, Md. : 1950)*, 192(4):1745–1752, February 2014.
- [98] K Trautwein-Weidner, A Gladiator, S Nur, P Diethelm, and S LeibundGut-Landmann. IL-17-mediated antifungal defense in the oral mucosa is independent of neutrophils. *Mucosal immunology*, 8(2):221–231, March 2015.
- [99] Andrew Johnston, Yi Fritz, Sean M Dawes, Doina Diaconu, Paul M Al-Attar, Andrew M Guzman, Cynthia S Chen, Wen Fu, Johann E Gudjonsson, Thomas S McCormick, and Nicole L Ward. Keratinocyte overexpression of IL-17C promotes psoriasiform skin inflammation. *Journal of immunology (Baltimore, Md. : 1950)*, 190(5):2252–2262, March 2013.
- [100] Heather R Conti, Natasha Whibley, Bianca M Coleman, Abhishek V Garg, Jillian R Jaycox, and Sarah L Gaffen. Signaling through IL-17C/IL-17RE is dispensable for immunity to systemic, oral and cutaneous candidiasis. *PloS one*, 10(4):e0122807, 2015.
- [101] André Gladiator, Nicolette Wangler, Kerstin Trautwein-Weidner, and Salome LeibundGut-Landmann. Cutting edge: IL-17-secreting innate lymphoid cells are essential for host defense against fungal infection. *Journal of immunology (Baltimore, Md. : 1950)*, 190(2):521–525, January 2013.
- [102] Heather R Conti, Alanna C Peterson, Lucas Brane, Anna R Huppler, Nydiaris Hernández-Santos, Natasha Whibley, Abhishek V Garg, Michelle R Simpson-Abelson, Gregory A Gibson, Anna J Mamo, Lisa C Osborne, Shrinivas Bishu, Nico Ghilardi, Ulrich Siebenlist, Simon C Watkins, David Artis, Mandy J McGeachy, and Sarah L Gaffen. Oral-resident natural Th17 cells and $\gamma\delta$ T cells control opportunistic *Candida albicans* infections. *The Journal of Experimental Medicine*, 211(10):2075–2084, September 2014.

- [103] Robert P Wilson, Megan L Ives, Geetha Rao, Anthony Lau, Kathryn Payne, Masao Kobayashi, Peter D Arkwright, Jane Peake, Melanie Wong, Stephen Adelstein, Joanne M Smart, Martyn A French, David A Fulcher, Capucine Picard, Jacinta Bustamante, Stephanie Boisson-Dupuis, Paul Gray, Polina Stepensky, Klaus Warnatz, Alexandra F Freeman, Jamie Rossjohn, James McCluskey, Steven M Holland, Jean-Laurent Casanova, Gulbu Uzel, Cindy S Ma, Stuart G Tangye, and Elissa K Deenick. STAT3 is a critical cell-intrinsic regulator of human unconventional T cell numbers and function. *The Journal of Experimental Medicine*, 212(6):855–864, June 2015.
- [104] Daniel J Cua and Cristina M Tato. Innate IL-17-producing cells: the sentinels of the immune system. *Nature reviews. Immunology*, 10(7):479–489, July 2010.
- [105] Elizabeth E Gray, Francisco Ramírez-Valle, Ying Xu, Shuang Wu, Zhihao Wu, Klaus E Karjalainen, and Jason G Cyster. Deficiency in IL-17-committed V γ 4(+) $\gamma\delta$ T cells in a spontaneous Sox13-mutant CD45.1(+) congenic mouse substrain provides protection from dermatitis. *Nature immunology*, 14(6):584–592, June 2013.
- [106] Jan D Haas, Sarina Ravens, Sandra Düber, Inga Sandrock, Linda Oberdörfer, Elham Kashani, Vijaykumar Chennupati, Lisa Föhse, Ronald Naumann, Siegfried Weiss, Andreas Krueger, Reinhold Förster, and Immo Prinz. Development of interleukin-17-producing $\gamma\delta$ T cells is restricted to a functional embryonic wave. *Immunity*, 37(1):48–59, July 2012.
- [107] Masahide Miyamoto, Olof Prause, Margareta Sjöstrand, Martti Laan, Jan Lötval, and Anders Lindén. Endogenous IL-17 as a mediator of neutrophil recruitment caused by endotoxin exposure in mouse airways. *Journal of immunology (Baltimore, Md. : 1950)*, 170(9):4665–4672, May 2003.
- [108] Patricia R Taylor, Sanhita Roy, Sixto M Leal, Yan Sun, Scott J Howell, Brian A Cobb, Xiaoxia Li, and Eric Pearlman. Activation of neutrophils by autocrine IL-17A-IL-17RC interactions during fungal infection is regulated by IL-6, IL-23, ROR γ t and dectin-2. *Nature immunology*, 15(2):143–151, February 2014.
- [109] A Fulurija, R B Ashman, and J M Papadimitriou. Neutrophil depletion increases susceptibility to systemic and vaginal candidiasis in mice, and reveals differences

between brain and kidney in mechanisms of host resistance. *Microbiology (Reading, England)*, 142 (Pt 12):3487–3496, December 1996.

- [110] Nora Branzk, Aleksandra Lubojemska, Sarah E Hardison, Qian Wang, Maximiliano G Gutierrez, Gordon D Brown, and Venizelos Papayannopoulos. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. *Nature immunology*, 15(11):1017–1025, November 2014.
- [111] Nikolaus Rieber, Anurag Singh, Hasan Öz, Melanie Carevic, Maria Bouzani, Jorge Amich, Michael Ost, Zhiyong Ye, Marlene Ballbach, Iris Schäfer, Markus Mezger, Sascha N Klimosch, Alexander N R Weber, Rupert Handgretinger, Sven Krappmann, Johannes Liese, Maik Engholm, Rebecca Schüle, Helmut Rainer Salih, László Maródi, Carsten Speckmann, Bodo Grimbacher, Jurgen Ruland, Gordon D Brown, Andreas Beilhack, Juergen Loeffler, and Dominik Hartl. Pathogenic Fungi Regulate Immunity by Inducing Neutrophilic Myeloid-Derived Suppressor Cells. *Cell host & microbe*, 17(4):507–514, April 2015.
- [112] Mihai G Netea, Leo A B Joosten, Jos W M van der Meer, Bart-Jan Kullberg, and Frank L van de Veerdonk. Immune defence against *Candida* fungal infections. *Nature reviews. Immunology*, 15(10):630–642, October 2015.
- [113] Christina E Zielinski, Federico Mele, Dominik Aschenbrenner, David Jarrossay, Francesca Ronchi, Marco Gattorno, Silvia Monticelli, Antonio Lanzavecchia, and Federica Sallusto. Pathogen-induced human TH17 cells produce IFN- γ or IL-10 and are regulated by IL-1 β . *Nature*, 484(7395):514–518, April 2012.
- [114] Simone Becattini, Daniela Latorre, Federico Mele, Mathilde Foglierini, Corinne De Gregorio, Antonino Cassotta, Blanca Fernandez, Sander Kelderman, Ton N Schumacher, Davide Corti, Antonio Lanzavecchia, and Federica Sallusto. T cell immunity. Functional heterogeneity of human memory CD4 T cell clones primed by pathogens or vaccines. *Science (New York, N.Y.)*, 347(6220):400–406, January 2015.
- [115] Katrina L Randall, Stephanie S-Y Chan, Cindy S Ma, Ivan Fung, Yan Mei, Mehmet Yabas, Andy Tan, Peter D Arkwright, Wafaa Al Suwairi, Saul Oswaldo Lugo Reyes, Marco A Yamazaki-Nakashimada, Maria de la Luz Garcia-Cruz, Joanne M Smart, Capucine Picard, Satoshi Okada, Emmanuelle Jouanguy, Jean-Laurent Casanova, Teresa Lambe, Richard J Cornall, Sarah Russell, Jane Oliaro,

- Stuart G Tangye, Edward M Bertram, and Christopher C Goodnow. DOCK8 deficiency impairs CD8 T cell survival and function in humans and mice. *The Journal of Experimental Medicine*, 208(11):2305–2320, October 2011.
- [116] Mayumi Nakagawa, Hannah N Coleman, Xuelian Wang, Jaclyn Daniels, James Sikes, and Uma M Nagarajan. IL-12 secretion by Langerhans cells stimulated with Candida skin test reagent is mediated by dectin-1 in some healthy individuals. *Cytokine*, 65(2):202–209, February 2014.
- [117] Ling Ni, Ingrid Gayet, Sandra Zurawski, Dorothee Duluc, Anne-Laure Flamar, Xiao-Hua Li, Amy O’Bar, Sandra Clayton, Anna Karolina Palucka, Gerard Zurawski, Jacques Banchereau, and SangKon Oh. Concomitant activation and antigen uptake via human dectin-1 results in potent antigen-specific CD8+ T cell responses. *Journal of immunology (Baltimore, Md. : 1950)*, 185(6):3504–3513, September 2010.
- [118] Jonathan L Linehan, Thamotharampillai Dileepan, Sakeen W Kashem, Daniel H Kaplan, Patrick Cleary, and Marc K Jenkins. Generation of Th17 cells in response to intranasal infection requires TGF- β 1 from dendritic cells and IL-6 from CD301b+ dendritic cells. *Proceedings of the National Academy of Sciences of the United States of America*, 112(41):12782–12787, October 2015.
- [119] Emma K Persson, Heli Uronen-Hansson, Monika Semmrich, Aymeric Rivollier, Karin Hägerbrand, Jan Marsal, Sigurdur Gudjonsson, Ulf Håkansson, Boris Reizis, Knut Kotarsky, and William W Agace. IRF4 transcription-factor-dependent CD103(+)CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity*, 38(5):958–969, May 2013.
- [120] Andreas Schlitzer, Naomi McGovern, Pearline Teo, Teresa Zelante, Koji Atarashi, Donovan Low, Adrian W S Ho, Peter See, Amanda Shin, Pavandip Singh Wasan, Guillaume Hoeffel, Benoit Malleret, Alexander Heiseke, Samantha Chew, Laura Jardine, Harriet A Purvis, Catharien M U Hilken, John Tam, Michael Poidinger, E Richard Stanley, Anne B Krug, Laurent Renia, Baalasubramanian Sivasankar, Lai Guan Ng, Matthew Collin, Paola Ricciardi-Castagnoli, Kenya Honda, Muzli-fah Haniffa, and Florent Ginhoux. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity*, 38(5):970–983, May 2013.

- [121] Shruti Naik, Nicolas Bouladoux, Jonathan L Linehan, Seong-Ji Han, Oliver J Harrison, Christoph Wilhelm, Sean Conlan, Sarah Himmelfarb, Allyson L Byrd, Clayton Deming, Mariam Quinones, Jason M Brenchley, Heidi H Kong, Roxanne Tussiwand, Kenneth M Murphy, Miriam Merad, Julia A Segre, and Yasmine Belkaid. Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. *Nature*, 520(7545):104–108, April 2015.
- [122] Kerstin Trautwein-Weidner, André Gladiator, Florian R Kirchner, Simone Beccattini, Thomas Rüllicke, Federica Sallusto, and Salome LeibundGut-Landmann. Antigen-Specific Th17 Cells Are Primed by Distinct and Complementary Dendritic Cell Subsets in Oropharyngeal Candidiasis. *PLoS pathogens*, 11(10):e1005164, October 2015.
- [123] Sophie Hambleton, Sandra Salem, Jacinta Bustamante, Venetia Bigley, Stephanie Boisson-Dupuis, Joana Azevedo, Anny Fortin, Muzlifah Haniffa, Lourdes Ceron-Gutierrez, Chris M Bacon, Geetha Menon, Céline Trouillet, David McDonald, Peter Carey, Florent Ginhoux, Laia Alsina, Timothy J Zumwalt, Xiao-Fei Kong, Dinakantha Kumararatne, Karina Butler, Marjorie Hubeau, Jacqueline Feinberg, Saleh Al-Muhsen, Andrew Cant, Laurent Abel, Damien Chaussabel, Rainer Doffinger, Eduardo Talesnik, Anete Grumach, Alberto Duarte, Katia Abarca, Dewton Moraes-Vasconcelos, David Burk, Albert Berghuis, Frédéric Geissmann, Matthew Collin, Jean-Laurent Casanova, and Philippe Gros. IRF8 mutations and human dendritic-cell immunodeficiency. *The New England journal of medicine*, 365(2):127–138, July 2011.
- [124] N Hernández-Santos, A R Huppler, A C Peterson, S A Khader, K C McKenna, and S L Gaffen. Th17 cells confer long-term adaptive immunity to oral mucosal *Candida albicans* infections. *Mucosal immunology*, 6(5):900–910, September 2013.
- [125] Marion Pepper, Jonathan L Linehan, Antonio J Pagán, Traci Zell, Thamotharampillai Dileepan, P Patrick Cleary, and Marc K Jenkins. Different routes of bacterial infection induce long-lived TH1 memory cells and short-lived TH17 cells. *Nature immunology*, 11(1):83–89, January 2010.
- [126] Douglas R McDonald. TH17 deficiency in human disease. *Journal of Allergy and Clinical Immunology*, 129(6):1429–1435, June 2012.

- [127] Anne Puel, Rainer Doffinger, Angels Natividad, Maya Chrabieh, Gabriela Barcenas-Morales, Capucine Picard, Aurélie Cobat, Marie Ouachée-Chardin, Antoine Toulon, Jacinta Bustamante, Saleh Al-Muhsen, Mohammed Al-Owain, Peter D Arkwright, Colm Costigan, Vivienne McConnell, Andrew J Cant, Mario Abinun, Michel Polak, Pierre-François Bougnères, Dinakantha Kumararatne, László Maródi, Amit Nahum, Chaim Roifman, Stéphane Blanche, Alain Fischer, Christine Bodemer, Laurent Abel, Desa Lilic, and Jean-Laurent Casanova. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *The Journal of Experimental Medicine*, 207(2):291–297, February 2010.
- [128] Sanne P Smeekens, Theo S Plantinga, Frank L van de Veerdonk, Bas Heinhuis, Alexander Hoischen, Leo A B Joosten, Peter D Arkwright, Andrew Gennery, Bart-Jan Kullberg, Joris A Veltman, Desa Lilic, Jos W M van der Meer, and Mihai G Netea. STAT1 hyperphosphorylation and defective IL12R/IL23R signaling underlie defective immunity in autosomal dominant chronic mucocutaneous candidiasis. *PloS one*, 6(12):e29248, 2011.
- [129] Amanda S MacLeod, Saskia Hemmers, Olivia Garijo, Marianne Chabod, Kerri Mowen, Deborah A Witherden, and Wendy L Havran. Dendritic epidermal T cells regulate skin antimicrobial barrier function. *The Journal of clinical investigation*, 123(10):4364–4374, September 2013.
- [130] E E Gray, K Suzuki, and J G Cyster. Cutting Edge: Identification of a Motile IL-17-Producing T Cell Population in the Dermis. *Journal of immunology (Baltimore, Md. : 1950)*, 186(11):6091–6095, May 2011.
- [131] S Naik, Shruti Naik, Nicolas Bouladoux, N Bouladoux, C Wilhelm, Christoph Wilhelm, Michael J Molloy, M J Molloy, R Salcedo, Rosalba Salcedo, Wolfgang Kastenmüller, W Kastenmuller, C Deming, Clayton Deming, M Quinones, Mariam Quinones, Lily Koo, L Koo, S Conlan, Sean Conlan, Sean Spencer, S Spencer, J A Hall, Jason A Hall, A Dzutsev, Amiran Dzutsev, Heidi Kong, H Kong, D J Campbell, Daniel J Campbell, G Trinchieri, Giorgio Trinchieri, Julia A Segre, J A Segre, Yasmine Belkaid, and Y Belkaid. Compartmentalized Control of Skin Immunity by Resident Commensals. *Science (New York, N.Y.)*, 337(6098):1115–1119, August 2012.

- [132] Nital Sumaria, Ben Roediger, Ben Roediger, Lai Guan Ng, Jim Qin, Rachel Pinto, Lois L Cavanagh, Elena Shklovskaya, Barbara Fazekas de St Groth, Barbara Fazekas de St Groth, James A Triccas, and Wolfgang Weninger. Cutaneous immunosurveillance by self-renewing dermal gammadelta T cells. *The Journal of Experimental Medicine*, 208(3):505–518, March 2011.
- [133] A S Woodward Davis, T Bergsbaken, M A Delaney, and M J Bevan. Dermal-Resident versus Recruited T Cell Response to Cutaneous Vaccinia Virus Infection. *Journal of immunology (Baltimore, Md. : 1950)*, 194(5):2260–2267, February 2015.
- [134] John S Cho, Eric M Pietras, Nairy C Garcia, Romela Irene Ramos, David M Farzam, Holly R Monroe, Julie E Magorien, Andrew Blauvelt, Jay K Kolls, Ambrose L Cheung, Genhong Cheng, Robert L Modlin, and Lloyd S Miller. IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice. *The Journal of clinical investigation*, 120(5):1762–1773, May 2010.
- [135] Caroline E Sutton, Stephen J Lalor, Cheryl M Sweeney, Corinna F Brereton, Ed C Lavelle, and Kingston H G Mills. Interleukin-1 and IL-23 Induce Innate IL-17 Production from $\gamma\delta$ T Cells, Amplifying Th17 Responses and Autoimmunity. *Immunity*, 31(2):331–341, August 2009.
- [136] Jonathan P Sherlock, Barbara Joyce-Shaikh, Scott P Turner, Cheng-Chi Chao, Manjiri Sathe, Jeff Grein, Daniel M Gorman, Edward P Bowman, Terrill K McClanahan, Jennifer H Yearley, Gérard Eberl, Christopher D Buckley, Robert A Kastelein, Robert H Pierce, Drake M LaFace, and Daniel J Cua. IL-23 induces spondyloarthritis by acting on ROR- γ t+ CD3+CD4–CD8– enthesal resident T cells. *Nature Medicine*, 18(7):1069–1076, July 2012.
- [137] André Gladiator and Salome LeibundGut-Landmann. Innate Lymphoid Cells: New Players in IL-17-Mediated Antifungal Immunity. *PLoS pathogens*, 9(12):e1003763, December 2013.
- [138] M Honjo, A Elbe, G Steiner, I Assmann, K Wolff, and G Stingl. Thymus-independent generation of Thy-1+ epidermal cells from a pool of Thy-1- bone marrow precursors. *The Journal of investigative dermatology*, 95(5):562–567, November 1990.

- [139] Yihua Cai, Feng Xue, Chris Fleming, Jie Yang, Chuanlin Ding, Yunfeng Ma, Min Liu, Huang-ge Zhang, Jie Zheng, Na Xiong, and Jun Yan. Differential developmental requirement and peripheral regulation for dermal V γ 4 and V γ 6T17 cells in health and inflammation. *Nature communications*, 5, June 2014.
- [140] Anna R Huppler, Shrinivas Bishu, and Sarah L Gaffen. Mucocutaneous candidiasis: the IL-17 pathway and implications for targeted immunotherapy. *Arthritis research & therapy*, 14(4):217, 2012.
- [141] Ansuman T Satpathy, Carlos G Briseño, Jacob S Lee, Dennis Ng, Nicholas A Manieri, Wumesh KC, Xiaodi Wu, Stephanie R Thomas, Wan-Ling Lee, Mustafa Turkoz, Keely G McDonald, Matthew M Meredith, Christina Song, Cynthia J Guidos, Rodney D Newberry, Wenjun Ouyang, Theresa L Murphy, Thaddeus S Stappenbeck, Jennifer L Gommerman, Michel C Nussenzweig, Marco Colonna, Raphael Kopan, and Kenneth M Murphy. Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. *Nature immunology*, 14(9):937–948, August 2013.
- [142] Nathan E Welty, Christopher Staley, Nico Ghilardi, Michael J Sadowsky, Botond Z Igyártó, and Daniel H Kaplan. Intestinal lamina propria dendritic cells maintain T cell homeostasis but do not affect commensalism. *The Journal of Experimental Medicine*, 210(10):2011–2024, September 2013.
- [143] Brian T Edelson, Wumesh KC, Richard Juang, Masako Kohyama, Loralyn A Benoit, Paul A Klekotka, Clara Moon, Jörn C Albring, Wataru Ise, Drew G Michael, Deepta Bhattacharya, Thaddeus S Stappenbeck, Michael J Holtzman, Sun-Sang J Sung, Theresa L Murphy, Kai Hildner, and Kenneth M Murphy. Peripheral CD103 +dendritic cells form a unified subset developmentally related to CD8 α +conventional dendritic cells. *The Journal of Experimental Medicine*, 207(4):823–836, April 2010.
- [144] Yosuke Kumamoto, Melissa Linehan, Jason S Weinstein, Brian J Laidlaw, Joseph E Craft, and Akiko Iwasaki. CD301b+ Dermal Dendritic Cells Drive T Helper 2 Cell-Mediated Immunity. *Immunity*, 39(4):733–743, October 2013.

- [145] Naomi McGovern, Andreas Schlitzer, Merry Gunawan, Laura Jardine, Amanda Shin, Elizabeth Poyner, Kile Green, Rachel Dickinson, Xiao-Nong Wang, Donovan Low, Katie Best, Samuel Covins, Paul Milne, Sarah Pagan, Khadija Aljefri, Martin Windebank, Diego Miranda-Saavedra, Diego Miranda Saavedra, Anis Larbi, Pavandip Singh Wasan, Kaibo Duan, Michael Poidinger, Venetia Bigley, Florent Ginhoux, Matthew Collin, and Muzlifah Haniffa. Human Dermal CD14+ Cells Are a Transient Population of Monocyte-Derived Macrophages. *Immunity*, 41(3):465–477, September 2014.
- [146] David M Graham, D M Graham, Ling Huang, L Huang, Kenneth R Robinson, K R Robinson, Mark A Messerli, and M A Messerli. Epidermal keratinocyte polarity and motility require Ca²⁺ influx through TRPV1. *Journal of cell science*, 126(20):4602–4613, October 2013.
- [147] Samuel Bertin, Yukari Aoki-Nonaka, Petrus Rudolf de Jong, Lilian L Nohara, Hongjian Xu, Shawna R Stanwood, Sonal Srikanth, Jihyung Lee, Keith To, Lior Abramson, Timothy Yu, Tiffany Han, Ranim Touma, Xiangli Li, José M González-Navajas, Scott Herdman, Maripat Corr, Guo Fu, Hui Dong, Yousang Gwack, Alessandra Franco, Wilfred A Jefferies, and Eyal Raz. The ion channel TRPV1 regulates the activation and proinflammatory properties of CD4+ T cells. *Nature immunology*, 15(11):1055–1063, October 2014.
- [148] Stephen M Ostrowski, Abdelmadjid Belkadi, Candace M Loyd, Doina Diaconu, and Nicole L Ward. Cutaneous Denervation of Psoriasiform Mouse Skin Improves Acanthosis and Inflammation in a Sensory Neuropeptide-Dependent Manner. *Journal of Investigative Dermatology*, 131(7):1530–1538, July 2011.
- [149] J Meng, S V Ovsepian, J Wang, M Pickering, A Sasse, K R Aoki, G W Lawrence, and J O Dolly. Activation of TRPV1 Mediates Calcitonin Gene-Related Peptide Release, Which Excites Trigeminal Sensory Neurons and Is Attenuated by a Retargeted Botulinum Toxin with Anti-Nociceptive Potential. *Journal of Neuroscience*, 29(15):4981–4992, April 2009.
- [150] Matthias A Engel, Iwona Izydorczyk, Sonja M Mueller-Tribbenesee, Christoph Becker, Markus F Neurath, and Peter W Reeh. Inhibitory CB1 and activating/desensitizing TRPV1-mediated cannabinoid actions on CGRP release in rodent skin. *Neuropeptides*, 45(3):229–237, June 2011.

- [151] Jennifer C Miller, Brian D Brown, Tal Shay, Emmanuel L Gautier, Vladimir Jojic, Ariella Cohain, Gaurav Pandey, Marylène Leboeuf, Kutlu G Elpek, Julie Helft, Daigo Hashimoto, Andrew Chow, Jeremy Price, Melanie Greter, Milena Bogunovic, Angelique Bellemare-Pelletier, Paul S Frenette, Gwendalyn J Randolph, Shannon J Turley, Miriam Merad, Immunological Genome Consortium, Claudia Jakubzick, Adam J Best, Jamie Knell, Ananda Goldrath, Jennifer Miller, Brian Brown, Daphne Koller, Nadia Cohen, Patrick Brennan, Michael Brenner, Aviv Regev, Anne Fletcher, Kutlu Elpek, Deepali Malhotra, Shannon Turley, Radu Jianu, David Laidlaw, Jim Collins, Kavitha Narayan, Katelyn Sylvia, Joon-soo Kang, Roi Gazit, Derrick J Rossi, Francis Kim, Tata Nageswara Rao, Amy Wagers, Susan A Shinton, Richard R Hardy, Paul Monach, Natalie A Bezman, Joseph C Sun, Charlie C Kim, Lewis L Lanier, Tracy Heng, Taras Kreslavsky, Michio Painter, Jeffrey Ericson, Scott Davis, Diane Mathis, and Christophe Benoist. Deciphering the transcriptional network of the dendritic cell lineage. *Nature immunology*, 13(9):888–899, July 2012.
- [152] Manyu Li, Sarah E Wetzel-Strong, Xiaoyang Hua, Stephen L Tilley, Erin Oswald, Matthew F Krummel, and Kathleen M Caron. Deficiency of RAMP1 Attenuates Antigen-Induced Airway Hyperresponsiveness in Mice. *PloS one*, 9(7):e102356, July 2014.
- [153] Yu-Lin Hsieh, Chih-Lung Lin, Hao Chiang, Yaw-Syan Fu, June-Horng Lue, and Sung-Tsang Hsieh. Role of Peptidergic Nerve Terminals in the Skin: Reversal of Thermal Sensation by Calcitonin Gene-Related Peptide in TRPV1-Depleted Neuropathy. *PloS one*, 7(11):e50805, November 2012.
- [154] Daria Augustyniak, Judyta Nowak, and Fionnuala T Lundy. Direct and indirect antimicrobial activities of neuropeptides and their therapeutic potential. *Current protein & peptide science*, 13(8):723–738, December 2012.
- [155] Heather R Conti and Sarah L Gaffen. Host responses to *Candida albicans*: Th17 cells and mucosal candidiasis. *Microbes and Infection*, 12(7):518–527, July 2010.
- [156] H R Conti, O Baker, A F Freeman, W S Jang, S M Holland, R A Li, M Edgerton, and S L Gaffen. New mechanism of oral immunity to mucosal candidiasis in hyper-IgE syndrome. *Mucosal immunology*, 4(4):448–455, February 2011.

- [157] Sanne P Smeekens, Frank L van de Veerdonk, Bart-Jan Kullberg, and Mihai G Netea. Genetic susceptibility to Candida infections. *EMBO Molecular Medicine*, 5(6):805–813, April 2013.
- [158] A Awasthi, L Riolo-Blanco, A Jager, T Korn, C Pot, G Galileos, E Bettelli, V K Kuchroo, and M Oukka. Cutting Edge: IL-23 Receptor GFP Reporter Mice Reveal Distinct Populations of IL-17-Producing Cells. *Journal of immunology (Baltimore, Md. : 1950)*, 182(10):5904–5908, May 2009.
- [159] Melissa A Kinnebrew, Charlie G Buffie, Gretchen E Diehl, Lauren A Zenewicz, Ingrid Leiner, Tobias M Hohl, Richard A Flavell, Dan R Littman, and Eric G Pamer. Interleukin 23 Production by Intestinal CD103+CD11b+ Dendritic Cells in Response to Bacterial Flagellin Enhances Mucosal Innate Immune Defense. *Immunity*, 36(2):276–287, February 2012.
- [160] Muzlifah Haniffa, Amanda Shin, Venetia Bigley, Naomi McGovern, Pearline Teo, Peter See, Pavandip Singh Wasan, Xiao-Nong Wang, Frano Malinarich, Benoit Malleret, Anis Larbi, Pearlie Tan, Helen Zhao, Michael Poidinger, Sarah Pagan, Sharon Cookson, Rachel Dickinson, Ian Dimmick, Ruth F Jarrett, Laurent Renia, John Tam, Colin Song, John Connolly, Jerry K Y Chan, Adam Gehring, Antonio Bertoletti, Matthew Collin, and Florent Ginhoux. Human Tissues Contain CD141hi Cross-Presenting Dendritic Cells with Functional Homology to Mouse CD103+ Nonlymphoid Dendritic Cells. *Immunity*, 37(1):60–73, July 2012.
- [161] Lars Stangenberg, L Stangenberg, D Burzyn, Dalia Burzyn, B A Binstadt, Bryce A Binstadt, Ralph Weissleder, R Weissleder, U Mahmood, Umar Mahmood, C Benoist, Christophe Benoist, Diane Mathis, and D Mathis. Denervation protects limbs from inflammatory arthritis via an impact on the microvasculature. *Proceedings of the National Academy of Sciences*, 111(31):11419–11424, August 2014.
- [162] T Joseph, J Kurian, D J Warwick, and P S Friedmann. Unilateral remission of psoriasis following traumatic nerve palsy. *The British journal of dermatology*, 152(1):185–186, January 2005.
- [163] M THOMPSON, E G L Bywaters Malcolm Thompson, and E G BYWATERS. Unilateral rheumatoid arthritis following hemiplegia. *Annals of the rheumatic diseases*, 21:370–377, December 1962.

- [164] M S Nash. Known and plausible modulators of depressed immune functions following spinal cord injuries. *The journal of spinal cord medicine*, 23(2):111–120, 2000.
- [165] D Rubin-Asher, G Zeilig, M Klieger, A Adunsky, and H Weingarden. Dermatological findings following acute traumatic spinal cord injury. *Spinal cord*, 43(3):175–178, March 2005.
- [166] B M Assas, J A Miyan, and J L Pennock. Cross-talk between neural and immune receptors provides a potential mechanism of homeostatic regulation in the gut mucosa. *Mucosal immunology*, 7(6):1283–1289, September 2014.
- [167] R Saraceno, C E Kleyn, G Terenghi, and C E M Griffiths. The role of neuropeptides in psoriasis. *The British journal of dermatology*, 155(5):876–882, November 2006.
- [168] Steffen Jung, Derya Unutmaz, Phillip Wong, Gen-ichiro Sano, Kenia De los Santos, Tim Sparwasser, Shengji Wu, Sri Vuthoori, Kyung Ko, Fidel Zavala, Eric G Pamer, Dan R Littman, and Richard A Lang. In Vivo Depletion of CD11c+ Dendritic Cells Abrogates Priming of CD8+ T Cells by Exogenous Cell-Associated Antigens. *Immunity*, 17(2):211–220, August 2002.
- [169] Peter Mombaerts, John Iacomini, Randall S Johnson, Karl Herrup, Susumu Tonegawa, and Virginia E Papaioannou. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell*, 68(5):869–877, March 1992.
- [170] Peter Mombaerts, Alan R Clarke, Michael A Rudnicki, John Iacomini, Shigeyoshi Itohara, Juan J Lafaille, Lili Wang, Yoshiaki Ichikawa, Rudolf Jaenisch, Martin L Hooper, and Susumu Tonegawa. Mutations in T-cell antigen receptor genes α and β block thymocyte development at different stages. *Nature*, 360(6401):225–231, November 1992.
- [171] Shigeyoshi Itohara, Peter Mombaerts, Juan Lafaille, John Iacomini, Andrew Nelson, Alan R Clarke, Martin L Hooper, Andrew Farr, and Susumu Tonegawa. T cell receptor δ gene mutant mice: Independent generation of $\alpha\beta$ T cells and programmed rearrangements of $\gamma\delta$ TCR genes. *Cell*, 72(3):337–348, February 1993.

- [172] N Ghilardi, N Kljavin, Q Chen, S Lucas, A L Gurney, and F J de Sauvage. Compromised Humoral and Delayed-Type Hypersensitivity Responses in IL-23-Deficient Mice. *Journal of immunology (Baltimore, Md. : 1950)*, 172(5):2827–2833, February 2004.
- [173] Frank Siebenhaar, Markus Magerl, Eva M J Peters, Sven Hendrix, Martin Metz, and Marcus Maurer. Mast cell-driven skin inflammation is impaired in the absence of sensory nerves. *Journal of Allergy and Clinical Immunology*, 121(4):955–961, April 2008.
- [174] Anthony W Bannon and Annika B Malmberg. *Models of Nociception: Hot-Plate, Tail-Flick, and Formalin Tests in Rodents*, volume Chapter 8. John Wiley & Sons, Inc., Hoboken, NJ, USA, May 2001.
- [175] Adam P Kardon, Erika Polgár, Junichi Hachisuka, Lindsey M Snyder, Darren Cameron, Sinead Savage, Xiaoyun Cai, Sergei Karnup, Christopher R Fan, Gregory M Hemenway, Carcha S Bernard, Erica S Schwartz, Hiroshi Nagase, Christoph Schwarzer, Masahiko Watanabe, Takahiro Furuta, Takeshi Kaneko, H Richard Koerber, Andrew J Todd, and Sarah E Ross. Dynorphin Acts as a Neuromodulator to Inhibit Itch in the Dorsal Horn of the Spinal Cord. *Neuron*, 82(3):573–586, May 2014.
- [176] I A Khasabova, D A Simone, and V S Seybold. Cannabinoids attenuate depolarization-dependent Ca²⁺ influx in intermediate-size primary afferent neurons of adult rats. *Neuroscience*, 115(2):613–625, December 2002.
- [177] H J Lo, J R Köhler, B DiDomenico, D Loebenberg, A Cacciapuoti, and G R Fink. Nonfilamentous *C. albicans* mutants are avirulent. *Cell*, 90(5):939–949, September 1997.
- [178] S C Cheng, Shih-Chin Cheng, Frank L van de Veerdonk, F L van de Veerdonk, M Lenardon, Megan Lenardon, M Stoffels, Monique Stoffels, Theo Plantinga, T Plantinga, S Smeekens, Sanne Smeekens, Lisa Rizzetto, L Rizzetto, Liliane Mukaremera, L Mukaremera, K Preechasuth, Kanya Preechasuth, D Cavalieri, Duccio Cavalieri, Thirumala-Devi Kanneganti, T D Kanneganti, J W M Van Der Meer, Jos W M van der Meer, Bart-Jan Kullberg, B J Kullberg, L A B Joosten, Leo A B Joosten, N A R Gow, Neil A R Gow, M G Netea, and Mihai G Netea. The dectin-1/inflammasome pathway is responsible for the induction of

- protective T-helper 17 responses that discriminate between yeasts and hyphae of *Candida albicans*. *Journal of leukocyte biology*, 90(2):357–366, August 2011.
- [179] Chantal A A van der Graaf, C A A van der Graaf, Mihai G Netea, M G Netea, I Verschueren, Ineke Verschueren, J W M Van Der Meer, Jos W M van der Meer, Bart-Jan Kullberg, and B J Kullberg. Differential Cytokine Production and Toll-Like Receptor Signaling Pathways by *Candida albicans* Blastoconidia and Hyphae. *Infection and immunity*, 73(11):7458–7464, October 2005.
- [180] C F d’Ostiani, G Del Sero, A Bacci, C Montagnoli, A Spreca, A Mencacci, P Ricciardi-Castagnoli, and L Romani. Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans*. Implications for initiation of T helper cell immunity in vitro and in vivo. *The Journal of Experimental Medicine*, 191(10):1661–1674, May 2000.
- [181] Caetano Reis e Sousa. Activation of dendritic cells: translating innate into adaptive immunity. *Current opinion in immunology*, 16(1):21–25, February 2004.
- [182] Wei Hu, Ty Dale Troutman, Ramakrishna Edukulla, and Chandrashekhar Pasare. Priming Microenvironments Dictate Cytokine Requirements for T Helper 17 Cell Lineage Commitment. *Immunity*, 35(6):1010–1022, December 2011.
- [183] Daniel H Kaplan, Botond Z Igyártó, and Anthony A Gaspari. Early immune events in the induction of allergic contact dermatitis. *Nature reviews. Immunology*, 12(2):114–124, February 2012.
- [184] Anne-Laure Flamar, Sandra Zurawski, Felix Scholz, Ingrid Gayet, Ling Ni, Xiao-Hua Li, Eynav Klechevsky, John Quinn, SangKon Oh, Daniel H Kaplan, Jacques Banchereau, and Gerard Zurawski. Noncovalent assembly of anti-dendritic cell antibodies and antigens for evoking immune responses in vitro and in vivo. *Journal of immunology (Baltimore, Md. : 1950)*, 189(5):2645–2655, September 2012.
- [185] H Liu, J Köhler, and G R Fink. Suppression of hyphal formation in *Candida albicans* by mutation of a STE12 homolog. *Science (New York, N.Y.)*, 266(5191):1723–1726, December 1994.
- [186] B R Braun and A D Johnson. Control of filament formation in *Candida albicans* by the transcriptional repressor TUP1. *Science (New York, N.Y.)*, 277(5322):105–109, July 1997.

- [187] J F Staab, S D Bradway, P L Fidel, and P Sundstrom. Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science (New York, N.Y.)*, 283(5407):1535–1538, March 1999.
- [188] Ivaylo I Ivanov, Brent S McKenzie, Liang Zhou, Carlos E Tadokoro, Alice Lepelley, Juan J Lafaille, Daniel J Cua, and Dan R Littman. The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*, 126(6):1121–1133, September 2006.
- [189] Yeonseok Chung, Seon Hee Chang, Gustavo J Martinez, Xuexian O Yang, Roza Nurieva, Hong Soon Kang, Li Ma, Stephanie S Watowich, Anton M Jetten, Qiang Tian, and Chen Dong. Critical Regulation of Early Th17 Cell Differentiation by Interleukin-1 Signaling. *Immunity*, 30(4):576–587, April 2009.
- [190] Aleh Bobr, Botond Z Igyártó, Krystal M Haley, Ming O Li, Richard A Flavell, and Daniel H Kaplan. Autocrine/paracrine TGF- β 1 inhibits Langerhans cell migration. *Proceedings of the National Academy of Sciences of the United States of America*, 109(26):10492–10497, June 2012.
- [191] Andrew E Armitage, Lucy A Eddowes, Uzi Gileadi, Suzanne Cole, Natasha Spottiswoode, Tharini Ashtalakshmi Selvakumar, Ling-Pei Ho, Alain R M Townsend, and Hal Drakesmith. Hepcidin regulation by innate immune and infectious stimuli. *Blood*, 118(15):4129–4139, October 2011.
- [192] F H van Enkevort, M G Netea, A R Hermus, C G Sweep, J F Meis, J W Van der Meer, and B J Kullberg. Increased susceptibility to systemic candidiasis in interleukin-6 deficient mice. *Medical mycology*, 37(6):419–426, December 1999.
- [193] P Bonifazi, T Zelante, C D’Angelo, A De Luca, S Moretti, S Bozza, K Perruccio, R G Iannitti, G Giovannini, C Volpi, F Fallarino, P Puccetti, and L Romani. Balancing inflammation and tolerance in vivo through dendritic cells by the commensal *Candida albicans*. *Mucosal immunology*, 2(4):362–374, July 2009.
- [194] Luigina Romani. Immunity to fungal infections. *Nature reviews. Immunology*, 11(4):275–288, April 2011.
- [195] Jessica Quintin, Sadia Saeed, Joost H A Martens, Evangelos J Giamarellos-Bourboulis, Daniela C Ifrim, Colin Logie, Liesbeth Jacobs, Trees Jansen, Bart-Jan Kullberg, Cisca Wijmenga, Leo A B Joosten, Ramnik J Xavier, Jos W M van der

- Meer, Hendrik G Stunnenberg, and Mihai G Netea. Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. *Cell host & microbe*, 12(2):223–232, August 2012.
- [196] C E Grubin, S Kovats, P deRoos, and A Y Rudensky. Deficient positive selection of CD4 T cells in mice displaying altered repertoires of MHC class II-bound self-peptides. *Immunity*, 7(2):197–208, August 1997.
- [197] James J Moon, H Hamlet Chu, Marion Pepper, Stephen J McSorley, Stephen C Jameson, Ross M Kedl, and Marc K Jenkins. Naïve CD4+ T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. *Immunity*, 27(2):203–213, August 2007.
- [198] Shizuo Akira, Satoshi Uematsu, and Osamu Takeuchi. Pathogen recognition and innate immunity. *Cell*, 124(4):783–801, February 2006.
- [199] Tong Liu, Yong-Jing Gao, and Ru-Rong Ji. Emerging role of Toll-like receptors in the control of pain and itch. *Neuroscience bulletin*, 28(2):131–144, April 2012.
- [200] Giorgio Santoni, Claudio Cardinali, Maria Beatrice Morelli, Matteo Santoni, Massimo Nabissi, and Consuelo Amantini. Danger- and pathogen-associated molecular patterns recognition by pattern-recognition receptors and ion channels of the transient receptor potential family triggers the inflammasome activation in immune cells and sensory neurons. *Journal of neuroinflammation*, 12:21, 2015.
- [201] F A Amaral, D Sachs, V V Costa, C T Fagundes, D Cisalpino, T M Cunha, S H Ferreira, F Q Cunha, T A Silva, J R Nicoli, L Q Vieira, D G Souza, and M M Teixeira. Commensal microbiota is fundamental for the development of inflammatory pain. *Proceedings of the National Academy of Sciences of the United States of America*, 105(6):2193–2197, February 2008.
- [202] Dorothea Terhorst, Rabie Chelbi, Christian Wohn, Camille Malosse, Samira Tamoutounour, Audrey Jorquera, Marc Bajenoff, Marc Dalod, Bernard Malissen, and Sandrine Henri. Dynamics and Transcriptomics of Skin Dendritic Cells and Macrophages in an Imiquimod-Induced, Biphasic Mouse Model of Psoriasis. *The Journal of Immunology*, October 2015.
- [203] Yan Gao, Simone A Nish, Ruoyi Jiang, Lin Hou, Paula Licona-Limón, Jason S Weinstein, Hongyu Zhao, and Ruslan Medzhitov. Control of T Helper 2 Responses

- by Transcription Factor IRF4-Dependent Dendritic Cells. *Immunity*, 39(4):722–732, October 2013.
- [204] Eva Bar, André Gladiator, Sonia Bastidas, Bernd Roschitzki, Hans Acha-Orbea, Annette Oxenius, and Salome LeibundGut-Landmann. A novel Th cell epitope of *Candida albicans* mediates protection from fungal infection. *Journal of immunology (Baltimore, Md. : 1950)*, 188(11):5636–5643, June 2012.
- [205] Clint S Schmidt, C Jo White, Ashraf S Ibrahim, Scott G Filler, Yue Fu, Michael R Yeaman, John E Edwards, and John P Hennessey. NDV-3, a recombinant alum-
adjuvanted vaccine for *Candida* and *Staphylococcus aureus*, is safe and immuno-
genic in healthy adults. *Vaccine*, 30(52):7594–7600, December 2012.
- [206] Philip J Mease, Mark C Genovese, Maria W Greenwald, Christopher T Ritchlin, André D Beaulieu, Atul Deodhar, Richard Newmark, JingYuan Feng, Ngozi Erondu, and Ajay Nirula. Brodalumab, an anti-IL17RA monoclonal antibody, in psoriatic arthritis. *The New England journal of medicine*, 370(24):2295–2306, June 2014.
- [207] Kristian Reich, Richard G Langley, Kim A Papp, Jean-Paul Ortonne, Kristina Unnebrink, Martin Kaul, and Joaquin M Valdes. A 52-week trial comparing briakinumab with methotrexate in patients with psoriasis. *The New England journal of medicine*, 365(17):1586–1596, October 2011.
- [208] Mark Lebwohl, Bruce Strober, Alan Menter, Kenneth Gordon, Jolanta Weglowska, Lluís Puig, Kim Papp, Lynda Spelman, Darryl Toth, Francisco Kerdell, April W Armstrong, Georg Stingl, Alexa B Kimball, Hervé Bachelez, Jashin J Wu, Jeffrey Crowley, Richard G Langley, Tomasz Blicharski, Carle Paul, Jean-Philippe Lacour, Stephen Tyring, Leon Kircik, Sergio Chimenti, Kristina Callis Duffin, Jerry Bagel, John Koo, Gary Aras, Joanne Li, Wenjie Song, Cassandra E Milmont, Yifei Shi, Ngozi Erondu, Paul Klekotka, Brian Kotzin, and Ajay Nirula. Phase 3 Studies Comparing Brodalumab with Ustekinumab in Psoriasis. *The New England journal of medicine*, 373(14):1318–1328, October 2015.
- [209] Richard G Langley, Boni E Elewski, Mark Lebwohl, Kristian Reich, Christopher E M Griffiths, Kim Papp, Lluís Puig, Hidemi Nakagawa, Lynda Spelman, Barour Sigurgeirsson, Enrique Rivas, Tsen-Fang Tsai, Norman Wasel, Stephen Tyring, Thomas Salko, Isabelle Hampele, Marianne Notter, Alexander Karpov,

Silvia Helou, Charis Papavassilis, ERASURE Study Group, and FIXTURE Study Group. Secukinumab in plaque psoriasis—results of two phase 3 trials. *The New England journal of medicine*, 371(4):326–338, July 2014.

- [210] Kenneth B Gordon, Kristina Callis Duffin, Robert Bissonnette, Jörg C Prinz, Yasmine Wasfi, Shu Li, Yaung-Kaung Shen, Philippe Szapary, Bruce Randazzo, and Kristian Reich. A Phase 2 Trial of Guselkumab versus Adalimumab for Plaque Psoriasis. *The New England journal of medicine*, 373(2):136–144, July 2015.