Title
Interactions of Human Immune Responses with Lipid Metabolism and the Skin Microbiome in Acne Vulgaris

Author
Do, Tran Hue

Publication Date
2020

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

The Mechanisms of Lipid and Bacterial Interactions with the Host Immune Response in Acne Vulgaris

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Molecular Biology

by

Tran Hue Do

2020

ABSTRACT OF THE DISSERTATION

The Mechanisms of

Lipid and Bacterial Interactions

with the Host Immune Response in Acne Vulgaris

by

Tran Hue Do

Doctor of Philosophy in Molecular Biology

University of California, Los Angeles, 2020

Professor Robert L. Modlin, Chair

Acne vulgaris is a great example to study the interaction among the environment, microbes, and host immune response. Acne vulgaris is a highly prevalent chronic inflammatory skin disease thought to be caused by increased in sebum secretion, bacterial colonization by the ubiquitous bacterium *Cutibacterium acnes (C. acnes),* and host inflammatory response. As a major factor implicated acne pathology, sebum and its composition, squalene, are unique to humans. Sebaceous lipids accumulate in the follicular duct are oxidized by *C. acnes* lipase leading to bacterial proliferation. The direct cause-and-effect relationship between the bacteria has been difficult to establish given that *C. acnes* is a ubiquitous bacterium and that there was no quantitative difference in the number of bacteria between subjects with and without acne. Recent genomic and phenotypic analyses on acne clinical samples revealed different phylotypes of *C. acnes* coexist in the pilosebaceous unit with other *Cutibacterium* spp. included *C. granulosum* and *C. humerusii*. Studies also showed strains phylotype IA1 are more abundant with the skin on acne patients (C_A) and phylotype II are more associated with healthy skin (C_H).

However, the mechanisms underlying the lipid and bacterial interaction to cause the onset of inflammation and subsequent development of acne are still not understood.

This dissertation begins with a detailed analysis using single cell RNA-seq to deconstruct the genetic and molecular profiles of individual cells to study differences in lesional and non-lesional skin of acne patients. Particularly we were interested in changes within the same cell types to understand how normal skin becomes inflamed acne skin. We identified that a TREM2 macrophage subpopulation, expressing a lipid gene metabolism program, is associated with acne lesions. We further discovered that squalene induces $TREM2^+$ macrophages in vitro to have enhanced phagocytosis but minimal antimicrobial activity against *C. acnes*. As a result, the squalene induced $TREM2^+$ macrophage perpetuates inflammation linking the excess lipid production in acne to inflammation in acne.

Next, we investigated clinical strains of *C. acnes*, C_A and C_H, to understand how the microbiome interact in acne lesions. These strains differ in their ability to trigger inflammation with C_A induces higher pro-inflammatory cytokine secretion compared to C_H in cell culture and mouse infection model. However, the virulence factor that accounts for the differences in immune response between C_A and C_H are still unknown. In this study, we showed that *C. acnes* activates the innate immune system through RNA species that are usually reserved for viral detection. Interestingly, RNA species from C_A and C_H have different bioanalyzer profiles and can trigger distinct immune response as seen with live bacteria. We also showed that C_A RNA stimulate the immune response through a TLR-8/IL-18/IL-12p40 pathway. Our in vitro data correlates well with our scRNA-seq data showing the abundance of IFN-γ and TLR-8 in acne lesions.

Taken together, the results enhanced our understanding of the impact of squalene, a sebum lipid, and resident microbes on the immunological functions of the skin. This work advanced our knowledge of the environment-microbe-host interplay occurring at the

pilosebaceous follicle and introduced a novel way of thinking about the mechanisms underlying the initiation and pathogenesis of acne vulgaris and inflammatory skin conditions.

The dissertation of Tran Hue Do is approved.

Steven Bensinger

Peter J. Bradley

Timothy E. O'Sullivan

Matteo Pellegrini

Robert L. Modlin, Committee Chair

University of California, Los Angeles

2020

TABLE OF CONTENTS

ABSTRACT OF THE DISSERTATION……...ii

COMMITTEE PAGE..……...v

TABLE OF CONTENTS……..……vi

LIST OF FIGURES, TABLES, AND SUPPLEMENTARY MATERIALS ……..……...vii

ACKNOWLEDGEMENTS……..……viii

VITA……..……..x

CHAPTER 1: Introduction……1

CHAPTER 2: Single cell Transcriptomic Analyses Reveal TREM2 Macrophages as a Possible Lipid Sensor Modulating Inflammation in Acne Lesion……..……...14

Abstract……14

Introduction……..……14

Results……..…16

Discussion……..……23

Materials and Methods……..……29

Figures and Figure Legends……..……34

CHAPTER 3: C. acnes RNA Stimulate IFN-γ Release in a TLR-8/IL12/IL-18 Pathway…….....62

Abstract……62

Introduction……..……63

Results……..……64

Discussion……..……67

Materials and Methods……70

CHAPTER 4: Conclusions……..…….....88

LIST OF FIGURES, TABLES, AND SUPPLEMENTARY MATERIALS

Figure 1. 1 Cell Types Recovered in Lesional and Non-lesional Acne.....34

Figure 1.2. Unique Myeloid Cell Type Associated with Acne Lesions 35

Figure 1.3 KCs in the hair follicles present in acne lesions is capable of squalene synthesis....37

Figure 1.4. Characterization of TREM2^{+} Macrophages.....39

Figure 1.5. Squalene Induce TREM2 Macrophage Differentiation.....41

Figure 1.6. Squalene/M-CSF Macrophage Have Increased Phagocytosis but No Antimicrobial Activity Against *C. acnes.*.....43

Figure 1.7. Model of Acne Inflammation with TREM2^{+} Macrophage.....44

Supplemental Table 1.1. Clinical characteristics of acne subjects.....45

Supplemental Table 1.2. 26 consensus signature genes from seven TREM2 macrophage studies.....46

Supplemental Fig. 1.1. Undetectable Expression of Canonical Sebocyte Markers.....47

Supplemental Fig. 1.2. Diversity of Fibroblasts and Endothelial Cells in Acne lesions.....48

Supplementary Fig. 1.3. Diversity of Lymphocytes in Acne Biopsies.....50

Supplemental Fig. 1.4. Detectable TREM2 signature not part of acne signature genes.....52

Fig. 2.1. C_A infection induces IFN-γ secretion at an early time point.....75

Fig. 2.2. C_A infection induces IFN-γ secretion at an early time point.....76

Fig. 2.3. TLR-8, IL-18, and IL-12p40 are required IFN-γ secretion.....78

Fig. 2.4. C_A RNA induces higher secretion of IFN-γ, IL12p40, and IL-18 compared to C_H RNA similar to the live bacteria response.....79

Fig. 2.5. C_A and C_H harbor different species of bacterial RNA.....80

Fig. 2.6. In vivo scRNA-seq data from acne lesions confirm higher expression of *TLR8*, *IL18*, and *IFNG*..... 81

Fig. 2.7. Proposed model of *C. acnes* infection with bacterial RNA stimulating TLR-8.....82

ACKNOWLEDGEMENTS

I would first like to begin by thanking Professor Robert Lazarus Modlin for his guidance and support throughout my time as a graduate student in his laboratory. Dr. Modlin has been an excellent mentor broadening my knowledge, helping me become an independent researcher, and always encouraging me to try the latest and most effective technologies. He also taught me to not be afraid to reach out to other experts in the field for advice and collaborations. I have grown tremendously during my graduate career and attribute a tremendous amount of this growth to Dr. Modlin's dedication as a mentor and his lead by example. Furthermore, I would like to thank the members of my committee, Professors Steven Bensinger, Peter J. Bradley, Timothy E. O'Sullivan, and Matteo Pellegrini for their helpful scientific discussion and constant support for my project.

I would also like to express my gratitude to numerous current and past members of the Modlin Laboratory for their support and assistance over the years. From teaching me how to perform various techniques to their intellectual contributions to their emotional support, my fellow lab members have contributed greatly to my development as a scientist. Dr. Laura Marinelli taught me the skills to work *C. acnes*. Aaron Choi, Armando Puente, Roksana Shirazi, Kindra Kelly Scumpia, and Bruno de Andrade Silva for their help in cell culture room and scientific discussion throughout the years. Also, I'd like to acknowledge Dr. Barry R. Bloom, who had given great input and constructive feedback on my project over the years.

Finally, I would like to thank many people for their continue support in my personal life. First and foremost, to my fiancé Jonathan Tsang, who has been there for me contributing to scientific discussion and caring for my well-being. I would also thank my family, Hue, Mung, and Angelie for their support and encouragement in this long MD/PhD journey. I would also like to

thank all the friends I have made and grown closer to at UCLA for making my time outside of lab enjoyable.

Chapter II is a version of a submitted manuscript: Tran H Do, Bruno J. de Andrade Silva, Xianying Xing, Feiyang Ma, Carol Cheng, Jenny Kim, Johann Gudjonsson, Matteo Pellegrini, and Robert L. Modlin. Single cell Transcriptomic Analyses Reveal TREM2 Macrophages as a Possible Lipid Sensor Modulating Inflammation in Acne Lesion. I was the primary researcher and author for the manuscript. The co-authors listed above either provided clinical samples or support for the research.

Chapter III is based on unpublished data currently being prepared for submission for publication: Tran H Do and Robert L. Modlin. *C. acnes* RNA Regulate IFN-γ Release in *Cutibacterium acnes* Infection. I was the primary researcher and author for the manuscript. Dr. Robert L. Modlin provided support for the research.

My graduate studies were funded by UCLA MSTP Training Grant (T32GM008042), Dermatology Scientist Training Program (T32AR071307), and support from the Modlin Laboratory.

VITA

EDUCATION

2016-present Ph.D. Candidate, Molecular Biology
University of California, Los Angeles
Molecular Biology Interdepartmental Doctoral Program
Los Angeles, CA

2010-2014 B. S. Chemical Biology
University of California, Berkeley
Department of Chemistry
Berkeley, CA

HONORS AND AWARD

2019 UCLA Dermatology Fund
UCLA Dermatology Department
Los Angeles, CA

2017-2020 UCLA T32 Dermatology Investigator Training Program
UCLA Dermatology Department
Los Angeles, CA

2016 Addressing Childhood Obesity Proposal Challenge Winner
Los Angeles County Medical Association
Los Angeles, CA

2012-2014 Initiative for Maximizing Student Development Grant Recipient
University of California, Berkeley
Berkeley, CA

2010-2014 Incentive Award Program Scholarship Recipient

University of California, Berkeley

Berkeley, CA

2010 Bay Area Affiliate Awards for Aspirations in Computing

WORK EXPERIENCE

2017-2019 Medical School Tutor

David Geffen School of Medicine

Los Angeles, CA

2012-2014 Peer Academic Counselor

University of California, Berkeley

Berkeley, CA

2011-2012 Writing Tutor

University of California, Berkeley

Berkeley, CA

PUBLICATIONS

Do, T.H, Silva B. A., Xing, X., Ma, F., Cheng, C., Kim, J. Gudjonsson, J., Pellegrini, M., Modlin, R.L. (2020). *Single cell Transcriptomic Analyses Reveal TREM2 Macrophages as a Possible Lipid Sensor Modulating Inflammation in Acne Lesion*. Manuscript in preparation.

Do, T.H., Modlin, R.L., *C. acnes RNA Regulate IFN-γ Release in Cutibacterium acnes Infection.* Manuscript in preparation.

Hughes, T.K., Wadsworth, M.H., Gierahn, T.M., Do, T…Shalek, A.K. *Highly Efficient, Massively Parallel Single-Cell RNA-Seq Reveals Cellular States and Molecular Features of Human Skin Pathology*. Manuscript in review.

CHAPTER 1

INTRODUCTION

The skin is the human largest organ and home to a vast microbial community, with 10^{10} bacteria across the $1.8m^2$ of the skin surface (Grice, Kong et al. 2008). It serves a vital role as our body's first line of defense against environment stimuli and potentially pathogenic microbes we encounter. The skin exists in a unique immunological state. It must respond to the environment, tolerate and avoid reacting to resident microbes under normal conditions while ready to defend against any invading pathogens. The ecology of the different organisms in the skin is highly influenced by the vastly different environments across the body (Grice and Segre 2011, Byrd, Belkaid et al. 2018). It hosts diverse communities of microorganisms on and within the skin and its appendages. Under normal conditions, these resident microbes adapt their environment and exist without eliciting an inflammatory response (Byrd, Belkaid et al. 2018). These microbes also acclimate to various host immunological factors and behavior. On the other hand, cells in the skin are tasked with the unique challenge of harboring the microbes without inducing unwarranted inflammation. But at the time of an invading microbe or a commensal becomes pathogenic and outcompetes the other microflora, the resident cells must act quickly and efficiently to protect the host from invading microbes (O'Neill and Gallo 2018). Of course, this execution is not perfect. Sometimes, the activation of immune pathways is prolonged due to the continued presence of the stimuli or the immune system failed to dampen the inflammation process. This leads to unwarranted inflammation resulting irreversible host tissue damage (Holland and Jeremy 2005). Inflammation localized to the pilosebaceous unit is often the defining feature of acne vulgaris.

Acne vulgaris is a chronic inflammatory skin disease that affects more than 5 million Americans annually (Lim, Collins et al. 2017). In the U.S, acne is the most diagnosed skin condition as well as the most common condition seen by a dermatologist (Lim, Collins et al. 2017). Most people experience acne during early adolescence, with boys are more likely be affected than girls, >95% versus 85%, respectively (Burton, Cunliffe et al. 1971). About 20% of young patients have moderate to severe acne that negatively affect their quality of life, more than 50% of patients continue to suffer with acne beyond their adolescent years (Karimkhani, Dellavalle et al. 2017, Thiboutot, Dreno et al. 2018). Patients are often (Ghodsi, Orawa et al. 2009) concerned about the redness, pain, post-inflammatory hyperpigmentation, and scars that are associated with inflammation-induced damage (Thiboutot, Dreno et al. 2018). The effects of acne are not limited to the visible lesions on the skin but also affecting adolescents and young contributing significantly to their low self-esteem (Nguyen, Koo et al. 2016), anxiety, depression, and increased risk of suicide (Purvis, Robinson et al. 2006, Vallerand, Lewinson et al. 2018). Acne is estimated to lead to suicidal ideation in 7% of patients (Picardi, Mazzotti et al. 2006). The disease develops from the pilosebaceous unit of the skin, which consists of hair shaft, hair follicle, sebaceous gland, and erector pili muscle. These units are most dense on scalp, face, neck, and shoulder, followed by torso and limbs (Wertz 2009). Palms, soles, and top of the feet are the only regions without any pilosebaceous units. The sebaceous gland connects to the hair follicle by a short sebaceous duct, in which the sebum flows. These are different from sebaceous follicle, which are less abundant and are not associated with major hair follicle. Acne vulgaris is an interplay of altered sebum secretion, hyperproliferation of keratinocytes, bacterial colonization by *Cutibacterium acnes (C. acnes)*, and activated host innate immunity (Holland and Jeremy 2005). Acne typically starts in early puberty with maturation of the androgen gland leading to increase in sebum production along with the appearance of pubic and axillary hair (Lucky 1998).

Sebum is secreted by the sebaceous gland and comprises of squalene, triglycerides, free fatty acids, wax ester, cholesterol, and cholesteryl ester. Sebum production can be activated by histamines, androgens, stress, and diet (Pelle, McCarthy et al. 2008, Zouboulis 2009). Sebaceous glands functions as its own endocrine organ responding to corticotropin-releasing hormone in times of stress mediating acerbation of acne (Zouboulis and Böhm 2004). They can also produce a variety of antimicrobial peptides, neuropeptides, and antibacterial lipids such as sapienic acid (Lovászi, Szegedi et al. 2017) . Increased sebum secretion from the pilosebaceous unit contributes to the pathogenesis of acne and likely triggers the inflammatory response that clinically present as comedones, papules, pustules, and nodules (Smith and Thiboutot 2008, Zouboulis, Jourdan et al. 2014, Lovaszi, Mattii et al. 2017). Acne generally occurs on areas with more sebaceous glands such as face, shoulders, upper chest, and back (Lovászi, Szegedi et al. 2017). Composition of the sebum also changes in acne patients with squalene representing a greater proportion of total sebaceous lipids with more than a 2-fold increase in production (Pappas, Johnsen et al. 2009). Mediated by leukotriene B4, squalene can stimulate keratinocyte proliferation and secretion of inflammatory responses (Alestas, Ganceviciene et al. 2006). Changes in sebum lipids either induce defective keratinocyte differentiation, leading to hyperkeratinization, or induce interleukin 1 (IL-1) secretion that leads to the formation of hyperkeratinization leading to comedone formation (Lovászi, Szegedi et al. 2017). Although the onset of inflammation is still highly debated, once inflammation is established, inflammatory acne lesions are characterized by upregulation of matrix metalloproteinases (MMP), β-defensins 4, IL-8, and granulysin (Trivedi, Gilliland et al. 2006). NF-kB pathways are also activated in acne lesions upregulating IL-8, IL-1β, and tumor necrosis factor (TNF) (Kang, Cho et al. 2005). Increased IL-8 attract inflammatory cells such as neutrophils and macrophages. IL-1β and TNF are known to upregulate ICAM-1 and VCAM-1, which are also increased on endothelial cells in inflammatory acne papules (Kang, Cho et al.

2005). IL-17A+ T cells and T helper 17 (Th17)-related cytokines are also present in acne lesions (Kelhala, Palatsi et al. 2014). Many of these pathways have been shown to be induced in the presence of *C. acnes*.

C. acnes is a microaerophilic, Gram-positive bacterium that is ubiquitous on human skin, representing 90% of the skin total bacteria (Fitz-Gibbon, Tomida et al. 2013). *C. acnes* derives nutrients for growth from sebum. During puberty, the marked increase in *C. acnes* colonization correlate with the maturation of sebaceous glands (McGinley, Webster et al. 1980). *C*. *acnes* co-exists in acne lesions with many other species of bacteria (Whiting 1979, Bialecka, Mak et al. 2005). *C. acnes* overgrowth is strongly related to the progression of acne vulgaris (Russell 2000, Nakatsuji, Tang et al. 2011), However, a direct cause-effect relationship between the presence of *C. acnes* and acne vulgaris have difficult to prove given that the amount of *C. acnes* on the skin was similar between patients with acne and normal skin. A metagenomic analysis showed that the strain phylotypes were different between the two groups (Fitz-Gibbon, Tomida et al. 2013). Full-genome sequencing with SNP-based phylotyping in conjunction with ribotyping based on 16S rRNA (Fitz-Gibbon, Tomida et al. 2013, Tomida, Nguyen et al. 2013) revealed that phylotype based on *tly* and *recA* genes, IA1, and ribotype (RT) 4 are associated with acne. The acne-associated types (C_A) were present in significant quantities in approximately 30–40% of patients with acne but rarely in individuals with healthy skin. Conversely, the phylotype II, RT 6 subgroup was found to be 99% associated with healthy skin (C_H) (Fitz-Gibbon, Tomida et al. 2013). These strains also have different inflammatory potential: C_A induces a pro-inflammatory, IFN-γ and IL-17 release while C_H induces a more anti-inflammatory, IL-10 response (Yu, Champer et al. 2016). *C. acnes* triggers toll-like receptor 2 (TLR-2) activation leading NF-κB activation and release of TNF and IL-8 (Takeuchi, Hoshino et al. 1999, Kim, Ochoa et al. 2002). *C. acnes* also promotes Th1 and Th17 response with IFN-γ and IL-17A secretion by CD4+ T cells, discovered both in vitro and in acne lesions (Agak, Qin et al. 2014, Yu, Champer et al.

2016, Agak, Kao et al. 2018). *C. acnes* can also activate NOD-, LRR- and pyrin-containing 3 (NLRP3) inflammasome resulting in IL-1β and IL-18 secretion (Kistowska, Gehrke et al. 2014, Li, Lin et al. 2019) as well as upregulate the expression of pro-inflammatory cytokines and antimicrobial peptide secretion in cultured sebocytes and keratinocytes (Graham, Farrar et al. 2004, Sanford, O'Neill et al. 2019).

There is a need to advance the understanding of acne pathophysiology to address the lack of new acne therapy. Topical treatments are effective in treating open and closed comedones with few inflammatory lesions. Benzoyl peroxide has antimicrobial activity against *C. acnes in vitro*, but it causes irritation, redness, and scaling at the concentrations required for topical therapy (Ozolins, Eady et al. 2004). These topical often work by application when applied to the whole area, but often comedones may not be readily visible. Topical retinoids work for both comedonal and inflammatory acne but are contraindicated in pregnant women, and all women of childbearing age must use effective contraception (Blasiak, Stamey et al. 2013). All topical retinoids induce local reactions and can increase skin sensitivity to ultraviolet light. Topical antibiotics, such as clindamycin, erythromycin, and tetracycline, are often used in combination therapy with either benzoyl peroxide or topical retinoids (Ozolins, Eady et al. 2004). However, efficacy of topical antibiotics might be declining with increased bacterial resistance. Systemic retinoids are effective but have multi-system side effects, including teratogenicity (Blasiak, Stamey et al. 2013). Oral antibiotic therapy is often inadequate because of systemic effects and a ~60% resistance rate in the target organism (Leyden, McGinley et al. 1975, Eady, Cove et al. 1989, Cooper 1998, Coates, Vyakrnam et al. 2002, Ross, Snelling et al. 2003). Combined oral contraceptive are effective for women doubling as acne treatment and birth control. However, the efficacy of oral contraceptive only works for some women and contraindicated in women with increased risk of thromboembolism (Arowojolu, Gallo et al. 2012). Over the last several decades, there has been little progress and minimal research into

advancing treatment through a better fundamental understanding of the pathophysiology of acne.

Acne vulgaris is an excellent model to study the interaction among local environment, microbiome, and the immune response. New insights into host-microbe interactions and the composition of the acne microbiome now make it possible to significantly advance the understanding of acne pathophysiology. We began our studies with the question of what are the mechanisms underlying the onset of inflammation in acne? We hypothesized that changes in sebum production and the presence of distinct *C. acnes* phylotypes differentially trigger inflammation. New technology such as scRNA-seq is a key innovation towards defining at single-cell resolution the diverse cell types/states and pathways that drive complex biological systems. We first used scRNA-seq to define the cell types and immune circuits present in acne lesions. We also dissected the mechanisms that underlie the induction of pro-inflammatory in lesional and non-lesional skin of acne patients. We then were able to differentiate monocytes to a specialized population of macrophages that are like those found in the lesion to study their ability to regulate inflammation. Finally, we investigate how different strains of *C. acnes* with different inflammatory potential to trigger inflammation.

References

Agak, G. W., S. Kao, K. Ouyang, M. Qin, D. Moon, A. Butt and J. Kim (2018). "Phenotype and Antimicrobial Activity of Th17 Cells Induced by Propionibacterium acnes Strains Associated with Healthy and Acne Skin." J Invest Dermatol **138**(2): 316-324.

Agak, G. W., M. Qin, J. Nobe, M. H. Kim, S. R. Krutzik, G. R. Tristan, D. Elashoff, H. J. Garban and J. Kim (2014). "Propionibacterium acnes Induces an IL-17 Response in Acne Vulgaris that Is Regulated by Vitamin A and Vitamin D." J Invest Dermatol **134**(2): 366-373.

Alestas, T., R. Ganceviciene, S. Fimmel, K. Müller-Decker and C. C. Zouboulis (2006). "Enzymes involved in the biosynthesis of leukotriene B 4 and prostaglandin E 2 are active in sebaceous glands." Journal of Molecular Medicine **84**(1): 75-87.

Arowojolu, A. O., M. F. Gallo, L. M. Lopez and D. A. Grimes (2012). "Combined oral contraceptive pills for treatment of acne." Cochrane Database of Systematic Reviews(7).

Bialecka, A., M. Mak, R. Biedron, M. Bobek, A. Kasprowicz and J. Marcinkiewicz (2005). "Different pro-inflammatory and immunogenic potentials of Propionibacterium acnes and Staphylococcus epidermidis: implications for chronic inflammatory acne." Arch Immunol Ther Exp (Warsz) **53**(1): 79-85.

Blasiak, R. C., C. R. Stamey, C. N. Burkhart, A. Lugo-Somolinos and D. S. Morrell (2013). "High-dose isotretinoin treatment and the rate of retrial, relapse, and adverse effects in patients with acne vulgaris." JAMA Dermatol **149**(12): 1392-1398.

Burton, J., W. Cunliffe, I. STAFFORD and S. SHUSTER (1971). "The prevalence of acne vulgaris in adolescence." British Journal of Dermatology **85**(2): 119-126.

Byrd, A. L., Y. Belkaid and J. A. Segre (2018). "The human skin microbiome." Nat Rev Microbiol **16**(3): 143-155.

Coates, P., S. Vyakrnam, E. A. Eady, C. E. Jones, J. H. Cove and W. J. Cunliffe (2002). "Prevalence of antibiotic-resistant propionibacteria on the skin of acne patients: 10-year surveillance data and snapshot distribution study." Br J Dermatol **146**(5): 840-848.

Cooper, A. J. (1998). "Systematic review of Propionibacterium acnes resistance to systemic antibiotics." Med J Aust **169**(5): 259-261.

Eady, E. A., J. H. Cove, K. T. Holland and W. J. Cunliffe (1989). "Erythromycin resistant propionibacteria in antibiotic treated acne patients: association with therapeutic failure." Br J Dermatol **121**: 51-57.

Fitz-Gibbon, S., S. Tomida, B. H. Chiu, L. Nguyen, C. Du, M. Liu, D. Elashoff, M. C. Erfe, A. Loncaric, J. Kim, R. L. Modlin, J. F. Miller, E. Sodergren, N. Craft, G. M. Weinstock and H. Li (2013). "Propionibacterium acnes strain populations in the human skin microbiome associated with acne." J Invest Dermatol **133**(9): 2152-2160.

Ghodsi, S. Z., H. Orawa and C. C. Zouboulis (2009). "Prevalence, severity, and severity risk factors of acne in high school pupils: a community-based study." Journal of investigative Dermatology **129**(9): 2136-2141.

Graham, G., M. Farrar, J. Cruse-Sawyer, K. Holland and E. Ingham (2004). "Proinflammatory cytokine production by human keratinocytes stimulated with Propionibacterium acnes and P. acnes GroEL." British Journal of Dermatology **150**(3): 421-428.

Grice, E. A., H. H. Kong, G. Renaud, A. C. Young, G. G. Bouffard, R. W. Blakesley, T. G. Wolfsberg, M. L. Turner and J. A. Segre (2008). "A diversity profile of the human skin microbiota." Genome research **18**(7): 1043-1050.

Grice, E. A. and J. A. Segre (2011). "The skin microbiome." Nature Reviews Microbiology **9**(4): 244-253.

Holland, D. B. and A. H. Jeremy (2005). "The role of inflammation in the pathogenesis of acne and acne scarring." Semin Cutan Med Surg **24**(2): 79-83.

Kang, S., S. Cho, J. H. Chung, C. Hammerberg, G. J. Fisher and J. J. Voorhees (2005). "Inflammation and extracellular matrix degradation mediated by activated transcription factors nuclear factor-κB and activator protein-1 in inflammatory acne lesions in vivo." The American journal of pathology **166**(6): 1691-1699.

Karimkhani, C., R. P. Dellavalle, L. E. Coffeng, C. Flohr, R. J. Hay, S. M. Langan, E. O. Nsoesie, A. J. Ferrari, H. E. Erskine, J. I. Silverberg, T. Vos and M. Naghavi (2017). "Global Skin Disease Morbidity and Mortality: An Update From the Global Burden of Disease Study 2013." JAMA Dermatol **153**(5): 406-412.

Kelhala, H. L., R. Palatsi, N. Fyhrquist, S. Lehtimaki, J. P. Vayrynen, M. Kallioinen, M. E. Kubin, D. Greco, K. Tasanen, H. Alenius, B. Bertino, I. Carlavan, B. Mehul, S. Deret, P. Reiniche, P. Martel, C. Marty, U. Blume-Peytavi, J. J. Voegel and A. Lauerma (2014). "IL-17/Th17 pathway is activated in acne lesions." PLoS One **9**(8): e105238.

Kim, J., M. T. Ochoa, S. R. Krutzik, O. Takeuchi, S. Uematsu, A. J. Legaspi, H. D. Brightbill, D. Holland, W. J. Cunliffe, S. Akira, P. A. Sieling, P. J. Godowski and R. L. Modlin (2002). "Activation of toll-like receptor 2 in acne triggers inflammatory cytokine responses." J Immunol **169**(3): 1535-1541.

Kistowska, M., S. Gehrke, D. Jankovic, K. Kerl, A. Fettelschoss, L. Feldmeyer, G. Fenini, A. Kolios, A. Navarini, R. Ganceviciene, J. Schauber, E. Contassot and L. E. French (2014). "IL-1beta drives inflammatory responses to propionibacterium acnes in vitro and in vivo." J Invest Dermatol **134**(3): 677-685.

Kistowska, M., B. Meier, T. Proust, L. Feldmeyer, A. Cozzio, T. Kuendig, E. Contassot and L. E. French (2015). "Propionibacterium acnes promotes Th17 and Th17/Th1 responses in acne patients." J Invest Dermatol **135**(1): 110-118.

Leyden, J. J., K. J. McGinley, O. H. Mills and A. M. Kligman (1975). "Propionibacterium levels in patients with and without acne vulgaris." J Invest Dermatol **65**(4): 382-384.

Li, X.-J., X.-Y. Lin, Z. Shen, Q. Deng, Y. Liu, S. Cheng and W. Liu (2019). "IL-18 and NLRP3 in the Process of Acnevulgaris." Sichuan da xue xue bao. Yi xue ban= Journal of Sichuan University. Medical science edition **50**(1): 66-70.

Lim, H. W., S. A. B. Collins, J. S. Resneck, Jr., J. L. Bolognia, J. A. Hodge, T. A. Rohrer, M. J. Van Beek, D. J. Margolis, A. J. Sober, M. A. Weinstock, D. R. Nerenz, W. Smith Begolka and J. V. Moyano (2017). "The burden of skin disease in the United States." J Am Acad Dermatol **76**(5): 958-972 e952.

Lovaszi, M., M. Mattii, K. Eyerich, A. Gacsi, E. Csanyi, D. Kovacs, R. Ruhl, A. Szegedi, L. Kemeny, M. Stahle, C. C. Zouboulis, S. Eyerich and D. Torocsik (2017). "Sebum lipids influence macrophage polarization and activation." Br J Dermatol **177**(6): 1671-1682.

Lovászi, M., A. Szegedi, C. C. Zouboulis and D. Törőcsik (2017). "Sebaceous-immunobiology is orchestrated by sebum lipids." Dermato-endocrinology **9**(1): e1375636.

Lovászi, M., A. Szegedi, C. C. Zouboulis and D. Törőcsik (2017). "Sebaceous-immunobiology is orchestrated by sebum lipids." Dermato-Endocrinology **9**(1).

Lucky, A. W. (1998). "A Review of Infantile and PediatricAcne." Dermatology **196**(1): 95-97.

McGinley, K., G. Webster, M. Ruggieri and J. Leyden (1980). "Regional variations in density of cutaneous propionibacteria: correlation of Propionibacterium acnes populations with sebaceous secretion." Journal of Clinical Microbiology **12**(5): 672-675.

Mouser, P. E., B. S. Baker, E. D. Seaton and A. C. Chu (2003). "Propionibacterium acnes-reactive T helper-1 cells in the skin of patients with acne vulgaris." J Invest Dermatol **121**(5): 1226-1228.

Nakatsuji, T., D. C. Tang, L. Zhang, R. L. Gallo and C. M. Huang (2011). "Propionibacterium acnes CAMP factor and host acid sphingomyelinase contribute to bacterial virulence: potential targets for inflammatory acne treatment." PLoS One **6**(4): e14797.

Nguyen, C. M., J. Koo and K. M. Cordoro (2016). "Psychodermatologic effects of atopic dermatitis and acne: a review on self-esteem and identity." Pediatric dermatology **33**(2): 129-135.

Norris, J. F. and W. J. Cunliffe (1988). "A histological and immunocytochemical study of early acne lesions." Br J Dermatol **118**(5): 651-659.

O'Neill, A. M. and R. L. Gallo (2018). "Host-microbiome interactions and recent progress into understanding the biology of acne vulgaris." Microbiome **6**(1).

Ozolins, M., E. A. Eady, A. J. Avery, W. J. Cunliffe, A. L. W. Po, C. O'Neill, N. B. Simpson, C. E. Walters, E. Carnegie and J. B. Lewis (2004). "Comparison of five antimicrobial regimens for treatment of mild to moderate inflammatory facial acne vulgaris in the community: randomised controlled trial." The Lancet **364**(9452): 2188-2195.

Pappas, A., S. Johnsen, J.-C. Liu and M. Eisinger (2009). "Sebum analysis of individuals with and without acne." Dermato-endocrinology **1**(3): 157-161.

Pelle, E., J. McCarthy, H. Seltmann, X. Huang, T. Mammone, C. C. Zouboulis and D. Maes (2008). "Identification of histamine receptors and reduction of squalene levels by an antihistamine in sebocytes." Journal of investigative dermatology **128**(5): 1280-1285.

Picardi, A., E. Mazzotti and P. Pasquini (2006). "Prevalence and correlates of suicidal ideation among patients with skin disease." J Am Acad Dermatol **54**(3): 420-426.

Purvis, D., E. Robinson, S. Merry and P. Watson (2006). "Acne, anxiety, depression and suicide in teenagers: A cross-sectional survey of New Zealand secondary school students." Journal of paediatrics and child health **42**(12): 793-796.

Ross, J. I., A. M. Snelling, E. Carnegie, P. Coates, W. J. Cunliffe, V. Bettoli, G. Tosti, A. Katsambas, G. P. D. P. JI, O. Rollman, L. Torok, E. A. Eady and J. H. Cove (2003). "Antibiotic-resistant acne: lessons from Europe." Br J Dermatol **148**(3): 467-478.

Russell, J. J. (2000). "Topical therapy for acne." Am Fam Physician **61**(2): 357-366.

Sanford, J. A., A. M. O'Neill, C. C. Zouboulis and R. L. Gallo (2019). "Short-Chain Fatty Acids from Cutibacterium acnes Activate Both a Canonical and Epigenetic Inflammatory Response in Human Sebocytes." J Immunol **202**(6): 1767-1776.

Smith, K. R. and D. M. Thiboutot (2008). "Thematic review series: skin lipids. Sebaceous gland lipids: friend or foe?" J Lipid Res **49**(2): 271-281.

Takeuchi, O., K. Hoshino, T. Kawai, H. Sanjo, H. Takada, T. Ogawa, K. Takeda and S. Akira (1999). "Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components." Immunity **11**(4): 443-451.

Thiboutot, D. M., B. Dreno, A. Abanmi, A. F. Alexis, E. Araviiskaia, M. I. Barona Cabal, V. Bettoli, F. Casintahan, S. Chow, A. da Costa, T. El Ouazzani, C. L. Goh, H. P. M. Gollnick, M. Gomez, N. Hayashi, M. I. Herane, J. Honeyman, S. Kang, L. Kemeny, R. Kubba, J. Lambert, A. M. Layton, J. J. Leyden, J. L. Lopez-Estebaranz, N. Noppakun, F. Ochsendorf, C. Oprica, B. Orozco, M. Perez, J. Piquero-Martin, J. A. See, D. H. Suh, J. Tan, V. T. Lozada, P. Troielli and L. F. Xiang (2018). "Practical management of acne for clinicians: An international consensus from the Global Alliance to Improve Outcomes in Acne." J Am Acad Dermatol **78**(2 Suppl 1): S1-S23 e21.

Tomida, S., L. Nguyen, B. H. Chiu, J. Liu, E. Sodergren, G. M. Weinstock and H. Li (2013). "Pan-genome and comparative genome analyses of propionibacterium acnes reveal its genomic diversity in the healthy and diseased human skin microbiome." mBio **4**(3): e00003-00013.

Trivedi, N. R., K. L. Gilliland, W. Zhao, W. Liu and D. M. Thiboutot (2006). "Gene array expression profiling in acne lesions reveals marked upregulation of genes involved in inflammation and matrix remodeling." J Invest Dermatol **126**(5): 1071-1079.

Vallerand, I., R. Lewinson, L. Parsons, M. Lowerison, A. Frolkis, G. Kaplan, C. Barnabe, A. Bulloch and S. Patten (2018). "Risk of depression among patients with acne in the UK: a population-based cohort study." The British journal of dermatology **178**(3): e194.

Wertz, P. W. (2009). Changes in epidermal lipids and sebum secretion with aging. Skin Aging Handbook, Elsevier**:** 91-104.

Whiting, D. A. (1979). "Acne." West J Med **131**(6): 551-557.

Yu, Y., J. Champer, G. W. Agak, S. Kao, R. L. Modlin and J. Kim (2016). "Different Propionibacterium acnes Phylotypes Induce Distinct Immune Responses and Express Unique Surface and Secreted Proteomes." J Invest Dermatol **136**(11): 2221-2228.

Zouboulis, C., E. Jourdan and M. Picardo (2014). "Acne is an inflammatory disease and alterations of sebum composition initiate acne lesions." Journal of the European Academy of Dermatology and Venereology **28**(5): 527-532.

Zouboulis, C. C. (2009). "Sebaceous gland receptors." Dermato-endocrinology **1**(2): 77-80.

Zouboulis, C. C. and M. Böhm (2004). "Neuroendocrine regulation of sebocytes–a pathogenetic link between stress and acne." Experimental dermatology **13**: 31-35.

CHAPTER 2

Single cell Transcriptomic Analyses Reveal TREM2 Macrophages as a Possible Lipid Sensor Modulating Inflammation in Acne Lesion

Abstract

The pathogenesis of acne involves the excess production of lipids by the sebaceous hair follicle, which is colonized by *Cutibacterium acnes (C. acnes)*, resulting in a host inflammatory response. The mechanisms by which these lipids modulate distinct cell populations promoting inflammation at the site of the disease remain unclear. Here, we used scRNA-seq analysis to comprehensively map cell populations and their gene expression profiles in inflamed lesions and non-lesional skin from acne patients. We identified that a TREM2 macrophage subpopulation, expressing a lipid gene metabolism program as well as *IL18*, is associated with acne lesions. We further discovered that squalene, excessively secreted by the human pilosebaceous unit in acne, induces $TREM2^+$ macrophages in vitro to have enhanced phagocytosis but no antimicrobial activity against *C. acnes*, yet secrete IL-18. Thus, the squalene induced $TREM2^+$ macrophage perpetuates inflammation linking the excess lipid production in acne to inflammation in acne. The ability of squalene to induce $TREM2^+$ macrophage differentiation may have important implications as a potential therapeutic target in acne vulgaris.

Introduction

Acne vulgaris is a highly prevalent skin inflammatory disease affecting more than 5 million Americans annually (Lim, Collins et al. 2017) and imposing a significant psychologic burden associated with low self-esteem (Nguyen, Koo et al. 2016), anxiety, depression, and

suicidal ideation (Purvis, Robinson et al. 2006, Vallerand, Lewinson et al. 2018). The disease develops from an interplay of sebum secretion, bacterial colonization by the commensal bacterium *Cutibacterium acnes (C. acnes)*, and host inflammatory responses. Increased sebum secretion from the pilosebaceous unit contributes to the pathogenesis of acne and likely triggers the inflammatory response that clinically present as comedones, papules, pustules, and nodules (Smith and Thiboutot 2008, Zouboulis, Jourdan et al. 2014, Lovaszi, Mattii et al. 2017). The main lipid components of sebum include squalene, triglycerides, free fatty acids, wax ester, cholesterol, and cholesteryl ester (Smith and Thiboutot 2008). These lipids can affect the growth of bacteria as well as host immune response. Lipids that accumulate in the follicular duct are oxidized by *C. acnes* lipase leading to bacterial proliferation and production of free fatty acids (Smith and Thiboutot 2008). Free fatty acids are a chemoattractant for macrophages and neutrophils resulting a cascade of immune cell recruitment and secretion of proinflammatory mediators. *C. acnes,* when supplied with a lipid substrate, can generate high levels of short chain fatty acids through anaerobic fermentation that modulate inflammatory gene expression from sebocytes, keratinocytes (KCs), and monocytes (Sanford, Zhang et al. 2016, Sanford, O'Neill et al. 2019). Squalene, a unique epidermal lipid in human sebum (Smith and Thiboutot 2008), also has multiple effects on the immune response (De Jong, Cheng et al. 2014, Lovaszi, Mattii et al. 2017) such that it is currently being used as an adjuvant, MF59, in FLUAD flu vaccine (O'Hagan, Ott et al. 2013). As a result, it has been suggested that lipids such as fatty acids and squalene may play a role in inducing the inflammatory process in acne.

Inflammation localized to the pilosebaceous unit is often the defining feature of acne. Patients are often concerned about the redness, pain, post-inflammatory hyperpigmentation, and scars that are associated with inflammation-induced damage (Thiboutot, Dreno et al. 2018). IL-1α/β are both expressed in early inflamed lesions and have been hypothesized to be the driver of the inflammatory cascade. The addition of IL-1α to KC culture was shown to activate

the hyperkeratinization process observed in comedones (Guy, Green et al. 1996). IL-1β can activate endothelial cells and signals for the migration of fibroblasts (Latkowski, Freedberg et al. 1995, Freedberg, Tomic-Canic et al. 2001, Kistowska, Gehrke et al. 2014). Elevated levels of $CD4^+$ T cells, $CD68^+$ macrophages, $CD83^+$ dendritic cells (DCs), and foamy macrophages have all been identified in acne lesions (Jerne 1984, Jeremy, Holland et al. 2003, Kelhala, Palatsi et al. 2014, Lovaszi, Mattii et al. 2017). However, the cellular components and mechanisms that initiate and perpetuate inflammation in acne are still unclear.

Single-cell RNA sequencing (scRNA-seq) has emerged as a powerful tool to deconstruct genetic and molecular profiles of individual cells, requiring few cells for analysis, such that it is readily possible to study immune cells from disease lesions (Papalexi and Satija 2018). In recent years, scRNA-seq methods are quickly becoming the technique of choice to study heterogeneous populations in complex tissues. The transcriptional signatures identified in these studies have enabled the identification of novel pathogenic biomarkers as well as reconstruct developmental trajectories to reveal cell fate decisions (Trapnell, Cacchiarelli et al. 2014). While RNA sequencing and microarrays have been performed on acne lesions, scRNA-seq studies provide more detailed transcriptional analysis of individual cells allowing for analysis of changes within the same cell types (Trivedi, Gilliland et al. 2006, Kelhala, Palatsi et al. 2014). As a result, we sought out to investigate these differences in acne that may have important implications for the understanding of the disease.

Results

Cell Types Recovered in Lesional and Non-lesional Acne

To elucidate cell heterogeneity and dynamic cellular changes in acne, we performed scRNA-seq on lesional (papules) and non-lesional skin from six individuals with active acne vulgaris (Supplementary Table 1.1). After application of quality control filters using Seurat

(Butler, Hoffman et al. 2018, Stuart, Butler et al. 2019), we subsequently included a total of 32,966 cells from lesional skin and 29,202 cells from non-lesional skin for the scRNA-seq analysis. Gene expression data obtained from cells from both lesional and non-lesional skin were aligned and projected onto a two-dimensional space UMAP (Uniform Manifold Approximation and Projection). Unsupervised clustering revealed eight major clusters corresponding to seven different cell types each cluster with a mixture of lesional and non-lesional cells (Fig. 1.1a-c). Within the myeloid, KCs, fibroblast, and endothelial cell clusters, the cells from lesional versus non-lesional samples were localized to distinct regions (Fig. 1.1b). Using differentially expressed genes and established canonical markers, we manually assigned cell type identities to endothelial cells (*SELE*, *PECAM*, *VWF*), fibroblasts (*COL1A1*, *COL3A1*, *COL6A2*), lymphoid cells (*TRBC2, CD3D*, *TRAC*), KC 1 (*KRT1*, *KRT10,* smooth muscle (*TAGLN*, *ACTA2*), myeloid cells (*LYZ*, *HLA-DRA*, *CD83*), KC 2 (MUCL1, KRT7, KRT18), and melanocytes (*MLANA*, *DCT*, *PMEL*) (Fig. 1.1d).

Unique Myeloid Cell Types Associated with Acne Lesions

To determine the range of biological diversity between lesional and non-lesional skin myeloid cells, we sub-clustered 2,638 myeloid cells and found six populations: three DC sub-clusters and three macrophage sub-clusters (Fig. 1.2a-d). The three DC sub-clusters were defined as $CD1C^+$ DC, Langerhans cells (LC), and $LAMP3^+$ DC (Fig. 1.2b). $CD1C^+$ DC expressed the cluster defining genes *CD1C* and *CLEC10A* (Fig. 1.2c, 2e). LC are antigen-presenting cells that reside in the epidermis tasked with maintaining tissue homeostasis. Here, LC expressed the classic markers *CD207* and *CD1A* (Fig. 1.2d, 2e), sharing a similar gene expression profile with migrating LCs including cytoskeleton remodeling (*ACTB*, *DSP*, *PFN1* and *PLEK2*) (Polak, Thirdborough et al. 2014, Artyomov, Munk et al. 2015). Cells in the $LAMP3^+$ DC sub-cluster are predominantly derived from lesional skin (Fig. 1.2c-d), and were

characterized by expression of *LAMP3,* as well as the DC maturation markers *CD80* and *CD83* (Fig. 1.2e), migration marker *CCR7*, and chemokines *CCL17* and *CCL19* consistent with similar cells identified in hepatocellular carcinoma (Polak, Thirdborough et al. 2014). The $LAMP3^+$ DC from acne lesions also expressed *CD274* (PD-L1) and *PDCD1LG2* (PD-L2) suggesting $LAMP3^+$ DC may employ the PD-1/PDL-1/PDL-2 axis to regulate peripheral tolerance (Weber, Meiler et al. 2011, Zhang, He et al. 2019).

We annotated two macrophage sub-clusters as M1-like macrophages and M2-like macrophages, distinguished based upon the expression of characteristic genes (M1-like: IL1A, *IL1B*, *TNF*, *IL6*, *IL12B*, and M2-like: *CD163*, *MRC1* (CD206), *TGFB1*, *IL10* (Fig. 1.2d, 2f). Analysis of the third and largest macrophage sub-cluster, which is over 95% associated with lesional skin, expressed triggering receptor expressed on myeloid cells 2 *(TREM2*) and *APOE* (Fig. 1.2f), along with a set of genes involved in lipid transport consistent with a defined population known as “TREM2 macrophages” (Keren-Shaul, Spinrad et al. 2017, Lavin, Kobayashi et al. 2017, Cochain, Vafadarnejad et al. 2018, Jaitin, Adlung et al. 2019, Wang, Dai et al. 2019, Xiong, Kuang et al. 2019, Xue, Tabib et al. 2020).

KCs in the Hair Follicles Present in Acne Lesions are Capable of Squalene Synthesis

Given that KCs constitute approximately 90% of cells in the epidermis, we wanted to profile how KCs differ between lesional and non-lesional acne specimens. We subclustered 8,271 KCs into five populations including spinous, basal, granular, eccrine gland, and hair follicle (Fig. 1.3a-d). Using known keratin-specific markers, we categorized KCs as spinous KC via *KRT1* and *KRT10* expression, *KRT5* and *KRT14* as basal KC, and *FLG* and *KRT2* as granular KC (Fig. 1.3d) (Fuchs and Raghavan 2002, Zhang, Yin et al. 2019). Cells localized to the eccrine gland were identified by *MUCL1* and *DCD (Gerber, Hevezi et al. 2013)* were only found in non-lesional skin (Fig. 1.3d). We were interested in the lesional-enriched sub-cluster of

KCs, which we designated as “hair follicle KC” based on its expression of *KRT6B* and *KRT6C* (Supplementary Fig. 1.1a) (Fuchs and Raghavan 2002). This sub-cluster highly expressed genes of the squalene and squalene epoxides synthesis pathway starting from *FDFT1* (squalene synthase) and *SQLE* (squalene epoxidase) whereas expression of LSS (lanosterol synthase), the enzyme required for the subsequent step in the cholesterol synthesis pathway, is reduced (Fig. 1.3e) (Singh, Gohil et al. 2019). The hair follicle sub-cluster also highly expressed *HIF1A*, a low oxygen-stress induced gene, hypothesized to contribute to the accumulation of squalene in sebocytes (Fig. 1.3e) (Smith and Thiboutot 2008). We did not observe any upregulation of genes for fatty acid synthesis (*SCD*) (Peck and Schulze 2016), wax ester synthesis (*AWAT1*, *AWAT2*) (Turkish, Henneberry et al. 2005), nor triglyceride synthesis (*DGAT1*, *DGAT2*) *(Chitraju, Walther et al. 2019)* (Fig. 1.3e). One of the terminal genes in cholesterol synthesis pathway, *DHCR24*, was upregulated in the keratinocytes, while *DHCR7* was not (Fig. 1.3e). Cholesterol synthesis requires both enzymes *DHCR24* and *DHCR7* to interact physically and functionally (Luu, Hart-Smith et al. 2015). Consistent with scRNA-seq data, *FDFT1* and *SQLE* encoded protein expressions observed by immunohistochemical staining were greater in the pilosebaceous unit in acne lesions compared to healthy controls (Fig. 1.3f). We were not able to locate sebocytes using canonical markers such as epithelial membrane antigen (EMA) (also known as mucin 1, *MUC1*) or androgen receptor (*AR*) (Supplementary Fig. 1.1b).

Diversity of Fibroblast and Endothelial Cells in Acne lesions

We found five sub-clusters of fibroblasts with two sub-clusters that are lesional-specific (Supplementary Fig. 1.2a-d). Fibroblast sub-cluster 1, composed predominantly of cells derived from acne lesions, was characterized by expression of genes encoding metalloproteinase proteins such as *MMP1*, *MMP3*, and *MMP9*, consistent with previous microarray studies

(Supplementary Fig. 1.2e) (Kang, Cho et al. 2005, Trivedi, Gilliland et al. 2006). There are also many lipid-related genes in fibroblast sub-cluster 3, a lesional-enriched sub-cluster, such as *APOE*, *ABCA1*, *ABCA8*, *APOL1*, *APOL2*, *APOL3*, and *PLIN2* (Supplementary Fig. 1.2e).

We found ten sub-clusters of endothelial cells based on their expression of *PECAM1* and *CDH5* (Supplementary Fig. 1.2f-j). To localize each sub-clusters, we used expression levels of *LYVE1* (lymphatic endothelial marker), *GJA4* and *GJA5* (venule endothelial markers), and *NR2F2* (arterial marker) (Supplementary Fig. 1.2j) (Sissaoui, Yu et al. 2020). Lesional enriched endothelial cell sub-clusters were sub-cluster 1 and sub-cluster 5, both of which are localized to the arterial region (Supplementary Fig. 1.2j).

Diversity of Lymphocytes in Acne Biopsy Specimens

We detected five populations of lymphocytes including naïve $CD4^+$ T cells (*CD40LG* and *CCR7)* (Lesley, Kelly et al. 2006), cytolytic T lymphocytes (CTL), regulatory T cells (Treg) (*FOXP3* and *CTLA4)*, NK cells, and B cells (*JCHAIN*, *CD79A*) in both lesional and non-lesional skin (Supplementary Fig. 1.3a-d). The CTL and NK sub-clusters variably expressed the mature CTL markers *GZMB*, *PRF1* and *GNLY*, with the majority of the CTL expressing the immature marker *GZMK* (Supplementary Fig. 1.3e). Whereas NK cells and some CTL expressed *NKG7*, the CTL all expressed *CD3D*. We found a lymphoid sub-cluster designated as “naïve T cells” that expressed Th17 cell related cytokine genes including *CCL20*, *CSF2*, *IL17A*, *IL17F*, *IL21*, *IL22*, *IL23*, and *IL26* as well as Th17 cell surface receptor genes such as *IL23R* and *CCR6* (Supplementary Fig. 1.3f). We detected similar levels of these Th17-associated gene expression in lesional and non-lesional skin.

Characterization of TREM2 macrophages

We further investigated the nature of the TREM2 macrophages in acne lesions by comparing the sub-cluster signature to a curated set of 100 defined macrophage signatures including the SAVANT database, bulk RNA-seq analysis of M1 and M2 macrophages as well as seven scRNA-seq studies of TREM2 macrophages (Xue, Schmidt et al. 2014, Keren-Shaul, Spinrad et al. 2017, Lavin, Kobayashi et al. 2017, Lopez, Montoya et al. 2017, Cochain, Vafadarnejad et al. 2018, Gerrick, Gerrick et al. 2018, Jaitin, Adlung et al. 2019, Wang, Dai et al. 2019, Xiong, Kuang et al. 2019, Xue, Tabib et al. 2020). The top 100 gene signature for the TREM2 macrophage sub-cluster was most similar to TREM2 macrophages identified by scRNA-seq in seven studies of diseases characterized by alterations in lipid metabolism, in particular the studies of lipid laden macrophages in obesity and foam cells in atherosclerosis (Fig. 1.4a) (Keren-Shaul, Spinrad et al. 2017, Lavin, Kobayashi et al. 2017, Cochain, Vafadarnejad et al. 2018, Jaitin, Adlung et al. 2019, Wang, Dai et al. 2019, Xiong, Kuang et al. 2019, Xue, Tabib et al. 2020). The TREM2 macrophages in acne lesions were similar to TREM2 macrophages in lung cancer, Alzheimer's disease and nonalcoholic fatty liver disease. We derived a list of 26 conserved genes that were present in at least four of the seven scRNA-seq studies of TREM2 macrophages (Supplementary Table 1.2), finding that 19 genes were present in the acne lesion TREM2 macrophage sub-cluster. Of the six conserved genes that were not acne signature genes, *LGALS3*, *TYROBP*, *AIF1*, *C1QC*, *CTSA,* and *SAT1* were expressed by >50% of cells in the acne TREM2 macrophage sub-cluster (Supplementary Fig. 1.4), but were also detected in the other myeloid sub-clusters.

We performed KEGG pathway analysis of the top 100 signature genes in the acne lesion TREM2 macrophage sub-cluster, revealing enrichment for pathways described as lysosomes, cholesterol metabolism, mineral absorption, phagosome, and PPAR signaling pathway (Fig. 1.4b). Focusing on cholesterol/lipid metabolism, we identified by gene ontology analysis that TREM2 macrophages express genes that are involved in lipid transport and binding (*APOC1*,

APOE, *CD14*, *LRP1*, *TREM2*), lipid intracellular transport (*NPC1*, *NPC2)*, lipid signaling (*NR1H3*), lipid efflux (*ABCA1*, *APOE*), lipid storage (*PLIN2*), and lipid metabolism (Fig. 1.4c-d). We also found that TREM2 macrophages in acne lesions expressed key pro-inflammatory cytokine genes *IL18* (Fig. 1.4e).

To validate expression of TREM2 in acne lesions, we performed immunohistochemistry, finding that TREM2 expression was greater in lesional and non-lesional skin from the same donor (Fig. 1.4f, Supplementary Fig. 1.2). By immunofluorescence staining, TREM2 colocalized with the macrophage marker CD68 in lesional skin compared to non-lesional skin (Fig. 1.4g). These results suggest TREM2 macrophages characterized by a lipid metabolic program and pro-inflammatory gene expression accumulate in acne lesions.

Squalene-induced TREM2 Expression in Macrophages

The presence of $TREM2^{+}$ macrophages in acne lesions suggests a role in the pathogenesis of the disease. We used Ingenuity Pathway Analysis (IPA) to analyze the top 100 genes from TREM2 macrophage cluster marker to identify cytokines that could drive the development of TREM2 macrophages. *IFNG*, *IL13, TNF, IL1B,* and *IL4* were the top 5 upstream regulators. Consistent with the detection of IL-4 as an upstream regulator, it has been reported that IL-4, in combination with macrophage colony-stimulating factor (M-CSF), induces TREM2 expression on macrophages in vitro (Turnbull, Gilfillan et al. 2006, Trapnell, Cacchiarelli et al. 2014). We tested the upstream regulators in culture, with and without M-CSF, for their ability to induce TREM2 expression on adherent macrophages. Given that we determined that squalene synthesis is upregulated in hair follicle keratinocytes, we were interested in whether squalene can induce TREM2 expression. After five days of culture, the media was washed, and cells were incubated for two additional days with media control, a panel of cytokines, and squalene. Media control cells showed minimal upregulation of TREM2 expression (Fig. 1.5c). In contrast,

subsequent co-culture of five-day M-CSF derived macrophages with IL-4 or squalene in the presence of M-CSF markedly upregulated TREM2 expression as compared to M-CSF alone (Fig. 1.5c). Squalene induced TREM2 expression in macrophages in a dose-dependent manner (Supplementary Fig. 1.3). The gene expression profile measured by qPCR of squalene-treated macrophages is very similar to those of TREM2 macrophages in acne lesions (Fig. 1.5d). The lipid efflux transporter, *ABCA1*, and fatty acid metabolism enzyme, LPL, SPP1, and TREM2 are upregulated in squalene-treated macrophages (Fig. 1.5d).

BODIPY staining revealed that the cytoplasm of squalene/M-CSF treated macrophages is full of lipids as compared to IFN-γ/LPS- and IL-4/M-CSF macrophages (Fig. 1.6a). We found squalene/M-CSF macrophages to have enhanced phagocytosis of latex beads and *C. acnes* when compared to IFN-γ/LPS and M-CSF macrophages (Fig. 1.6b-c). Squalene/M-CSF macrophages did not have measurable antimicrobial activity against *C. acnes* (Fig. 1.6d). We also observed squalene/M-CSF to secrete higher IL-1β and IL-18 when stimulated with live *C. acnes* as compared to IFN-γ/LPS and IL-4/M-CSF macrophages (Fig. 1.6d) consistent with the expression of these genes in TREM2 macrophages in acne lesions (Fig. 1.6e).

Discussion

Acne is a multifactorial disease involving excess lipid production from the sebaceous hair follicle and increased colonization by the commensal *C. acnes*, resulting in a host inflammatory response that underlies the clinical appearance of erythematous papules. We investigated the cellular response at the site of disease in acne by performing scRNA-seq on skin biopsy specimens from patients. We identified a subset of $TREM2^{+}$ macrophages in acne lesions, characterized by a gene program the encodes lipid uptake, metabolisms, storage, and efflux, as well as expression of proinflammatory cytokines. A key component of human sebum, squalene, induced the $TREM2^{+}$ macrophage program in vitro resulting in the enhanced

phagocytosis of *C. acnes* with the production of IL-18 (Fig. 1.6c), without a concomitant antimicrobial response (Fig. 1.6d). These data indicate that the excess lipid production triggers the induction and activation of TREM2$^+$ macrophages resulting in inflammation that characterizes the clinical presentation of acne vulgaris.

TREM2 is a transmembrane glycoprotein that can interact with lipoproteins, phospholipids, and anionic ligands (Daws, Sullam et al. 2003, Cannon, O'Driscoll et al. 2012, Wang, Cella et al. 2015), facilitated by the lipid transporter APOE. The TREM2$^+$ macrophages in acne lesions were characterized by the upregulation of 24 genes involved in lipid transport and metabolism including *APOE*. Other genes in the TREM2$^+$ macrophage lipid metabolism include *ABCA1*, the key efflux transporter of lipids, which is upregulated in the activation of liver X receptor (LXR) (Costet, Luo et al. 2000). We also see evidence of lipid processing in TREM2 macrophages with the increase expression of cathepsins (*CTSB*, *CTSD*), lysosomal acid lipase A (*LIPA*), acetyl-coA acetyltransferase 1 (ACAT1, gene *SOAT1*), intracellular lipid transporters such as Niemann-Pick disease (*NPC1*, *NPC2*) as well as a marker for intracellular storage of lipid droplets perilipin 2 (*PLIN2*) (Fig. 1.4d). Excess lipids activate LXRs (gene *NR1H3*) to upregulate *ABCA1* as well as lipid carrier *APOE* to facilitate efflux (Fig. 1.4d). The uptake of lipids by macrophages is mediated by micropinocytosis and various cell surface receptors. Instead of limiting lipid uptake of lipids, they largely depend on cholesterol efflux pathways to maintain cellular lipid homeostasis *(Lovaszi, Mattii et al. 2017, Batista-Gonzalez, Vidal et al. 2019)*. The gene expression suggests there is an active effort by these macrophages to upregulate metabolism, storage, and the efflux response to deal with the lipid accumulation. The presence of TREM2$^+$ macrophages in acne lesions along with the finding that squalene, a lipid that is abundant in acne, induces and activates TREM2$^+$ macrophages, indicating that TREM2$^+$ macrophages are part of the host response to extracellular lipids.

TREM2^{+} macrophages have recently been a major focus in Alzheimer's disease as studies revealed that variants of TREM2 increase the risk of early-onset disease and its increased lipids (such as Aβ deposits) phagocytosis may contribute to Aβ aggregates and tau pathology (Ruiz, Dols-Icardo et al. 2014, Slattery, Beck et al. 2014, Yao, Coppola et al. 2019). TREM2^{+} macrophages have also been detected in a wide range of inflammatory diseases characterized by alternations in lipid metabolism including obesity, atherosclerosis, lung cancer, and nonalcoholic fatty liver disease (Keren-Shaul, Spinrad et al. 2017, Lavin, Kobayashi et al. 2017, Cochain, Vafadarnejad et al. 2018, Jaitin, Adlung et al. 2019, Wang, Dai et al. 2019, Xiong, Kuang et al. 2019, Xue, Tabib et al. 2020), thus representing a conserved and general macrophage response to excess extracellular lipids. Using the patterns of upregulated cluster specific marker genes of the seven scRNA-seq studies of these TREM2-associated diseases, we derived a list of 26 conserved genes that were present in at least four of the seven TREM2 macrophage cluster markers (Supplementary Table 1.2). This included 19 genes that were present in the acne TREM2 macrophage gene signature. While six of the 26 common genes were not part of the acne TREM2 macrophage signature but were strongly expressed by these and other macrophage sub-clusters in acne. Only *SYNGR1* (synaptogyrin 1) was absent in acne TREM2 macrophages, yet this gene encodes a membrane protein associated with presynaptic vesicles in neuronal cells (Verma, Kubendran et al. 2005). The 15 core signature genes encode for a lipid metabolism program that involves *ACAA2, CEBPA, GALC, GBA, GLB1, HSD3B7, LEPROT, LIPA, LPL, LRP1, NCEH1, SCD, SOAT1, SPHK1*. The 70 genes defined as lipid metabolism genes in the acne TREM2+ macrophage cluster that were not part of the TREM2 macrophage seven studies consensus included genes that encode proteins involved in lipid transport (*APOC1, CD14*), metabolism (*GLUL, NCEH1*), storage (*PLIN2*), and signaling (*NR1H3*). All 70 genes, except for *PRXL2B (*encodes prostamide/prostaglandin F Synthase),

are present in at least two of the consensus studies. This indicates TREM2$^+$ lipid-associated signature is conserved throughout various inflammatory and metabolic diseases.

TREM2 expression in vitro is induced by M-CSF and IL-4, factors often used to differentiate monocytes to M2 macrophages (Turnbull, Gilfillan et al. 2006). This is evident in our macrophage sub-cluster as TREM2$^+$ macrophages express similar levels of *CD163*, *MRC1*, *TGFB1*, and *IL10* as M2-like macrophages (Fig. 1.2f). Using pseudotime analysis in combination with IPA, we identified TNF, IFNG, IL4, and IL1B (Fig. 1.5a) as potential regulators that have been observed experimentally to upregulate genes in TREM2$^+$ macrophages. We found IL-4, consistent with previous studies (Turnbull, Gilfillan et al. 2006) and squalene induced TREM2 expression in vitro in presence of M-CSF (Fig. 1.5c). We noted that TREM2 is downregulated in response to IFN-γ consistent with a previous study (Turnbull, Gilfillan et al. 2006), while M-CSF, TNF-α, and IL-1β has a minimal effect on TREM2 expression (Fig. 1.5c). We also observed squalene/M-CSF-treated macrophage expressed similar pattern of lipid-related genes and secrete IL-1β and IL-18 similar to TREM2 macrophages in acne lesion (Fig. 1.4e). IL-1β is known to drive the host inflammatory responses in *C. acnes* infected monocytes and mouse model (Kistowska, Gehrke et al. 2014). Free fatty acids and triglycerides can activate macrophages to synthesize IL-18 (Murphy, Kraakman et al. 2016). Serum IL-18 levels in obese individuals was found to correlate strongly with metabolic syndrome and insulin resistance (Esposito, Pontillo et al. 2002, Hung, McQuillan et al. 2005).The cytoplasm of these macrophages is filled with lipids, as seen with BODIPY stain (Fig. 1.5a), which are characteristics of foam cells found in acne lesions (Lovaszi, Mattii et al. 2017). These squalene-treated macrophages exhibited enhanced phagocytosis, yet no antimicrobial activity against *C. acnes* compared to IFN-γ/LPS and IL-4/M-CSF-treated macrophages. Squalene had been shown to be an autoantigen in the activation of T cells as well as promote the differentiation of macrophages in the absence of *C. acnes* (De Jong, Cheng et al. 2014, Lovaszi, Mattii et al.

2017). These results suggest that the presence of excess squalene may enhance the macrophage's ability to phagocytose but compromise its ability to kill bacteria. Without its antimicrobial activity, *C. acnes* growth will remain unchecked in the hair follicle and possibly in phagolysosomes, contributing to inflammation.

We found evidences of squalene production and lipid metabolisms in other cells in acne lesions. A sub-cluster of hair follicle KC expressed high levels of *FDFT1* and *SQLE*. *FDFT1* catalyzes the conversion of two molecules of farnesyl pyrophosphate (FPP) to squalene while *SQLE* is the first oxygenation step in cholesterol biosynthesis. *SQLE* is thought to be one of the rate-limiting enzymes in this pathway converting squalene to squalene epoxide in order to continue the biosynthesis pathway of lanosterol and eventually cholesterol *(Singh, Gohil et al. 2019)*. The buildup of squalene in sebaceous gland with little conversion to lanosterol have been attributed to the anaerobic environment of the pilosebaceous unit during comedone formation (Smith and Thiboutot 2008). Evidently, we found high expression of *HIF1A* in the hair follicle KC sub-cluster. As KCs in the hair follicle experience hypoxia, they try to increase expression of *SQLE* to continue the cholesterol synthesis pathway but instead create a bottleneck halting the lanosterol synthesis, which we observed as a low expression of *LSS* (Fig. 1.3e). Other enzymes involved in fatty acid synthesis (*SCD*), wax ester synthesis (*AWAT1, AWAT2)*, triglyceride synthesis (*DGAT1, DGAT2)*, and cholesterol synthesis (*DHCR7)* were not upregulated in the hair-follicle keratinocytes (Fig. 1.3e) suggesting higher amounts of squalene products are made in the acne-associated hair-follicle compared to the other sebum-associated products. Although DHCR24 is upregulated, the terminal step of cholesterol synthesis requires both DHCR7 and DHCR24 to physically and functionally bind (Luu, Hart-Smith et al. 2015). We also detected a lesional-enriched sub-cluster of fibroblasts that also upregulated genes related to lipid transport and storage. This finding suggests that other cells in acne lesions are also finding ways to deal with the excess lipids.

Thus, we proposed that acne vulgaris initiates inflammation with the production squalene and squalene epoxides in the hair follicles. The accumulation of squalene and its derivatives upregulate the expression of TREM2 on macrophages. TREM2 expression enhances the phagocytosis ability of the macrophages to uptake lipids and bacteria. However, the surplus of lipids overwhelmed and immobilized the macrophages making them unable to kill the intracellular bacteria. $TREM2^{+}$ macrophages upregulate expression and processing of IL-18 to activate the immune response. At the same time, some $TREM2^{+}$ macrophages may undergo apoptosis releasing excess bacteria and lipids which further activate the immune response leading to inflammation (Fig. 2.2).

One limitation of our scRNA-seq data is the failure to detect sebocytes. Given the lack of scRNA-seq signature for sebocytes and possible lysis of sebocytes during tissue processing, we were unable to locate a sebocyte population, even when we used MUC1 and AR (Supplementary Fig. 1.1). This could also be a limitation of scRNA-seq data where some transcripts are not detected in all cells, resulting in dropouts or a false-negative read count (*20*). Given the limitation of our data, the hair follicle KC sub-cluster we attributed the high expression of *FDFT1* and *SQLE* to these cells, which could be sebocytes. Our analysis did not detect differences in the T cell compartment between skin biopsy specimens from acne lesions as compared to non lesional skin, differing from one of two cohorts in a microarray study (Kelhala, Palatsi et al. 2014). Some of the Th17 cytokines such as *IL17A, IL17F, IL21, IL22*, present in acne lesions, are not readily detected by the single cell platform, 10X Genomics. Also, the failure to detect gene changes in acne lesions compared to non-lesional skin may be due to the acne severity in our subjects. Of the six acne subjects, three had mild and three had moderate acne. This was possibly due to our strict exclusion criteria of no acne medication or hormonal regulation medication or implants in the past three months. As a result, the acne biopsies may not have been in a chronic inflammation stage and, possibly, were still in the early inflammatory

stage. It would be relevant to determine how immune cells in acne lesions vary with increased severity of acne.

Our results underline the importance of squalene in the initiation of inflammation in acne, it would be interesting to see whether squalene could be a potential target for acne therapy. Squalene is a unique lipid component of human sebum (Lovaszi, Mattii et al. 2017) and also found in greater proportions on acne patients compared to no acne controls (Pappas, Johnsen et al. 2009). Current animal models of acne do not fully mimic the diseased features in humans. Some studies have used topical application of human sebum, to promote the survival of bacteria after intradermal injection in mice (Kolar, Tsai et al. 2019). A recent phase-three trial using small molecule inhibitor of acetyl coenzyme A (CoA) carboxylase (ACC) to affect fatty acids biosynthesis failed to see a reduction in the number of inflammatory lesions (Bissonnette, Poulin et al. 2017). Interestingly, the small molecular inhibitor blocks the synthesis free fatty acids, wax esters, and triglycerides in the sebum but has no effects on squalene or cholesterol synthesis (Bissonnette, Poulin et al. 2017). Thus, squalene represents an attractive therapeutic target given its central role in the formation of sebum, a key factor involved in acne development.

Materials and Methods

Healthy Blood donors, skin samples, and single cell library preparation

The study was performed in accordance with protocols approved by the institutional review board at University of California, Los Angeles. All patients provided written informed consent. Healthy blood and acne donors were recruited from the University of California, Los Angeles. Patients were excluded from the study if they had been using acne medication, hormonal regulation medication, or hormonal-related implants in the past three months. Skin samples were obtained from 3mm punch biopsies in the back. Briefly, skin was digested enzymatically at 2 hours with 0.4% Dispase II at 37°C with agitation. The epidermis was separated from the

dermis. Epidermis was then treated with 0.25% Trypsin and 10U/mL of DNAse I for 30 minutes. Dermis was homogenized and treated with 0.4% Collagenase II and 10U/mL DNase I for 2 hours. Isolated cells were loaded into the Chromium instrument (10X Genomics) according to manufacturer's protocol and analyzed using Seurat (*19, 20*). Pseudo-time trajectories for macrophage cluster were constructed using Monocle 2, an R package (*45*).

Immunohistochemical and Immunofluorescence staining

Frozen tissue sections were blocked with normal horse serum before incubation 2 hours with mAbs against FDFT1, SQLE, and TREM2 (Invitrogen) followed by incubation with biotinylated horse anti–mouse IgG for 90 minutes. Slides were counterstained with hematoxylin and mounted in crystal mounting medium (Biomeda) and were visualized using the ABC Elite system (Vector Laboratories). Skin sections were examined with a Leica microscope.

Cryosections (4mm) from acne patients was incubated with antibodies against and CD68 (BD) followed by Alexa Fluor 594 isotype specific secondary (Invitrogen). Sections were washed, incubated with monoclonal antibodies for TREM2 (Thermo Scientific) followed by and Alexa Fluor 647 isotype-specific secondary (Invitrogen). Slides were mounted in crystal mounting medium (Biomedia) then preserved with Prolong Gold with DAPI (Invitrogen). Colocalization analysis was performed with Leica Application Suite X (LAS X).

Differentiated macrophages were fixed with 4% paraformaldehyde onto a poly-L-lysine coated microscope slide. Sections were washed and incubated with BOPIDY at for 1 hour at room temperature. Slides were then preserved with Prolong Gold with DAPI (Invitrogen). Colocalization analysis was performed with Leica Application Suite X (LAS X).

Gene overlap, functional, and upstream regulator analyses

Gene overlap used the top 100 genes of TREM2 macrophage sub-cluster and various macrophage signatures. Significance (-log(p-value)) of the overlap was calculated using hypergeometric test assuming 22,792 unique human genes. KEGG pathway analysis was performed using top 100 genes of the TREM2 macrophage sub-cluster via Enrichr (*60, 61*). Lipid specific Gene Ontology terms was performed using the ClueGO plugin (Cytoscape) (*62*). Upstream Regulator Analysis in Ingenuity Pathways Analysis (Qiagen) with the genes obtained from pseudo-time analysis.

Macrophage Characterization

Monocytes were isolated from healthy donor blood using a Ficoll (GE Healthcare) density gradient and adhered for 2 hours with RPMI supplemented with 1% fetal calf serum (FCS). Monocytes were seeded at 500,000 cells/well in 24-well plate and differentiated in M-CSF (50 ng/ml) (R&D System) for 5 day in RPMI with 10% FCS at 37°C. Macrophages were then stimulated with LPS (100ng/mL) + IFN-γ (20ng/mL) for 1 day, M-CSF (50 ng/ml), IL-4 (20ng/mL), squalene (5 μM), TNF-a (1μg/ml) (Thermo Scientific), IL-1β (0.1 μg/mL) (Thermo Scientific), IFN-γ (1 μg/mL) (Thermo Scientific). Squalene was sonicated for 30 minutes in RPMI prior to adding to macrophage culture. Adherent cells were detached with 1mM EDTA in PBS. Cells were stained with APC-conjugated mAbs against TREM2 (R&D System). Isotype controls were performed in parallel. Cells were acquired with LSR II flow cytometer (BD) and analyzed with FlowJo (BD).

RNA isolation and reverse transcription-real time quantitative PCR analysis

Total RNA was isolated at 12 hours and 24 hours using TRIzol (Invitrogen) according to manufacturer's protocol. First strand cDNA synthesis was performed with iScript cDNA synthesis kit (BioRad). Total RNA was subjected to reverse transcription-quantitative

polymerase chain reaction (RT-qPCR) via Bio-Rad IQ5 system using iQ SYBR Green Supermix (BioRad). The relative quantities of gene tested per sample were calculated against GAPDH using ΔΔCT. Human specific probes were generated by RT-qPCR amplification using the following primer sets: ABCA1 for 5'-AACAGTTTGTGGCCCTTTTG-3', ABCA1 rev 5'-AGTTCCAGGCTGGGGTACTT-3', ABCG1 for 5'- CCTTTCCTATTCGGTTCCTGA-3', ABCG1 rev 5'- TTCCTTTCAGGAGGGTCTTG-3', APOE For 5'-CCAGCGGAGGTGAAGGAC-3', APOE Rev 5'-CGCTTCTGCAGGTCATCG-3', LPL For 5'-GAGATTTCTCTGTATGGCACC-3', LPL rev 5'-CTGCAAATGAGACACTTTCTC-3', SCD1 For 5'-TGCCCACCTCTTCGGATATC-3', SCD1 Rev 5'-GATGTGCCAGCGGTACTCACT-3', SPP1 For 5'-GCCGCTGTAACCTCTTCGG-3', SPP1 Rev 5'-GTCTTCGGCCAATCTGGCTTT-3', TREM2 For 5'-GCCTGACATGCCTGATCCTC-3', TREM2 Rev 5'-AGGACCTTCCTGAGGGTGTC-3', GAPDH For 5'-GGATTTGGTCGTATTGGG-3', GAPDH Rev 5'-GGAAGATGGTGATGGGATT-3'.

Bacterial Culture

Single colonies of *C. acnes* HL096PA1 were inoculated into 5 mL of Reinforced Clostridial Media (RCM) (Oxoid) and cultured at 37°C under anaerobic conditions until early logarithmic growth phase (OD_{600} = 0.1 – 0.3). Bacteria pellets were harvested by centrifugation at 5,000xg for 10 minutes and washed with PBS three times. *C. acnes* were labeled using PKH26 general cell membrane labeling kit (Millipore) according to manufacturer's protocol.

ELISA

In vitro macrophages generated as described above with LPS (100ng/mL) + IFN-γ (20ng/mL) for 1 day, M-CSF (50 ng/ml), IL-4 (20ng/mL) and squalene (5 μM) for 2 days. Cytokine and squalene-treated macrophages were washed three times with PBS. *C. acnes* was added at MOI 10 to 500,000 macrophages per well in a 24-well plate. After 24 hours of

stimulation, IL-1β (R&D Systems) and IL-18 (R&D Systems) release was measured by ELISA according the manufacturer's protocol.

Phagocytosis Activity

Macrophages were incubated with PKH26-labelled *C. acnes* or latex beads (Millipore Sigma) for 24 hours. Cells were prepared with cold PBS washing and extracellular bacteria were killed with 300 μg/mL gentamicin (Sigma Aldrich). Cells were then acquired with LSR II flow cytometer (BD) and analyzed with FlowJo (BD).

Antimicrobial Assays

Macrophages were incubated with bacteria at MOI 10 for 1 hour and 24 hours at 37°C. Cells were washed 3 times with cold PBS. Extracellular bacteria were killed with 300 μg/mL gentamicin (Sigma Aldrich). Cells were lysed with a 10 minutes treatment with 0.5% saponin. A dilution series was plated on Brucella agar with 5% Sheep Blood (Thermo Scientific) to determine viable intracellular bacteria by CFU (colony forming unit) counting after 5 days of incubation at 37°C using Anaeropack (Thermo Scientific). Antimicrobial activity was calculated by subtracting the 24-hour CFU count with the 1-hour CFU count and divided by the 1-hour CFU count to determine the percentage of killing.

Statistical Analysis.

Statistical analyses were calculated using GraphPad Prism version 8.0, and p values ≤ 0.05 were assigned as significant. For comparisons involving two groups, a paired Student t test or one-way ANOVA with Sidak posttest was performed. For more than two comparison groups, a one-way ANOVA with Tukey posttest, a two-way ANOVA with Tukey posttest, or a Friedman test with Dunn posttest was performed. Data are represented in figures as mean ± SEM.

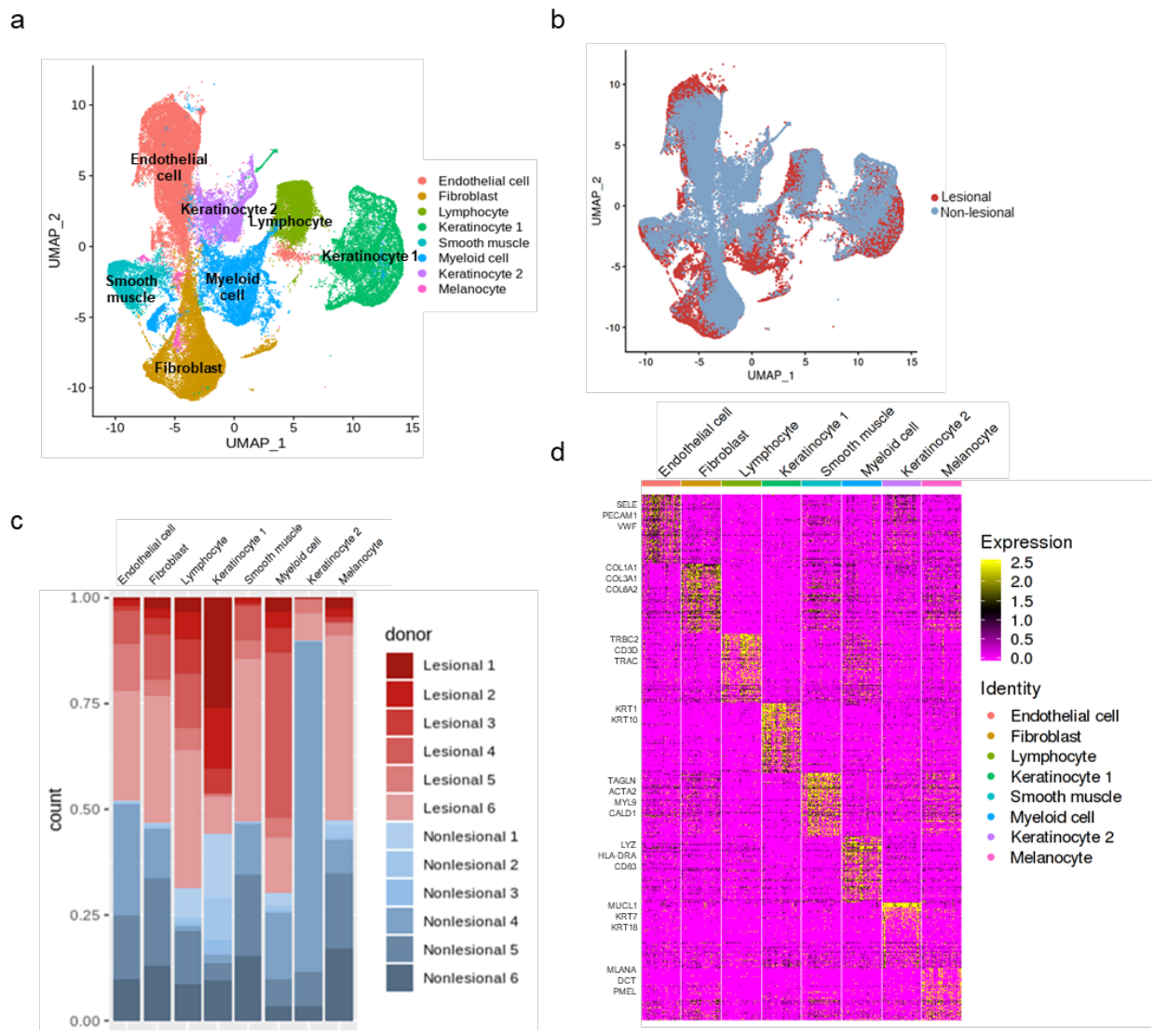


Fig. 1.1. Cell Types Recovered in Lesional and Non-lesional Acne

a. UMAP plot for 62,168 cells from acne patients colored by cell type cluster.
b. UMAP plot for 62,168 cells colored by lesional types with 32,966 cells from lesional skin and 29,202 cells from non-lesional skin. Lesional cells are shown in red while non-lesional cells are shown in blue.
c. Stacked bar-plot showing the donor and lesion type composition for each of the cell types. Lesional cells are shown in red while non-lesional cells are shown in blue.
d. Heatmap showing normalized gene expression values for a curated list of cell type specific genes. High expression values are shown in yellow while low expression values are show in purple.

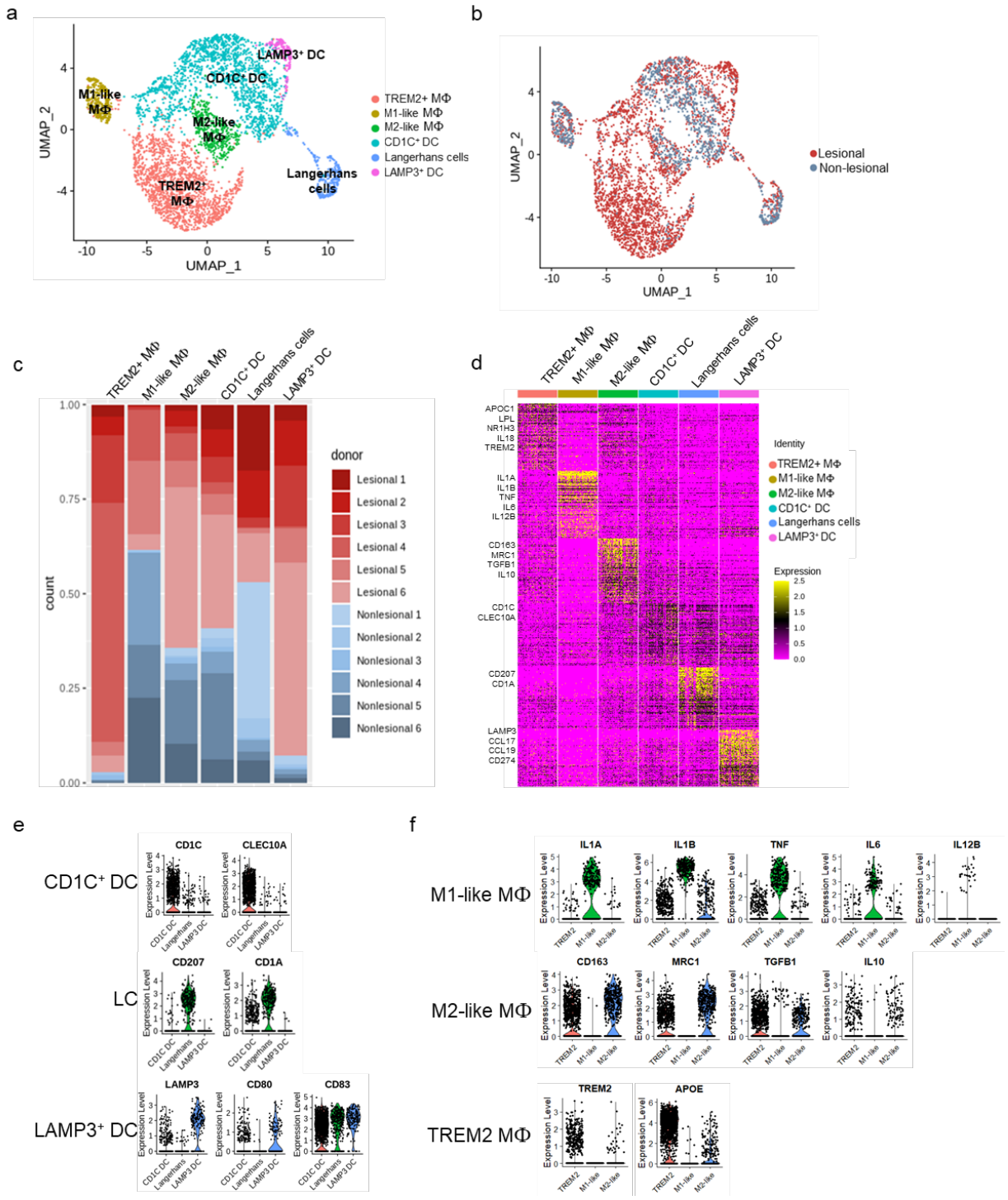


Fig. 1.2. Unique Myeloid Cell Type Associated with Acne Lesions

a. UMAP plot for 4,370 myeloid cells from acne patients colored by cell types.

b. UMAP plot for 4,370 myeloid cells colored by lesional types with 3,152 myeloid cells from lesional skin and 1,218 myeloid cells from non-lesional skin. Lesional cells are shown in red while non-lesional cells are shown in blue.

c. Stacked bar-plot showing the donor and lesion type composition for each of the cell types. Lesional cells are shown in red while non-lesional cells are shown in blue.

d. Heatmap showing normalized gene expression values for a curated list of cell type specific genes. High expression values are shown in yellow while low expression values are show in purple.

e. Violin plots showing the normalized expression levels for genes in DC sub-clusters.

f. Violin plots showing the normalized expression levels for genes in macrophage sub-clusters.

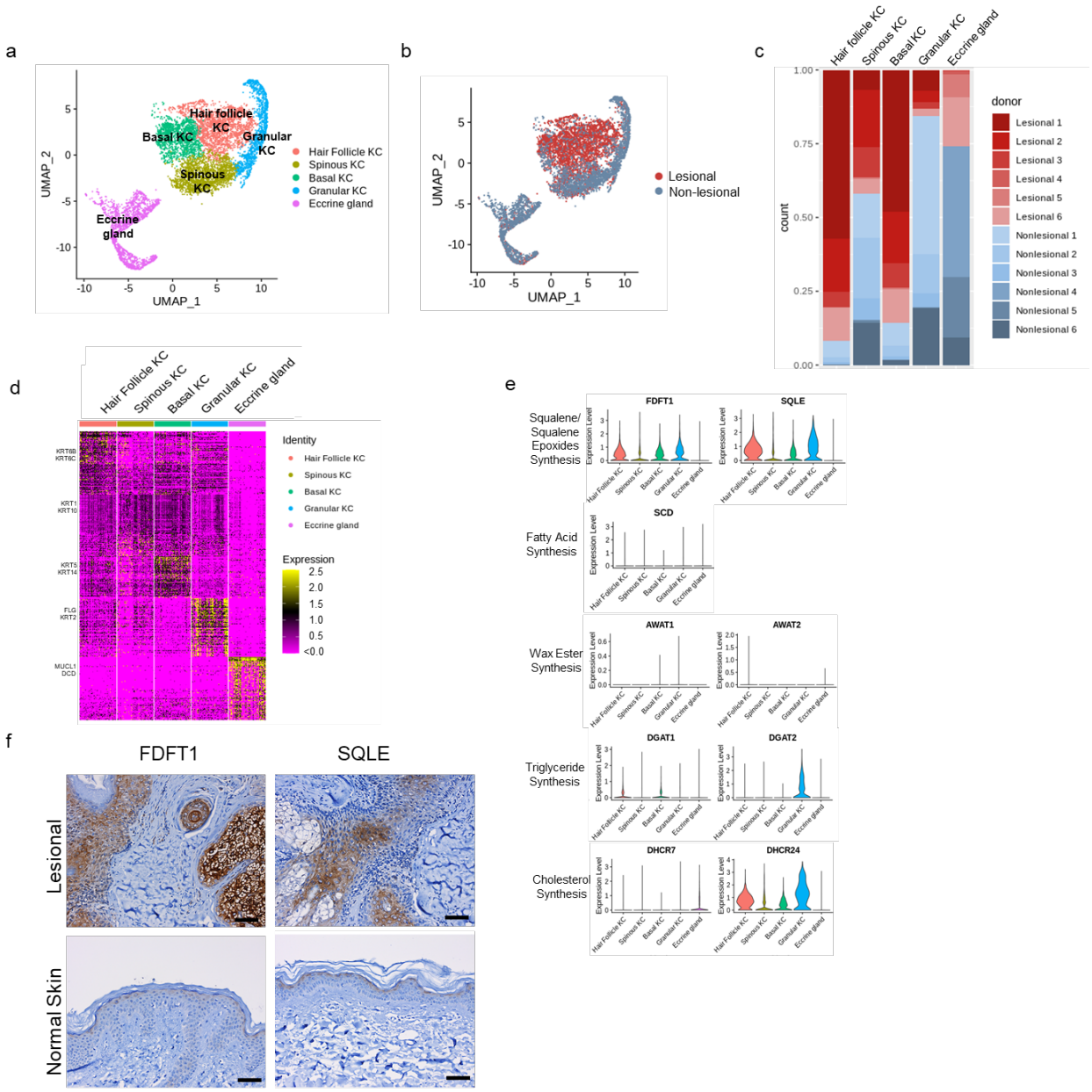


Fig. 1.3. KCs in the hair follicles present in acne lesions is capable of squalene synthesis

a. UMAP plot for 8,271 KCs from acne patients colored by cell types.

b. UMAP plot for 8,271 keratinocytes colored by lesional types with 4,473 keratinocytes from lesional skin and 3,798 keratinocytes from non-lesional skin. Lesional cells are shown in red while non-lesional cells are shown in blue.

c. Stacked bar-plot showing the donor and lesion type composition for each of the cell types. Lesional cells are shown in red while non-lesional cells are shown in blue.

d. Heatmap showing normalized gene expression values for a curated list of cell type specific genes. High expression values are shown in yellow while low expression values are show in purple.

e. Violin plots showing the normalized expression levels of curated list of lipid biosynthesis pathways for keratinocyte sub-clusters.

f. Immunohistochemistry staining of FDFT1 and SQLE encoded protein. Scale bar = 100 micron

Fig. 4.

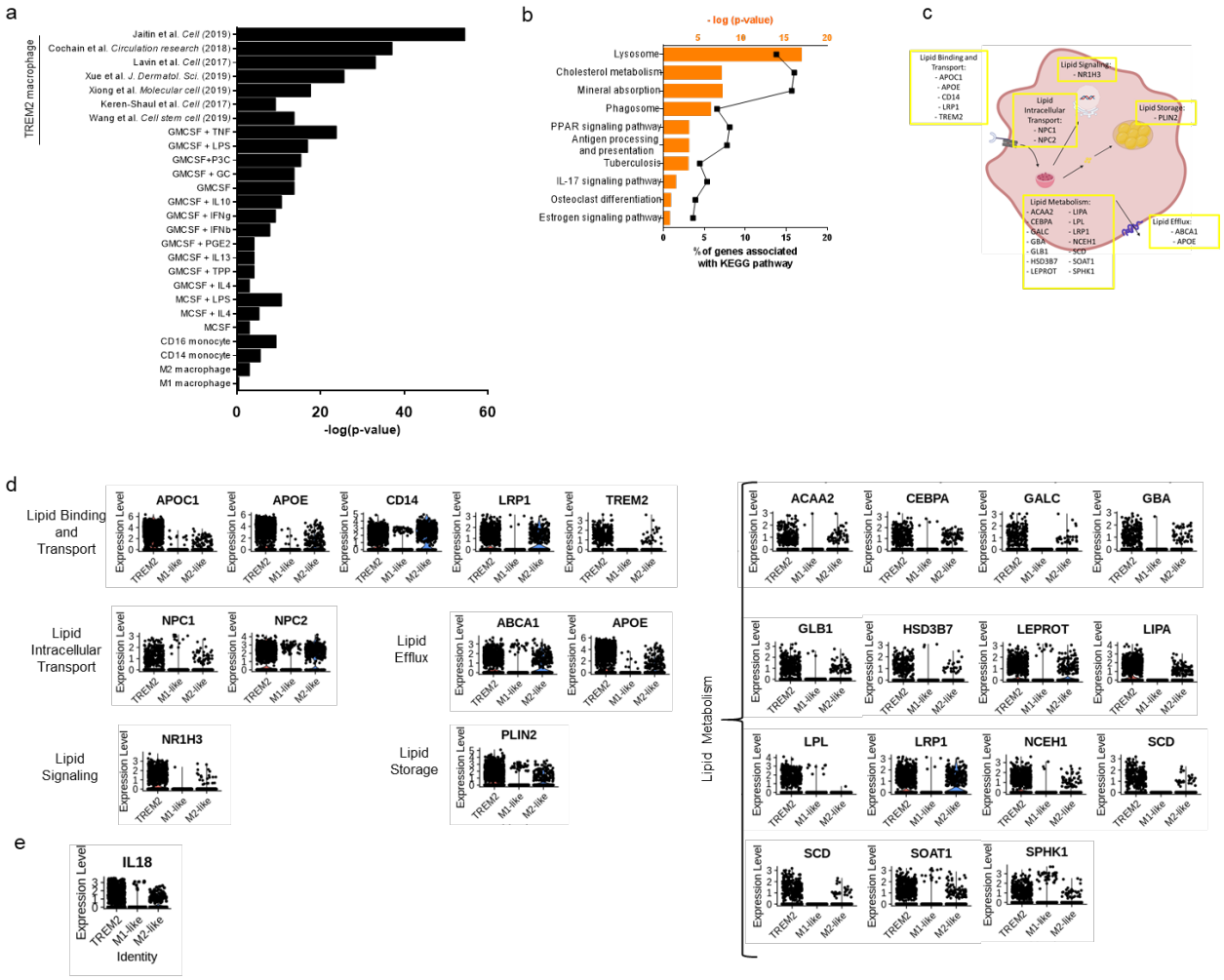

a
TREM2 macrophage
Jaitin et al. Cell (2019)
Cochain et al. Circulation research (2018)
Lavin et al. Cell (2017)
Xue et al. J. Dermatol. Sci. (2019)
Xiong et al. Molecular cell (2019)
Keren-Shaul et al. Cell (2017)
Wang et al. Cell stem cell (2019)
GMCSF + TNF
GMCSF + LPS
GMCSF+P3C
GMCSF + GC
GMCSF
GMCSF + IL10
GMCSF + IFNg
GMCSF + IFNb
GMCSF + PGE2
GMCSF + IL13
GMCSF + TPP
GMCSF + IL4
MCSF + LPS
MCSF + IL4
MCSF
CD16 monocyte
CD14 monocyte
M2 macrophage
M1 macrophage
0
20
40
60
-log(p-value)
b
- log (p-value)
5
10
15
20
Lysosome
Cholesterol metabolism
Mineral absorption
Phagosome
PPAR signaling pathway
Antigen processing and presentation
Tuberculosis
IL-17 signaling pathway
Osteoclast differentiation
Estrogen signaling pathway
% of genes associated with KEGG pathway
c
Lipid Binding and Transport:
- APOC1
- APOE
- CD14
- LRP1
- TREM2
Lipid Signaling:
- NR1H3
Lipid Intracellular Transport:
- NPC1
- NPC2
Lipid Storage:
- PLIN2
Lipid Metabolism:
- ACAA2
- CEBPA
- GALC
- GBA
- GLB1
- HSD3B7
- LEPROT
- LIPA
- LPL
- LRP1
- NCEH1
- SCD
- SOAT1
- SPHK1
Lipid Efflux:
- ABCA1
- APOE
d
Lipid Binding and Transport
APOC1
APOE
CD14
LRP1
TREM2
Lipid Intracellular Transport
NPC1
NPC2
Lipid Efflux
ABCA1
APOE
Lipid Signaling
NR1H3
Lipid Storage
PLIN2
Lipid Metabolism
ACAA2
CEBPA
GALC
GBA
GLB1
HSD3B7
LEPROT
LIPA
LPL
LRP1
NCEH1
SCD
SCD
SOAT1
SPHK1
Expression Level
TREM2
M1-like
M2-like
e
IL18
Identity
f
TREM2
Lesional
Normal Skin
g
Lesional
Non-lesional
TREM2
CD68
MERGE

Fig. 1.4. Characterization of TREM2^{+} Macrophages
a. Bar plot comparing the top 100 marker genes of the acne TREM2 macrophage subcluster s with top 100 genes of a selected list of macrophage signatures. Significance (-log(p-value)) of the overlap was calculated using hypergeometric test assuming 22,792 unique human genes.
b. KEGG pathway analysis using the top 100 marker genes of the TREM2 macrophage subcluster via Enrichr (*49, 50*).
c. Diagram illustrating the categories of lipid processing pathways based on gene ontology analysis
d. Violin plots showing normalized expression of categories of lipid processing genes based on gene ontology analysis.
e. Violin plot of normalized expression for IL18 in the macrophage sub-clusters.
f. Immunohistology staining of normal skin (control) and acne lesions for TREM2 expression.
g. Immunofluorescence staining in lesional and non-lesional acne skin biopsy samples for TREM2 and CD68 expression. Scale bar = 20 micron.

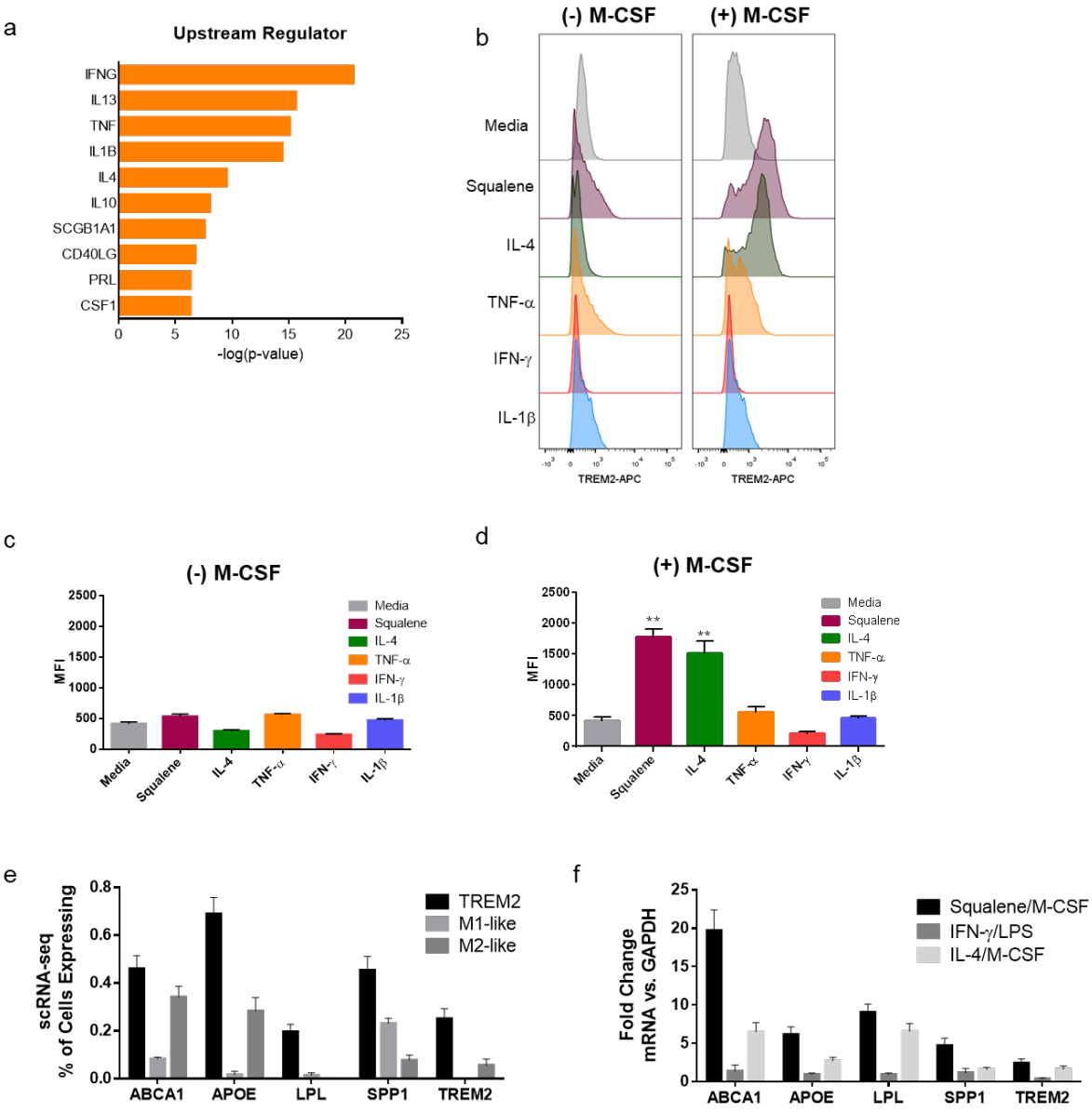

a
Upstream Regulator
IFNG
IL13
TNF
IL1B
IL4
IL10
SCGB1A1
CD40LG
PRL
CSF1
0
5
10
15
20
25
-log(p-value)
b
(-) M-CSF
(+) M-CSF
Media
Squalene
IL-4
TNF-α
IFN-γ
IL-1β
TREM2-APC
TREM2-APC
c
(-) M-CSF
MFI
2500
2000
1500
1000
500
0
Media
Squalene
IL-4
TNF-α
IFN-γ
IL-1β
d
(+) M-CSF
**
**
e
scRNA-seq
% of Cells Expressing
0.8
0.6
0.4
0.2
0.0
TREM2
M1-like
M2-like
ABCA1
APOE
LPL
SPP1
TREM2
f
Fold Change
mRNA vs. GAPDH
25
20
15
10
5
0
Squalene/M-CSF
IFN-γ/LPS
IL-4/M-CSF
ABCA1
APOE
LPL
SPP1
TREM2

Fig. 1.5. Squalene Induce TREM2 Macrophage Differentiation

a. Top 10 upstream regulators identified by IPA for top 100 marker genes of TREM2 macrophage subcluster.

b. Histogram showing the expression of surface TREM2 expression with IL-4 (20ng/mL), squalene (5 mM), TNF-a (1mg/ml), IL-1b (0.1 mg/mL), IFN-g (1 mg/mL), with or without M-CSF for 48 hours.

c. Mean fluorescence intensity (MFI) of TREM2 expression with the different stimuli (n=3) without M-CSF

d. Mean mluorescence intensity (MFI) of TREM2 expression with the different stimuli (n=3) with M-CSF

e. Comparing scRNA-seq average percent of cell expressing lipid genes for donor with more than 10 cells expressing the marker genes with real-time PCR of fold change of relative gene expression to GAPDH.

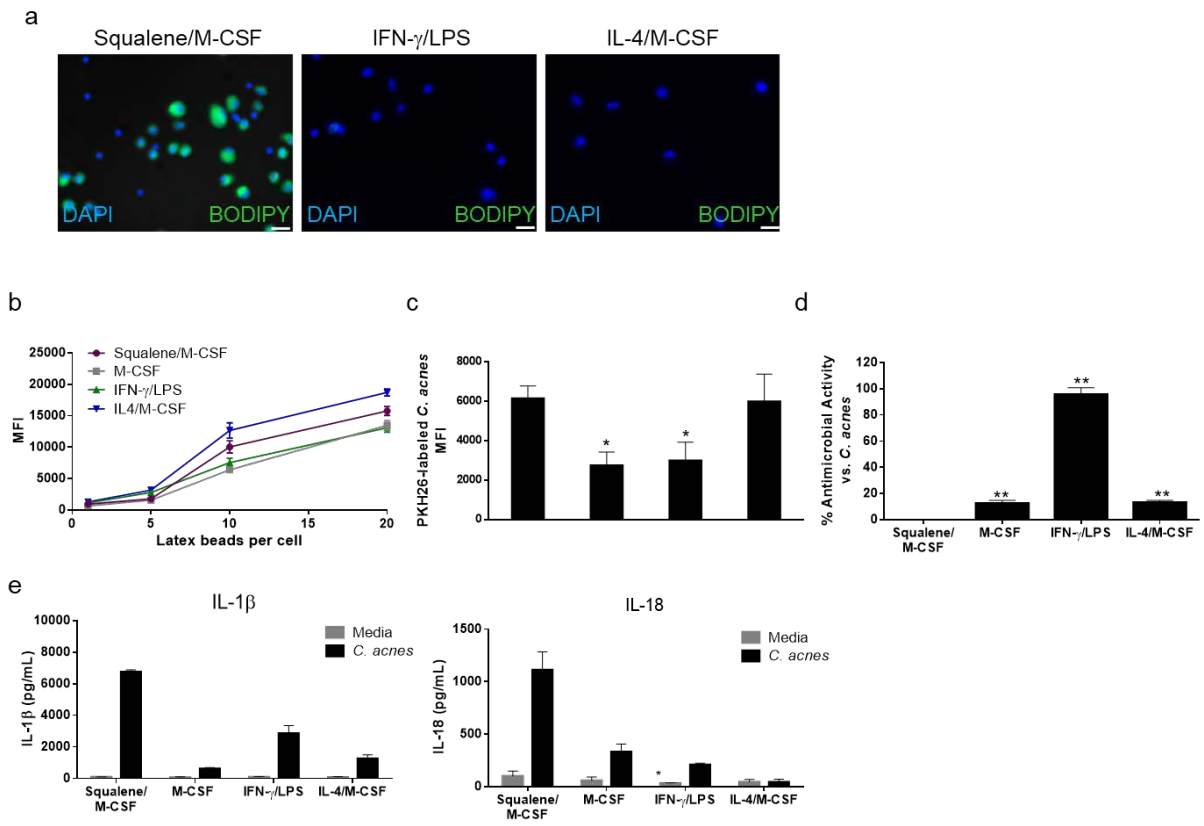


Fig. 1.6. Squalene/M-CSF Macrophage Have Increased Phagocytosis but No Antimicrobial Activity Against *C. acnes*

a. Immunocytochemical staining of in vitro stimulated macrophages. DAPI is in blue and BODIPY is in green. Scale bar = 20 microns.

b. In vitro macrophages uptake of PE-labeled latex beads at varying concentrations, as measured by flow cytometry using MFI

c. In vitro macrophages uptake PKH-26-labeled *C. acnes,* as measured by flow cytometry using MFI. Results are shown as mean ± SEM (n=3). *p <0.05. **p <0.01. Significance was calculated using one-way ANOVA with Tukey posttest

d. Cytokine and squalene-treated macrophages were stimulated with *C. acnes* for at 1 and 24 hours. Antimicrobial activity was calculated by subtracting 24-hour CFU (colony counting unit) count with 1-hour CFU count to determine the percentage of killing. Results are shown as mean ± SEM (n=3). *p <0.05. **p <0.01. Significance was calculated using one-way ANOVA with Tukey posttest.

e. In vitro cytokine and squalene-treated macrophages were stimulated with media or *C. acnes* for 24 hours. IL-1β and IL-18 in culture supernatant were measured by ELISA.

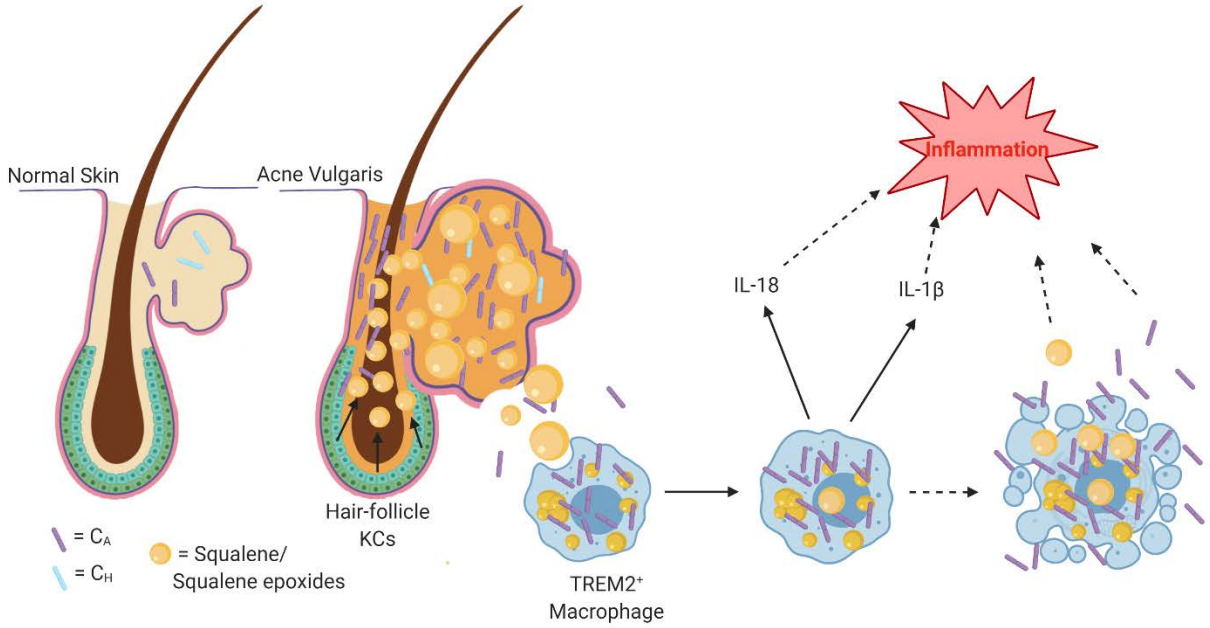


Fig. 1.7. Model of Acne Inflammation with TREM2⁺ Macrophage

Activation of hair-follicle KCs leads to production squalene and squalene epoxides. The build-up of squalene and its derivatives upregulate the expression of TREM2 on macrophages. TREM2 expression enhances the phagocytic capacity of the macrophages to uptake lipids and bacteria. However, these macrophages becomes immobilized and unable to kill the intracellular bacteria. TREM2+ macrophages upregulate expression and processing of IL-1β and IL-18 to recruit other immune cells. At the same time, some TREM2⁺ macrophages may undergo apoptosis releasing excess bacteria and squalene further activate the immune response leading to inflammation

Donor	Age	Gender	Race/Ethnicity	Severity
1	21	Female	Asian	Moderate
2	29	Female	White	Mild
3	26	Male	White	Mild
4	18	Male	Hispanic	Moderate
5	21	Female	Hispanic	Mild
6	20	Female	Asian	Moderate
Mean	22.5 ± 3.8			

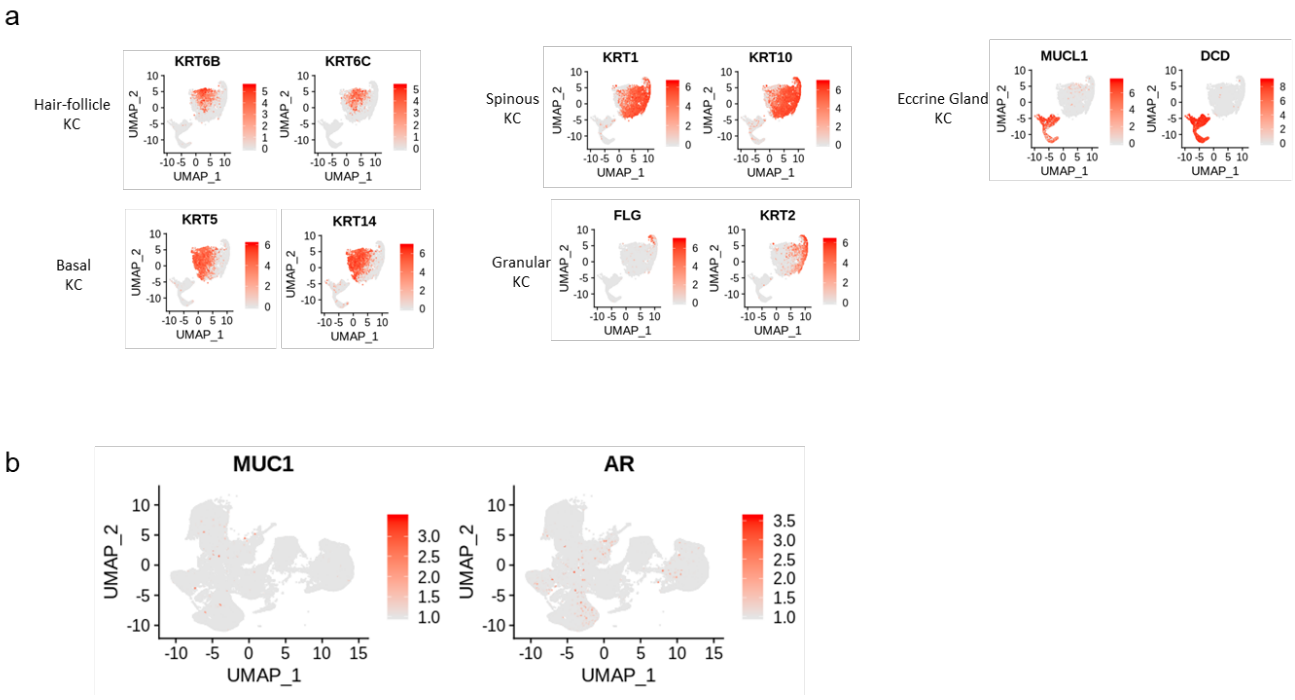
Supplemental Table 1.1. Clinical characteristics of acne subjects.

A table of clinical characteristics of acne patients. Acne severity at the biopsy site (back) was assessed by Global Evaluation Acne scale by a UCLA dermatologist.

Gene Name	Number of TREM2 studies that the gene present and is significant
APOE	7
CTSB	6
TREM2	6
CD68	5
GPNMB	5
LPL	5
SPP1	5
LGALS3	5
TYROBP	5
C1QA	4
C1QB	4
CAPG	4
CSTB	4
CTSD	4
CTSS	4
CYBB	4
LAMP1	4
LIPA	4
NPC2	4
PLD3	4
PRDX1	4
AIF1	4
C1QC	4
CTSA	4
SAT1	4
SYNGR1	4

Supplemental Table 1.2. 26 consensus signature genes from seven TREM2 macrophage studies

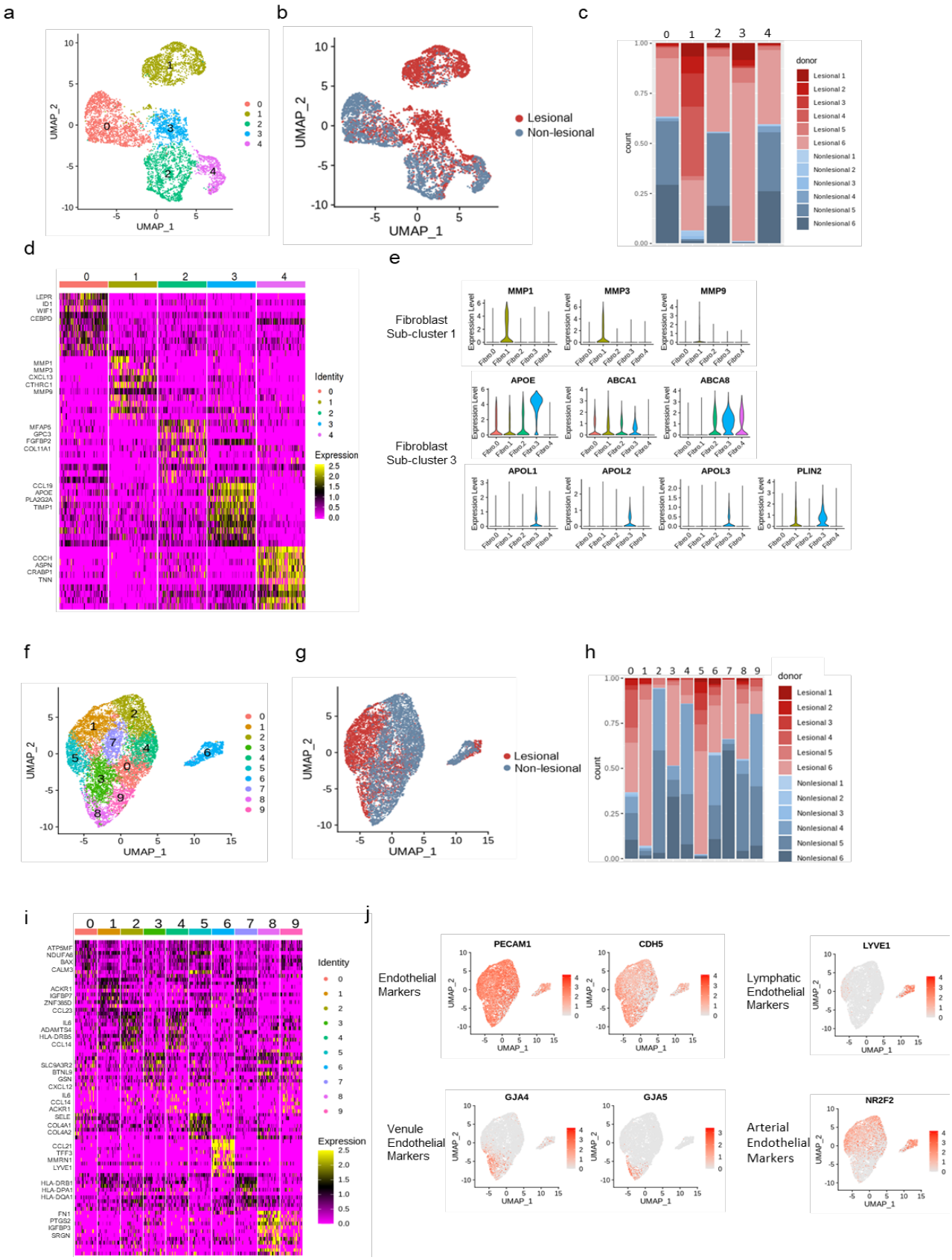
A table of genes in at least four of the seven TREM2 macrophage scRNA-seq studies



Supplemental Fig. 1.1. Undetectable Expression of Canonical Sebocyte Markers

a. UMAP plots showing normalized expression of curated set of genes to identify keratinocyte sub-clusters

b. UMAP plots showing normalized expression of MUC1 encoding epithelial membrane antigen (EMA) and AR. Higher expressions are shown in red, according to the scale shown.


a
b
c
d
e
f
g
h
i
j
UMAP_1
UMAP_2
Lesional
Non-lesional
donor
count
Identity
Expression
Fibroblast Sub-cluster 1
Fibroblast Sub-cluster 3
MMP1
MMP3
MMP9
APOE
ABCA1
ABCA8
APOL1
APOL2
APOL3
PLIN2
Endothelial Markers
Venule Endothelial Markers
Lymphatic Endothelial Markers
Arterial Endothelial Markers
PECAM1
CDH5
LYVE1
GJA4
GJA5
NR2F2

Supplementary Fig. 1.2. Diversity of Fibroblasts and Endothelial Cells in Acne lesions

a. UMAP plot for 6,735 fibroblasts from acne patients colored by sub-clusters.

b. UMAP plot for 6,735 fibroblasts colored by lesional types with 4,103 fibroblast from lesional skin and 2,632 fibroblast from non-lesional skin. Lesional cells are shown in red while non-lesional cells are shown in blue.

c. Stacked bar-plot showing the donor and lesion type composition for each of the cell types. Lesional cells are shown in red while non-lesional cells are shown in blue.

d. Heatmap showing normalized gene expression values for a curated list of sub-cluster specific genes. High expression values are shown in yellow while low expression values are show in purple.

e. Violin plot showing normalized expression of a curated list MMPs, and lipid-related genes in fibroblast sub-cluster 1 and 3 genes.

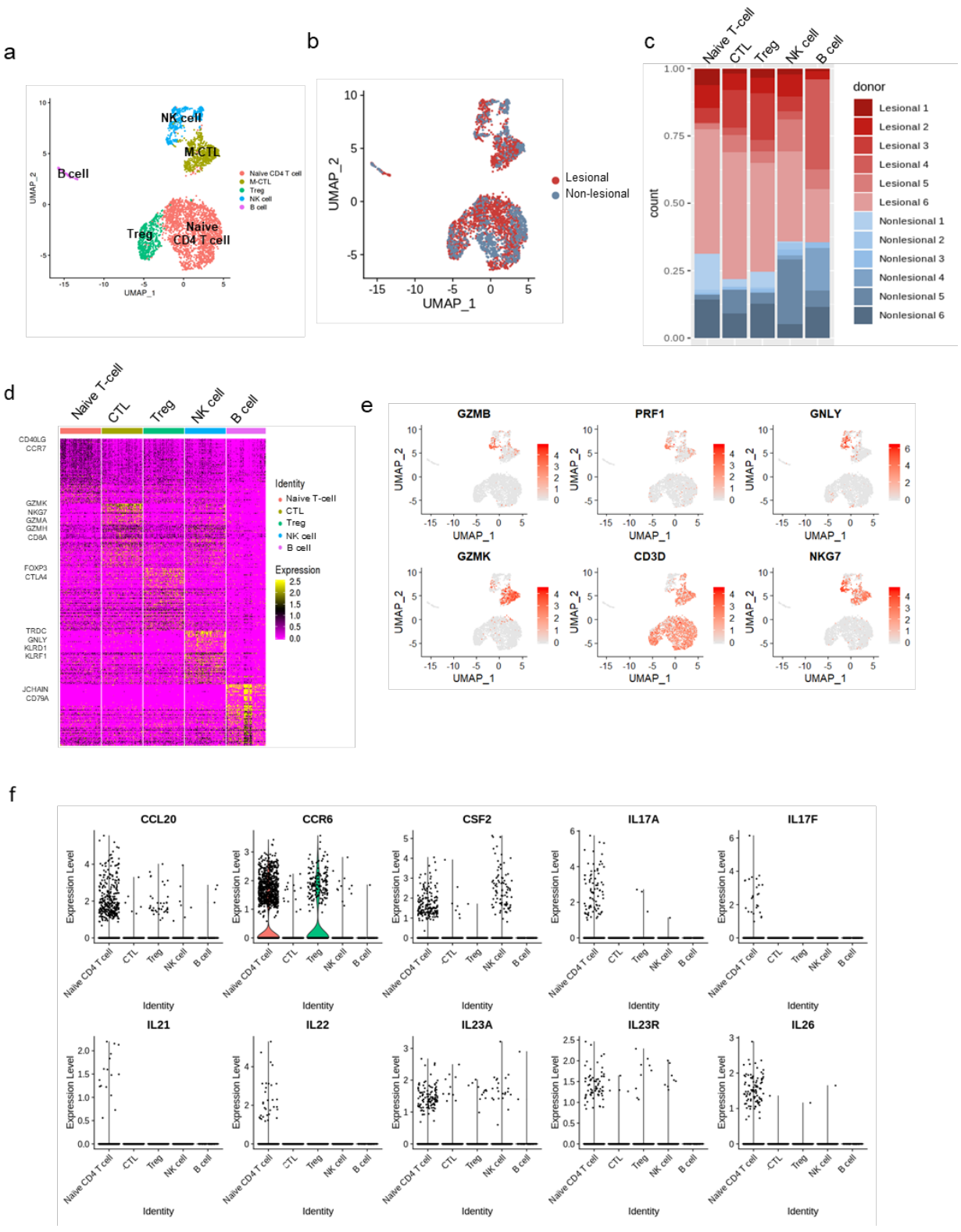
f. UMAP plot for 10,827 endothelial cells from acne patients colored by cell type cluster.

g. UMAP plot for 10,827 fibroblast colored by lesional types with 5,257 endothelial cells from lesional skin and 5,570 endothelial cells from non-lesional skin. Lesional cells are shown in red while non-lesional cells are shown in blue.

h. Stacked bar-plot showing the donor and lesion type composition for each of the sub-clusters. Lesional cells are shown in red while non-lesional cells are shown in blue.

i. Heatmap showing normalized gene expression values for a curated list of sub-cluster specific genes. High expression values are shown in yellow while low expression values are show in purple.

j. UMAP plots showing normalized expression of a curated list endothelial markers. Higher expressions are shown in red, according to the scale shown.


a
NK cell
M-CTL
B cell
Treg
Naive CD4 T cell
Naive CD4 T cell
M-CTL
Treg
NK cell
B cell
UMAP_1
UMAP_2
b
Lesional
Non-lesional
UMAP_1
UMAP_2
c
Naive T-cell
CTL
Treg
NK cell
B cell
count
donor
Lesional 1
Lesional 2
Lesional 3
Lesional 4
Lesional 5
Lesional 6
Nonlesional 1
Nonlesional 2
Nonlesional 3
Nonlesional 4
Nonlesional 5
Nonlesional 6
d
Naive T-cell
CTL
Treg
NK cell
B cell
CD40LG
CCR7
GZMK
NKG7
GZMA
GZMH
CD8A
FOXP3
CTLA4
TRDC
GNLY
KLRD1
KLRF1
JCHAIN
CD79A
Identity
Naive T-cell
CTL
Treg
NK cell
B cell
Expression
e
GZMB
PRF1
GNLY
GZMK
CD3D
NKG7
UMAP_1
UMAP_2
f
CCL20
CCR6
CSF2
IL17A
IL17F
IL21
IL22
IL23A
IL23R
IL26
Expression Level
Identity
Naive CD4 T cell
CTL
Treg
NK cell
B cell

Supplementary Fig. 1.3. Diversity of Lymphocytes in Acne Biopsies

a. UMAP plot for 3,893 lymphocytes from acne patients colored by cell types.

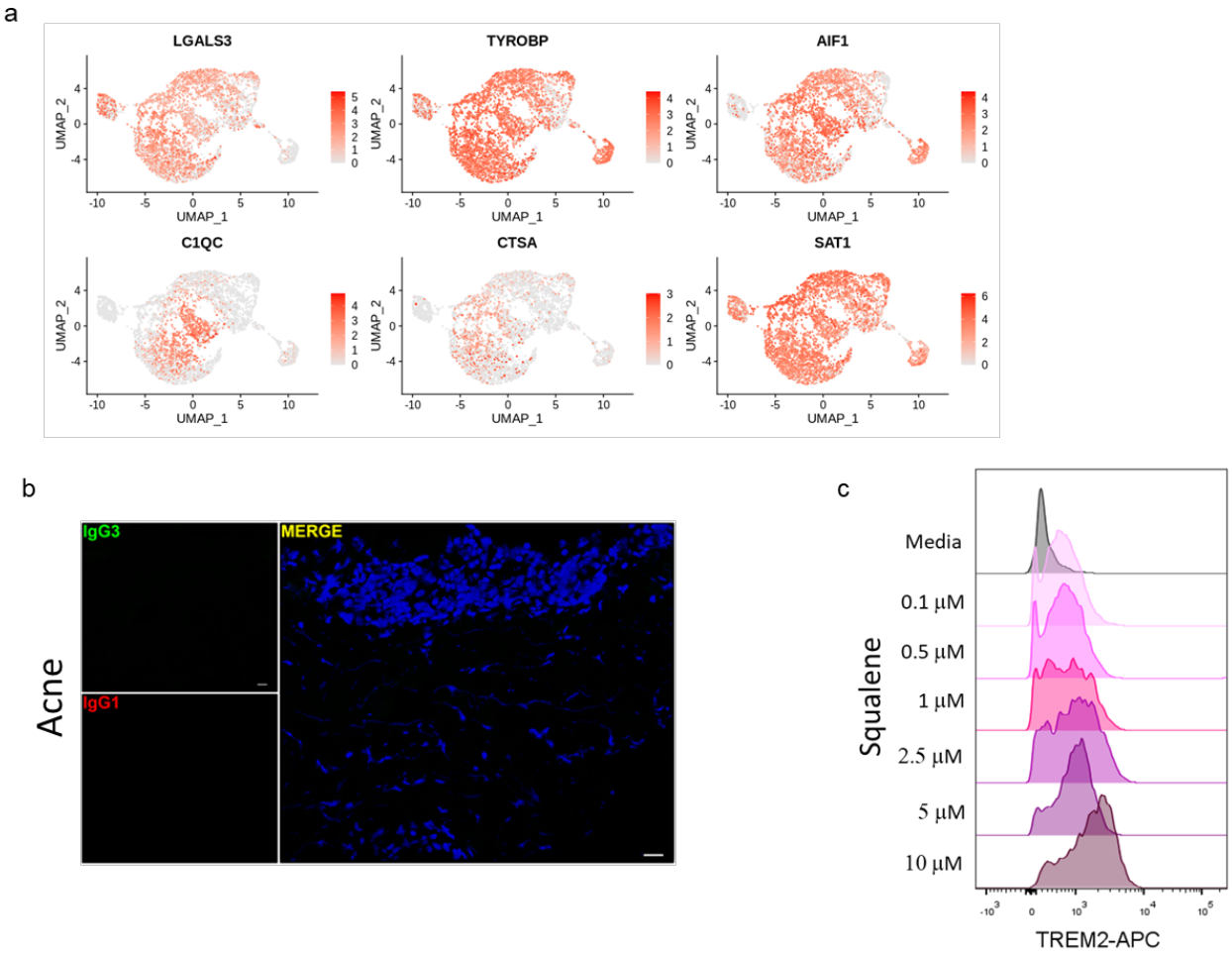
b. UMAP plot for 3,893 lymphocytes colored by lesional types with 2,751 lymphocytes from lesional skin and 1,142 lymphocytes from non-lesional skin. Lesional cells are shown in red while non-lesional cells are shown in blue.

c. Stacked bar-plot showing the donor and lesion type composition for each of the cell types. Lesional cells are shown in red while non-lesional cells are shown in blue.

d. Heatmap showing normalized gene expression values for a curated list of sub-cluster specific genes. High expression values are shown in yellow while low expression values are show in purple.

e. UMAP plots showing normalized expression of lymphocyte and NK cell markers. Higher expressions are shown in red, according to the scale shown.

f. Violin plot showing normalized expression of a curated list Th17-related genes.



Supplemental Fig. 1.4. TREM2 Signature Genes and Expression Levels

a. UMAP plots showing normalized expression of six conserved genes that were not acne signature genes from seven TREM2 macrophage scRNA-seq studies (24-30). Higher expressions are shown in red, according to the scale shown.

b. Immunofluorescence staining of isotype for TREM2 (IgG3) and CD68 (IgG1)

c. Histogram showing the expression of surface TREM2 expression with increasing concentration of squalene.

References

Artyomov, M. N., A. Munk, L. Gorvel, D. Korenfeld, M. Cella, T. Tung and E. Klechevsky (2015). "Modular expression analysis reveals functional conservation between human Langerhans cells and mouse cross-priming dendritic cells." Journal of Experimental Medicine **212**(5): 743-757.

Batista-Gonzalez, A., R. Vidal, A. Criollo and L. J. Carreno (2019). "New Insights on the Role of Lipid Metabolism in the Metabolic Reprogramming of Macrophages." Front Immunol **10**: 2993.

Bissonnette, R., Y. Poulin, J. Drew, H. Hofland and J. Tan (2017). "Olumacostat glasaretil, a novel topical sebum inhibitor, in the treatment of acne vulgaris: A phase IIa, multicenter, randomized, vehicle-controlled study." Journal of the American Academy of Dermatology **76**(1): 33-39.

Butler, A., P. Hoffman, P. Smibert, E. Papalexi and R. Satija (2018). "Integrating single-cell transcriptomic data across different conditions, technologies, and species." Nature biotechnology **36**(5): 411-420.

Cannon, J. P., M. O'Driscoll and G. W. Litman (2012). "Specific lipid recognition is a general feature of CD300 and TREM molecules." Immunogenetics **64**(1): 39-47.

Chitraju, C., T. C. Walther and R. V. Farese (2019). "The triglyceride synthesis enzymes DGAT1 and DGAT2 have distinct and overlapping functions in adipocytes." Journal of lipid research **60**(6): 1112-1120.

Cochain, C., E. Vafadarnejad, P. Arampatzi, J. Pelisek, H. Winkels, K. Ley, D. Wolf, A. E. Saliba and A. Zernecke (2018). "Single-Cell RNA-Seq Reveals the Transcriptional Landscape and Heterogeneity of Aortic Macrophages in Murine Atherosclerosis." Circ Res **122**(12): 1661-1674.

Costet, P., Y. Luo, N. Wang and A. R. Tall (2000). "Sterol-dependent transactivation of theABC1 promoter by the liver X receptor/retinoid X receptor." Journal of Biological Chemistry **275**(36): 28240-28245.

Daws, M. R., P. M. Sullam, E. C. Niemi, T. T. Chen, N. K. Tchao and W. E. Seaman (2003). "Pattern recognition by TREM-2: binding of anionic ligands." The Journal of Immunology **171**(2): 594-599.

De Jong, A., T.-Y. Cheng, S. Huang, S. Gras, R. W. Birkinshaw, A. G. Kasmar, I. Van Rhijn, V. Peña-Cruz, D. T. Ruan and J. D. Altman (2014). "CD1a-autoreactive T cells recognize natural skin oils that function as headless antigens." Nature immunology **15**(2): 177.

Esposito, K., A. Pontillo, M. Ciotola, C. Di Palo, E. Grella, G. Nicoletti and D. Giugliano (2002). "Weight loss reduces interleukin-18 levels in obese women." The Journal of Clinical Endocrinology & Metabolism **87**(8): 3864-3866.

Freedberg, I. M., M. Tomic-Canic, M. Komine and M. Blumenberg (2001). "Keratins and the keratinocyte activation cycle." Journal of Investigative Dermatology **116**(5): 633-640.

Fuchs, E. and S. Raghavan (2002). "Getting under the skin of epidermal morphogenesis." Nature Reviews Genetics **3**(3): 199-209.

Gerber, P. A., P. Hevezi, B. A. Buhren, C. Martinez, H. Schrumpf, M. Gasis, S. Grether-Beck, J. Krutmann, B. Homey and A. Zlotnik (2013). "Systematic identification and characterization of novel human skin-associated genes encoding membrane and secreted proteins." PloS one **8**(6).

Gerrick, K. Y., E. R. Gerrick, A. Gupta, S. J. Wheelan, S. Yegnasubramanian and E. M. Jaffee (2018). "Transcriptional profiling identifies novel regulators of macrophage polarization." PloS one **13**(12).

Guy, R., M. R. Green and T. Kealey (1996). "Modeling acne in vitro." Journal of investigative dermatology **106**(1): 176-182.

Hung, J., B. M. McQuillan, C. M. Chapman, P. L. Thompson and J. P. Beilby (2005). "Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance." Arteriosclerosis, thrombosis, and vascular biology **25**(6): 1268-1273.

Jaitin, D. A., L. Adlung, C. A. Thaiss, A. Weiner, B. Li, H. Descamps, P. Lundgren, C. Bleriot, Z. Liu, A. Deczkowska, H. Keren-Shaul, E. David, N. Zmora, S. M. Eldar, N. Lubezky, O. Shibolet, D. A. Hill, M. A. Lazar, M. Colonna, F. Ginhoux, H. Shapiro, E. Elinav and I. Amit (2019). "Lipid-Associated Macrophages Control Metabolic Homeostasis in a Trem2-Dependent Manner." Cell **178**(3): 686-698 e614.

Jeremy, A. H., D. B. Holland, S. G. Roberts, K. F. Thomson and W. J. Cunliffe (2003). "Inflammatory events are involved in acne lesion initiation." J Invest Dermatol **121**(1): 20-27.

Jerne, N. K. (1984). "Idiotypic networks and other preconceived ideas." Immunol Rev **79**: 5-24.

Kang, S., S. Cho, J. H. Chung, C. Hammerberg, G. J. Fisher and J. J. Voorhees (2005). "Inflammation and extracellular matrix degradation mediated by activated transcription factors nuclear factor-κB and activator protein-1 in inflammatory acne lesions in vivo." The American journal of pathology **166**(6): 1691-1699.

Kelhala, H. L., R. Palatsi, N. Fyhrquist, S. Lehtimaki, J. P. Vayrynen, M. Kallioinen, M. E. Kubin, D. Greco, K. Tasanen, H. Alenius, B. Bertino, I. Carlavan, B. Mehul, S. Deret, P. Reiniche, P. Martel, C. Marty, U. Blume-Peytavi, J. J. Voegel and A. Lauerma (2014). "IL-17/Th17 pathway is activated in acne lesions." PLoS One **9**(8): e105238.

Keren-Shaul, H., A. Spinrad, A. Weiner, O. Matcovitch-Natan, R. Dvir-Szternfeld, T. K. Ulland, E. David, K. Baruch, D. Lara-Astaiso, B. Toth, S. Itzkovitz, M. Colonna, M. Schwartz and I. Amit (2017). "A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease." Cell **169**(7): 1276-1290 e1217.

Kistowska, M., S. Gehrke, D. Jankovic, K. Kerl, A. Fettelschoss, L. Feldmeyer, G. Fenini, A. Kolios, A. Navarini and R. Ganceviciene (2014). "IL-1β drives inflammatory responses to Propionibacterium acnes in vitro and in vivo." Journal of Investigative Dermatology **134**(3): 677-685.

Kolar, S. L., C.-M. Tsai, J. Torres, X. Fan, H. Li and G. Y. Liu (2019). "Propionibacterium acnes–induced immunopathology correlates with health and disease association." JCI Insight **4**(5).

Latkowski, J.-A. M., I. M. Freedberg and M. Blumenberg (1995). "Keratinocyte growth factor and keratin gene regulation." Journal of dermatological science **9**(1): 36-44.

Lavin, Y., S. Kobayashi, A. Leader, E. D. Amir, N. Elefant, C. Bigenwald, R. Remark, R. Sweeney, C. D. Becker, J. H. Levine, K. Meinhof, A. Chow, S. Kim-Shulze, A. Wolf, C. Medaglia, H. Li, J. A. Rytlewski, R. O. Emerson, A. Solovyov, B. D. Greenbaum, C. Sanders, M. Vignali, M. B. Beasley, R. Flores, S. Gnjatic, D. Pe'er, A. Rahman, I. Amit and M. Merad (2017). "Innate Immune Landscape in Early Lung Adenocarcinoma by Paired Single-Cell Analyses." Cell **169**(4): 750-765 e717.

Lesley, R., L. M. Kelly, Y. Xu and J. G. Cyster (2006). "Naive CD4 T cells constitutively express CD40L and augment autoreactive B cell survival." Proceedings of the National Academy of Sciences **103**(28): 10717-10722.

Lim, H. W., S. A. B. Collins, J. S. Resneck, Jr., J. L. Bolognia, J. A. Hodge, T. A. Rohrer, M. J. Van Beek, D. J. Margolis, A. J. Sober, M. A. Weinstock, D. R. Nerenz, W. Smith Begolka and J. V. Moyano (2017). "The burden of skin disease in the United States." J Am Acad Dermatol **76**(5): 958-972 e952.

Lopez, D., D. Montoya, M. Ambrose, L. Lam, L. Briscoe, C. Adams, R. L. Modlin and M. Pellegrini (2017). "SaVanT: a web-based tool for the sample-level visualization of molecular signatures in gene expression profiles." BMC genomics **18**(1): 824.

Lovaszi, M., M. Mattii, K. Eyerich, A. Gacsi, E. Csanyi, D. Kovacs, R. Ruhl, A. Szegedi, L. Kemeny, M. Stahle, C. C. Zouboulis, S. Eyerich and D. Torocsik (2017). "Sebum lipids influence macrophage polarization and activation." Br J Dermatol **177**(6): 1671-1682.

Luu, W., G. Hart-Smith, L. J. Sharpe and A. J. Brown (2015). "The terminal enzymes of cholesterol synthesis, DHCR24 and DHCR7, interact physically and functionally." Journal of lipid research **56**(4): 888-897.

Murphy, A. J., M. J. Kraakman, H. L. Kammoun, D. Dragoljevic, M. K. Lee, K. E. Lawlor, J. M. Wentworth, A. Vasanthakumar, M. Gerlic and L. W. Whitehead (2016). "IL-18 production from the NLRP1 inflammasome prevents obesity and metabolic syndrome." Cell metabolism **23**(1): 155-164.

Nguyen, C. M., J. Koo and K. M. Cordoro (2016). "Psychodermatologic effects of atopic dermatitis and acne: a review on self-esteem and identity." Pediatric dermatology **33**(2): 129-135.

O'Hagan, D. T., G. S. Ott, G. V. Nest, R. Rappuoli and G. D. Giudice (2013). "The history of MF59® adjuvant: a phoenix that arose from the ashes." Expert review of vaccines **12**(1): 13-30.

Papalexi, E. and R. Satija (2018). "Single-cell RNA sequencing to explore immune cell heterogeneity." Nature Reviews Immunology **18**(1): 35.

Pappas, A., S. Johnsen, J.-C. Liu and M. Eisinger (2009). "Sebum analysis of individuals with and without acne." Dermato-endocrinology **1**(3): 157-161.

Peck, B. and A. Schulze (2016). "Lipid desaturation–the next step in targeting lipogenesis in cancer?" The FEBS journal **283**(15): 2767-2778.

Polak, M. E., S. M. Thirdborough, C. Y. Ung, T. Elliott, E. Healy, T. C. Freeman and M. R. Ardern-Jones (2014). "Distinct molecular signature of human skin Langerhans cells

denotes critical differences in cutaneous dendritic cell immune regulation." Journal of Investigative Dermatology **134**(3): 695-703.

Purvis, D., E. Robinson, S. Merry and P. Watson (2006). "Acne, anxiety, depression and suicide in teenagers: A cross-sectional survey of New Zealand secondary school students." Journal of paediatrics and child health **42**(12): 793-796.

Ruiz, A., O. Dols-Icardo, M. J. Bullido, P. Pastor, E. Rodríguez-Rodríguez, A. L. de Munain, M. M. de Pancorbo, J. Pérez-Tur, V. Álvarez and A. Antonell (2014). "Assessing the role of the TREM2 p. R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia." Neurobiology of aging **35**(2): 444. e441-444. e444.

Sanford, J. A., A. M. O'Neill, C. C. Zouboulis and R. L. Gallo (2019). "Short-Chain Fatty Acids from Cutibacterium acnes Activate Both a Canonical and Epigenetic Inflammatory Response in Human Sebocytes." J Immunol **202**(6): 1767-1776.

Sanford, J. A., L.-J. Zhang, M. R. Williams, J. A. Gangoiti, C.-M. Huang and R. L. Gallo (2016). "Inhibition of HDAC8 and HDAC9 by microbial short-chain fatty acids breaks immune tolerance of the epidermis to TLR ligands." Science immunology **1**(4).

Singh, V., N. Gohil, I. Mani, G. Bhattacharjee and K. Khambhati (2019). "Engineering strategies in microorganisms for the enhanced production of Squalene: Advances, challenges, and opportunities." Frontiers in Bioengineering and Biotechnology **7**: 50.

Sissaoui, S., J. Yu, A. Yan, R. Li, L. J. Zhu, A. Kucukural, O. Yukselen and N. D. Lawson (2020). "Genomic Characterization of Endothelial Enhancers Reveals a Multifunctional Role for NR2F2 in Regulation of Arteriovenous Gene Expression." Circulation Research.

Slattery, C. F., J. A. Beck, L. Harper, G. Adamson, Z. Abdi, J. Uphill, T. Campbell, R. Druyeh, C. J. Mahoney and J. D. Rohrer (2014). "R47H TREM2 variant increases risk of typical early-onset Alzheimer's disease but not of prion or frontotemporal dementia." Alzheimer's & Dementia **10**(6): 602-608. e604.

Smith, K. R. and D. M. Thiboutot (2008). "Thematic review series: skin lipids. Sebaceous gland lipids: friend or foe?" J Lipid Res **49**(2): 271-281.

Stuart, T., A. Butler, P. Hoffman, C. Hafemeister, E. Papalexi, W. M. Mauck, 3rd, Y. Hao, M. Stoeckius, P. Smibert and R. Satija (2019). "Comprehensive Integration of Single-Cell Data." Cell **177**(7): 1888-1902 e1821.

Thiboutot, D. M., B. Dreno, A. Abanmi, A. F. Alexis, E. Araviiskaia, M. I. Barona Cabal, V. Bettoli, F. Casintahan, S. Chow, A. da Costa, T. El Ouazzani, C. L. Goh, H. P. M. Gollnick, M. Gomez, N. Hayashi, M. I. Herane, J. Honeyman, S. Kang, L. Kemeny, R. Kubba, J. Lambert, A. M. Layton, J. J. Leyden, J. L. Lopez-Estebaranz, N. Noppakun, F. Ochsendorf, C. Oprica, B. Orozco, M. Perez, J. Piquero-Martin, J. A. See, D. H. Suh, J. Tan, V. T. Lozada, P. Troielli and L. F. Xiang (2018). "Practical management of acne for clinicians: An international consensus from the Global Alliance to Improve Outcomes in Acne." J Am Acad Dermatol **78**(2 Suppl 1): S1-S23 e21.

Trapnell, C., D. Cacchiarelli, J. Grimsby, P. Pokharel, S. Li, M. Morse, N. J. Lennon, K. J. Livak, T. S. Mikkelsen and J. L. Rinn (2014). "Pseudo-temporal ordering of individual cells reveals dynamics and regulators of cell fate decisions." Nature biotechnology **32**(4): 381.

Trivedi, N. R., K. L. Gilliland, W. Zhao, W. Liu and D. M. Thiboutot (2006). "Gene array expression profiling in acne lesions reveals marked upregulation of genes involved in inflammation and matrix remodeling." J Invest Dermatol **126**(5): 1071-1079.

Turkish, A. R., A. L. Henneberry, D. Cromley, M. Padamsee, P. Oelkers, H. Bazzi, A. M. Christiano, J. T. Billheimer and S. L. Sturley (2005). "Identification of Two Novel Human Acyl-CoA Wax Alcohol Acyltransferases MEMBERS OF THE DIACYLGLYCEROL ACYLTRANSFERASE 2 (DGAT2) GENE SUPERFAMILY." Journal of biological chemistry **280**(15): 14755-14764.

Turnbull, I. R., S. Gilfillan, M. Cella, T. Aoshi, M. Miller, L. Piccio, M. Hernandez and M. Colonna (2006). "Cutting edge: TREM-2 attenuates macrophage activation." J Immunol **177**(6): 3520-3524.

Vallerand, I., R. Lewinson, L. Parsons, M. Lowerison, A. Frolkis, G. Kaplan, C. Barnabe, A. Bulloch and S. Patten (2018). "Risk of depression among patients with acne in the UK: a population-based cohort study." The British journal of dermatology **178**(3): e194.

Verma, R., S. Kubendran, S. K. Das, S. Jain and S. K. Brahmachari (2005). "SYNGR1 is associated with schizophrenia and bipolar disorder in southern India." Journal of human genetics **50**(12): 635-640.

Wang, E. C. E., Z. Dai, A. W. Ferrante, C. G. Drake and A. M. Christiano (2019). "A Subset of TREM2(+) Dermal Macrophages Secretes Oncostatin M to Maintain Hair Follicle Stem Cell Quiescence and Inhibit Hair Growth." Cell Stem Cell **24**(4): 654-669 e656.

Wang, Y., M. Cella, K. Mallinson, J. D. Ulrich, K. L. Young, M. L. Robinette, S. Gilfillan, G. M. Krishnan, S. Sudhakar and B. H. Zinselmeyer (2015). "TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model." Cell **160**(6): 1061-1071.

Weber, C., S. Meiler, Y. Döring, M. Koch, M. Drechsler, R. T. Megens, Z. Rowinska, K. Bidzhekov, C. Fecher and E. Ribechini (2011). "CCL17-expressing dendritic cells drive atherosclerosis by restraining regulatory T cell homeostasis in mice." The Journal of clinical investigation **121**(7): 2898-2910.

Xiong, X., H. Kuang, S. Ansari, T. Liu, J. Gong, S. Wang, X. Y. Zhao, Y. Ji, C. Li, L. Guo, L. Zhou, Z. Chen, P. Leon-Mimila, M. T. Chung, K. Kurabayashi, J. Opp, F. Campos-Perez, H. Villamil-Ramirez, S. Canizales-Quinteros, R. Lyons, C. N. Lumeng, B. Zhou, L. Qi, A. Huertas-Vazquez, A. J. Lusis, X. Z. S. Xu, S. Li, Y. Yu, J. Z. Li and J. D. Lin (2019). "Landscape of Intercellular Crosstalk in Healthy and NASH Liver Revealed by Single-Cell Secretome Gene Analysis." Mol Cell **75**(3): 644-660 e645.

Xue, D., T. Tabib, C. Morse and R. Lafyatis (2020). "Transcriptome landscape of myeloid cells in human skin reveals diversity, rare populations and putative DC progenitors." J Dermatol Sci **97**(1): 41-49.

Xue, J., S. V. Schmidt, J. Sander, A. Draffehn, W. Krebs, I. Quester, D. De Nardo, T. D. Gohel, M. Emde, L. Schmidleithner, H. Ganesan, A. Nino-Castro, M. R. Mallmann, L. Labzin, H. Theis, M. Kraut, M. Beyer, E. Latz, T. C. Freeman, T. Ulas and J. L. Schultze (2014). "Transcriptome-based network analysis reveals a spectrum model of human macrophage activation." Immunity **40**(2): 274-288.

Yao, H., K. Coppola, J. E. Schweig, F. Crawford, M. Mullan and D. Paris (2019). "Distinct Signaling Pathways Regulate TREM2 Phagocytic and NFκB Antagonistic Activities." Frontiers in cellular neuroscience **13**.

Zhang, Q., Y. He, N. Luo, S. J. Patel, Y. Han, R. Gao, M. Modak, S. Carotta, C. Haslinger, D. Kind, G. W. Peet, G. Zhong, S. Lu, W. Zhu, Y. Mao, M. Xiao, M. Bergmann, X. Hu, S. P. Kerkar, A. B. Vogt, S. Pflanz, K. Liu, J. Peng, X. Ren and Z. Zhang (2019). "Landscape and Dynamics of Single Immune Cells in Hepatocellular Carcinoma." Cell **179**(4): 829-845 e820.

Zhang, X., M. Yin and L.-j. Zhang (2019). "Keratin 6, 16 and 17—Critical Barrier Alarmin Molecules in Skin Wounds and Psoriasis." Cells **8**(8): 807.

Zouboulis, C., E. Jourdan and M. Picardo (2014). "Acne is an inflammatory disease and alterations of sebum composition initiate acne lesions." Journal of the European Academy of Dermatology and Venereology **28**(5): 527-532.

CHAPTER 3

C. acnes RNA Stimulate IFN-γ Release in a TLR-8/IL-12/IL-18 Dependent Pathway

Abstract

Acne is a chronic inflammatory skin disease thought to be driven in part by the activation of the immune response to *Cutibacterium acnes* (*C. acnes*), a ubiquitous skin commensal. It has been reported that the relative abundance of *C. acnes* is similar in the follicles of acne patients compared to healthy controls. This suggests the presence of the bacteria alone does not lead to inflammation. Metagenomic analyses of the skin microbiome revealed a diverse population of *C. acnes* strains that are distinct between acne and healthy individuals. These strains also differed in their potential to trigger inflammation with strains enriched on the skin of acne patients (C_A) induces higher pro-inflammatory cytokine compared to strains associated with clear or healthy skin (C_H). However, the virulence factor that accounts for the differences in immune response between C_A and C_H are still unknown. In this study, we showed that *C. acnes* activates the innate immune system through RNA species that are usually reserved for viral detection. Interestingly, RNA species from C_A and C_H had different bioanalyzer profiles and triggered distinct immune response as seen with live bacteria. We also showed that C_A RNA stimulate the immune response through a TLR-8/IL-18/IL-12p40 pathway. Our in vitro data correlated well with our single cell RNA-seq data showing the abundance of IFN-γ and TLR-8 in acne lesions. Our study suggests that RNA from C_A triggers inflammatory response that contribute to the pathogenesis and clinical presentation of acne.

Introduction

Microbes play a large part in the pathogenesis of disease (Rosenthal, Goldberg et al. 2011). On the skin surface, the interplay within the microbial community is essential for the maintenance of healthy skin. *Cutibacterium acnes* (*C. acnes*) (formerly known as *Propionibacterium acnes*) is a Gram-positive, microaerophilic bacillus that is considered to be one of the factors driving inflammation in acne (Bhate and Williams 2013). However, the direct cause-and-effect relationship between the bacteria and the disease has been difficult to establish given that *C. acnes* is a ubiquitous bacterium and that there was no quantitative difference in the number of bacteria between subjects with and without acne (Barnard, Shi et al. 2016). *C. acnes* has been shown to coexist in the pilosebaceous unit with other *Cutibacterium* spp. included *C. granulosum* and *C. humerusii* (Grice and Segre 2011).

Recent genomic and phenotypic analyses provide new insights into the cellular physiology underlying the heterogeneity of the bacteria. Certain *C. acnes* strains, designated type IA1 or IC, identified by multi-locus sequence typing (MLST), were present in significant quantities in approximately 30–40% of patients with acne (C_A). Conversely, the phylotype II, RT 6 subgroup was found to be 99% associated with healthy skin (C_H) (Fitz-Gibbon, Tomida et al. 2013, Tomida, Nguyen et al. 2013). The two divergent phylotypes also exhibited differences in inflammatory potential with C_A inducing higher pro-inflammatory cytokine secretion from human peripheral blood mononuclear cells (PBMCs), Th17 clones, and murine model (Yu, Champer et al. 2016, Agak, Kao et al. 2018, Kolar, Tsai et al. 2019).

Acne is characterized by inflammation localized to the pilosebaceous follicle, which promotes redness, pain, post-inflammatory hyperpigmentation, and scars that are associated with inflammation-induced damage (Thiboutot, Dreno et al. 2018). Although acne is a disease of the skin, the effects of acne go beyond the skin manifestations as studies have found that the clinical presence of inflammatory skin lesions impose a significant psychologic burden on

adolescents and young adults associated with low self-esteem (Nguyen, Koo et al. 2016), anxiety, depression, and suicidal ideation (Purvis, Robinson et al. 2006, Vallerand, Lewinson et al. 2018).

Given the increasing recognition that commensal and mutualistic microorganisms are necessary for the maintenance of a healthy human physiology, it is essential that we understand how the different phylotypes in *C. acnes* modulate the inflammatory microenvironment. Thus, if we were able to identify the bacterial component from C_A that promote inflammation, it may be possible to prevent inflammatory sequelae and promote skin homeostasis.

Results:

C_A infection induces IFN-γ secretion at an early time point

We hypothesized that the pro-inflammatory potential of C_A is associated with a strain-specific virulence factor. To investigate this, we stimulated peripheral blood mononuclear cells (PBMCs) with three C_A isolates: HL5PA1, HL43PA1, and HL96PA1 and three C_H isolates: HL42PA3, HL110PA3, and HL110PA4. While we found C_A-treated PBMCs secrete higher amounts of pro-inflammatory, IFN-γ, TNF-α, and IL-1β, we noted the largest difference, approximately three-fold, in IFN-γ secretion among the C_A and C_H strains compared to 1.5-fold difference for TNF-α and IL-1β (Fig. 2.1a-c).

To determine whether IFN-γ plays a role in innate and adaptive immune response in *C. acnes* infection, we studied the time course of IFN-γ secretion. Within six hours to *C. acnes*, significant IFN-γ secretion could be detected in cell supernatant compared to tetanus toxoid, a known adaptive immune response activator, which was not detectable until 24 hours after induction (Fig. 2.1d). IFN-γ secretion continued to increase throughout the seven-day period (Fig. 2.1d).

NK Cells are the main producer of IFN-γ

We next determined which cells in PBMCs are responsible for the early IFN-γ response in *C. acnes* infection. We depleted $CD3^+$ T cells, $CD56^+$ NK cells, and $CD14^+$ monocytes in PBMC culture as well as isolated and enriched for $CD3^+$, $CD56^+$, and $CD14^+$ cells in culture and stimulated them with *C. acnes* (Fig. 2.2a-b). NK (CD56+) depletion significant decreased IFN-γ secretion while isolated NK cells were able to induce IFN-γ response (Fig. 2.2b). Intra-cellular flow cytometry confirmed $CD56^+CD3^-$ NK cells to be the major producer of IFN-γ (Fig. 2.2c-d).

TLR-8, IL-18, and IL-12p40 are required for IFN-γ secretion

Since IL-18 and IL-12/IL-23 are known to synergistically trigger secretion of IFN-γ (Ziblat, Nuñez et al. 2018), we investigated their kinetics in response to *C. acnes* infection. We detected a robust IL-18 and IL-12p40 response as early as 6 hours and the levels are steadily maintained up until 24 hours as IFN- γ exponentially increased (Fig. 2.3a). Using neutralizing antibodies to IL-18 and IL-12p40, determined that the inhibition of IL-18 and IL-12p40 also impeded IFN-γ secretion (Fig. 2.3b). TLR-8 activation in monocytes is known to secrete IL-18 and IL-12/IL-23 (Gorski, Waller et al. 2006, Keegan, Krutzik et al. 2018, Ziblat, Nuñez et al. 2018), which activate NK cells to produce IFN-γ (Gorski, Waller et al. 2006). Using a TLR8- inhibitor and an antagonist, we found TLR8 inhibition significantly decreased IFN-γ secretion. TLR-2 and TLR-4 have been shown to play a role in *C. acnes* infection (Liu, Krutzik et al. 2005). We tested whether TLR-2, TLR-4, or TLR-8 play a role in IFN-γ secretion. The activation of TLR-2 and TLR-4 did not induce IFN-γ as expected and thus, their inhibition had no effect on IFN-γ secretion (Fig. 2.3d-e) These results suggest IFN-γ secretion in a *C. acnes* infection is regulated by TLR8, IL-18, and IL-12p40.

C_A RNA induces higher secretion of IFN-γ, IL12p40, and IL-18 compared to C_H RNA similar to the live bacteria response

To determine which bacterial components were able to induce an IFN-γ response, we added live, heat-killed, RNase-treated, and DNAse-treated bacteria to PBMC culture. HK bacteria and RNase-treated bacteria did not induce an IFN-γ response (Fig. 2.4a). We also isolated bacterial supernatant, RNA, and DNA as well as placed PBMCs in a transwell (Fig. 2.4b). We observed that bacterial supernatant and transwell experiment did not induce an IFN-γ response. Interestingly, when we transfected bacterial RNA and DNA with DOTAP, a cationic surfactant reagent to facilitate uptake, into PBMCs, we observed the secretion of IFN-γ at a similar level as the live bacteria (Fig. 2.4b). Bacterial RNA transfection had a more robust response compared DNA transfection (Fig. 2.4b).

To address whether C_A and C_H RNA induce different immune responses, we isolated RNA from C_A and C_H and transfected them with DOTAP into PBMCs or monocyte-derived macrophages (MDMs). C_A RNA induced higher IFN-γ, IL-18, and IL-12p40 secretion in PBMCs as seen with live bacteria (Fig. 2.4c-e). Although we did not detect any IFN-γ secretion in MDMs, we observed similar increase in IL-18 and IL-12p40 secretion in C_A-treated cells (Fig. 2.4f).

C_A and C_H harbor different species of bacterial RNA

To identify differences in RNA species between the bacterial strains, we measured their lengths using a bioanalyzer. The bioanalyzer traces showed C_A RNA predominantly ranges from 25-200 nucleotides (nt) while C_H RNA has peaks at a range of 25-200 nt and 1000-4000 nt (Fig. 2.5a-b). We also isolated RNA species from other commensal inhabitants of the pilosebaceous gland such as: *C. humerusii*, and *C. granulosum* and their RNAs were similar to C_H (Fig. 2.5c).

In vivo scRNA-seq data from acne lesions confirms higher expression of TLR8, IL18, and IFNG

Lastly, we looked at whether the IL-18 to IFN-γ pathway may play a role in acne lesions. From our single cell RNA-seq data of acne lesions, we found more lesional cells expressing IFN-γ compared to non-lesional cells (Fig. 2.6 a). Lymphocyte, specifically cytotoxic T lymphocyte (CTL) was found to be the major producer of *IFNG* (Fig. 2.6b-c). *IL18* was found in to be highly expressed in lesional macrophages that express TREM2 (triggering receptor expressed on myeloid cells 2). We also investigated the expression of the different TLRs in lesional and non-lesional skin. *TLR8* expression is differentially expressed in lesional vs. nonlesional skin of acne patients. TREM2 macrophages also highly expressed *TLR8*. Finally, we looked at the z-scores calculated based on the expression of each cell type normalized across all samples to confirm that there are differences in lesional and non-lesional expression of *TLR8*, *IFNG*, and *IL18* as described above (Fig. 2.6h). Indeed, TREM2 lesional-specific macrophage express higher levels of TLR8, IFNG, and IL18 (Fig. 1.13h). Moreover, CTL, naïve T cells, and NK cells lesional cells all express higher levels of *IFNG* (Fig. 2.6h). These results in scRNA-seq are very similar to what we observed in vitro. This suggests there are evidence of IFN-γ, IL-18, and TLR-8 activation in acne lesions.

Discussion

The interaction between microbiota and the immune system is critical for host defense. To maintain homeostasis, the immune system must ensure that commensal microbial load is tolerated while remains reactive to pathogenic microbial invasion. These communications are mediated by recognition of specific bacterial virulence factors with pattern recognition receptors expressed on host cells. While it has been shown that *C. acnes* clinical strains can induce a pro-inflammatory response that strongly correlated with their clinical association (Yu, Champer et al. 2016, Agak, Kao et al. 2018, Kolar, Tsai et al. 2019), these studies have not been able to

identify distinct virulence-associated bacterial components that can explain the distinct immune response of the different strains. Here, we identified that RNA species from C_A can activate the immune response that are usually reserved for viral detection. Interestingly, C_A and C_H harbor different species of RNA that can trigger distinct immune response as seen with live bacteria.

We were interested in IFN-γ given its role in both innate and adaptive immune response (Ivashkiv 2018). Our experiments suggest IFN-γ plays a role in bridging the innate and adaptive response in *C. acnes* infection given that it increased early and continued to rise throughout the 72-hour (Fig. 2.1d). We identified NK cells to be the main producer of IFN-γ in vitro (Fig. 2.2d). TLR8 is a human a pattern recognition receptor (PRR) that can recognize RNA and activates pathways to cleave IL-18 and secrete IL-12p40 (Bergstrom, Aune et al. 2015, Coch, Hommertgen et al. 2019). Experiments with neutralizing antibodies and antagonist treatment showed that IL-12p40, IL-18, and TLR8 are required for IFN-γ secretion (Fig. 2.3b-d). These findings were also verified as clinically relevant with our scRNA-seq experiment. We found that more lesional cells express *IFNG* (Fig. 2.6a); however, these most of *IFNG* expression came from CTL in the lesion rather than $CD56^+$ NK cells as we observed in PBMC in vitro (Fig. 2.2c). This could be due to the nature of the infection. Our time point for IFN-γ secretion is 24-hours to look for early immune activation of *C. acnes*. The acne lesions we sampled could be exhibiting the immune response a later time point with CTL secretion of IFN-γ. We also verified in the lesion that *TLR8* expression was most distinct for lesional and non-lesional compared to *TLR2* and *TLR4* (Fig. 2.3f). We also found *IL18* is highly expressed in lesional macrophages (Fig. 2.6f, h). Both *IL18* and *TLR8* expression were most abundant in TREM2 macrophages (Fig. 2.6e, j). TREM2 macrophages were most similar to macrophages found in diseases characterized by alteration in lipid metabolism such as obesity and atherosclerosis (Keren-Shaul, Spinrad et al. 2017, Lavin, Kobayashi et al. 2017, Cochain, Vafadarnejad et al. 2018, Jaitin, Adlung et al. 2019, Wang, Dai et al. 2019, Xiong, Kuang et al. 2019, Xue, Tabib et al. 2020). In vitro data

suggests that differentiated TREM2$^+$ macrophages had enhanced phagocytosis of *C. acnes* but no antimicrobial response against the bacteria. Instead TREM2$^+$ macrophages upregulate processing of IL-18 to activate the immune response. Since in vitro data suggests TREM2$^+$ macrophages have no antimicrobial activities, bacteria inside the macrophage could be releasing excess RNA species that activate TLR8. Macrophages then secrete IL-18 and IL-12p40 to activate NK, CD8, CD4 T cells to secrete IFN-γ for immune cell activation promoting inflammation (Fig. 2.7).

We also found that the ligand for IFN-γ induction required bacteria-host interaction because there was no cytokine secretion when PBMCs were stimulated with bacterial supernatant in a transwell experiment (Fig. 1.11b), where the cells are not able to directly interact with the bacteria. We hypothesized bacterial RNA as a possible ligand for IFN-γ induction when we found RNAse-treated host cells did not mount an immune response (Fig. 2.4a). We further confirmed bacterial RNA role in inducing an IFN-γ response when we transfected C_A and C_H RNA with a liposomal transfection reagent, DOTAP. Secretion levels of IFN-γ, IL-18, and IL-12p40 were similar to the response to live bacteria in PBMCs and MDMs (Fig. 2.4c-g). Interestingly, we found C_A to have high levels of short RNA (25-200nt) fragments and undetectable amount of 1000-4000 nt length corresponding to ribosomal RNA, differing from C_H and other *Cutibacterium* spp (Fig. 2.5a-c). Nucleic acids, particular RNA, can act as a robust immune activator because detection of these RNA signifies that the microorganism encountered is metabolically active and potentially presents a greater threat than its dead counterpart. Immunologist has coined this phenomenon viability-associated pathogen-associated molecular patterns (vita-PAMPs) (Underhill and Goodridge 2012). These RNAs are present only in viable bacteria and often reserved for pathogenic strains meant to elicit robust innate immune response. When macrophage phagocytose live bacteria, bacterial RNA may leak out of the phagolysosome and be detected by cytosolic receptors. In simpler terms, bacterial

RNA is used by the innate immune system as a proxy to identify pathogenic, viable bacteria (Underhill and Goodridge 2012). This could suggest why the transwell experiment did not result in the induction of IFN-γ secretion. The macrophages needed to phagocytose bacteria in order to activate the TLR-8 response. It would be interesting to sequence RNA from C_A and C_H and compare the sequences of each species of RNA.

Taken together, our results showed *that C. acnes* RNA plays a role in modulating inflammation and that RNase has the ability to inhibit IFN-γ secretion. It would be interesting to test whether RNase can also inhibit other inflammatory cytokine caused by *C. acnes* infection. These results suggest that the C_A RNA-based element may be key to how host cells distinguish C_A versus C_H. The identification of RNA as contributing to inflammation in *C. acnes* infection provides a potenteial target to develop novel therapeutics for acne vulgaris.

Materials and Methods

Healthy Blood donors, skin samples, and single cell library preparation

The study was performed in accordance with protocols approved by the institutional review board at University of California, Los Angeles. All patients provided written informed consent. Healthy blood and acne donors were recruited from the University of California, Los Angeles. Patients were excluded from the study if they had been using acne medication, hormonal regulation medication, or hormonal-related implants in the past three months. Skin samples were obtained from 3mm punch biopsies in the back. Briefly, skin was digested enzymatically at 2 hours with 0.4% Dispase II at 37°C with agitation. The epidermis was separated from the dermis. Epidermis was then treated with 0.25% Trypsin and 10U/mL of DNAse I for 30 minutes. Dermis was homogenized and treated with 0.4% Collagenase II and 10U/mL DNase I for 2 hours. Isolated cells were loaded into the Chromium instrument (10X

Genomics) according to manufacturer's protocol and analyzed using Seurat (*19, 20*). Pseudo-time trajectories for macrophage cluster were constructed using Monocle 2, an R package (*45*).

Whole blood was obtained from healthy donors who provided written informed consent (UCLA Institutional Review Board). PBMCs were isolated by Ficoll-Paque (Sigma) density gradient centrifugation and cultured in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (FBS) (Seradigm) at a density of $2.5x10^6$/mL in a 24-well flat-bottomed plate.

Macrophages were generated from CD14 positive cells isolated using CD14 microbeads (Miltenyi Biotec) according the manufacture protocol. CD14 positive cells were cultured with M-CSF (50ng/mL) or GM-CSF (50ng/mL) in RPMI 1640 supplemented with 10% FBS for 5-6 days at a density of $5x10^5$/mL in a 24-well plate.

Reagents for Cell Stimulation.

19kD, a TLR2/1L synthetic lipopeptide derived from the 19kDa mycobacterial lipoprotein was obtained from EMC Microcollections and used at 10 ug/ml. LPS *E. coli* (Sigma) was used at a concentration of 2 μg/ml. TL8-506 (TLR8 agonist) (Invivogen) were used according to manufacturer protocol. Tetanus toxoid (Invitrogen) was used at a concentration of 5 μg/mL. Total RNA was added at (5ug/mL)

Bacteria.

C. acnes were obtained from BEI resources and include HL005PA1, HL043PA1, HL096PA1, HL042PA3, HL110PA3, HL110PA4. Colonies were grown on brucella agar with 5% sheep blood, hemin, and vitamin K (Thermo Fisher Scientific Remel Products, Lenexa, KS) at 37C for 5-7days under anaerobic conditions in sealed containers containing oxygen-absorbing carbon dioxide-generating Anaeropack (Mitsubishi Gas Chemical Co., Inc, Tokyo, Japan),

Cultures inoculated from single colonies were grown under the same conditions in Reinforced Clostridial Medium (Oxoid, Basingstoke, England).

Bacterial treatment

Live bacteria were heat-killed at 95˚C for 10 mins and cooled on ice before addition of bacteria to cell culture. Live bacteria were digested using RNase cocktail (Invitrogen) or DNase I (Invitrogen) and incubated at 37C for 1 hour. Nucleases were inactivated with 0.1% SDS.

Bacterial total RNA extraction

Total RNA from *C. acnes* pellets was extracted as described (Su, Chan et al. 2014, Hia, Chionh et al. 2015) using phenol:chloroform:isoamyl alcohol and sodium acetate pH 5.2. Aqueous phase was collected, and total RNA was precipitated with 1:1 isopropanol at -20C for 1 hour. RNA pellet was washed with ethanol, air-dried, and resuspended in RNase-free water. RNA profiles were analyzed on RNA 6000 Nano chips using Agilent Bioanalyzer (Agilent Technologies) at the Technology Center for Genomics & Bioinformatics (TCGB) Core at UCLA. All RNA samples were used immediately or stored at -80C.

Cytokine Quantification

Cell culture supernatants were harvested at 24h unless otherwise noted. Cytokines measured by sandwich ELISA using antibody pairs were as follows: IL-18, IL-1β, IL-12p40 (R&D Duoset), IFN-γ (BD), IL-6, TNF-α (Invitrogen).

Intracellular Flow Cytometry

PBMCs were stimulated with C_A for 19 hours and a protein transport inhibitor was added during the last 5 hours. Cells were stained with CD3 BV605, CD4 BV421, CD8 FITC, CD56 PE,

or IFN-γ-APC (Biolegend). Cells with were fixed using BD Perm/Fix Buffer (BD) according to manufacturer's protocol. Data was acquired on BD LSRII analyzer.

Statistical Analysis.

Statistical analyses were calculated using GraphPad Prism version 8.0, and p values ≤ 0.05 were assigned as significant. For comparisons involving two groups, a paired Student t test or one-way ANOVA with Sidak posttest was performed. For more than two comparison groups, a one-way ANOVA with Tukey posttest, a two-way ANOVA with Tukey posttest, or a Friedman test with Dunn posttest was performed. Data are represented in figures as mean ± SEM.

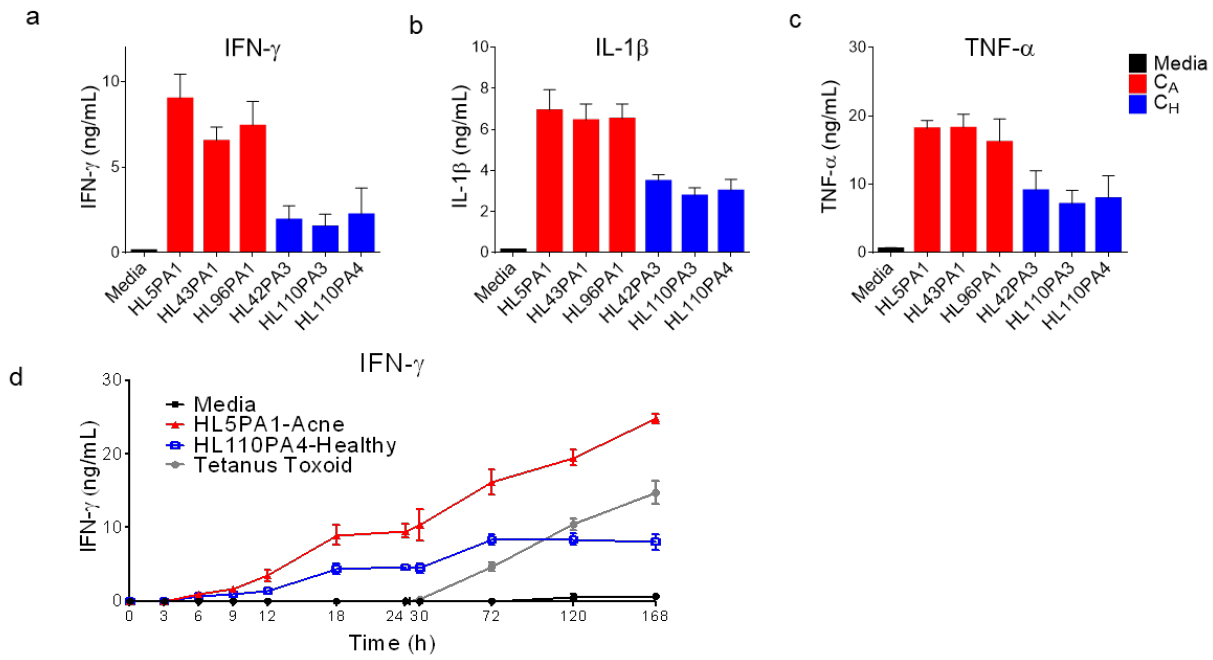


Fig. 2.1. C_A infection induces IFN-γ secretion at an early time point

(a-c) PBMCs were stimulated with *C. acnes* C_A or C_H at an MOI of 0.5. Various cytokines were measured at 24h via ELISA. (d) PBMCs were stimulated with *C. acnes* C_A or C_H at MOI 0.5 and tetanus at 5 µg/ml and various time points were collected from 3 hours to 7 days. N = 3 for each experiment.

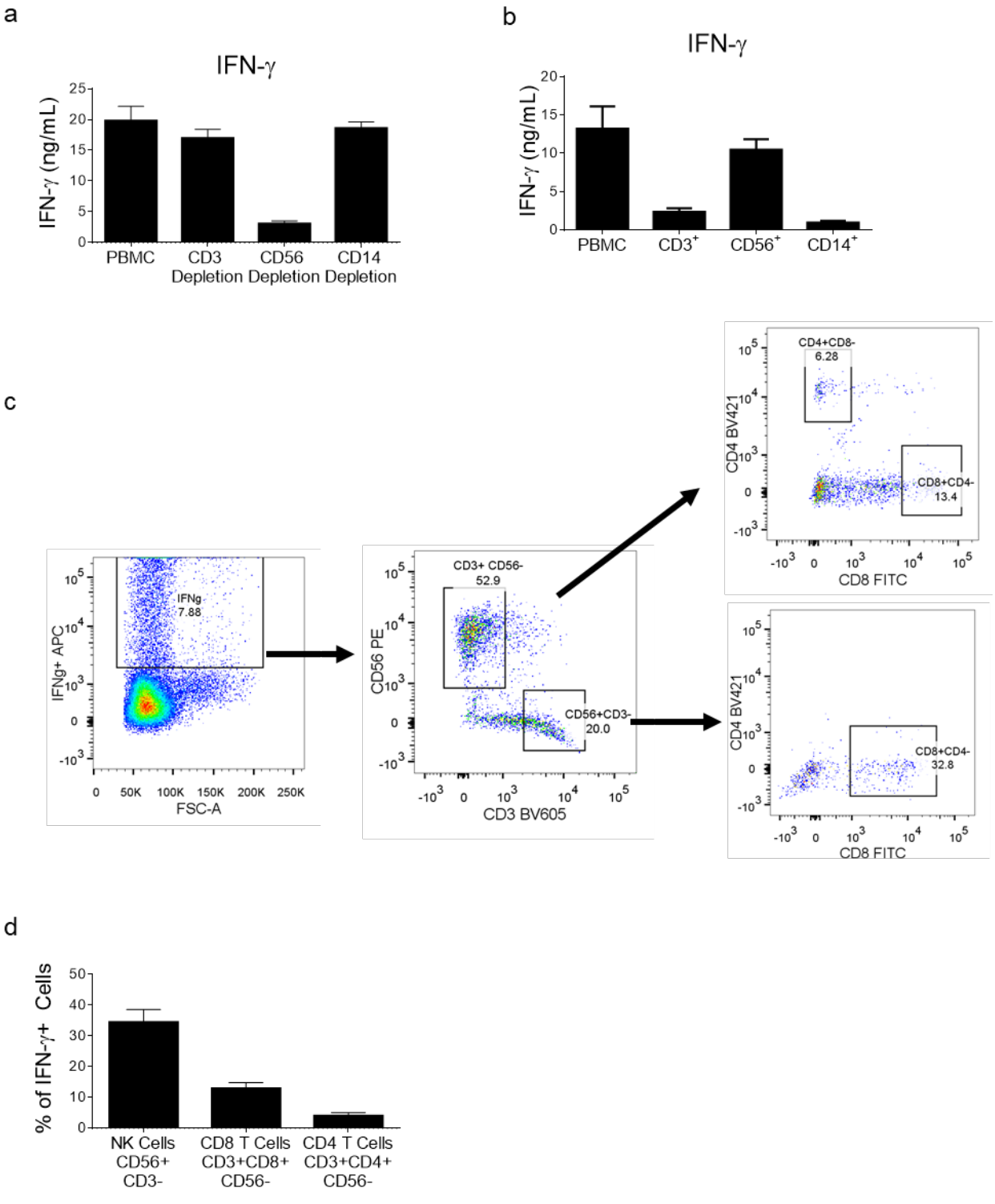

a
IFN-γ
IFN-γ (ng/mL)
PBMC
CD3 Depletion
CD56 Depletion
CD14 Depletion
b
IFN-γ
IFN-γ (ng/mL)
PBMC
CD3+
CD56+
CD14+
c
IFNg 7.88
IFNg+ APC
FSC-A
CD3+ CD56- 52.9
CD56 PE
CD3 BV605
CD56+CD3- 20.0
CD4+CD8- 6.28
CD8+CD4- 13.4
CD4 BV421
CD8 FITC
CD8+CD4- 32.8
d
% of IFN-γ+ Cells
NK Cells CD56+ CD3-
CD8 T Cells CD3+CD8+ CD56-
CD4 T Cells CD3+CD4+ CD56-

Fig. 2.2. C_A infection induces IFN-γ secretion at an early time point

(a-b) CD3, CD56, and CD14 were depleted or isolated use magnetic microbeads (Miltenyi) were stimulated with *C. acnes* C_A MOI 0.5. IFN-γ was measured at 24h via ELISA PBMCs were stimulated with C_A for 19 hours and the last 5 hours was the presence of protein transport inhibitor. Cells were stained with CD3 BV605, CD4 BV421, CD8 FITC, CD56 PE, or IFN-γ-APC (Biolegend). Cells with were fixed using BD Perm/Fix Buffer (BD) according to manufacturer's protocol. Data was acquired on BD LSRII analyzer. (c) An example traces of IFN-γ intracellular flow cytometry. (d) Summary with percentage of cells for n =3.

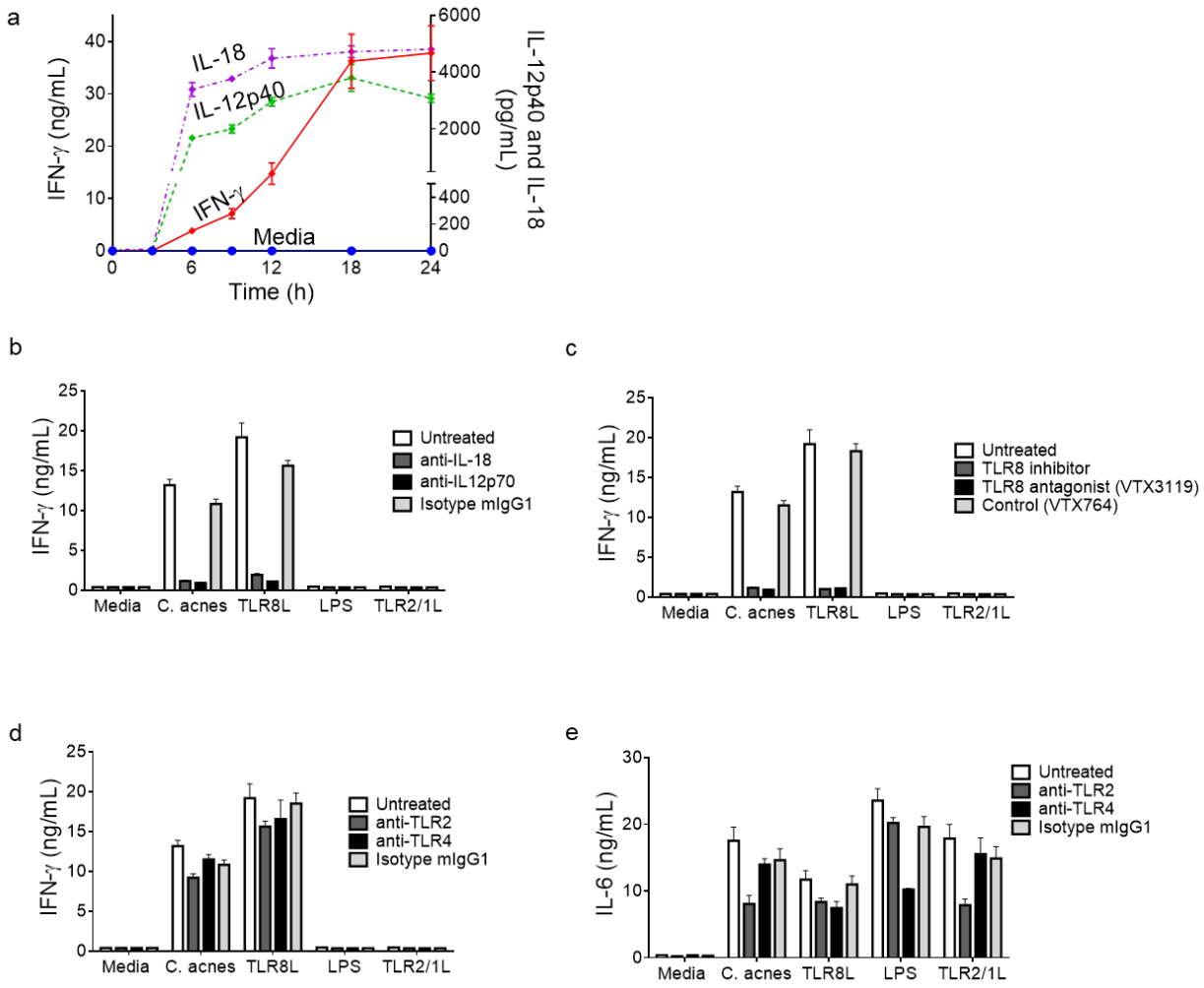


Fig. 2.3. TLR-8, IL-18, and IL-12p40 are required IFN-γ secretion

(a) PBMCs were stimulated with *C. acnes* C_A at MOI 0.5 for various time points were collected as listed from 3 hours to 24 hours. N = 3 for each experiment. (b) CD3, CD56, and CD14 were depleted or isolated use magnetic microbeads (Miltenyi) were stimulated with *C. acnes* C_A MOI 0.5. IFN-γ and IL-6 were measured at 24h by ELISA. n ≥3.

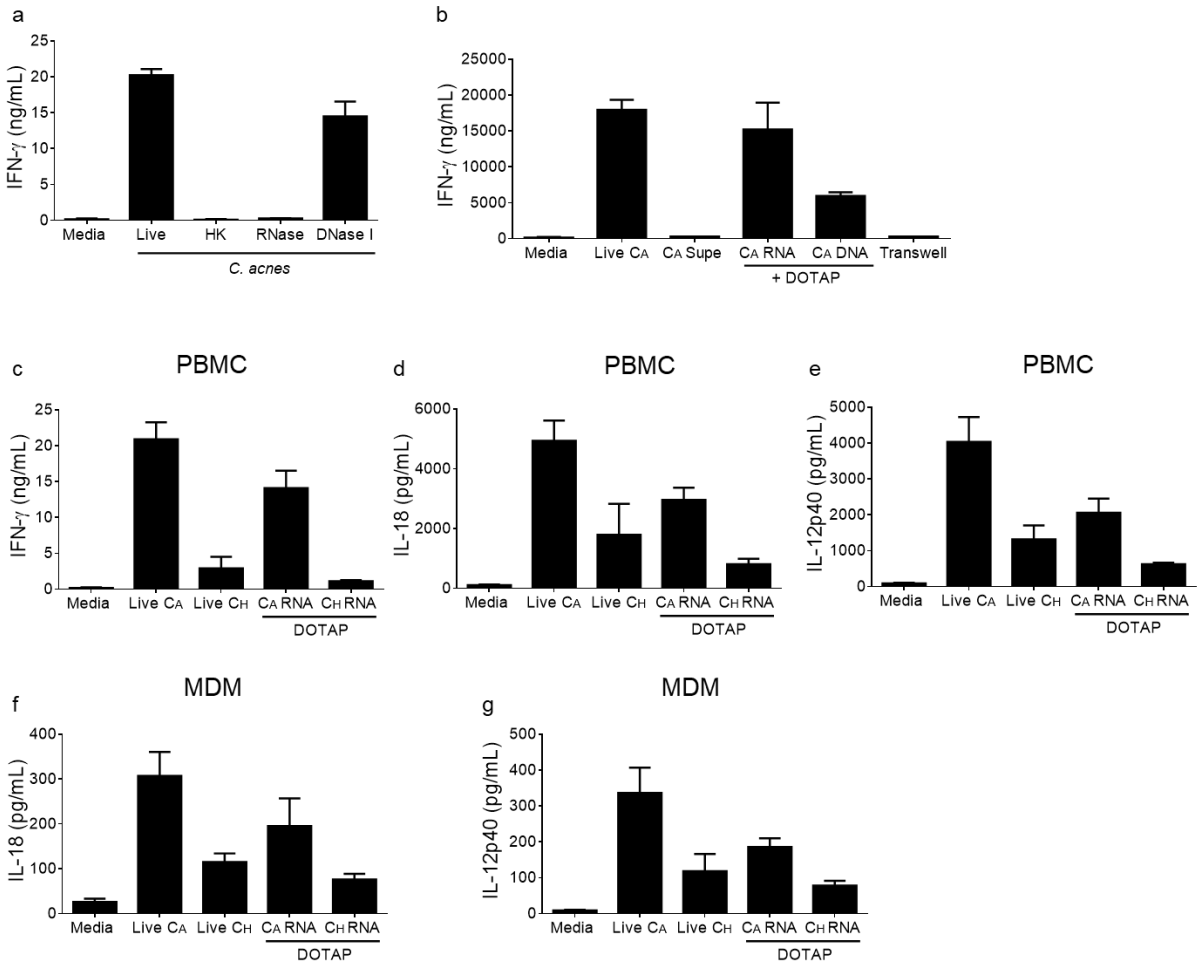


Fig. 2.4. C_A RNA induces higher secretion of IFN-γ, IL12p40, and IL-18 compared to C_H RNA similar to the live bacteria response

(a) PBMCs were stimulated with untreated, heat-killed (HK), RNase cocktail, or DNase I treated live C_A (HL5PA1) for 24h. (b) PBMCs were stimulated with live C_A, C_A bacteria-free supernatant (C_A supe), C_A RNA, C_A DNA, or transwell. IFN-g was measured by ELISA at 24h. (C-E) PBMCs stimulated with live, total RNA (5μg/mL) with DOTAP from C_A and C_H. MDMs were stimulated with either live *C. acnes* or *C. acnes* total RNA (5μg/mL) for 24h. Cytokines were measured in the 24h supernatant by ELISA.

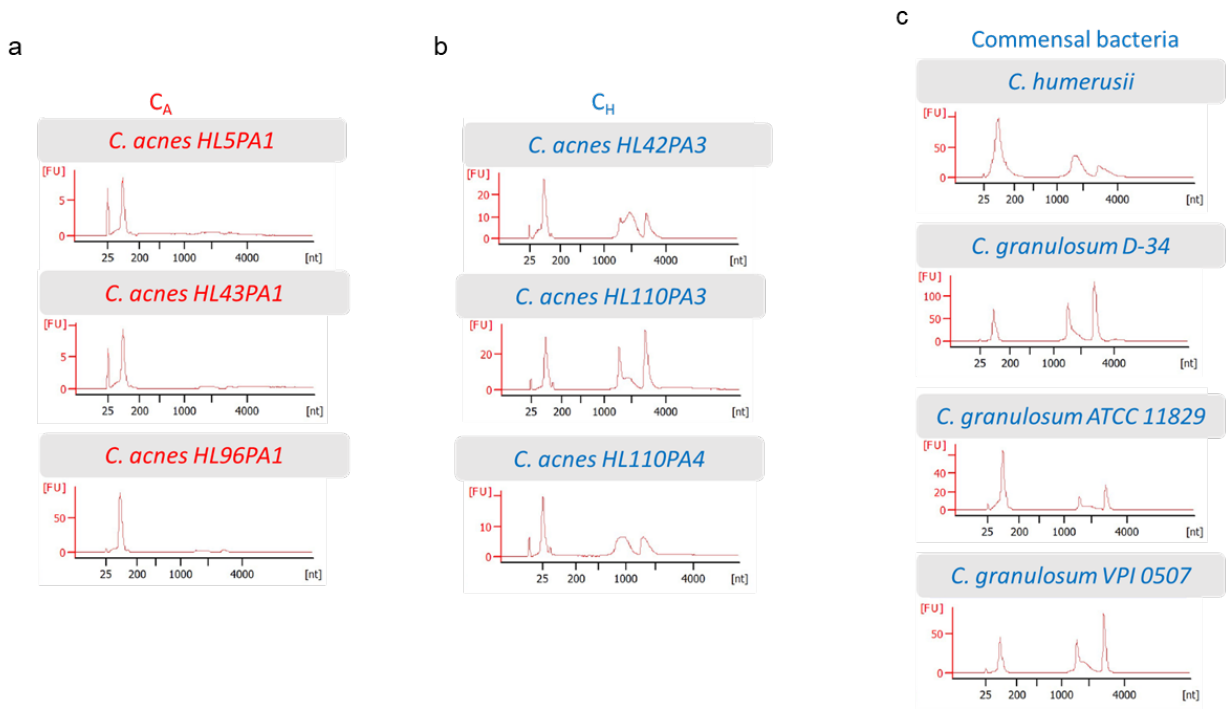


Fig. 2.5. C_A and C_H harbor different species of bacterial RNA

Total RNA from various bacteria was isolated and loaded on to the Agilent Bioanalyzer using RNA 6000 Nano chip.

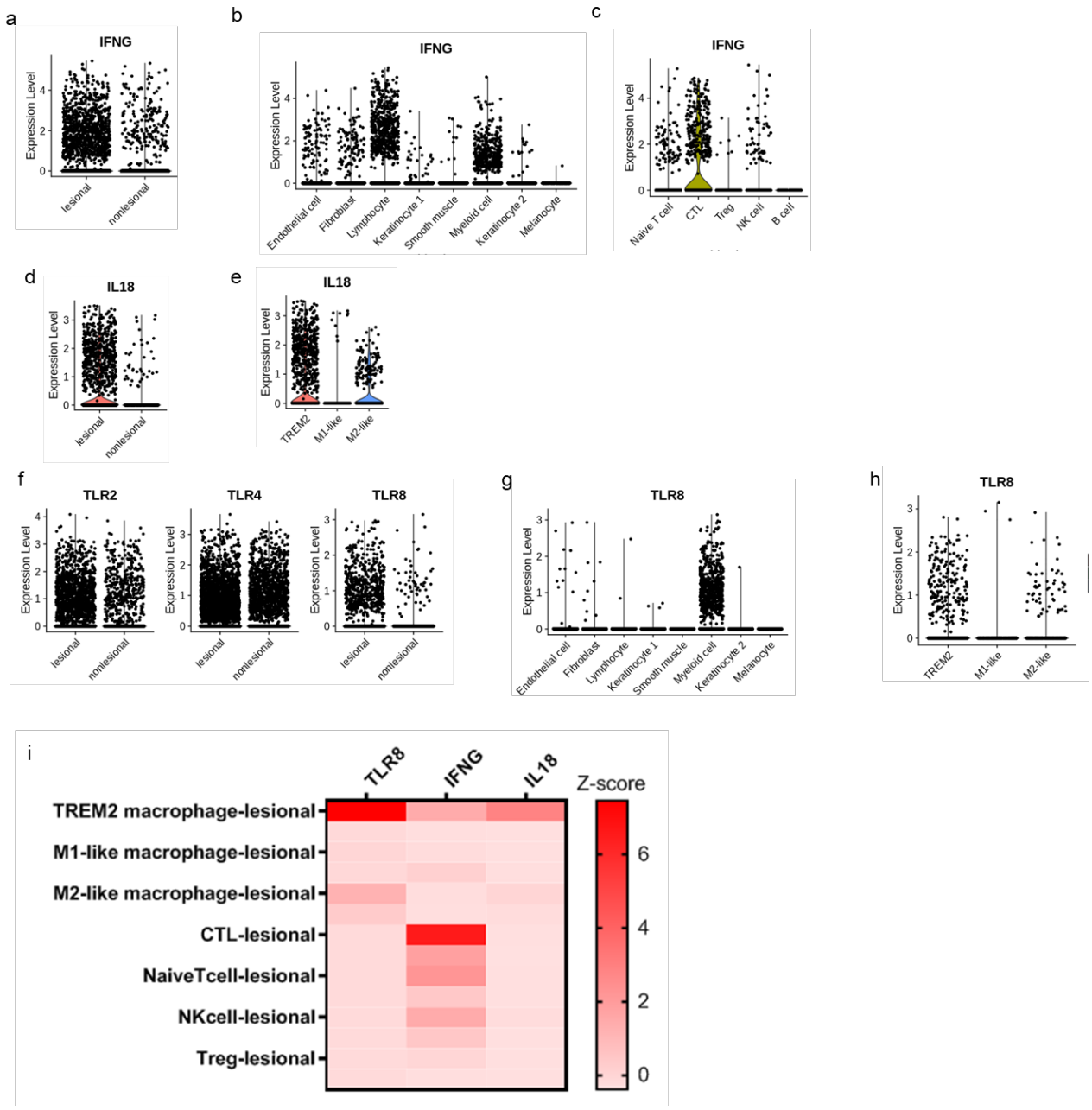


Fig. 2.6. In vivo scRNA-seq data from acne lesions confirms higher expression of *TLR8*, *IL18*, and *IFNG*

Violin plots showing the normalized expression levels for *IFNG* in (a) all cell types lesional versus non-lesional (b) grouped by cell types (c) grouped by sub-clusters of lymphocytes. Violin plots showing normalized expression levels for *IL18* in (d) all cell types lesional versus non-lesional (e) grouped by sub-clusters of macrophages. Violin plots showing the normalized expression levels of (f) *TLR2*, *TLR4*, *TLR8* in lesional and non-lesional cells (g) grouped by cell types (h) grouped by sub-clusters of macrophages. (i) Heatmap of z-scores calculated based on the expression of each cell type normalized across all samples for *TLR8*, *IFNG* and *IL18.*

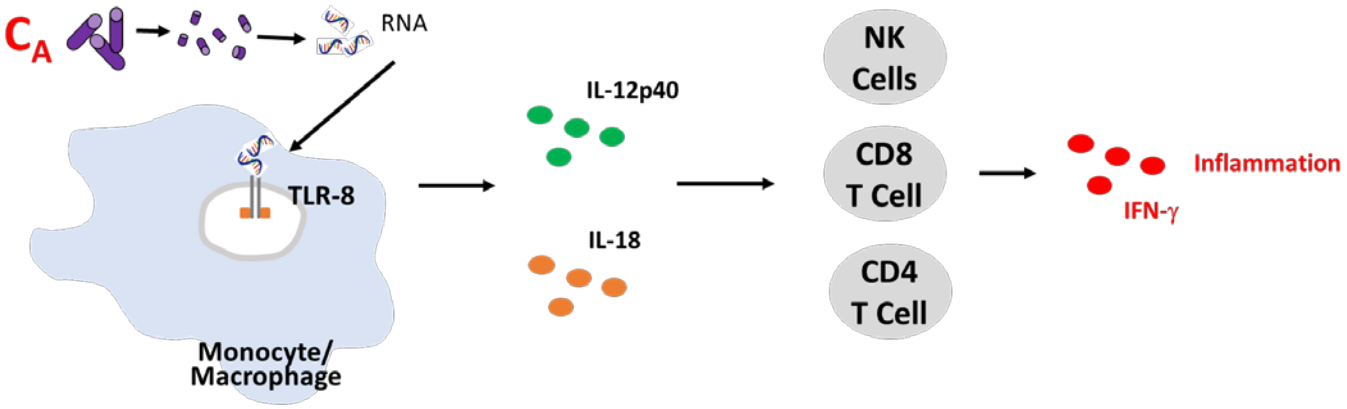


Fig. 2.7. **Proposed model of *C. acnes* infection with bacterial RNA stimulating TLR-8**

C_A RNA can activate TLR-8 receptor on monocytes/macrophages to secrete IL-12p40 and IL-18 to stimulate NK, CD8 T, and CD4 T cells. The cells secrete IFN-γ which can activate the immune response and promote inflammation.

References

Agak, G. W., S. Kao, K. Ouyang, M. Qin, D. Moon, A. Butt and J. Kim (2018). "Phenotype and Antimicrobial Activity of Th17 Cells Induced by Propionibacterium acnes Strains Associated with Healthy and Acne Skin." J Invest Dermatol **138**(2): 316-324.

Barnard, E., B. Shi, D. Kang, N. Craft and H. Li (2016). "The balance of metagenomic elements shapes the skin microbiome in acne and health." Sci Rep **6**: 39491.

Bergstrom, B., M. H. Aune, J. A. Awuh, J. F. Kojen, K. J. Blix, L. Ryan, T. H. Flo, T. E. Mollnes, T. Espevik and J. Stenvik (2015). "TLR8 Senses Staphylococcus aureus RNA in Human Primary Monocytes and Macrophages and Induces IFN-beta Production via a TAK1-IKKbeta-IRF5 Signaling Pathway." J Immunol **195**(3): 1100-1111.

Bhate, K. and H. C. Williams (2013). "Epidemiology of acne vulgaris." Br J Dermatol **168**(3): 474-485.

Coch, C., B. Hommertgen, T. Zillinger, J. Dassler-Plenker, B. Putschli, M. Nastaly, B. M. Kummerer, J. F. Scheunemann, B. Schumak, S. Specht, M. Schlee, W. Barchet, A. Hoerauf, E. Bartok and G. Hartmann (2019). "Human TLR8 Senses RNA From Plasmodium falciparum-Infected Red Blood Cells Which Is Uniquely Required for the IFN-gamma Response in NK Cells." Front Immunol **10**: 371.

Cochain, C., E. Vafadarnejad, P. Arampatzi, J. Pelisek, H. Winkels, K. Ley, D. Wolf, A. E. Saliba and A. Zernecke (2018). "Single-Cell RNA-Seq Reveals the Transcriptional Landscape and Heterogeneity of Aortic Macrophages in Murine Atherosclerosis." Circ Res **122**(12): 1661-1674.

Fitz-Gibbon, S., S. Tomida, B. H. Chiu, L. Nguyen, C. Du, M. Liu, D. Elashoff, M. C. Erfe, A. Loncaric, J. Kim, R. L. Modlin, J. F. Miller, E. Sodergren, N. Craft, G. M. Weinstock and H. Li (2013). "Propionibacterium acnes strain populations in the human skin microbiome associated with acne." J Invest Dermatol **133**(9): 2152-2160.

Gorski, K. S., E. L. Waller, J. Bjornton-Severson, J. A. Hanten, C. L. Riter, W. C. Kieper, K. B. Gorden, J. S. Miller, J. P. Vasilakos, M. A. Tomai and S. S. Alkan (2006). "Distinct indirect pathways govern human NK-cell activation by TLR-7 and TLR-8 agonists." Int Immunol **18**(7): 1115-1126.

Grice, E. A. and J. A. Segre (2011). "The skin microbiome." Nature Reviews Microbiology **9**(4): 244-253.

Hia, F., Y. H. Chionh, Y. L. Pang, M. S. DeMott, M. E. McBee and P. C. Dedon (2015). "Mycobacterial RNA isolation optimized for non-coding RNA: high fidelity isolation of 5S rRNA from Mycobacterium bovis BCG reveals novel post-transcriptional processing and a complete spectrum of modified ribonucleosides." Nucleic Acids Res **43**(5): e32.

Ivashkiv, L. B. (2018). "IFNgamma: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy." Nat Rev Immunol **18**(9): 545-558.

Jaitin, D. A., L. Adlung, C. A. Thaiss, A. Weiner, B. Li, H. Descamps, P. Lundgren, C. Bleriot, Z. Liu, A. Deczkowska, H. Keren-Shaul, E. David, N. Zmora, S. M. Eldar, N. Lubezky, O. Shibolet, D. A. Hill, M. A. Lazar, M. Colonna, F. Ginhoux, H. Shapiro, E. Elinav and I. Amit (2019). "Lipid-Associated Macrophages Control Metabolic Homeostasis in a Trem2-Dependent Manner." Cell **178**(3): 686-698 e614.

Keegan, C., S. Krutzik, M. Schenk, P. O. Scumpia, J. Lu, Y. L. J. Pang, B. S. Russell, K. S. Lim, S. Shell, E. Prestwich, D. Su, D. Elashoff, R. M. Hershberg, B. R. Bloom, J. T. Belisle, S. Fortune, P. C. Dedon, M. Pellegrini and R. L. Modlin (2018). "Mycobacterium tuberculosis Transfer RNA Induces IL-12p70 via Synergistic Activation of Pattern Recognition Receptors within a Cell Network." J Immunol **200**(9): 3244-3258.

Keren-Shaul, H., A. Spinrad, A. Weiner, O. Matcovitch-Natan, R. Dvir-Szternfeld, T. K. Ulland, E. David, K. Baruch, D. Lara-Astaiso, B. Toth, S. Itzkovitz, M. Colonna, M. Schwartz and I.

Amit (2017). "A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease." Cell **169**(7): 1276-1290 e1217.

Kolar, S. L., C.-M. Tsai, J. Torres, X. Fan, H. Li and G. Y. Liu (2019). "Propionibacterium acnes–induced immunopathology correlates with health and disease association." JCI Insight **4**(5).

Lavin, Y., S. Kobayashi, A. Leader, E. D. Amir, N. Elefant, C. Bigenwald, R. Remark, R. Sweeney, C. D. Becker, J. H. Levine, K. Meinhof, A. Chow, S. Kim-Shulze, A. Wolf, C. Medaglia, H. Li, J. A. Rytlewski, R. O. Emerson, A. Solovyov, B. D. Greenbaum, C. Sanders, M. Vignali, M. B. Beasley, R. Flores, S. Gnjatic, D. Pe'er, A. Rahman, I. Amit and M. Merad (2017). "Innate Immune Landscape in Early Lung Adenocarcinoma by Paired Single-Cell Analyses." Cell **169**(4): 750-765 e717.

Liu, P. T., S. R. Krutzik, J. Kim and R. L. Modlin (2005). "Cutting edge: all-trans retinoic acid down-regulates TLR2 expression and function." J Immunol **174**(5): 2467-2470.

Nguyen, C. M., J. Koo and K. M. Cordoro (2016). "Psychodermatologic effects of atopic dermatitis and acne: a review on self-esteem and identity." Pediatric dermatology **33**(2): 129-135.

Purvis, D., E. Robinson, S. Merry and P. Watson (2006). "Acne, anxiety, depression and suicide in teenagers: A cross-sectional survey of New Zealand secondary school students." Journal of paediatrics and child health **42**(12): 793-796.

Rosenthal, M., D. Goldberg, A. Aiello, E. Larson and B. Foxman (2011). "Skin microbiota: microbial community structure and its potential association with health and disease." Infect Genet Evol **11**(5): 839-848.

Su, D., C. T. Chan, C. Gu, K. S. Lim, Y. H. Chionh, M. E. McBee, B. S. Russell, I. R. Babu, T. J. Begley and P. C. Dedon (2014). "Quantitative analysis of ribonucleoside modifications in tRNA by HPLC-coupled mass spectrometry." Nat Protoc **9**(4): 828-841.

Thiboutot, D. M., B. Dreno, A. Abanmi, A. F. Alexis, E. Araviiskaia, M. I. Barona Cabal, V. Bettoli, F. Casintahan, S. Chow, A. da Costa, T. El Ouazzani, C. L. Goh, H. P. M. Gollnick, M. Gomez, N. Hayashi, M. I. Herane, J. Honeyman, S. Kang, L. Kemeny, R. Kubba, J. Lambert, A. M. Layton, J. J. Leyden, J. L. Lopez-Estebaranz, N. Noppakun, F. Ochsendorf, C. Oprica, B. Orozco, M. Perez, J. Piquero-Martin, J. A. See, D. H. Suh, J. Tan, V. T. Lozada, P. Troielli and L. F. Xiang (2018). "Practical management of acne for clinicians: An international consensus from the Global Alliance to Improve Outcomes in Acne." J Am Acad Dermatol **78**(2 Suppl 1): S1-S23 e21.

Tomida, S., L. Nguyen, B. H. Chiu, J. Liu, E. Sodergren, G. M. Weinstock and H. Li (2013). "Pan-genome and comparative genome analyses of propionibacterium acnes reveal its genomic diversity in the healthy and diseased human skin microbiome." mBio **4**(3): e00003-00013.

Underhill, D. M. and H. S. Goodridge (2012). "Information processing during phagocytosis." Nat Rev Immunol **12**(7): 492-502.

Vallerand, I., R. Lewinson, L. Parsons, M. Lowerison, A. Frolkis, G. Kaplan, C. Barnabe, A. Bulloch and S. Patten (2018). "Risk of depression among patients with acne in the UK: a population-based cohort study." The British journal of dermatology **178**(3): e194.

Wang, E. C. E., Z. Dai, A. W. Ferrante, C. G. Drake and A. M. Christiano (2019). "A Subset of TREM2(+) Dermal Macrophages Secretes Oncostatin M to Maintain Hair Follicle Stem Cell Quiescence and Inhibit Hair Growth." Cell Stem Cell **24**(4): 654-669 e656.

Xiong, X., H. Kuang, S. Ansari, T. Liu, J. Gong, S. Wang, X. Y. Zhao, Y. Ji, C. Li, L. Guo, L. Zhou, Z. Chen, P. Leon-Mimila, M. T. Chung, K. Kurabayashi, J. Opp, F. Campos-Perez, H. Villamil-Ramirez, S. Canizales-Quinteros, R. Lyons, C. N. Lumeng, B. Zhou, L. Qi, A. Huertas-Vazquez, A. J. Lusis, X. Z. S. Xu, S. Li, Y. Yu, J. Z. Li and J. D. Lin (2019).

"Landscape of Intercellular Crosstalk in Healthy and NASH Liver Revealed by Single-Cell Secretome Gene Analysis." Mol Cell **75**(3): 644-660 e645.

Xue, D., T. Tabib, C. Morse and R. Lafyatis (2020). "Transcriptome landscape of myeloid cells in human skin reveals diversity, rare populations and putative DC progenitors." J Dermatol Sci **97**(1): 41-49.

Yu, Y., J. Champer, G. W. Agak, S. Kao, R. L. Modlin and J. Kim (2016). "Different Propionibacterium acnes Phylotypes Induce Distinct Immune Responses and Express Unique Surface and Secreted Proteomes." J Invest Dermatol **136**(11): 2221-2228.

Ziblat, A., S. Y. Nuñez, X. L. Raffo Iraolagoitia, R. G. Spallanzani, N. I. Torres, J. M. Sierra, F. Secchiari, C. I. Domaica, M. B. Fuertes and N. W. Zwirner (2018). "Interleukin (IL)-23 stimulates IFN-γ secretion by CD56bright natural killer cells and enhances IL-18-driven dendritic cells activation." Frontiers in immunology **8**: 1959.

CHAPTER 4

Conclusions

In this dissertation, we began with a hypothesis that changes in sebum production and the presence of distinct *C. acnes* phylotypes differentially trigger inflammation in lesions and in vitro. We performed scRNA-seq on lesional and non-lesional skin biopsies of acne individuals. We identified several interesting sub-clusters of cells that are specific to acne lesions. Keratinocytes in the hair follicle, localized by the expression of *KRT6B* and *KRT6C*, were found to contribute to the production of squalene in the hair follicle with the expression of *FDFT1* and *SQLE*. As the first oxygenation step in cholesterol biosynthesis, SQLE is thought to be one of the rate-limiting enzymes in this pathway. The conversion from squalene to squalene epoxide is required for the lanosterol synthesis and eventually cholesterol (*36*). Since SQLE requires oxygen but the anerobic environment in pilosebaceous unit limits its function, the result is a buildup of squalene in sebaceous gland (*2*). Anaerobic environment is evident in the expression of *HIF1A* in the hair follicle KC sub-cluster. This suggest keratinocytes contribute to the production of squalene synthesis in the hair follicle.

We also uncovered a subset of specialized TREM2 macrophages in acne lesions. These macrophages are similar to macrophages found in high lipid environment that are associated with disease of dysfunction lipid metabolism such as including obesity, atherosclerosis, lung cancer, and nonalcoholic fatty liver disease (Keren-Shaul, Spinrad et al. 2017, Lavin, Kobayashi et al. 2017, Cochain, Vafadarnejad et al. 2018, Jaitin, Adlung et al. 2019, Wang, Dai et al. 2019, Xiong, Kuang et al. 2019, Xue, Tabib et al. 2020). The uptake of lipids by macrophages is mediated by micropinocytosis and various cell surface receptors. Instead of limiting uptake of lipids, macrophages largely depend on cholesterol efflux pathways to maintain cellular lipid

homeostasis (Batista-Gonzalez, Vidal et al. 2019). TREM2 is a transmembrane glycoprotein that can interact with lipoproteins, phospholipids, and anionic ligands (Kober and Brett 2017) and can uptake lipids that binds to APOE (Yeh, Wang et al. 2016). ABCA1, the key efflux transporter of lipids, is upregulated in the activation of liver X receptor (LXR) (Costet, Luo et al. 2000). We saw evidence of lipid processing in TREM2 macrophages with the increase expression of cathepsins (*CTSB*, *CTSD*), lysosomal acid lipase A (*LIPA*), acetyl-coA acetyltransferase 1 (ACAT1, gene *SOAT1*), intracellular lipid transporters such as Niemann-Pick disease (*NPC1*, *NPC2*) as well as a marker for intracellular storage of lipid droplets perilipin 2 (*PLIN2*). Excess lipids activate LXRs (gene *NR1H3*) to upregulate *ABCA1* as well as lipid carrier *APOE* to facilitate efflux. Macrophages mainly use ABCA1 to maintain a balance between uptake and export of lipids. For macrophages to upregulate lipid metabolism and efflux pathways suggest there is an active effort process lipid. This further suggests these macrophages are loaded with lipids. The presence of $TREM2^+$ macrophages in acne lesions along with the finding that squalene, a lipid that is abundant in acne, induces and activates $TREM2^+$ macrophages. This indicates that $TREM2^+$ macrophages are part of the host inflammatory response to extracellular lipids.

Squalene, a component that is unique to human sebum, induced the expression of TREM2 in macrophages in vitro. These macrophages expressed similar pattern of genes to TREM2 macrophage in acne lesions. We observed that their cytoplasm was filled with lipids validating our hypothesis of uptake of lipids. Foam cells, which have been found in acne lesions (Lovaszi, Mattii et al. 2017), happen when macrophages overloaded with lipids that they give a “foamy” appearance on histology. These differentiated $TREM2^+$ macrophages had enhanced phagocytosis of *C. acnes* but no antimicrobial response against the bacteria. Squalene-treated $TREM2^+$ macrophages IL-1β and IL-18 both are known to recruit neutrophils and recruit Th1 response. These results indicate that excess lipid production, specifically squalene, could

initiate the inflammatory cascade by neutralizing the macrophage's ability to kill bacteria, allowing for the bacterial escape, and activating the innate and adaptive immune response with the release of inflammatory cytokines.

Our results underline pivotal role for squalene in the initiation of inflammation in acne. Squalene is an unsaturated hydrocarbon and a precursor to cholesterol. While other tissue can rapidly convert squalene into cholesterol, human sebocytes accumulate sebum (Lovászi, Szegedi et al. 2017). It was initially thought of an inert lubricant of the skin. However, squalene can be neutralized with UV irradiation to induce reactive oxygen species in the skin and has been reported to induce comedone formation in the skin (Chiba, Yoshizawa et al. 2000). It was also reported that acne patients had higher levels and higher proportion of squalene on their skin compared to healthy controls (Pappas, Johnsen et al. 2009). Squalene represents an attractive therapeutic approach to prevent inflammation. A small molecule inhibitor, acetyl coA carboxylase inhibitor, to target sebum synthesis underwent a phase 3 trial to treat acne. However, the primary endpoint saw no reduction in inflammatory lesions. Interestingly, the small molecule inhibits all the other sebaceous lipid synthesis except for squalene and cholesterol (Bissonnette, Poulin et al. 2017). It would be interesting to investigate how squalene synthesis affect resident cells and other strains of *C. acnes* in the skin.

In chapter 3, we investigated how different strains of C_A versus C_H differ in their inflammatory potential trigger inflammation in immune cells. Specifically, we looked for distinct virulence-associated bacterial components that could explain the distinct inflammatory potential of the different strains of C. acnes. Given the important role of IFN-γ in both innate and adaptive immune response (Ivashkiv 2018), we identified the source of IFN-γ to be NK cells in vitro and NK cells and CTL in acne lesions. The ability of *C. acnes* to induce IFN-γ was found to be

dependent on the TLR8-mediated release of IL12 and IL-18, a cytokine known to trigger the release of IFN--γ from lymphocytes.

RNA was found to play an important role in IFN-γ induction. IFN-γ was elicited only by viable *C. acnes* and was abrogated after pretreatment with RNase but not DNase. Transfection of RNA with DOTAP elicited IFN-γ, IL-18, and IL-12p40 release from PBMCs and MDMs. The difference of cytokine release was similar to those with live bacteria where C_A RNA elicit greater IFN-γ secretion than C_H RNA. The RNA species also varied in sizes with C_A harbored a short (25-200nt) RNA species while C_H harbored both short RNA species and a long (1000-4000 nt) RNA species.

Immune cells employ multiple ways to detect pathogenic invaders. A recent pathway was observed in macrophages to distinguish between live and dead *E.* coli through the detection of vita-PAMPs (Underhill and Goodridge 2012). When live bacteria are phagocytosed and degraded in the phagolysosome, they release RNA, which is thought to leak out into the cytoplasm. Cytosolic receptors can then detect released bacterial RNA. On the other hand, when dead bacteria are engulfed, their RNAs are rapidly degraded so no RNA is detected (Underhill and Goodridge 2012). This could explain why PBMCs required direct contact with C_A for IFN-γ secretion. Myeloid cells would have to first encapsulate the bacteria in a lysosome before the RNA are released. We plan to sequence the short RNA fragments from both phylotype of bacteria to compare their differences.

Our in vitro data demonstrating activation of TLR-8/IL-18/IFN-γ pathway was validated in acne lesions with increased *IL18* and *TLR8* expression on lesion specific TREM2 macrophages. *TLR8* expression was most distinct for lesional and non-lesional compared to *TLR2* and *TLR4*. We also found *IL18* is highly expressed in lesional macrophages. Both *IL18* and *TLR8* expression were most abundant in TREM2 macrophages. TREM2 macrophages in acne lesions

were most similar to macrophages found in diseases characterized by alteration in lipid metabolism such as obesity and atherosclerosis (Keren-Shaul, Spinrad et al. 2017, Lavin, Kobayashi et al. 2017, Cochain, Vafadarnejad et al. 2018, Jaitin, Adlung et al. 2019, Wang, Dai et al. 2019, Xiong, Kuang et al. 2019, Xue, Tabib et al. 2020). Since in vitro data suggests TREM2+ macrophages have no antimicrobial activities, bacteria inside the macrophage could be upregulating its virulence factor transcription leading to excess RNA in the environment that is causing the upregulation of TLR8. This excess RNA could be due to the proliferation and, thus a high turnover rate, of the pathogenic *C. acnes.*

In conclusion, this dissertation describes several novel findings in the pathogenesis of acne vulgaris. The presence of TREM2 macrophages have often associate with inflammatory diseases. Here, we showed TREM2 macrophages could also be associated with an infectious disease, acne vulgaris, where the presence of TREM2 indicate a dysfunction of lipid metabolism. We have also identified that RNA species from C_A can activate the immune response. The RNA recognition through TLR-8 are usually reserved for viral detection. The differences in C_A and C_H RNA species, which trigger distinct immune response as seen with live bacteria, suggest bacterial RNA could activate the immune response. This work not only advances our understanding of acne vulgaris but also skin immunology. Environment can play an important role in activating the immune response that has not been realized before. While we often categorize bacteria based on various PAMPs they may have based on the morphology, bacteria may employ different strategies to evade the immune system as well as host immune cells employing different strategies to detect bacteria that we may not have previously known.

References

Batista-Gonzalez, A., R. Vidal, A. Criollo and L. J. Carreno (2019). "New Insights on the Role of Lipid Metabolism in the Metabolic Reprogramming of Macrophages." Front Immunol **10**: 2993.

Bissonnette, R., Y. Poulin, J. Drew, H. Hofland and J. Tan (2017). "Olumacostat glasaretil, a novel topical sebum inhibitor, in the treatment of acne vulgaris: A phase IIa, multicenter, randomized, vehicle-controlled study." Journal of the American Academy of Dermatology **76**(1): 33-39.

Chiba, K., K. Yoshizawa, I. Makino, K. Kawakami and M. Onoue (2000). "Comedogenicity of squalene monohydroperoxide in the skin after topical application." The Journal of toxicological sciences **25**(2): 77-83.

Cochain, C., E. Vafadarnejad, P. Arampatzi, J. Pelisek, H. Winkels, K. Ley, D. Wolf, A. E. Saliba and A. Zernecke (2018). "Single-Cell RNA-Seq Reveals the Transcriptional Landscape and Heterogeneity of Aortic Macrophages in Murine Atherosclerosis." Circ Res **122**(12): 1661-1674.

Costet, P., Y. Luo, N. Wang and A. R. Tall (2000). "Sterol-dependent transactivation of theABC1 promoter by the liver X receptor/retinoid X receptor." Journal of Biological Chemistry **275**(36): 28240-28245.

Ivashkiv, L. B. (2018). "IFNgamma: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy." Nat Rev Immunol **18**(9): 545-558.

Jaitin, D. A., L. Adlung, C. A. Thaiss, A. Weiner, B. Li, H. Descamps, P. Lundgren, C. Bleriot, Z. Liu, A. Deczkowska, H. Keren-Shaul, E. David, N. Zmora, S. M. Eldar, N. Lubezky, O. Shibolet, D. A. Hill, M. A. Lazar, M. Colonna, F. Ginhoux, H. Shapiro, E. Elinav and I. Amit (2019). "Lipid-Associated Macrophages Control Metabolic Homeostasis in a Trem2-Dependent Manner." Cell **178**(3): 686-698 e614.

Keren-Shaul, H., A. Spinrad, A. Weiner, O. Matcovitch-Natan, R. Dvir-Szternfeld, T. K. Ulland, E. David, K. Baruch, D. Lara-Astaiso, B. Toth, S. Itzkovitz, M. Colonna, M. Schwartz and I. Amit (2017). "A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease." Cell **169**(7): 1276-1290 e1217.

Kober, D. L. and T. J. Brett (2017). "TREM2-Ligand Interactions in Health and Disease." J Mol Biol **429**(11): 1607-1629.

Lavin, Y., S. Kobayashi, A. Leader, E. D. Amir, N. Elefant, C. Bigenwald, R. Remark, R. Sweeney, C. D. Becker, J. H. Levine, K. Meinhof, A. Chow, S. Kim-Shulze, A. Wolf, C. Medaglia, H. Li, J. A. Rytlewski, R. O. Emerson, A. Solovyov, B. D. Greenbaum, C. Sanders, M. Vignali, M. B. Beasley, R. Flores, S. Gnjatic, D. Pe'er, A. Rahman, I. Amit and M. Merad (2017). "Innate Immune Landscape in Early Lung Adenocarcinoma by Paired Single-Cell Analyses." Cell **169**(4): 750-765 e717.

Lovaszi, M., M. Mattii, K. Eyerich, A. Gacsi, E. Csanyi, D. Kovacs, R. Ruhl, A. Szegedi, L. Kemeny, M. Stahle, C. C. Zouboulis, S. Eyerich and D. Torocsik (2017). "Sebum lipids influence macrophage polarization and activation." Br J Dermatol **177**(6): 1671-1682.

Lovászi, M., A. Szegedi, C. C. Zouboulis and D. Törőcsik (2017). "Sebaceous-immunobiology is orchestrated by sebum lipids." Dermato-endocrinology **9**(1): e1375636.

Pappas, A., S. Johnsen, J.-C. Liu and M. Eisinger (2009). "Sebum analysis of individuals with and without acne." Dermato-endocrinology **1**(3): 157-161.

Underhill, D. M. and H. S. Goodridge (2012). "Information processing during phagocytosis." Nat Rev Immunol **12**(7): 492-502.

Wang, E. C. E., Z. Dai, A. W. Ferrante, C. G. Drake and A. M. Christiano (2019). "A Subset of TREM2(+) Dermal Macrophages Secretes Oncostatin M to Maintain Hair Follicle Stem Cell Quiescence and Inhibit Hair Growth." Cell Stem Cell **24**(4): 654-669 e656.

Xiong, X., H. Kuang, S. Ansari, T. Liu, J. Gong, S. Wang, X. Y. Zhao, Y. Ji, C. Li, L. Guo, L. Zhou, Z. Chen, P. Leon-Mimila, M. T. Chung, K. Kurabayashi, J. Opp, F. Campos-Perez, H. Villamil-Ramirez, S. Canizales-Quinteros, R. Lyons, C. N. Lumeng, B. Zhou, L. Qi, A. Huertas-Vazquez, A. J. Lusis, X. Z. S. Xu, S. Li, Y. Yu, J. Z. Li and J. D. Lin (2019). "Landscape of Intercellular Crosstalk in Healthy and NASH Liver Revealed by Single-Cell Secretome Gene Analysis." Mol Cell **75**(3): 644-660 e645.

Xue, D., T. Tabib, C. Morse and R. Lafyatis (2020). "Transcriptome landscape of myeloid cells in human skin reveals diversity, rare populations and putative DC progenitors." J Dermatol Sci **97**(1): 41-49.

Yeh, F. L., Y. Wang, I. Tom, L. C. Gonzalez and M. Sheng (2016). "TREM2 Binds to Apolipoproteins, Including APOE and CLU/APOJ, and Thereby Facilitates Uptake of Amyloid-Beta by Microglia." Neuron **91**(2): 328-340.