Annual Symposium

Thursday, February 29, 2024

Kahn Auditorium

Biomedical Science Research Building

Michigan Medicine, Ann Arbor, MI

Event Program

Our annual symposium is sponsored by:

NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases

MEDICAL SCHOOL UNIVERSITY OF MICHIGAN

UNIVERSITY OF MICHIGAN DEPARTMENT OF DERMATOLOGY

POSTER PRESENTATIONS

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2. Sex-dependent activation of keratinocytes by IL-36 cytokines Mrinal Sarkar

3. Single-cell RNA Sequencing Identifies Fibrotic Fibroblasts and TGF$\mathcal{B}$ Dysregulation in a Model of Cutaneous Lupus by Nazy Gharaee-Kermani

4. CRISPR/Cas9-mediated genome editing in postnatal epidermis drives squamous tumor development by LiJyun Syu

5. Immunological Differences Between Palmoplantar and Non-palmoplantar Skin by Enze Zing

6. Exploring VGLL3-TEAD interaction as a potential therapeutic target in lupus by Vincent van Drogelen

7. Unique epithelial proliferative transcriptomic signature in proton pump inhibitor-responsive Pediatric Eosinophilic Esophagitis by Sahiti Marella

8. Neutrophil secrete exosome-associated DNA (SEAD) to promote resolution by Arya Subhash

9. Expansion of Th17 cells in CD3/CD28-activated human PBMC: Monocyte apoptosis and skin-homing T-cell bias by Zhaolin Zhang

10. Single-Cell Analysis Reveals Distinct Subpopulations of T Cells and Elevated IL26 and IL17 Levels in Palmoplantar Pustulosis Lesions by Tran Do

11. Dermal Fibroblasts Cultured from Aged Human Skin Display a Pro-inflammatory Phenotype by Yilei Cui

12. Co-transcription Factors YAP and TAZ Regulate Dermal Extracellular Matrix homeostasis and Scar Formation in Mouse Skin by Alexandre Ermilov

5th Annual UM-Skin Biology & Diseases Resource-Based Center Symposium
Kahn Auditorium, Biomedical Science Research Building, University of Michigan
109 Zina Pitcher Place, Ann Arbor, MI
Thursday February 29, 2024
8:00 am – 12 noon
Agenda

Breakfast & refreshments available in the BSRB Seminar Rooms: 7:30 am-9:30 am.
Required: Registration

No food and/or drinks are allowed in Kahn auditorium

8:00 - 8:05 am **Welcome & Overview**
Johann Gudjonsson, M.D., Ph.D.

FAC Research Updates & Initiatives

8:05 – 8:20 am Alex Tsoi, PhD & Rachael Bogle

"Bioinformatics on Spatial analysis capabilities: data and platforms"

8:20 – 8:35 am Mrinal Sarkar, Ph.D.
"Overcoming Transfection Resistance: CRISPR's Path to Gene Editing Success"

Research Updates: UM-SBDRC Pilot Awards

8:35 – 8:45 am Olesya Plazyo, Ph.D.
" VGLL3/ IL-7 axis in autoimmunity"

8:45 – 8:55 am Tom Kerppola, Ph.D.
"Stress and innate immunity – moderation of cytokine transcription by the multifunctional Keap1 protein."

8:55 – 9:05 am Claudia Loebel, M.D., Ph.D. & Brendon Baker, Ph.D.
"Lineage-specific ECM labeling in skin fibrosis models"

9:05 – 9:15 am **Pilot Q&A**

9:15 – 9:30 am **Break**

9:30 – 9:45 am Pei-Suen (Eliza) Tsou, Ph.D.
"*Interplay between calcium signaling and mitochondrial oxidative stress in scleroderma fibrosis"*

9:45-10:00 am Andrzej Dlugosz, M.D.
"Animal Modeling Core: Advances in Fluorescent Cell Fate Tracking"

10:00-10:45 am **Beverage Break & Research Posters in Second floor atrium (in-person only)**

10:50-11:50 am **Keynote Address**

Michel Gilliet, MD, Professor & Chair of Dermatology, University of Lausanne, Switzerland

"Immune Profiling for Precision Medicine of Inflammatory Skin Diseases"

11:50 - 12 noon **Closing Remarks & Poster Awards**

12 pm–1:00 pm **Lunch by Katherines Catering will be served in the Seminar Rooms**

Poster #1: Epidermal ZBP1 stabilizes mitochondrial Z-DNA to drive UV-induced IFN signaling in autoimmune photosensitivity

Benjamin Klein[1], Mack B. Reynolds[2], Bin Xu[1], Mehrnaz Gharaee-Kermani[1,3], Yiqing Gao[1], Celine C. Berthier[4], Svenja Henning[1], Shannon N. Loftus[1], Kelsey E. McNeely[1], Amanda M. Victory[1], Craig J. Dobry[3], Grace A. Hile[3], Feiyang Ma[1,3], Jessica L. Turnier[5], Johann E. Gudjonsson[3], Mary X. O' Riordan[2], J. Michelle Kahlenberg[1,3]*

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[3] Department of Dermatology, University of Michigan, Ann Arbor, Michigan
[4] Division of Nephrology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, United States
[5] Division of Pediatric Rheumatology, Department of Pediatrics, University of Michigan, Ann Arbor

Photosensitivity is observed in numerous autoimmune diseases and drives poor quality of life and disease flares. Elevated epidermal type I interferon (IFN) production primes for photosensitivity and enhanced inflammation, but the substrates that sustain and amplify this cycle remain undefined. Here, we show that IFN-induced Z-DNA binding protein 1 (ZBP1) stabilizes ultraviolet (UV)B-induced cytosolic Z-DNA derived from oxidized mitochondrial DNA. ZBP1 is significantly upregulated in the epidermis of adult and pediatric patients with autoimmune photosensitivity. Strikingly, lupus keratinocytes accumulate extensive cytosolic Z-DNA after UVB, and transfection of keratinocytes with Z-DNA results in stronger IFN production through cGAS-STING activation compared to B-DNA. ZBP1 knockdown abrogates UV-induced IFN responses, whereas overexpression results in a lupus-like phenotype with spontaneous Z-DNA accumulation and IFN production. Our results highlight Z-DNA and ZBP1 as critical mediators for UVB-induced inflammation and uncover how type I IFNs prime for cutaneous inflammation in photosensitivity.

Poster #2: Sex-dependent activation of keratinocytes by IL-36 cytokines

Mrinal K. Sarkar[1], Anthony Coon[1], Christopher Cole[1], Feiyang Ma[2], Bethany Perez White[3], Craig Dobry[1], Lam C. Tsoi[1], Nicole L. Ward[4], J. Michelle Kahlenberg[2], Johann E. Gudjonsson[1]

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[4]Department of Dermatology, Vanderbilt University Medical Center, Nashville, TN

Pustular psoriasis is a devastating subtype of psoriasis associated with significant morbidity and mortality. Pustular psoriasis is the only form of psoriasis that shows prominent sex bias, with the majority of cases found in women. Pustular psoriasis, and to a lesser extent plaque psoriasis, is characterized by the prominent involvement of the IL-36 family of cytokines, which consists of three pro-inflammatory cytokines: IL-36A, IL-36B, and IL-36G, and the IL-36 receptor antagonist (IL-36RA/*IL36RN*). All three IL-36 members and the IL-36 receptor antagonist are increased (5-10 fold) in psoriatic skin. Single-cell and spatial-seq data demonstrated that IL-36 responses are primarily localized to the supraspinous compartment of the epidermis and strongly correlate with and act downstream of both IL-17A and TNF responses. CRISPR/Cas9 targeted knocking out of each IL-36 family member in keratinocytes demonstrates that both *IL36G* and *IL36R* KOs, but not *IL36A* KO, suppress both IL-17A and TNF responses ($p<0.001$). Notably, the suppressive role of *IL36G* KO occurred in the absence of neutrophil proteases, which have been considered primary activators of the IL-36 axis in the skin. Bulk RNA-seq data from primary keratinocytes (n=47) showed marked sex bias in IL-36G response, with female keratinocytes having a more robust pro-inflammatory response to IL-36G compared to male keratinocytes($p<0.001$). Furthermore, female keratinocytes showed higher expression of the type I IFN, *IFNK* ($p<0.001$; FC-3.84), and IFN signature genes, such as *MX1* ($p<0.001$; FC-4.78), indicating an association between IFN-κ and the IL-36 axis in female keratinocytes. These data provide novel insights into IL-36 biology, demonstrate its role in amplifying IL-17 and TNF responses in the epidermis, and suggest that the sex bias of IL-36 response may contribute to the marked female bias of pustular forms of psoriasis.

Poster #3: Single-cell RNA Sequencing Identifies Fibrotic Fibroblasts and TGFβ Dysregulation in a Model of Cutaneous Lupus

Mehrnaz Gharaee-Kermani[1,2], Allison C. Billi[1], Jacob WS. Martens[2], Marisa C. Hildebrandt[1], Michelle Kahlenberg[1,2], Johann E. Gudjonsson[1,2].

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Background: Fibrosis is characterized by collagen deposition, fibro/myofibroblast accumulation, and extracellular matrix remodeling and often leads to organ disfunction. Cutaneous lupus erythematosus (CLE) subtypes, particularly discoid lupus erythematosus (DLE), are associated with significant fibrosis with no effective treatment. However, the mechanisms that drive scar formation in DLE are unknown. We have shown that epidermal-directed overexpression of murine *Vgll3* causes skin lesions suggestive of DLE, and thus, we aimed to explore the role of Vgll3 in cutaneous fibrosis.

Methods: 2–3-month-old male and female transgenic (TG) mice overexpressing *Vgll3* in the epidermis under the K5 promoter were compared to wildtype (WT) C57Bl/6 mice (n=3-5 per group). Fibrotic biomarkers in TG mice and WT compared, as well as fibrotic biomarkers, of human DLE and scleroderma compared with healthy control. We used single-cell-RNA-sequencing (scRNA-seq) from lesional/nonlesional and WT skin to investigate the transcriptomes of the potential cellular fibrotic players such as fibroblasts (FBs) and myofibroblasts (MYOFBs). ScRNA-seq data analyzed using Seurat. Several subclusters of FBs, MYOFBs, and T cells were identified and fibrotic markers for each FBs subcluster were analyzed. Gene expression of fibrosis-associated genes in FBs/MYOFBs, T-cells, from lesional/nonlesional and control were compared.

Results: Male and female transgenic Vgll3 TG mice exhibit cutaneous inflammation and a significant increase in fibrotic markers (*Acta2, Col1a1, Col1a2, Tgfb1, Ctgf)* and pro-fibrotic cytokines compared to non-lesional skin. scRNA-seq of lesional and nonlesional skin from TG mice vs WT skin showed a higher inflammatory infiltrate in lesional TG compared to nonlesional TG, and no inflammatory infiltrate in WT skin, which was verified by immunohistochemistry. Myeloid cells in lesional TG>nonlesional TG>WT skin had increased inflammatory gene expression (*Cxcr4, Cxcl2, Il1b, Tnf, Cd14*), and CD4+ T cells in lesional TG skin exhibited greater *Gata3,* and *Tgfb1* expression than nonlesional TG and WT skin. Additionally, lesional TG skin

exhibit higher expression of *Tgfb1, Col1a1,* and *Col1a2* compared to nonlesional TG and WT mouse skin. We identified a fibroblast (FB) subcluster unique to lesional TG skin representing adventitial Pi16+ FB, which express chemokines (*CCL7, CCL8, CCL11, CCL19*). Finally, increased expression of Hippo and TGFβ pathway-regulated genes in FBs and keratinocytes from lesional/nonlesional TG skin was seen.

Conclusion: Overall, we have linked *Vgll3*-driven dysregulation of the Hippo and TGFβ pathways to fibrotic phenotypes, which may provide a critical novel target for the alleviation of disfiguring fibrosis in CLE. Our results indicate that VGLL3-Hippo/TEAD inhibition may attenuate the fibrotic phenotype in DLE.

Poster #4: CRISPR/Cas9-mediated genome editing in postnatal epidermis drives squamous tumor development

Li-Jyun Syu1, Shreya Mishra1, Rong Wu2, Dawn Wilbert1, Russell Ryan, Kathleen Cho, Andrzej Dlugosz

1. Dermatology, University of Michigan Michigan Medicine, Ann Arbor, MI, UnitedStates.
2. University of Michigan Michigan Medicine, Ann Arbor, MI, United States.

Tumorigenesis is driven by accumulation of somatic alterations in multiple cancer driver genes, including tumor suppressors and oncogenes. Conventional mouse models have yielded important insights into the molecular basis of cancer, but engineering several genetic alterations in vivo is expensive, time-consuming, and labor-intensive. Here we show that CRISPR/Cas9 technology can be used to disrupt multiple tumor suppressor genes in epidermal cells of adult mice, leading to squamous papilloma development. Transgenic "*PRN*" mice were generated that constitutively express sgRNAs targeting the tumor suppressor genes *Trp53*, *Rb1*, and *Nf1*. To disrupt gene expression in postnatal mouse epidermis we generated *iK14;Cas9;PRN* mice carrying a tamoxifen-activated epidermal Cre allele (*K14-CreERT*), a Cre-inducible Cas9 allele (*R26-LSL-Cas9*), and the *PRN* allele. Mice were treated with tamoxifen at 7.5 weeks to induce Cas9 expression and monitor for tumor development. Four out of 5 *iK14;Cas9;PRN* mice developed focal hyperkeratotic skin tumors 5 to 9.5 months after starting tamoxifen treatment, with H&E histology showing squamous papillomas. Sanger sequencing revealed successful editing of *Trp53* in 6/8, *Rb1* in 8/8, and *Nf1* in 8/8 tumors. Surprisingly, two *iK14;Cas9;PRN* mice and one *Cas9;PRN* mouse developed liver lesions with histology resembling a lymphoid or other hematopoietic malignancy suggesting ectopic and 'leaky' Cas9 expression, even in the absence of Cre recombinase. Our data establish the utility of CRISPR/Cas9 for editing multiple target genes simultaneously in epidermis using multi-guide transgenic mice, setting the stage for functional validation of candidate cutaneous cancer drivers using an approach considerably less cumbersome than conventional mouse modeling. Further modifications are required to ensure tight control of Cas9 expression, which is needed to avoid tumor development in non-targeted tissues.

Poster #5: Immunological Differences Between Palmoplantar and Non-palmoplantar Skin

Xing E, Chopp L, Ma F, Wasikowski R,Billi AC, Xing X, Plazyo O, Tsoi LC, Gudjonsson JE

Palmoplantar (PP) skin is functionally and histologically distinct from non-PP skin. Inflammatory skin diseases may present differently when involving the PP skin compared to the rest of the body and diseases in acral skin are frequently treatment-resistant with topical and systemic therapies. These observations suggest PP skin embodies a unique immunological niche that is functionally distinct. We aimed to identify these differences by performing single-cell RNA-sequencing onCD45+ immune cells isolated from matched palm and hip biopsies from6healthydonors.No significant differences were seen in immune cell numbers between PP and non-PP skin. Next, myeloid, T cell, mast cell, and contaminating fibroblast populations were isolated. The myeloid populations between the skin sites were distinct, and Langerhans cells (LCs) were the primary myeloid cell type in both PP and non-PP skin. Two LC subtypes were identified, with L0 making up a higher proportion of myeloid cells in the hip, while L1 was relatively enriched in the palm. Transcriptomic analysis also suggested functional differences between the LC subtypes, with L0 skewing towards adaptive immunity and L1 exhibiting more innate immune activity. While the T cell subset proportions were similar between the two sites, they also appeared functionally distinct, with palmar CD4 and CD8 T cells exhibiting increased activation compared to those from the hip. These results define PP skin as a unique immunological site. They further suggest that PP presentations of common inflammatory dermatoses should be studied as disease entities independent from their non-PP counterparts and may require alternative therapeutic management.

Poster #6: Exploring VGLL3-TEAD interaction as a potential therapeutic target in lupus

Vincent van Drongelen[1], Kelly Z. Young[1], Olesya Plazyo[1], Emma Griffin[1], Jennifer Fox[1], Nicole L. Ward[2], J. Michelle Kahlenberg[1], Andrzej A. Dlugosz[1], Johann E. Gudjonsson[1], Allison C. Billi[1]

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Nine out of 10 individuals with systemic lupus erythematosus are women, and skin is one of the most frequently affected organs. We previously found that expression of the transcription coregulator VGLL3 is increased in keratinocytes of healthy women, where it promotes expression of inflammatory genes. Mice that have epidermal overexpressing of VGLL3 develop cutaneous and systemic lupus-like autoimmune disease. However, whether VGLL3 targeting in lupus can serve as therapeutic target remains unknown. Here, we have utilized transgenic mouse models and human skin to investigate whether VGLL3 can be used for therapeutic intervention in lupus. Utilizing an explant culture approach, we are testing drugs predicted to block VGLL3-TEAD interaction for therapeutic effect using biopsies from our lupus mouse models and from human patient skin. Exploiting our novel transgenic mouse models, we have demonstrated that in established disease normalization of epidermal VGLL3 expression ameliorates both skin and systemic manifestations of VGLL3-induced murine lupus, including near-normalization of lupus-specific anti-dsDNA autoantibody titers. Through IP-mass spectrometry, we determined VGLL3-TEAD binding in VGLL3 overexpressing cells. Via proximity ligation assay we confirmed that VGLL3 interacts with TEAD transcription factors in the epidermis of healthy individuals and patients with lupus and we established that TEAD binding capacity is required for epidermal VGLL3 to cause murine lupus. The results of this work are anticipated to provide vital preclinical data supporting the approach of VGLL3-TEAD inhibition in lupus patients with skin involvement.

Poster #7: Unique epithelial proliferative transcriptomic signature in proton pump inhibitor-responsive Pediatric Eosinophilic Esophagitis

*Sahiti Marella[1], *Ankit Sharma[1], Varsha Ganesan[1], Gila Idelman[1], Talaya McCright-Gill[2], Nancy Gonzalez[2], Alexandros Polydorides[3], Paul Foster[4], Simon Hogan[1], Mirna Chehade[2].

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Background and Aims: Clinical studies identified two distinct eosinophilic esophagitis (EoE) treatment phenotypes, proton pump inhibitor (PPI)-responsive (PPI-R) and PPI-unresponsive (PPI-UR), which are clinically, endoscopically, and histologically indistinguishable at diagnosis. However, comprehensive analyses of EoE in treatment-naïve individuals at diagnosis and following PPI therapy have not previously been performed.

Methods: Esophageal biopsies were collected from healthy control patients (n = 5), and treatment naïve PPI-R and PPI-UR EoE patients (n = 10) prior to and following PPI therapy. Endotype characteristics were assessed by the EoE Endoscopic Reference Score, EoE Histology Scoring System and RNA sequencing.

Results: Evaluation of the esophageal biopsies revealed similar inflammatory and histological alterations and endoscopic features between PPI-R and PPI-UR EoE individuals at diagnosis. RNAseq analyses revealed 2296 differentially expressed genes (DEGs) in R-EoE and 4084 DEGs in UR-EoE at diagnosis. The DEGs in both endotypes were enriched for the prototypic EoE transcriptome genes (*CCL26, CCL24, TNAIP6, ALOX15, FFAR3, IL13*). R-EoE DEGs were enriched for innate and interferon-signaling pathways and UR-EoE DEGs were enriched for chemokine and apoptosis signaling. Mapping DEGs in both endotypes revealed 1189 common genes, 407 unique genes in R-EoE, which were enriched for biological processes involved in hydrogen peroxide catabolism and neuropeptide signaling, and 2195 unique genes in UR-EoE, which were enriched for DNA replication, cell cycle and cell division.

Conclusions: Treatment-naïve PPI-R and PPI-UR EoE patients at diagnosis possess a common immune and inflammatory transcriptional signature and distinct signatures enriched for processes related to neuropeptide signaling and cell cycle and division.

Poster #8: Neutrophil secrete exosome-associated DNA (SEAD) to promote resolution

Subhash B. Arya[1], Samuel P. Collie[2], Yang Xu[3], Martin Fernandez[4], Venkatesha Basrur[5], Jonathan Sexton[7,8], Shyamal Mosalaganti[1,8], Pierre A. Coulombe[8.9.10], and Carole A. Parent[1,3,8.10]

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Neutrophils are the first line of defense in response to injury or infection. Once neutrophils reach affected sites, they contain the area by phagocytosing pathogens and releasing powerful proteases from cytoplasmic granules. In addition, neutrophils release their genomic DNA, studded with toxic enzymes, that go on to ensnare and kill microbes in neutrophil extracellular traps (NETs). Excessive NET release aggravates inflammation, delays resolution, and subsequently leads to host tissue injury during various autoimmune diseases, and infections. Neutrophils migrating towards shallow chemoattractant gradients emanating from injured/infected tissues amplify their recruitment range by releasing the secondary chemoattractant leukotriene B_4 (LTB_4). LTB_4 is synthesized from arachidonic acid through the action of the cytosolic enzyme 5-lipoxygenase (5-LO), the endoplasmic reticulum/nuclear envelope-resident protein 5-lipoxygenase activating protein (FLAP) and leukotriene A_4 hydrolase. The LTB_4 synthesizing enzymes and LTB_4 are packaged in and released from extracellular vesicles called exosomes. We recently showed that the biogenesis of LTB_4-containing exosomes originates at the nuclear envelope (NE) of activated neutrophils. We found that the neutral Sphingomyelinase 1 (nSMase1)-dependent generation of ceramide-rich lipid-ordered membrane microdomains is required for FLAP and 5-LO clustering at the NE, and subsequent NE-budding. With nano-scale microscopic resolution achieved by 4x isotopic expansion of samples, we found 5-LO-positive intraluminal vesicles within NE-resident Lamin B Receptor (LBR)-positive limiting membranes, generating NE-derived multivesicular bodies (NE-MVBs). Interestingly, we discovered that both NE-derived MVBs and exosomes are distinct from conventional plasma membrane-derived CD63-positive MVB/exosomes. We also observed the presence of DNA in the lumen of the NE-MVBs. Using SYTOXgreen, a membrane-impermeable DNA-binding dye, we visualized in real time the secretion of DNA from the back

of chemotaxing neutrophils – a process that was dependent on nSMase1 activity and on the presence of LBR, which is known to bind DNA. We further determined that decondensed chromatin is present in the lumen of NE-MVBs and found that increasing chromatin decondensation, by inhibiting histone deacetylases (HDAC), increases NET secretion. Finally, we determined that DNase-induced dissolution of secreted exosome associated DNA (SEAD) leads to erratic neutrophil migration with a loss of directionality in vitro and mi mice skin inflammation it leads to defect in resolution. Taken together, our study provides novel mechanistic insights into an inside-out pathway of DNA and exosome secretion originating from the NE of activated neutrophils, involved in physiological regulation of neutrophil chemotaxis.

Poster #9: Expansion of Th17 cells in CD3/CD28-activated human PBMC: Monocyte apoptosis and skin-homing T-cell bias

Z Zhang, PE Stuart, RP Nair, H Zhang, R Wasikowski, J E Gudjonsson, LC Tsoi, J T Elder, Dept of Derm, University of Michigan

T-cells undergo polyclonal expansion in cutaneous psoriasis (PsC) and psoriatic arthritis, with persistence of "driver clones" after effective treatment (JCI 127:4031; JI 172:1935). A recent scRNA-seq study found roughly equal compartments of clonally expanded and polyclonal Th17 and Tc17 cells in PsC (Science 371:364). We and others have shown that Th17 expansion from human PBMC requires contact between monocytes and memory T-cells in the context of TCR ligation (PNAS 104:17034; SID 139:1245). We stimulated PBMC (n=153) with anti-CD3/CD28 beads for 0 or 24h followed by flow cytometry (CD3+CD45RO+, CD4/CD8 x CLA+/CLA-). Activation-related DEGs featured marked up-regulation of Th17 signature mRNAs (*IL17A*, *IL17F*, *IL22*, and *CCL22*) along with the Th1 cytokine *IFNG*, with a corresponding induction of IL-17A and IL-22 proteins by flow cytometry. These findings were confirmed by cluster analysis of scRNA-seq libraries of CD3/CD28-activated PBMC (n=4 subjects). Stratified analysis of skin homing in CD3/CD28-activated cells revealed 2.9 to 12.1-fold upregulation of *IL17A*, *IL17F*, *IL22*, and *CCL22* in CLA+ vs CLA-, without a corresponding difference in *IFNG*. *IL17A* and *IL17F* were overexpressed in activated T-cells from psoriatics vs. controls (each 1.9-fold, $p=4.7x10^{-4}$). As revealed by scRNA-seq of lesional psoriatic skin, *IL17A* was overexpressed (2.2-fold, p= 0.003) in skin-homing (FUT7+) vs non-skin-homing (FUT7-) T-cells, whereas *IFNG* was not. As reported in a recent CITE-seq study (Front Immunol 12:636720), our bulk- and sc-RNA-seq experiments revealed dramatic disappearance of monocytes within 24h of CD3/CD28 activation, which was confirmed by imaging flow cytometry and morphologically identified as apoptosis by time-lapse microscopy. Taken together with data showing IL-23 expression by inflammatory monocyte-like cells in dermal clusters in PsC (JID 141:1707), our experiments suggest a contact-dependent interplay between activated T-cells and monocyte-derived cells in dermal clusters, which maintains polyclonal activation of skin-homing Th17 cells in psoriatic lesions.

Poster #10: Single-Cell Analysis Reveals Distinct Subpopulations of T Cells and Elevated IL26 and IL17 Levels in Palmoplantar Pustulosis Lesions

Tran H. Do[1], Rachael Wasikowski[1], Xianying Xing[1], Mehrnaz Gharaee-Kermani[1], Madalina Raducu[2], Jennifer Fox[1], J. Michelle Kahlenberg[3], Robert L. Modlin[4], Ozge Uluckan[2], Lam C. Tsoi[1], Johann E. Gudjonsson[1]

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4. Department of Dermatology, UCLA, Los Angeles, CA, USA

Palmoplantar pustulosis (PPP) is a chronic, debilitating inflammatory skin disorder characterized by erythematous pustules, and desquamation in the palms and soles. Despite its significant impact on the quality of life for affected individuals, the understanding of the pathogenesis of PPP remains limited. In this study, skin biopsies from three PPP patients and five controls were subjected to single-cell analysis, analyzing both lesional and non-lesional samples. Focusing on the immune cell population, there was a significant increase in B cells among the lymphocytes in lesional samples compared to non-lesional ones. Additionally, the T cell landscape exhibited a unique profile in PPP, with regulatory T cells (Treg) and T helper 17 cells (Th17) exclusively present in PPP samples. Further characterization of Th17 cells uncovered two distinct populations, each exhibiting differential cytokine expression and regulation. One subset, termed "regulatory Th17", expressed the immune regulatory molecules FOXP3 and CTLA4. Interestingly, this regulatory Th17 subpopulation demonstrated elevated levels of IL17F and IL26 in lesional cells, suggesting a potential role in the inflammatory cascade associated with PPP. Immunohistochemistry analysis further confirmed elevated levels of IL-26 and IL-17 in lesional skin. Cell-cell interaction analyses unveiled IL26 signaling, associated with activation of the IL-10 signaling pathway, originating from the "regulatory Th17" subset. These comprehensive findings shed light on the intricate cellular dynamics within PPP lesions, highlighting the potential role of IL26 and IL17 and providing valuable insights for understanding the disease's immune landscape and identifying potential therapeutic targets.

Poster #11: Dermal Fibroblasts Cultured from Aged Human Skin Display a Pro-inflammatory Phenotype

Yilei Cui, Mai Shi, John J Voorhees, Yi Zhao, Gary J Fisher

University of Michigan, Ann Arbor, United States and Tsinghua University, Beijing, China.

The dermal extracellular matrix (ECM) provides mechanical support to the skin and a microenvironment that is critical for the function of dermal cells. Dermal fibroblasts synthesize, organize, and reside within the dermal ECM. During aging, the ECM becomes fragmented. This fragmentation is associated with the clinical manifestations of aging, i.e., thin, fragile skin, and deleterious alterations of fibroblast function, most notably reduced expression of ECM structural genes and increased expression of pro-inflammatory mediators. We have investigated the degree to which the functional alterations of fibroblasts in aged skin reflect adaptation to the aged dermal ECM microenvironment versus cell-autonomous age-driven responses. Primary dermal fibroblasts from young (20-30 years, 6 females, 6 males) and aged (>80 years, 6 females, 6 males) individuals were placed in standard monolayer culture and harvested at 70% confluence (P1) for RNAseq transcriptome analysis. 477 genes were differentially expressed (DEGs); 300 upregulated and 177 downregulated in aged versus young. Interestingly, upregulated DEGs and protein-protein interactions were enriched in cytokine and chemokine-mediated signaling, including cytokine-cytokine receptor interactions, TNF signaling pathway, and chemokine activity (all $p<10^{-5}$). Down-regulated DEGs and protein-protein interactions were enriched in the regulation of lipid biosynthesis, and fatty acid metabolism. Notably, P1 fibroblasts from young and aged skin expressed similar levels of genes that encode ECM structural proteins including collagens (42 genes expressed), proteoglycans (29 genes expressed), and glycoproteins (157 genes expressed). The above findings indicate that aging causes fibroblasts to acquire a cell-autonomous pro-inflammatory phenotype. In contrast, fibroblasts cultured from aged skin retain the capacity to express "youthful" levels of ECM structural genes, suggesting that diminished expression of these genes *in vivo* reflects adaptation to the aged dermal microenvironment.

Poster #12: Co-transcription Factors YAP and TAZ Regulate Dermal Extracellular Matrix homeostasis and Scar Formation in Mouse Skin

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The collagen-rich dermal extracellular matrix (ECM) provides mechanical support to the skin and a microenvironment that is critical for the function of dermal cells. Dermal fibroblasts are the primary cell type that produce and maintain the dermal ECM. During early postnatal life, collagen fibrils are formed as the dermal ECM matures and expands to accommodate growth. During adult life, dermal ECM homeostasis maintains structural integrity and restoration after wounding. Two highly homologous transcriptional co-factors *Yap* and *Taz* are vital downstream effectors of the hippo signaling pathway, which controls organ size and tissue homeostasis. In addition, *Yap/Taz* regulates cellular responses to mechanical forces. We have investigated the roles of *Yap/Taz* in fibroblasts during dermal ECM maturation and post-wound scarring using conditional Cre-LoxP deletion of *Yap* and *Taz*. Deletion of *Yap/Taz* in dermal fibroblasts three days after birth led to significantly decreased dermal ECM collagen fibril production, deposition, and organization. Dermal ECM density and type I collagen gene expression were substantially reduced (67%, $p<0.035$, and 88%, $p<0.001$, respectively). Spatial RNAseq of the dermis confirmed reduced expression of the predominant collagen genes COL1A1 (76% reduced, $p<0.0001$), COL1A2 (73% reduced, $p<0.0001$), and COL3A1 (58% reduced, $p<0.0004$). Notably, the hippo signaling pathway was negatively enriched, whereas cytokines inflammatory response pathways were positively enriched. Furthermore, fibrotic scarring, following incisional tail wounding was substantially reduced (50% reduced dermal thickness, $p>0.01$) in *YAP/Taz* knockout mice, compared to age and sex-matched littermate controls. Bleomycin-induced dermal fibrosis was also significantly reduced (33%, $p<0.01$). The above data demonstrate the critical role of *Yap/Taz* in dermal ECM homeostasis and identify the *Yap/Taz* pathway as a potential target to reduce fibrotic scarring.