LC2017
15th International Workshop on Langerhans Cells

October 6 – 8, 2017
New York, NY, USA

Program & Abstracts

Dear Colleagues,

Welcome to New York City and to LC2017, the 15th in a series of biannual workshop conferences addressing cutaneous immunology dating back to 1989. This conference originally focussed primarily on epidermal Langerhans cells, but more recently it has grown to encompass all antigen-presenting cells in the skin and other aspects of cutaneous immunity and pathology. The skin, with its diverse spectrum of immune cells, remains an attractive target for immunotherapeutic approaches directed against inflammatory and infectious skin diseases and for exploiting the cutaneous immune system for immunotherapy. Previous Langerhans Cell Workshops have impressively documented the developments and achievements in this field, and we trust the current LC2017 program will again address these important topics.

The biannual Langerhans Cell Workshops foster productive interactions between established and young investigators, often leading to important advances in cutaneous immunology and Langerhans cell immunobiology in particular. LC2017 will again be a very interactive and fruitful scientific meeting.

The Langerhans Cell Workshops have always given fresh impetus to the immunological field of dendritic cells. We would very much like to see the field not only maintain but also increase this momentum and ongoing productivity. We therefore welcome your participation in the 15th International Workshop on Langerhans Cells in New York, taking place from Friday morning, October 6th through early afternoon on Sunday, October 8th, 2017.

Sincerely,

Jim Young, Conference Chair
Miriam Merad, Dan Kaplan, Niki Romani, and Kenji Kabashima

COMMITTEES

Local Organizing Committee

James W. Young, New York, NY, USA, LC 2017 Chair
Miriam Merad, New York, NY, USA
Daniel Kaplan, Pittsburgh, PA, USA

International Organizing Committee

Björn Clausen, Mainz, Germany
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Sandrine Henri, Marseille, France
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Eynav Klechevsky, St.Louis, MO, USA
Keisuke (Chris) Nagao, USA
Nikolaus Romani, Innsbruck, Austria
Patrizia Stoitzner, Innsbruck, Austria
Marcel B.M. Teunissen, Amsterdam, The Netherlands

Past Langerhans Cell Workshops

1989 Oslo, Norway
1991 Lyon, France
1993 Dallas, TX, USA
1995 Scheveningen, The Netherlands
1997 Fuschl / Salzburg, Austria
1999 New York, USA
2001 Stresa, Italy
2003 Tokyo, Japan
2005 Funchal, Madeira, Portugal
2007 Berne, Switzerland
2009 Funchal, Madeira, Portugal
2011 Innsbruck, Austria
2013 Amsterdam, The Netherlands
2015 Kyoto, Japan

Participation fees

	Early bird period (payment received by September 4, 2017)	Payment after September 4, 2017
Registration fee	$495	$600
Students, Post-Docs, Residents*	$250	$400

*Students/ Post-Docs and Residents are required to provide proof of their student or training status.

Conference Venue

Zuckerman Research Center Auditorium
Memorial Sloan Kettering Cancer Center
417 E 68th Street
New York, NY 10065

Contact

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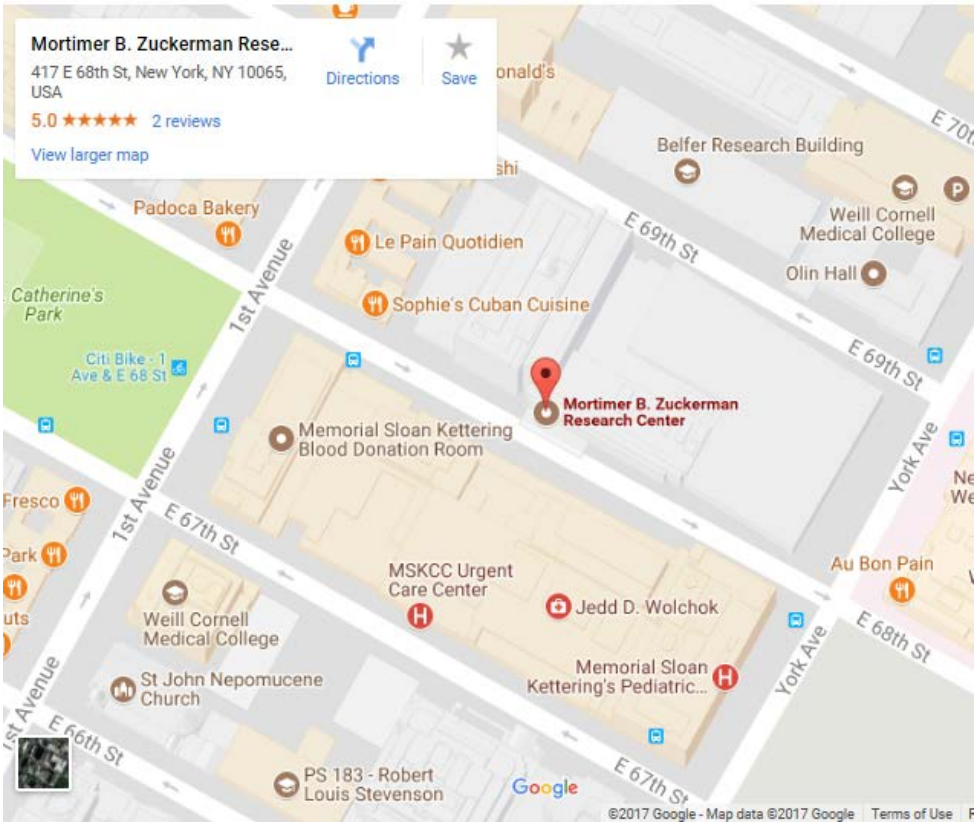

LC2017 SYNOPSIS

FRIDAY OCT 6		SATURDAY OCT 7		SUNDAY OCT 8	
9h00	*DEPT OF MEDICINE GRAND ROUNDS IN SAME AUDITORIUM ADJOURNS AT 9h00*	8h00-9h00	Coffee/tea/light breakfast food (catered on site at MSKCC by outside vendor)	8h00-8h45	**Coffee/tea/light breakfast**
9h00-9h45	**Badge/program pickup, on-site registration, coffee/tea/light breakfast**	9h00-10h30	**SESSION 4 PSORIASIS**	8h45-10h30	**SESSION 8 ANTIGEN PROCESSING/PRESENTATION** (includes HIV, nanoparticle loading, melanophages, liposomal delivery of Ag, etc.)
9h45-10h00	**WELCOME, introduction, orientation, etc. (Jim Young)**	10h30-11h00	**Coffee/tea break**	10h30-11h00	**Coffee/tea break**
10h00-12h45	**SESSION 1 ONTOGENY, ORGANIZATION, AND FUNCTIONAL DIFFERENTIATION OF THE LC/DC SYSTEM**	11h00-12h30	**SESSION 5 CUTANEOUS IMMUNITY**	11h00-13h00	**SESSION 9 LCs IN DISEASE**
12h45-13h45	**LUNCH**	12h30-13h30	**LUNCH**	13h00	**FAREWELL – Jim Young Incl announcement about DC2018 in Germany**
13h45-15h00	**SESSION 2 REGULATORY T CELLS AND LC/DCs**	13h30-15h30	**SESSION 6 LCs, TUMOR MICROENVIRONMENT, TUMOR IMMUNITY, VACCINES**		
15h00-15h30	**Coffee/tea break**	15h30-16h00	**Coffee/tea break**		
15h30-18h00	**SESSION 3 INFLAMMATORY SKIN DISEASES, INCLUDING ATOPY**	16h00-17h30	**SESSION 7 LCs IN TOLERANCE, HOMEOSTASIS, TRANSPLANTATION, ETC.**		
18h00-19h00 or 19h30 or 20h00	**POSTER SESSION WITH SOCIAL HOUR**	17h30-18h30 or 19h00 or 19h30	**POSTER SESSION WITH SOCIAL HOUR**		

OPENING – WELCOME AND INTRODUCTION TO LC 2017

9:00  Coffee/Tea/ Light Breakfast & Badge/Program Pick-up; On-site Registration

9:45 Chair: James W. Young – Memorial Sloan Kettering Cancer Center, New York, NY, USA

SESSION 1 — ONTOGENY, ORGANIZATION, AND FUNCTIONAL DIFFERENTIATION OF THE LC/DC SYSTEM

10:00 Muzlifah Haniffa – Newcastle University, Newcastle upon Tyne, UK
Deconstructing the human skin using single cell technologies

10:30 Eynav Klechevsky – Washington University, Saint Louis, MO, USA
Human Langerhans Cell Subsets in control of cutaneous immunity

11:00 Juliana Idoyaga – Stanford University, Stanford, CA, USA
A high-dimensional phenotypic map of human skin DC subsets

11:30 Victoria Zyulina – Institute of Pathophysiology and Immunology, Medical University Graz, Austria
miR-424 regulates human in vitro DC subset specification

11:45 Martin Zenke – RWTH Aachen University Medical School, Aachen, Germany
An Id2-Zeb2-E47 centered gene network controls tissue residence of mouse dendritic cells by inducing mesenchymal-to-epithelial transition

12:00 Andreas Schlitzer – University of Bonn, Bonn, Germany
Cellular reprogramming of human monocytes is regulated by time-dependent IL4 signalling and NCOR2

12:15 Mathias Hochgerner – Medical University of Vienna, Vienna, Austria
BMPR1a is essential for correct development and function of Langerhans Cells in mice

12:30 Maria Gschwandtner – Medical University of Vienna, Vienna, Austria.
Human dendritic cells are identified by the reticulum-associated protein Reticulon 1A

12:45  Lunch

SESSION 2 — REGULATORY T CELLS AND LC/DCs

13:45 Alexander Rudensky – Memorial Sloan Kettering Cancer Center, New York, NY, USA
Differentiation and function of regulatory T cells

14:30 Tommaso Sconocchia – Medical University of Graz, Graz, Austria
Enhanced proliferation and immunosuppressive functions of human induced regulatory T cells by BMP7-dependent Langerhans cells in vitro

14:45 Sayuri Yamazaki – Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan
The critical role of dendritic cell subset in expanding Foxp3+ regulatory T cells in the murine skin after ultraviolet B exposure

15:00  Coffee/Tea Break

SESSION 3 — INFLAMMATORY SKIN DISEASES, INCLUDING ATOPY

15:30 Emma Guttman – Mount Sinai, New York, NY, USA
Atopic dermatitis is driven by a network of T-cells and dendritic cells

16:00 Florian Winau – Harvard Medical School, Boston, MA, USA
Revisiting the importance of CD1a on Langerhans cells

16:30 Shruti Naik – The Rockefeller University, New York, NY, USA
Memory of Inflammation in Epidermal Stem Cells

17:00 William Shipman – Weill Cornell/Rockefeller/Sloan-Kettering, New York, NY, USA
Langerhans cells limit ultraviolet radiation (UVR)-induced skin inflammation and lupus photosensitivity using mouse and human systems

17:15 I-hsin Su – Nanyang Technological University, Singapore
Ezh2 regulates Langerhans cell migration and host protection against allergic contact dermatitis in mouse

17:30 Christine Moussion – Genentech, San Francisco, CA, USA
Neutrophils prime extra-cellular matrix to facilitate mouse dendritic cells transmigration

17:45 Richard Granstein – Weill Cornell Medicine, New York, NY USA
Exposure of murine Langerhans cells (LCs) to IL-6 biases antigen presentation toward an IL-17A response

18:00  Poster Session with Social Hour

8:00  Coffee/Tea/Light Breakfast

SESSION 4 — PSORIASIS

9:00 James Krueger – The Rockefeller University, New York, NY, USA
Psoriasis and dendritic cell involvement

9:30 Björn Clausen – University Medical Center, Mainz, Germany
Cellular and molecular players driving psoriatic skin disease in mice

10:00 Izabela Borek – Medical University of Graz, Graz, Austria
Bone morphogenetic protein signature defines psoriatic niche and instructs human inflammatory Langerhans cells

10:15 Tae-Gyun Kim – Yonsei University College of Medicine, Seoul, South Korea
Skin-specific CD301b+ dermal dendritic cells drive IL-17-mediated psoriasis-like immune response

10:30  Coffee/Tea Break

SESSION 5 — CUTANEOUS IMMUNITY

11:00 Kenji Kabashima – Kyoto University, Kyoto, Japan
Role of skin dendritic cells in the elicitation phase of acquired immune responses

11:30 Daniel Kaplan – University of Pittsburgh, Pittsburg, PA, USA
Non-hematopoetic control of skin immunity

12:00 Siqi Liu – The Rockefeller University, New York, NY, USA
Impaired epidermal to dendritic T cell signaling slows wound repair in aged mice

12:15 Qingtai Su – Baylor Institute for Immunology Research, Dallas, TX, USA
Keratinocytes affect biology of Langerhans cells through mRNA transfer

12:30  Lunch

SESSION 6 — LCs, TUMOR MICROENVIRONMENT, TUMOR IMMUNITY, VACCINES

13:30 Patrizia Stoitzner – Medical University of Innsbruck, Innsbruck, Austria
Skin dendritic cells in cancer immunity: can they be harnessed for immunotherapy?

14:00 Niroshana Anandasabapathy – Brigham and Woman's Hospital, Boston, MA, USA
Skin dendritic cells in cancer immunity: can they be harnessed for immunotherapy?

SESSION 6 — LCs, TUMOR MICROENVIRONMENT, TUMOR IMMUNITY, VACCINES

14:30 David Chung – Memorial Sloan Kettering Cancer Center, New York, NY, USA
Langerhans-type dendritic cells electroporated with TRP-2 mRNA stimulate cellular immunity against melanoma: Results of a phase I vaccine trial

14:45 Lydia Bellmann – Medical University of Innsbruck, Innsbruck, Austria
Reinforcing dendritic cells for cancer immunotherapy: diverse ways and means to target antigens to human skin

15:00 Nicolas Pinto – Instituto de Biología y Medicina Experimental, Buenos Aires, Argentina
Galectin 7 promotes chemical skin carcinogenesis through induction of myeloid regulatory cells in mice

15:15 Toshiro Hirai – University of Pittsburgh, Pittsburgh, PA, USA
TGFβ activation by keratinocytes promotes systemic and tissue-resident memory CD8 T cells during Vaccinia virus skin infection

15:30  Coffee/Tea Break

SESSION 7 — LCs in TOLERANCE, HOMEOSTASIS, TRANSPLANTATION, ETC

16:00 Clare Bennett – University College London, London, UK
Defining a novel role for LC in the epidermis – LC are required for cutanous GVHD by directly activating a unique programme of pathogenicity in effector T cells

16:30 Matthew Collin – Newcastle University, Newcastle upon Tyne, UK
The role of donor myeloid cells in human graft versus host disease of the skin

17:00 Sakeen Kashem – University of Minnesota, Minneapolis, MN, USA
Expression of αvβ8 by Langerhans cells is required for Th17 differentiation and tethering of LC in the epidermis

17:15 Heather West – University College London, London, UK
Replacement of the Langerhans cell network in a murine model of acute graft-versus-host disease results in a loss of tolerance in the skin

17:30  Poster Session with Social Hour

8:00 Coffee/Tea/Light Breakfast

SESSION 8 — ANTIGEN PROCESSING/PRESENTATION (includes HIV, nanoparticle loading, melanophages, liposomal delivery of Ag, etc.)

8:45 Sandrine Henri – Centre d'Immunologie Marseille-Luminy, Marseille, France
Unveiling melanophage dynamics : Insights from a new mouse model

9:15 Yonatan Ganor – Cochin Institute, INSERM U1016, CNR UMR8104, Paris Descartes University, Paris, France
Calcitonin gene-related peptide diverts viruses away from the endo-lysosomal pathway in human Langerhans cells

9:30 Bernardien Nijmeijer – Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
HIV-1 enhances sexual transmission of Hepatitis C virus by human mucosal Langerhans cells

9:45 Christoph Rademacher – Max Planck Institute of Colloids and Interfaces, Potsdam, Germany
Small molecule-mediated liposomal delivery to human Langerhans cells

10:00 Carla Ribeiro – Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
Autophagy restricts HIV-1 infection of human dendritic cell subsets and drives protective antiviral T-cell immunity

10:15 Nienke H. van Teijlingen – Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
Vaginal dysbiosis-associated bacteria Prevotella strongly increases HIV-1 uptake in human vaginal Langerhans cells – scavengers or ticking time bombs?

10:30 Coffee/Tea Break

SESSION 9 — LCs in DISEASE

11:00 Miriam Merad – Mount Sinai Hospital, New York, NY, USA
Langerhans cell histiocytosis: human and mouse models

11:30 Carl E. Allen – Texas Children's Hospital, Houston, TX, USA
Langerhans Cell Histiocytosis: A human model of DC differentiation and function

12:00 Paul Milne – Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom.
The haematopoietic origin of human Langerhans cell histiocytosis

SESSION 9 — LCs in DISEASE

12:15 Sergei Koralov – NYU School of Medicine, New York, NY, USA
Analysis of molecular etiology and bacterial triggers of Cutaneous T cell lymphoma

12:45 James W. Young – Memorial Sloan Kettering Cancer Center, New York, NY, USA
Primary T cells from cutaneous T-cell lymphoma skin explants display an exhausted immune checkpoint profile

13:00 FAREWELL – Including announcement about DC2018 in Germany

Muzlifah Haniffa – Newcastle University, Newcastle upon Tyne, UK

Deconstructing the human skin using single cell technologies

Space for your notes:

Eynav Klechevsky – Washington University, Saint Louis, MO, USA

Human Langerhans Cell Subsets in control of cutaneous immunity

Space for your notes:

Juliana Idoyaga – Stanford Hospital, Stanford, CA, USA

A high-dimensional phenotypic map of human skin DC subsets

Space for your notes:

O1) miR-424 regulates human in vitro DC subset specification

Victoria Zyulina [viktoria.zyulina@medunigraz.at]

Zyulina Victoria, Strobl Herbert

Institute of Pathophysiology and Immunology, Medical University Graz, Austria

MicroRNAs (miRNAs) are regulators of various biological processes and are differentially expressed by DC subsets. It was previously shown that deficiency of Dicer, an enzyme critical for miRNA processing, causes strongly dysregulated epidermal Langerhans cell (LC) homeostasis.

To identify individual effector miRNAs, that are potentially involved in human DC subset development and functions, we performed microarray-based miRNA profiling of LC-type and interstitial-type DCs (intDCs) generated in vitro from human CD34+ cord blood hematopoietic progenitor cells (in presence of either TGF- β1 or IL-4 respectively). Among approximately 20 differentially regulated miRNAs from the screen, miR-424 exhibited the strongest inverse expressed pattern between these two DC subsets (intDCs > LCs).

miR-424 has previously been shown to be activated by transcription factor PU.1 and to promote human M-CSF-dependent monocyte differentiation. Both DC subsets can be generated from monocytic precursors, and PU.1 promotes both LC and intDCs differentiation in vitro. Given its inverse expression, we analyzed whether miR-424 is differentially required for LC vs intDCs differentiation. We transduced CD34+ hematopoietic progenitor cells with anti-miR-424 or control plasmid and induced them to differentiate into either intDCs or LCs. Anti-miR-424 exhibited profound inverse effects. It inhibited intDCs and promoted LC differentiation. Therefore, miR-424 is required for IL-4-dependent intDCs differentiation but is dispensable for TGF-β1-dependent LC differentiation.

O2) *An Id2-Zeb2-E47 centered gene network controls tissue residence of mouse dendritic cells by inducing mesenchymal-to-epithelial transition*

Martin Zenke [martin.zenke@rwth-aachen.de]

Kristin Seré[1], Corinna Rösseler[1], Maike Kosanke[1], Saskia Mitzka[1], Gülcan Aydin[1], Jan Hapala[1], Thomas Hieronymus[1], Julia Ober-Blöbaum[1], Hubert Smeets[2], Barbara L. Kee[3], Emma L. Rawlins[4], Viktor Janzen[5] and Martin Zenke[1]

[1] Department of Cell Biology, Institute for Biomedical Engineering, RWTH Aachen University Medical School and Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany
[2] Department of Genetics and Cell Biology, Maastricht University Medical Center, and Research Schools CARIM and GROW, Maastricht University Medical Center, Maastricht, The Netherlands
[3] Committee on Immunology and Department of Pathology, The University of Chicago, Chicago, IL 60637, USA
[4] Wellcome Trust/CRUK Gurdon Institute, Wellcome Trust/MRC Stem Cell Institute, Department of Pathology, University of Cambridge, Cambridge CB2 1QN, UK
[5] Department of Internal Medicine III, Hematology/Oncology/Rheumatology, University of Bonn, Bonn, Germany

The transcription factor Id2 (inhibitor of DNA binding/differentiation) is mandatory for development of specific dendritic cell (DC) subsets. Id2−/− mice lack Langerhans cells (LC) and classical DC type 1 (cDC1). Here we investigated how Id2 exerts its function.

In an Id2 lineage tracing mouse model we show that Id2 is absent in prenatal LC precursors and upregulated during the first days after birth when LC precursors enter epidermis. Surprisingly, LC precursors are present in Id2-/- mice and seed the epidermis but fail to settle down and are not retained in epidermis. We found that Id2 triggers mesenchymal-to-epithelial transition (MET) in LC differentiation.

MET and its reversed program, epithelial-to-mesenchymal transition (EMT) are mechanisms traditionally described in embryology and tumor biology. MET and EMT coordinate the switch from a migratory to a sessile cellular phenotype and vice versa. MET by Id2 in LC differentiation triggers induction of epithelial molecules in LC, such as E-cadherin, claudin and EpCam, and thus ensures stable anchorage of LC in the epithelial environment of epidermis.

On the molecular level we found that helix-loop-helix transcription factor E47 prevents MET by inducing the EMT transcription factor Zeb2 and by inhibiting cell adhesion molecules. Deletion of E47 in Id2−/− E47−/− double knockout mice rescues LC development. We also show that Id2 and Zeb2 expression is regulated by TGFbeta signaling, which has a major impact on LC residence in epidermis.

Taken together, we describe an Id2-Zeb2-E47 centered gene network, which is regulated by TGFbeta signaling and required for residence of LC. Finally, we extend this concept also to lymphoid tissue resident cDC1.

O3) Cellular reprogramming of human monocytes is regulated by time-dependent IL4 signalling and NCOR2

Andreas Schlitzer [andreas.schlitzer@uni-bonn.de]

Jil Sander[1], Susanne V. Schmidt[2], Branko Cirovic[3], Naomi McGovern[4,5], Olympia Papantonopoulou[1], Anna-Lena Hardt[1], Anna C. Aschenbrenner[1], Christoph Kreer[6], Thomas Quast[7], Alexander M. Xu[8], Lisa M. Schmidleithner[1], Heidi Theis[1], Thi Huong Lan Do[1], Hermi Rizal Bin Sumatoh[4], Mario A. R. Lauterbach[2], Jonas Schulte-Schrepping[1], Patrick Günther[1], Jia Xue[1], Kevin Baßler[1], Thomas Ulas[1], Kathrin Klee[1], Stefanie Herresthal[1], Wolfgang Krebs[1], Bianca Martin[2], Eicke Latz[2,9,10], Kristian Händler[1], Michael Kraut[1], Waldemar Kolanus[7], Marc Beyer[1,11], Christine S. Falk[12], Bettina Wiegmann[13], Sven Burgdorf[6], Nicholas A. Melosh[8], Evan W. Newell[4], Florent Ginhoux[4], Joachim L. Schultze[1,14], Andreas Schlitzer[3,4]

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[14] Platform for Single Cell Genomics and Epigenomics (PRECISE) at the German Center for Neurodegenerative Diseases and the University of Bonn

The clinical and therapeutic value of human in vitro generated monocyte-derived dendritic cell (moDC) and macrophages is well established. However, in line with recent findings regarding myeloid cell ontogeny and due to our limited understanding of their physiological counterparts, transcriptional regulation and heterogeneity, the full potential of these important cellular systems is still underestimated.

In this study, we use cutting edge high-dimensional analysis methods to better understand the transcriptional organization, phenotypic heterogeneity and functional differences between human ex vivo isolated and in vitro generated mononuclear phagocytes with the aim to better realize their full potential in the clinic.

We demonstrate that human monocytes activated by MCSF or GMCSF most closely resemble inflammatory macrophages identified in vivo, while IL4 signalling in the presence of GMCSF generates moDCs resembling inflammatory DCs in vivo, but not steady state cDC1 or cDC2. Moreover, these reprogramming regimes lead to activated monocytes that present with profoundly different transcriptomic, metabolic, phenotypic and functional profiles. Furthermore, we demonstrate that CD14+ monocytes are integrating multiple exogenous activation signals such as GMCSF and

IL4 in a combinatorial and temporal fashion, resulting in a high-dimensional cellular continuum of reprogrammed monocytes dependent on the mode and timing of cytokine exposure. Utilizing nanostraw-based knockdown technology, we demonstrate that the IL4-dependent generation of moDCs relies on the induction, nuclear localization and function of the transcriptional regulator NCOR2.

Finally, we unravel unappreciated heterogeneity within the clinically moDCs population and propose a novel high-dimensional phenotyping strategy to better tailor clinical quality control strategies for patient need and culture conditions to enhance therapeutic outcome.

O4) BMPR1a is essential for correct development and function of Langerhans Cells in mice

Mathias Hochgerner [mathias.hochgerner@meduniwien.ac.at]

Hochgerner Mathias[1,2], Bauer Thomas[1], Borek Izabela[2], Warsi Sarah[3], Sibilia Maria[1], Karlsson Stefan[3], Strobl Herbert[2]

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[2] Institute of Pathophyiology and Immunology, Mecial University of Graz
[3] Division of Molecular Medicine and Gene Therapy, Lund University

Development and function of Langerhans Cells (LCs) are critically dependent on the cytokine TGF-β1. However, our lab could recently show that Bone Morphogenic Protein 7 (BMP-7) allows for the in vitro generation of LCs from human CD34+ cord blood stem cells, replacing TGF-β1. In order to investigate the role of BMPs in LC biology in vivo, we generated several conditional knock-out mouse models.

In BMPR1aΔvav-mice, the BMP receptor type 1 (BMPR1a, Alk3) is deleted already in early LC precursors, allowing the investigation of LC differentiation in vivo. In line with our in vitro findings, these mice show disturbed LC differentiation. While the overall density of the epidermal DC network is unchanged, the number of cells expressing CD207 is reduced and existing CD207+ cells show decreased expression levels of this terminal differentiation marker. Furthermore, bone marrow from BMPR1aΔvav-mice generates less DCs in in vitro cultures. Curiously, when deleting the complementary type-2 receptor BMPR2 in the same manner, the phenotype is similar but far less pronounced. In BMPR2Δvav-mice LCs also display lower levels of CD207, but the overall number of CD207+ cells in the epidermis is unaffected.

In BMPR1aΔCD11c-mice, BMPR1a is deleted during late DC/LC differentiation. In these mice, the LC network appears to be unaffected in the steady state. However, in two different models of skin inflammation BMPR1aΔCD11c-mice displayed stronger and longer lasting inflammation. A migration assay revealed that LCs from these mice migrate faster and show a higher expression of MHCII and CD86.

Taken together, our results provide further evidence supporting the theory that BMP receptor signaling plays an important role in establishing an intact LC network. Furthermore, BMP-signaling in DCs/LCs seems to play a major role in counteracting and resolving inflammation. Further investigation of this previously unknown anti-inflammatory properties might lead to novel therapies for inflammatory skin diseases.

O5) Human dendritic cells are identified by the reticulum-associated protein Reticulon 1A

Maria Gschwandtner [maria.gschwandtner@meduniwien.ac.at]

Gschwandtner Maria[1], Kienzl Philip[2], Tajpara Poojabahen[2], Schuster Christopher[2], Mildner Michael[1], Elbe-Bürger Adelheid[2], Tschachler Erwin[1]

[1] Department of Dermatology, Research Division of Biology and Pathobiology of the Skin, Medical University of Vienna, Vienna, Austria.
[2] Department of Dermatology, Division of Immunology, Allergy and Infectious Diseases, Medical University of Vienna, Vienna, Austria

Reticulon 1 (RTN1) is an endoplasmic reticulum (ER)-associated protein that has initially been identified in neuronal tissues and is functionally involved in ER shaping and membrane transport. The purpose of our study was the investigation of the RTN1 expression pattern in skin. Surprisingly, the main isoform RTN1A was not identified in the skin nervous system but in dendritic cells. HLA-DR+CD1a+CD207+CD11cweak Langerhans cells were the only cells in the epidermis and HLA-DR+CD11c+ dendritic cells were the main cells in the dermis expressing this protein. RTN1A+ dendritic cells were also demonstrated in other human tissues such as the gingiva, trachea, tonsil, thymus and peripheral blood. RTN1A protein partially co-localized with the ER marker protein disulfide isomerase and could not be identified on the cell surface. During differentiation of MUTZ-3 cells into Langerhans cells, expression of RTN1A mRNA and protein preceded the established Langerhans cell markers CD1a and CD207. In line with this observation, RTN1A was expressed by around 80% of Langerhans cell precursors in embryonic skin implying its involvement in the differentiation of Langerhans cells and dendritic cells from precursor cells.
Our findings show that RTN1A is a novel marker for cells of the dendritic cell lineage. This finding will serve as a starting point for the identification of novel roles of RTN1A in the immune system and in the description of a novel player contributing to dendritic cell maturation and function.

Alexander Rudensky – Memorial Sloan Kettering Cancer Center, New York, NY, USA

Differentiation and function of regulatory T cells

Space for your notes:

O6) Enhanced proliferation and immunosuppressive functions of human induced regulatory T cells by BMP7-dependent Langerhans cells in vitro.

Tommaso Sconocchia [tommaso.sconocchia@medunigraz.at]

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Langerhans cells (LCs) play a key role in immuno-stimulation. Recent evidence suggests that they also have a significant impact on immune tolerance since LCs induce regulatory T (iTreg) cells. To date, the generation of LCs in vitro involves the stimulation of CD34+ hematopoietic progenitor cells (HPC) with a cocktail of cytokines in which the transforming growth factor beta 1 (TGF-β1) plays an essential role. Recent studies have shown that LCs can also be differentiated from CD34+ HPC by substituting TGF-β1 with the bone morphogenetic protein 7 (BMP7). BMP7-dependent LCs produce lower amounts of TGF-β1 than TGF-β1-dependent LCs as well as higher amounts of cytokines in response to microbial activation. TGF-β1-dependent LCs versus BMP7-dependent LCs share phenotypic characteristics with LCs from non-inflamed versus inflamed psoriatic epidermis, respectively. Therefore, we compared their capacity to instruct iTreg generation. Specifically, we evaluated the effect of LCs on allogeneic, naïve T cell proliferation and iTreg production by a mixed lymphocyte reaction assay. Following cell sorting, selected LCs and CFSE-labeled naïve T cells were co-cultured and analyzed by flow cytometry. BMP7-dependent LCs displayed an enhanced naïve T cell stimulatory capacity compared to TGF-β1-dependent LCs. Also, the extent of the iTreg content of five consecutive experiments was 20.5±2.4% utilizing BMP7-dependent LCs, which was significantly higher than that induced by TGF-β1-dependent LCs 14.2±1.2% (P=0.001). Subsequently, iTregs were labeled with a violet dye and cultured at a concentration ranging between 1.25x10^4-1x10^5, with a fixed number of CFSE-labeled autologous naïve T cells (10^5), in the presence of beads coated with anti-CD28 and anti-CD3 monoclonal antibodies. Then, CFSE-labeled T cell proliferation was measured by flow cytometry. iTregs derived from BMP7-dependent LCs were significantly more immunosuppressive than those derived from TGF-β1-dependent LCs. These data, for the first time, suggest that BMP7-dependent LCs are better iTreg inducers than TGF-β1-dependent LCs.

O7) The critical role of dendritic cell subset in expanding Foxp3+ regulatory T cells in the murine skin after ultraviolet B exposure

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Ultraviolet B (UVB) irradiation is known to induce immune tolerance and has been proven to be clinically effective for immunological skin diseases such as psoriasis. We previously reported that thymus-derived Foxp3+ regulatory T (Treg) cells with Treg-specific CpG hypomethylation were expanded to ~ 60% of the CD4+ T cells in the murine skin after UVB exposure and formed clusters with dendritic cells (DCs). This indicates involvement of DCs in the expansion of Treg cells. It remains obscure, however, whether skin DCs are involved in UVB-induced immune tolerance. In this study, using mice, we investigated the roles of DC subsets for UVB-mediated immune tolerance, and found a critical skin DC subset to expand Treg cells after UVB exposure. The skin DC subset up-regulated a set of genes associated with immunological tolerance and induced proliferation of Treg cells. These results indicate that there is a specialized DC subset in the UVB-exposed skin to expand Treg cells, which suppress autoimmunity. Further understanding of the functions of the DC subset in the UVB-exposed skin would enable expanding antigen-specific Treg cells for the treatment of autoimmunity, allergy and graft rejection in the skin.

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Atopic dermatitis is driven by a network of T-cells and dendritic cells

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Revisiting the importance of CD1a on Langerhans cells

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Memory of Inflammation in Epidermal Stem Cells

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O8) Langerhans cells limit ultraviolet radiation (UVR)-induced skin inflammation and lupus photosensitivity using mouse and human systems.

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Photosensitivity, defined as the development of skin lesions upon exposure to light, often due to UVR, is a defining characteristic of lupus erythematosus (LE). Photosensitive lesions can be aesthetically disfiguring and can exacerbate systemic manifestations, but the pathogenesis of photosensitivity in LE remains poorly understood. Langerhans cells (LCs), dendritic antigen presenting cells of the epidermis, can play regulatory roles in various types of skin injury and inflammation. As LCs are closely associated with keratinocytes, we hypothesized that LCs can limit UVR-induced keratinocyte injury and overall skin inflammation.

We show that LangerinDTA mice, constitutively depleted of LCs, have increased keratinocyte apoptosis, increased monocyte accumulation, blunted epidermal hyperplasia, and increased epidermal permeability after UVR exposure. In vitro experiments show that LCs directly limit UVR-induced keratinocyte apoptosis when co-cultured together. Two lupus mouse models, MRL-Faslpr and B6.Sle1Yaa mice, show increased sensitivity to UVR and exhibit a dysfunctional LC-keratinocyte axis. Human LCs are also able to protect keratinocytes from UVR-induced apoptosis and human LE skin shows signs of a dysfunctional LC-keratinocyte axis.

These results delineate a novel LC-keratinocyte axis that limits photosensitivity. Our results have implications for better understanding UVR skin effects and suggest that targeting this axis may be a new approach to treating photosensitivity in LE and other rheumatic diseases.

O9) Ezh2 regulates Langerhans cell migration and host protection against allergic contact dermatitis in mouse

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Enhancer of Zeste homolog 2 (Ezh2) is a well-established epigenetic repressor that can down-regulate leukocyte inflammatory responses, but it is unknown whether Ezh2 also plays non-epigenetic roles in control of host immunity. Here we demonstrate that Ezh2 regulates Langerhans cell (LC) transmigration across the epidermal basement membrane via a non-epigenetic mechanism that involves direct methylation of talin1. Ezh2 deficiency impaired disassembly of adhesion structures in LCs, leading to defective integrin-dependent emigration from the epidermis. Using a mouse model of allergic contact dermatitis, we further show that reduced migration of Ezh2-deficient LCs to skin-draining lymph nodes results in breakdown of cutaneous tolerance and subsequent development of an exaggerated contact hypersensitivity response. Moreover, tolerance was restored in Ezh2-deficient mice upon mobilization of Langerin- dermal dendritic cells (DCs) via high-dose treatment with a weak hapten. Collectively, these data reveal a novel role for Ezh2 in governing LC-mediated immune responses by regulating their migration.

O10) Neutrophils prime extra-cellular matrix to facilitate mouse dendritic cells transmigration

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Upon inflammation Dendritic cells (DCs) translocate from peripheral tissues to the draining lymph node where they present processed antigens to T cells. Most skin DCs reside in the dermis and crawl easily through the interstitium toward the afferent lymphatic vessels. Lately this migratory step became a potent new paradigm for studying the cell biology of interstitial motility. Langerhans cells (LCs) are an exceptional DC subtype, as they colonize the epidermis. Hence, they need to cross the basement membrane (BM) of the epidermal-dermal junction before proceeding towards the lymphatic vessels. BM penetration is the prototype of a tissue invasion event, which has been extensively studied in culture but rarely addressed in a physiological setting. We used LC invasion as a novel experimentally tractable model to study the cell biology of BM penetration. Invasion requires cell-cell contact detachment, cytoskeletal dynamics, substrate adhesion and peri-cellular proteolysis to act in concert in order for the cell to transmigrate. Integrins are receptors that help polarizing the degradative machinery of invading cells. The use of mice harboring DCs conditionally deleted for the integrin adaptor Talin-1 permits to assess how adhesion and invasion are interrelated. This study highlights that Integrin-mediated adhesion to the Extra-cellular matrix (ECM) is crucial for LCs to switch from an epithelial phenotype engaging E-Cadherin contact with keratinocytes to a migratory phenotype. In inflammatory conditions, the step of invasion of the BM is less dependent on adhesion, as an early priming of the ECM by Polynuclear Neutrophils facilitates the infiltration of Talin-1 deficient DCs. Intravital imaging as well as a reductionist in vitro setup where LCs invade decellularized skin and a characterization of the BM by Atomic Force Microscopy are used in this study to discover principles of cell migration equally relevant for immunology, embryonic development and cancer biology.

O11) Exposure of murine Langerhans cells (LCs) to IL-6 biases antigen presentation toward an IL-17A response

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Our laboratory has reported that exposure of endothelial cells (ECs) to the neuropeptide calcitonin gene-related peptide endows ECs with the ability, acting as bystanders, to bias the outcome of LC antigen presentation to responsive T cells away from Th1 responses and towards Th17 responses. Experiments indicate that induced IL-6 production by ECs mediates most of this effect. To determine whether IL-6 action on LCs alone or T cells alone is sufficient for this phenomenon, we exposed epidermal BALB/c LCs or, separately, T cells from DO11.10 mice (BALB/c background; DO11.10 mice have T cell receptor peptides engineered to respond to a fragment of chicken ovalbumin, cOVA323–339) to IL-6 or medium alone in culture for 3 hours. Then, all cells were washed x 4. IL-6-exposed or medium-exposed LC were cultured with DO11.10 T cells not exposed to IL-6 along with antigen. Supernatants were harvested 72 hours later and analyzed by ELISA for cytokine content. Exposure of LCs to IL-6 led to significantly enhanced production of IL-6 and IL-17A with significantly reduced IFNgamma production. When analogous experiments were set-up treating responding T cells with IL-6 instead of LCs, no effect was observed. In a preliminary experiment, exposure of LCs to monoclonal anti-CD126 antibodies before and during treatment with IL-6 significantly inhibited enhancement of IL-17A and IL-6 production by responding DO11.10 T cells while having no effect on the level of T cell responses in the absence of IL-6 treatment, suggesting that presentation of IL-6 by the IL-6 receptor alpha chain may be important for this effect. These experiments show that exposure of LCs to IL-6 polarizes the outcome of antigen presentation to responsive T cells away from an IFNgamma response and toward an IL-17A response. This finding may have implications for a greater understanding of the pathophysiology of inflammatory skin disorders.

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Psoriasis and dendritic cell involvement

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Cellular and molecular players driving psoriatic skin disease in mice

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O12) Bone morphogenetic protein signature defines psoriatic niche and instructs human inflammatory Langerhans cells

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Several mouse models of psoriasis suggest a positive feedback loop between dendritic cells [DCs] and keratinocytes. Epithelial factors imprinting inflammatory Langerhans cells [LCs] phenotype are under investigation, and the role of LCs in psoriasis remains controversial. Histological comparison of healthy and psoriatic skin was performed. Gene expression profile of TGF-β1 vs. BMP7-driven LCs generated from human CD34+ cells was analyzed using cDNA microarray, followed by flow cytometry phenotyping and ultrastructural analysis. To determine functional properties of BMP7-LCs vs. TGF-β1-LCs cell kinetics studies, co-culture with naive CD4 T-cells and cytokine measurements were performed. Additionally, in vivo experiments with Junf/fJunBf/fK5creER mice were carried out to assess the role of BMP signaling in psoriatic skin inflammation.

While in the healthy skin bone morphogenetic protein 7 [BMP7] expression is confined to basal keratinocytes, psoriatic epidermis exhibits strong BMP7 expression throughout all epidermal layers and shows strong pSMAD1/5/8 signature. DNA microarray profiling of in vitro TGF-β1-LCs vs. BMP7-LCs revealed upregulation of inflammation-related genes in BMP7-LCs. In an in vitro model of LC differentiation, BMP7 induced a population of CD1a+/CD207+ cells with inflammatory characteristics: (1) up-regulation of CD206, CD36 and CD1c and loss of epithelial adhesion molecule TROP2, (2) lack of Birbeck granules and increased frequency of MHCII-dependent antigen loading compartments, (3) high cytokine production in response to microbial activation, (4) strong allogeneic CD4 T-cell stimulatory capacity. Additionally, a substantial percentage of BMP7-LCs stained positive for the proliferation marker Ki67. While Ki67+ LCs were frequently observed throughout psoriatic epidermis, Ki67+LCs in the steady-state epidermis were confined to basal keratinocytes layer. Moreover, inhibition of BMP signaling via intradermal Noggin injection resulted in decreased epidermal thickening in Junf/fJunBf/fK5creERmice in comparison to the control injected mice.

In summary, psoriatic inflammation is characterized by strong BMP7-pSMAD1/5/8 signature, and BMP7 represents an instructive factor for proliferative LCs with distinct inflammatory profile.

O13) Skin-specific CD301b+ dermal dendritic cells drive IL-17-mediated psoriasis-like immune response

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Conventional dendritic cells (cDCs) are composed of heterogeneous subsets commonly arising from DC-committed progenitors. A population of CD301b-expressing DCs has recently been identified in non-lymphoid barrier tissues such as skin. However, whether CD301b+ DCs in the skin represent ontogenetically unique subpopulation of migratory cDCs has not been fully addressed. Here, we demonstrated that CD301b+ dermal DCs were distinct subpopulation of FLT3L-dependent CD11b+ cDC2 lineage which required an additional GM-CSF cue for the adequate development. Although the majority of lymphoid resident cDC2 lacked CD301b expression, dermal migratory cDC2 contained a substantial fraction of CD301b+ subset. Similar to CD301b− population, CD301b+ dermal DC development was closely regulated by FLT3 signaling, suggesting their common origin from FLT3L-responsive cDC progenitors. However, FLT3L-driven cDC progenitor culture was not sufficient but additional GM-CSF treatment was required to produce CD301b+ cDC2. The development of CD301b+ cDC2 in vivo was augmented by exogenous GM-CSF, while the repopulation of CD301b+ dermal cDC2 was significantly abrogated by GM-CSF neutralization. Functionally, CD301b+ cDC2 was capable of producing a high level of IL-23, and the depletion of CD301b+ cDC2 effectively prevented IL-17-mediated psoriasiform dermatitis. Therefore, our findings highlight the differentiation program of a distinct CD301b+ dermal cDC2 subset in the skin and its involvement in psoriatic inflammation.

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Role of skin dendritic cells in the elicitation phase of acquired immune responses

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Non-hematopoetic control of skin immunity

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O14) Impaired epidermal to dendritic T cell signaling slows wound repair in aged mice

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Skin has a remarkable ability to heal wounds through re-epithelialization, a repair process fueled by epithelial adult stem cells. Following injury, wound-edge epidermal keratinocytes proliferate and migrate into the wound site to initiate wound closure in young mice. In contrast, aged skin heals wounds poorly, increasing susceptibility to infections. We found that aged keratinocytes display significantly reduced proliferation and migration at the wound edge. Importantly, the aged wound edge failed to properly activate and maintain dendritic epidermal T cells (DETCs), a skin resident gammadeltaT cell that is important for wound repair. Mechanistically, we identified a family of transcripts named Skint (selection and upkeep of intraepithelial T cells) that were robustly induced in the wound edge basal keratinocytes of young mice but not in that of aged animals. We then identified Stat3 as the transcription factor that regulates Skint upregulation at the wound edge and mediates proper DETC function. Notably, when epidermal Stat3, Skints, or DETCs are silenced in young skin, re-epithelialization following wounding is perturbed. These findings underscore epithelial-immune crosstalk perturbations in general, and Stat3 and Skints in particular, as critical mediators in the age-related decline in wound repair.

O15) Keratinocytes affect biology of Langerhans cells through mRNA transfer

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Dendritic cell (DC) subsets might acquire specific immune functions based on their tissue of residence. Langerhans cells (LCs), a specific DC population located in the epidermis, are in close contact with epidermal keratinocytes (KCs), providing us with an easily accessible model to dissect the effect of epithelial cells on infiltrating DCs. Using microarray analysis, RT-qPCR, histology and flow cytometry, we showed that many of the KC-specific molecules, such as keratins and adhesion molecules, can be detected in LCs at mRNA and protein levels. To determine whether these KC-specific genes are accessible for transcription in LCs, we performed ATAC-seq on flow-purified LCs. We found that chromatin loci of these genes were in the closed conformation in LCs. Therefore, these data support the active transport of mRNAs from the KCs to LCs. Furthermore, we also showed that KC-specific expression of Cre, driven by the KRT14 promoter, can lead to genetic recombination and expression of YFP in the LCs. Additionally, YFP+ LCs could be readily identified in LC-depleted KRT14-YFP mice reconstituted with WT bone marrow, which further supports the material transfer from KCs to LCs. Of note, we found that tunneling nanotubes could be one of the potential mechanisms mediating the material transfer. The transfer of material between epithelial cells and epithelia-associated DCs was not limited to mice or to KC-to-LC transfer.

Taken together, these data suggest that the epithelial environment might have a long-term effect on DC biology and that genetic tools specifically targeting epithelial cells also affect tissue-resident immune cells, which adds another layer of complexity to data interpretation. The material transfer from epithelial cells to DCs might have evolved to maintain epithelial integrity and promote tolerance to self-antigens.

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Skin dendritic cells in cancer immunity: can they be harnessed for immunotherapy?

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Co-opting of homeostatic tissue DC development in the tumor microenvironment

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O16) Langerhans-type dendritic cells electroporated with TRP-2 mRNA stimulate cellular immunity against melanoma: Results of a phase I vaccine trial

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Purpose: We conducted a phase I vaccine trial to determine safety, toxicity, and immunogenicity of autologous Langerhans-type dendritic cells (LCs), electroporated with murine tyrosinase-related peptide-2 (mTRP2) mRNA in patients with resected AJCC stage IIB, IIC, III, or IV (MIa) melanoma.

Experimental Design: Nine patients received a priming immunization plus four boosters at three week intervals. Vaccines comprised 10x10e6 mRNA-electroporated LCs, based on absolute number of CD83+CD86(bright)HLA-DR(bright)CD14-neg LCs by flow cytometry. Initial vaccines used freshly generated LCs, whereas booster vaccines used viably thawed cells from the cryopreserved initial product. Post-vaccination assessments included evaluation of delayed-type hypersensitivity (DTH) reactions after booster vaccines and immune response assays at one and three months after the final vaccine.

Results: All patients developed mild DTH reactions at injection sites after booster vaccines, but there were no toxicities exceeding grade 1 (CTCAE, v4.0). At one and three months post-vaccination, antigen-specific CD4 and CD8 T cells increased secretion of proinflammatory cytokines (IFN-gamma, IL-2, and TNF-α), above pre-vaccine levels, and also upregulated the cytotoxicity marker CD107a. Next-generation deep sequencing of the TCR-V-β CDR3 documented fold-increases in clonality of 2.11 (range 0.85-3.22) for CD4 and 2.94 (range 0.98-9.57) for CD8 T cells at one month post-vaccines. Subset analyses showed overall lower fold-increases in clonality in three patients who relapsed (CD4: 1.83, CD8: 1.54) versus non-relapsed patients (CD4: 2.31, CD8: 3.99).

Conclusions: TRP2 mRNA-electroporated LC vaccines are safe and immunogenic. Responses are antigen-specific in terms of cytokine secretion, cytolytic degranulation, and increased TCR clonality, which correlates with clinical outcomes.

O17) Reinforcing dendritic cells for cancer immunotherapy: diverse ways and means to target antigens to human skin

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Dendritic cells (DC) are essential for the induction of primary immune responses, and hence preferred targets for immunization against cancer. Skin DC express C-type lectin receptors such as Langerin or DEC-205 for recognition of (pathogen-derived) antigens. In situ, Langerin is expressed mainly on Langerhans cells (LC), whereas DEC-205 is expressed by dermal DC and LC. We aim to load skin-resident DC (i) with antibody-antigen fusion proteins directed against these C-type lectin receptors or (ii) with antigens encapsulated in liposomes coated with a Langerin ligand.

(i) Monoclonal antibodies (mAb) were injected intradermally into human skin explants for targeting of skin DC subtypes corresponding to their C-type lectin receptor expression. Langerin mAb was detected exclusively in epidermal LC, whereas DEC-205 mAb targeted both CD1a+dermal DC and LC. A model antigen (EBNA1) fused to DEC-205 mAb elicited EBNA1-specific T cell responses in autologous PBMC. (ii) Liposomes coated with a Langerin ligand showed exclusive binding to LC in cell suspensions obtained from healthy human skin. These liposomes were rapidly incorporated into LC as visualized by confocal microscopy. For the future, liposomes provide a flexible delivery platform that will allow us to encapsulate antigens to investigate their potential for targeted delivery.

Our results show that specific skin DC subsets can be targeted in our ex vivo approach. Furthermore, this study will provide a deeper insight into DC-targeted cancer vaccines, their uptake, intracellular trafficking and antigen processing in human skin DC, especially LC. Those tumor-antigen:anti-DC antibody constructs or LC-specific liposomes loaded with tumor antigens will allow to boost patient´s pre-existing immunity. Ultimately, this DC-based immunotherapy can be used to increase the response rates when used in combination with immune checkpoint inhibitors.

O18) Galectin 7 promotes chemical skin carcinogenesis through induction of myeloid regulatory cells in mice

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Skin immunity is finely regulated by a broad network of cytokines and growth factors present in the epidermis and dermis to maintain tissue homeostasis. Disruption of this cellular and molecular balance may trigger different skin inflammatory diseases including psoriasis and dermatitis or neoplastic transformations like squamous cell carcinoma. Galectins (Gals), a family of beta-galactoside proteins that signal via glycosylated receptors, have emerged as key regulators of immune cell homeostasis. Galectin-7 (Gal7) is abundant in keratinocytes and is tightly regulated in response to skin environmental stress, suggesting that its altered expression may contribute to skin disease. The aim of this study was to evaluate the role of Gal7 during skin carcinogenesis. Using bioinformatic analysis (Enrichr resource and Cytoscape´s plugins ClueGo/CluePedia) we found that several oncogenic drivers (SOX2, NRAS, FOS, CD44 and RAC1 among others) were up-regulated in transgenic mice (Tg46) over-expressing Gal7 under the K14 promoter as, compared to wild type (WT) and Gal7-deficient (Lgals7-/-) mice. This expression profile positively correlated with a higher number of skin papillomas developed in the skin of Tg46 mice compared to WT or Lgals7-/- animals. Notably, these mice were more susceptible to two-stage induced-carcinogenesis and developed papillomas at day 45, whereas WT and Lgals7-/- animals developed skin lesions at day 53 and 60 respectively. Interestingly, Tg46 mice overexpressing Gal7 in keratinocytes exhibited a higher percentage of CD11b+Ly6G-Ly6C+ myeloid-derived suppressor cells (MDSCs) in the spleen compared to their WT counterpart. MDSCs purified from Tg46 mice showed enhanced immunosuppressive activity as compared to WT and Lgals7-/- MDSCs in in vitro lymphoproliferation assay. This enhanced immunosuppressive effect may account for increased tumor susceptibility in vivo. In conclusion, altered expression of Gal7 may contribute to skin carcinogenesis by favoring dysregulation of oncogenic drivers and promoting expansion of immunosuppressive MDSCs.

O19) TGFβ activation by keratinocytes promotes systemic and tissue-resident memory CD8 T cells during Vaccinia virus skin infection

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Cutaneous vaccination and cutaneous viral infections efficiently produce both skin CD8 T cell resident memory (TRM) and systemic memory that together provide efficient pathogen protection. In the epidermis, TGFβ is required for differentiation of CD8 TRM. We have previously shown that two RGD-binding integrins expressed by keratinocytes, αvβ6 and αvβ8, are required for activation of latent TGFβ in the epidermis. CD8 T cells recruited into the epidermis by application of DNFB in mice lacking these integrins (Itgb6-/- Itgb8ΔKC mice) failed to persist into the memory phase. To determine whether differentiation of memory precursors requires integrin-mediated activation of epidermal TGFβ in an infection model, we examined the kinetics of antigen-specific CD8 TRM differentiation in Itgb6-/- Itgb8ΔKC mice using B8R tetramers and a Vaccinia virus (VV) skin infection model. On day +14, shortly after peak of infection, numbers of CD103+ CD69+ cells were reduced in Itgb6-/- Itgb8ΔKC mice with a greater reduction by day56, indicating that differentiation as well as persistence of TRM are impaired in the absence of epidermal activation of TGFβ. Notably, numbers of splenic B8R+ CD8 T cells expanded normally in Itgb6-/- Itgb8ΔKC mice with a peak on day +14, but were reduced by day +56. This was associated with an expansion of KLRG1hi CD127lo short lived effector cells (SLEC) and a reduction in KLRG1lo CD127hi memory precursor cells. Similar alterations in number and phenotype were not observed following i.v. VV infection. This finding is consistent with a model in which local activation of TGFβ in the epidermis during cutaneous viral infection inhibits SLEC expansion thereby enhancing the development of systemic CD8 T cell memory. This phenomenon may explain why the skin is a particularly efficient route for vaccination and suggests potential approaches to augment vaccine adjuvant efficiency.

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Defining a novel role for LC in the epidermis – LC are required for cutanous GVHD by directly activating a unique programme of pathogenicity in effector T cells

Space for your notes:

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The role of donor myeloid cells in human graft versus host disease of the skin

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O20) Expression of αvβ8 by Langerhans cells is required for Th17 differentiation and tethering of LC in the epidermis

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Langerhans cells (LCs) reside in the epidermis where they capture cutaneous antigens and migrate to the skin draining lymph nodes (sLN) to prime and differentiate T cells. Using a murine skin infection model, our group has recently demonstrated that Langerhans cells were both necessary and sufficient to drive T helper 17 (Th17) differentiation in response to epicutaneous Candida albicans infection in vivo. Th17 differentiation requires a combination of IL-1β, IL-6, IL-23 and TGFβ. LC-derived IL-6 but not IL-1β, IL-23 or TGFβ was required for Th17 differentiation. Although LC-derived TGFβ was not required for Th17 differentiation, we found that activation of latent TGF-β by the integrin αvβ8 expressed by LC was required for efficient Th17 differentiation during C. albicans infection. Notably, the constitutive absence of TGFβ or αvβ8 on LC did not affect Treg numbers or phenotype. We have previously shown that activation of latent TGFβ by αvβ8 expressed by keratinocytes in Itgb8ΔKC mice is required to inhibit spontaneous migration of LCs from the epidermis to sLN. LC migration under steady state and inflammatory conditions, however, was not affected in Itgb8ΔLC mice. In contrast, tamoxifen induced ablation of αvβ8 in Itgb8TAMΔLC mice prevented LC migration. Over time, LC were observed in the epidermis near the stratum granulosum and were significantly reduced from both the epidermis and sLN. Thus, activation of TGFβ via αvβ8 expressed by LC is required for efficient Th17 differentiation in the setting of C. albicans infection. αvβ8 on LC is also required for appropriate epidermal localization of LC but this is likely compensated by other factors in the setting of a constitutive loss of this integrin.

O21) Replacement of the Langerhans cell network in a murine model of acute graft-versus-host disease results in a loss of tolerance in the skin

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Langerhans cells (LC) are the resident mononuclear phagocytes of the epidermis and self-renew throughout life. Functionally, LC are critical for the induction of tolerance in the skin and mediate this response by modulating T cell function. However, loss of embryonic-derived host LC from the epidermis results in repopulation of the empty niche by donor monocyte-derived cells. It is currently unknown how the turnover of LC impacts on their tolerance function in the skin.

To investigate the impact of LC replacement in the skin, we utilised a murine model of acute GVHD, where alloreactive CD8+ T cells kill recipient LC, forcing their replacement from donor bone marrow. Ten weeks later, after resolution of clinical GVHD, we investigated the impact of LC turnover on tolerance in the skin.

We induced tolerance in a contact hypersensitivity (CHS) model by topically applying the innocuous chemical 2,4-dinitrothiocyanobenzene (DNTB). Mice that had recovered from GVHD showed delayed kinetics of CHS resolution and tolerance to DNTB was not induced. To test the hypothesis that loss of tolerance was due to a lack of CD4+ T cell priming in lymph nodes (LN), we compared DNTB-induced LC and dermal dendritic cell (DC) migration post-GVHD. This demonstrated reduced migration of LC and langerin-expressing dermal DC to the LN following GVHD, but despite this, migrating cells were sufficient to prime both effector and regulatory T cell populations. Consequently, to determine whether tolerance was lost in situ in the epidermis, we characterised skin lymphocyte populations following DNTB challenge and identified an increase in dermal T cell number and reduced expression of the T cell retention integrin CD103.

We therefore propose a model whereby donor LC fail to induce tolerance following GVHD due to dysregulation of LC – T cell interactions in the skin, leading to a loss of retention of regulatory T cells.

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Unveiling melanophage dynamics : Insights from a new mouse model

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O22) Calcitonin gene-related peptide diverts viruses away from the endo-lysosomal pathway in human Langerhans cells

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Sexually transmitted human immunodeficiency virus type 1 (HIV-1) and herpes simplex virus (HSV) target Langerhans cells (LCs) in genital epithelia. Langerin-mediated HIV-1 internalization induces endo-lysosomal viral degradation. Yet, infectious virions escaping degradation are transferred to CD4+ T-cells during early first phase trans-infection. We recently reported that the neuro-immune dialogue between LCs and peripheral neurons, via the mucosally secreted neuropeptide calcitonin gene-related peptide (CGRP), inhibits LCs-mediated HIV-1 trans-infection. Herein, we investigated the mechanism of CGRP-induced inhibition of trans-infection, focusing on the role of HIV-1 degradation in LCs. In untreated LCs, HIV-1 degradation indeed occurs in endo-lysosomes, and functionally blocking such degradation with lysosomotropic agents results in increased trans-infection. CGRP induces faster HIV-1 degradation, but without affecting the kinetics of endo-lysosomal degradation. Unexpectedly, we reveal that CGRP completely shifts HIV-1 degradation towards the proteasome. Such proteasomal degradation is more efficient, thereby inhibiting first phase trans-infection. In turn, proteasomal inhibitors abrogate CGRP-induced inhibition. These results provide the first evidence for HIV-1 proteasomal degradation in LCs and suggest that the current paradigm of exclusive HIV-1 endo-lysosomal degradation should be refined. Next, we investigated how HSV infects LCs and whether CGRP modulates such infection. In untreated LCs, both lysosomotropic agents and proteasome inhibitors decrease HSV-1 productive infection, suggesting HSV-1 entry by fusion both within endo-lysosomes and the plasma membrane. CGRP decreases HSV-1 infection, which remains sensitive to proteasomal, but not endo-lysosomal, inhibition. Together, our results establish a common mechanism whereby CGRP bypasses the endo-lysosomal pathway in human LCs, to decrease infection with both HIV-1 (inducing efficient degradation via the proteasome) and HSV-1 (blocking endocytosis-mediated entry). These results extend the anti-viral activity of CGRP. As HSV infection enhances susceptibility to HIV-1 acquisition, CGRP-containing formulations might turn out clinically useful as novel microbicides with wide-range activity, whereby a single nerve-derived molecule could simultaneously limit HIV-1/HSV co-infections.

O23) HIV-1 enhances sexual transmission of Hepatitis C virus by human mucosal Langerhans cells

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Sexual transmission of Hepatitis C virus (HCV), until recently, was thought to be rare. However, there has been a significant rise in the incidence of HCV infection among HIV-infected men-who-have-sex-with-men (MSM) and studies suggest that HCV can be sexually transmitted within this population. The mechanism underlying this sexual transmission are unclear. We hypothesized that HIV-1 replication in HIV-1-infected MSM leads to mucosal changes that allow HCV entry and subsequent dissemination to hepatocytes via an unknown mechanism. Therefore, we analyzed the immune cells within mucosal anal biopsies from HIV-1 infected MSM individuals as a potential entry route for HCV during sexual contact. Notably, we detected Langerhans cells (LCs) within the mucosal anal tissue. As human LCs have been shown to be involved in limiting dissemination upon sexual contact by degrading HIV-1 and preventing HIV-1 transmission, we investigated their role in HCV infection and transmission using human primary isolated LCs and the ex vivo tissue transmission model. Immature LCs were neither infected nor transmitted HCV to hepatocytes in vitro and ex vivo. As sexual transmission is mostly observed within HIV-1 infected individuals, we pre-exposed tissues with HIV-1 and, strikingly, HIV-1 pre-exposure significantly increased HCV transmission by LCs. Studies have shown that the activation state of LCs changes susceptibility to HIV-1, leading to LC infection and subsequent HIV-1 transmission. Strikingly, activation of LCs did not lead to infection by HCV but these activated LCs, in contrast to immature LCs from same donor, were efficient in transmitting HCV to hepatocytes. Our data strongly suggest that HIV-1 replication in mucosal tissues in HIV-1 infected MSM changes LC function, which causes HCV capture and subsequent transmission to hepatocytes. This novel transmission mechanism by LCs implicates also that the activation state of LCs is an important determinant for HCV susceptibility.

O24) Small molecule-mediated liposomal delivery to human Langerhans cells

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Langerhans cells are attractive target cells for immune modulatory agents. Antibody-based delivery of antigens has provided significant insight into their ability to promote a cytotoxic T cell response and thereby generating interest in the role of LCs of human and murine origin. As an alternative delivery platform to antibodies, nanoparticles such as liposomes provide flexibility and high loading capacities. However, liposomal formulations are often insufficiently taken up by the targeted cells. They can be subject to bystander cell internalization, hence potentially imposing the aspired targeted effect. To overcome this limitation, liposomes are decorated with small molecules specific for human Langerin and thereby enable specific delivery of cargo e.g. antigens to LCs. The ligand we developed shows specific binding to Langerin-expressing cells and mediates selective uptake of these targeted liposomes followed by flow cytometry and confocal microscopy. Additionally, our targeted delivery devices were able to address Langerhans cells in the context of cell suspensions derived from human skin. The high specificity and fast kinetics upon uptake render our nanoparticles a highly suitable platform for the delivery of immunomodulatory agents in the future.

O25) Autophagy restricts HIV-1 infection of human dendritic cell subsets and drives protective antiviral T-cell immunity

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Langerhans cells (LCs) form an innate antiviral barrier against HIV-1 during sexual transmission. LCs restrict HIV-1 infection; C-type lectin receptor (CLR) Langerin captures HIV-1 into Birbeck granules, which prevents infection, but the underlying molecular mechanisms remain unclear.

Here, we show that autophagy potently restricts HIV-1 infection of human LCs, whereas HIV-1 escapes this restriction in subepithelial DC-SIGN+ dendritic cells (DCs). TRIM5α mediates assembly of autophagy-activating scaffold to Langerin-HIV complexes, which targets HIV-1 for autophagic degradation and prevents infection of LCs. By contrast, HIV-1 binding to CLR DC-SIGN in DCs leads to disassociation of TRIM5α from DC-SIGN, which abrogates autophagy restriction. Hence, these data strongly suggest that restriction by autophagy is controlled by CLR-dependent uptake of HIV-1, dictating protection or infection of human DC subsets.

Remarkably, we have recently identified a novel gene polymorphism in a component of the autophagy machinery which renders HIV-1 unable to escape autophagy restriction in DC-SIGN+ DCs. This autophagy-associated polymorphism is associated with decreased plasma viral load and improved survival in HIV-1 infected individuals from Amsterdam Cohort Studies, underscoring the in vivo relevance of the autophagy mechanisms in chronic HIV-1 patients. Our preliminary data suggest that healthy donors homozygous for this autophagy-associated polymorphism display decreased HIV-1 susceptibility; we observed block of HIV-1 infection of emigrated DC subsets and reduced transmission to CD4+ T cells in ex vivo skin explant tissue model. Moreover, our recent data suggest that this autophagy-associated polymorphism is associated with augmented autophagy levels in LCs and DC-SIGN+ DCs as well as rapid and higher magnitude of CD8+ T cell responses.

These findings substantiate the pivotal role of autophagy machinery in not only limiting HIV-1 establishment, but also in driving protective T-cell immunity to HIV-1. Host-directed therapies that harness these novel autophagy-directed mechanisms could thus provide superior DC-mediated antiviral immunity to HIV-1 in humans.

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O26) Vaginal dysbiosis-associated bacteria Prevotella strongly increases HIV-1 uptake in human vaginal Langerhans cells – scavengers or ticking time bombs?

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HIV-1 and AIDS remain the leading cause of death among African women of reproductive age and the major route of HIV-1 infection in women is sexual transmission via the vaginal mucosa. Dysbiosis of the vaginal microbiome has been associated with an increased risk for HIV-1 acquisition, but the underlying mechanisms remain unclear. Here we have selected vaginal microbiota ranging from healthy (i.e. Lactobacilli) to dysbiotic (i.e. Prevotella) and investigated their effect on vaginal HIV-1 transmission in a human vaginal ex vivo explant model as well as in in vitro isolated human vaginal LCs.

Pre-exposure of vaginal explants to Lactobacilli and other microbiota did not affect HIV-1 susceptibility but, notably, Prevotella strongly increased HIV-1 uptake by vaginal LCs. Confocal and flow cytometer techniques confirmed the presence of large aggregates of HIV-1 virions within langerin-positive vesicles in Prevotella-stimulated vaginal LCs, even days after exposure. Remarkably, this colossal HIV-1 uptake by vaginal LCs did not result in infection of LCs, neither was HIV-1 transmitted to CD4+ T cells in co-culture experiments. Thus, our data highlight the unique protective function of vaginal LCs in HIV-1 infection across mucosal barriers, as this function remains intact, even after exposure to Prevotella. However, vesicular HIV-1 in Prevotella-stimulated LCs remained infective, posing a potential risk as ticking time bomb.

These data provide the first functional evidence in human vaginal tissues for the increased risk of HIV-1 acquisition during vaginal dysbiosis and show a remarkable differential role for the interaction between Prevotella and vaginal LCs. Therapies that exploit the anti-viral function of vaginal LCs could serve the design of preventative strategies to combat HIV-1 infection in women.

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Langerhans cell histiocytosis: human and mouse models

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Langerhans Cell Histiocytosis: A human model of DC differentiation and function

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O27) The haematopoietic origin of human Langerhans cell histiocytosis

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Langerhans cell Histiocytosis (LCH) is caused by activating somatic mutations in the ERK pathway, ~60% of patient lesions harbour the BRAFV600E mutation. Although LCH cells are LC-like, the identity of LCH precursors is unclear. Therefore, the purpose of this study was to understand the cellular origin(s) of LCH.

Forty patients with LCH were recruited to the study. BRAFV600E was detected in flow cytometry sorted BM haematopoietic stem cells and myeloid progenitors and peripheral blood CD14+ monocytes and CD1c+ DCs by allele-specific PCR. In vitro, CD1c+ DCs produced CD1a+ langerin-high LC-like cells with Birbeck granules and multilamellar structures (pathognomonic of neonatal self-resolving LCH), with GM-CSF and TGFb/BMP7. In contrast, CD14+ monocytes expressed only moderate langerin and no Birbeck granules unless further stimulated with notch ligation. Illumina microarray gene expression studies during differentiation in vitro confirmed that CD1c+ DCs and monocytes remained distinct without transdifferentiation despite expression of similar surface markers. By unsupervised gene expression analysis, LCH cells and lesional CD1c+ DCs clustered together, distinct from healthy tissue LCs and CD1c+ DCs. In contrast, lesional CD14+ cells clustered with healthy tissue CD14+ monocyte-macrophages. By gene enrichment scores, LCH cells were most enriched for the global CD1c+ DC signature, exceeding that of LCs and CD14+ tissue cells. Neither CD1c+ DCs nor CD14+ cells isolated from LCH lesions had significant levels of somatic mutation compared with LCH cells (0.2-0.5% vs 20-75%).

Together these data confirm a haematopoietic origin of LCH, consistent with a model wherein CD14+ monocytes and/or CD1c+ DCs (cDC2), carrying somatic mutation, differentiate into LCH cells. The results also delineate possible developmental pathways of epidermal LC reconstitution post-inflammation.

O28) Primary T cells from cutaneous T-cell lymphoma skin explants display an exhausted immune checkpoint profile

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Cutaneous T-cell lymphoma (CTCL) develops from clonally expanded CD4+ T cells in a background of chronic inflammation. Dendritic cells (DCs) are essential for T cell activation; yet despite their extensive presence in skin, these cutaneous T cells do not respond with effective anti-tumor immunity. We evaluated primary T-cell and DC émigrés from brief cultures of epidermal and dermal explants of skin biopsies from CTCL patients (n = 37) and healthy donors (n = 5). Compared with healthy skin, CD4+ CTCL populations contained more T cells expressing PD-1, CTLA-4, and LAG-3; and CD8+ CTCL populations comprised more T cells expressing CTLA-4 and LAG-3. CTCL populations also contained more T cells expressing the inducible T-cell costimulator (ICOS), a marker of T cell activation. DC émigrés from healthy or CTCL skin biopsies expressed PD-L1, indicating that maturation during migration resulted in PD-L1 expression irrespective of disease. Most T cells did not express PD-L1. Exhaustion of activated T cells is therefore a hallmark of both CD4+ and CD8+ T cells directly isolated from the lesional skin of patients with CTCL, regardless of disease stage. These results justify identification of antigens driving T-cell exhaustion and development of immunotherapeutic interventions to reverse T-cell exhaustion in CTCL.

POSTER PRESENTATIONS

P1) Human skin explants and CyTOF analysis as reliable tools to characterize dendritic cell receptors as therapeutic targets
Marcela Alcantara

P2) Murine skin DC subsets induce distinct humoral immune responses by regulating Tfh cell responses
Aurelie Bouteau

P3) Human Langerin-driven cre induces genetic recombination in a subset of central nervous system neurons
Aurelie Bouteau

P4) Capturing the tissue-resident CD1a-reactive T cell population from human skin
Rachel Cotton

P5) The impact of octenidine on Langerhans cells in a human skin model
Adelheid Elbe-Bürger

P6) MDA5 - an attractive candidate to target Langerhans cells in human skin?
Adelheid Elbe-Bürger

P7) The role of Langerhans cells in allergen uptake following epicutaneous administration with Viaskin® on human skin
Lucie Mondoulet

P8) Effect of chronic stress in a mouse model of contact hypersensitivity
Anna MacKerracher

P9) Hypoxia inducible factor-1 alpha downregulates Langerhans cell functions in a murine irritant dermatitis model
Saeko Nakajima

P10) Histone H3K4 trimethylation determines human Langerhans cell transcriptional programmes.
Marta E. Polak

P11) Langerhans cells in hypospadias: is the skin immune system compromised in patients with this congenital malformation?
Nikolaus Romani

P12) Laser-assisted skin immunisation to target dendritic cells in human skin
Nikolaus Romani

P13) Regulation of murine skin and gut anti-microbial peptide responses by dendritic cells
Christiane Ruedl

POSTER PRESENTATIONS

P14) Human antigen presenting cells and keratinocytes internalize extracellular vesicles released from the skin commensal yeast Malassezia sympodialis
Annika Scheynius

P15) Towards human Langerhans cell specific immunomodulation by Langerin targeting nanoparticles
Jessica Schulze

P16) Deciphering the myeloid cell compartment in transplantable melanoma mouse models
Patrizia Stoitzner

P17) Keratinocytes derived TGFβ is not required for immune homeostasis in mouse skin
Yukari Zenke [yukazen@luke.ac.jp]

P1) Human skin explants and CyTOF analysis as reliable tools to characterize dendritic cell receptors as therapeutic targets

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Until now, human dendritic cell (DC)-based therapeutic strategies have mainly relied on ex vivo generated DC differentiated from CD14+ blood monocytes in the presence of GMCSF and IL4. Unfortunately, monocyte-derived DC (moDC) have demonstrated limited efficacy in clinical trials. Thus, identifying alternative therapeutic strategies that harness human DC subsets localized in tissues is likely to yield improved treatments. In situ DC subsets can be targeted by anti-receptor monoclonal antibodies (mAb) carrying antigens and adjuvants. This approach has shown robust results in murine models, increasing the potency of vaccines by at least 100-fold. Several targeting receptors have been identified in mice, including DEC205, Langerin, CLEC9A, and more recently XCR1. Similarly, several receptors have been proposed in humans and tested in vitro in blood DC or moDC. However, to bring this strategy to the clinic, it is imperative to understand the expression of these receptors in DC subsets localized at sites of vaccination. We use mass cytometry (CyTOF) and unbiased analysis to examine the expression of uptake receptors by skin DC subsets. We found evidence that surface receptors expression differs significantly between blood and skin DC subsets. We then used human skin explants combined with CyTOF analysis as a model to study anti-receptor mAb capture in situ. Our data offer new insights into strategies to harness distinct skin DC subsets for immunotherapeutics.

P2) Murine skin DC subsets induce distinct humoral immune responses by regulating Tfh cell responses

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Dendritic cells (DC) are classified into multiple subsets based on their origin and function, specifically with respect to T helper cell induction and differentiation. In contrast, the analogous functions of DC subsets on B cells and humoral immune responses are currently unknown. To better understand the role of different DC subsets at inducing humoral immune responses, we developed a system that allows us to limit antigen availability and presentation to certain skin DC subsets. Using this system, our recent findings revealed differential induction of humoral immune responses by Langerhans cells (LCs) and CD103+ DCs. While the antibody response induced by LCs was of higher titers and affinity, involved germinal center (GC) formation and was dependent on CD40L and IL-10 signaling, the one initiated by CD103+ DCs reached lower titers and was generated in a GC-independent manner. Furthermore, we found that both high antigen dose and CD103+ DCs inhibited the GC-dependent humoral immune response induced by LCs. Our preliminary data support a model whereby LCs and CD103+ DCs induce distinct humoral immune responses through their regulation of Tfh cell responses.

Thus, antigen dose and the targeting of certain DC subsets should be considered for the generation of effective vaccines.

P3) Human Langerin-driven cre induces genetic recombination in a subset of central nervous system neurons

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HuLangerin-Cre-YFPf/f mice were generated to specifically mark epidermal Langerhans cells (LCs). During histological characterization of these mice, we found that, in addition to LCs, CD11b+ CD103+ dendritic cells in the gut-associated lymphoid tissues and an uncharacterized cell population in the central nervous system (CNS) also expressed YFP. The cells in the CNS were significantly dimmer for YFP than the other cell types. In this study, we aimed to characterize the YFP+ cells in the CNS. Since a subset of LCs share common precursors with microglia, we first analyzed the expression of standard microglial markers by YFP+ cells using histology and flow cytometry. We found that the YFP+ cells were positive for Iba-1 but negative for all other microglial markers, including CD11b, CD45.2, F4/80 and CX3CR1. Further characterization found that the YFP+ cells were negative for the astrocyte marker GFAP, but they expressed mature neuronal marker NeuN and showed neuronal morphology, identifying them as neurons. The YFP-expressing cells were negative for somatostatin and parvalbumin, specific markers of the two major subsets of CNS interneurons. The YFP+ cells were detectable in the CNS of the newborn mice, and their abundance remained relatively constant throughout adulthood. However, the attempt to delete the YFP+ cells by breeding these mice to floxed “stop” DTR or DTA mice remained unsuccessful.

Thus, these mice might be used to study the ontogeny, migration and cellular interactions of a specific subset of CNS interneurons.

P4) Capturing the tissue-resident CD1a-reactive T cell population from human skin

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CD1a is an antigen-presenting molecule that binds lipids in its hydrophobic pocket for display to T cells. Recently discovered, CD1a-reactive T cells remarkably bind CD1a without directly contacting the cargo in the CD1a pocket. Altered-self lipids, environmental lipids, or small molecules in CD1a fine-tune the activation of these T cells by interfering with or permitting CD1a-TCR contacts. The CD1a system is uniquely concentrated in human skin: CD1a has constitutively high surface expression on epidermal Langerhans cells; epidermal lipids are permissive for CD1a-TCR binding; and CD1a-reactive T cells in circulation have a skin-homing Th22-like signature. Studies from our group and others implicate CD1a-reactive T cells in allergic and inflammatory skin diseases. However, capturing CD1a-reactive T cells from their tissue microenvironment remains a major gap. There are no known surface markers or TCR gene patterns to discriminate CD1a-reactive cells from other skin-resident T cells. To obtain skin T cells from healthy individuals, we cultured surgical discard skin on 3D matrices with IL-2 and IL-15, recovering millions of T cells migrating from the skin per donor. To license CD1a-dependent responses, we co-cultured skin T cells with in vitro derived CD1a++ Langerhans-like cells. We measured the frequency of CD1a-depdendent cells by IL-22 production in activation assays with CD1a+ K562 cells +/- CD1a-blocking antibody. By IL-22 ELISPOT, CD1a-dependent responses across donors (n=13) were variable, with 7/13 exhibiting CD1a-dose-dependent and blockable numbers of CD1a-dependent IL-22-producing cells. A second group (3/13) had an elevated frequency in IL-22-producing T cells at baseline that was CD1a-dose dependent but only partially reduced by CD1a-blocking, suggesting that CD1a-reactive T cells make up a variable fraction of IL-22+ skin T cells by donor. Using a newly developed IL-22 capture reagent, we are positively selecting CD1a-dependent IL-22-secreting cells for further phenotyping and high throughput sequencing of TCRα/δ and TCRβ genes. By adapting a described skin 3D culture method, we are capturing CD1a-reactive T cells from their tissue microenvironment.

P5) The impact of octenidine on Langerhans cells in a human skin model

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Prevention of infections by using antiseptics is a key element in professional wound management. Ideal agents for the topical treatment of skin wounds should have antimicrobial efficacy without negative influence on wound-healing. Octenidine (OCT) has become a widely used antiseptic in modern professional wound care, but little is known about its effects on skin physiology. Therefore, the impact of standard OCT concentrations was tested on Langerhans cells (LCs) and non-immune skin cells upon topical application on ex vivo untreated and tape-stripped (simulation of wound situation) human skin explants at defined culture periods. Hematoxylin and eosin as well as immunofluorescence staining revealed that OCT altered neither human skin architecture nor the viability of skin cells upon 72 hours of culture compared to controls. Immunohistochemistry of epidermal sheets showed that not only the epidermis of explants but also CD207+ LCs in OCT-treated skin remained morphologically intact and comparable to controls all throughout the culture duration. Moreover, OCT inhibited the upregulation of the maturation marker CD83 on LCs and prevented their emigration in tape-stripped skin. Furthermore, OCT had strong anti-inflammatory capacity as demonstrated by the inhibition of IL-8 and IL-33 upregulation in skin explant cultures. VEGF, a potent angiogenic and essential growth factor for vascular endothelial cells, was not changed by OCT. In conclusion, our data provide novel insights into the host response to OCT in the biologically relevant environment of viable human (wounded) skin, suggesting, in addition to its known antimicrobial activity, also an anti-inflammatory action that might contribute to its observed positive wound healing influence resulting in better scar quality.

P6) MDA5 - an attractive candidate to target Langerhans cells in human skin?

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Together with keratinocytes (KCs) and the dense network of Langerhans cells (LCs), the epidermis is an ideal portal for vaccine delivery. Pattern recognition receptor agonists, in particular polyinosinic-polycytidylic acid [p(I:C)], a synthetic analog of virus dsRNA, are promising adjuvant candidates for therapeutic vaccination to generate protective T cell immunity. As not much is known about initial events of PRR agonists on skin cells in their natural environment, we investigated the expression of dsRNA receptors and the activation of downstream signaling pathways in LCs and KCs upon incubation with p(I:C) in a skin explant model. Both cell types were able to take up rhodamine-labeled p(I:C). In contrast to KCs, which expressed all known dsRNA sensing receptors at a constitutive and inducible level, LCs exclusively expressed MDA5 in untreated skin and freshly isolated cells. Stimulation with p(I:C) downregulated MDA5 expression in LCs in barrier-disrupted skin samples and upregulated its expression in single cells. Comparative assessments of downstream signaling pathways induced by p(I:C) revealed activation of MAVS, and differential nuclear translocation of IRF3 and NF-kappaB (p65) in LCs and KCs. Consequently, p(I:C)-treatment of LCs induced IFN-alpha and IFN-beta but not TNF-alpha mRNA expression, while in KCs p(I:C) significantly up-regulated IFN-beta and TNF-alpha but not IFN-alpha mRNA. In addition, release of IL-6, IL-8 and TNF-alpha protein was induced by p(I:C) in KCs but not in LCs, showing that different dsRNA-sensing receptors and/or downstream signaling pathways are activated in both cell types. Our data suggest that MDA5 may be an attractive adjuvant target for epicutaneous delivery of therapeutic vaccines with the goal to target LCs.

P7) The role of Langerhans cells in allergen uptake following epicutaneous administration with Viaskin® on human skin

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Allergen applied to the skin during epicutaneous immunotherapy (EPIT) with Viaskin® patches induces tolerance in sensitized mice. In previous work, we showed that allergen delivered by Viaskin is mainly taken-up by Langerhans cells (LCs) and transported to regional lymph nodes. The aim of this study was to determine whether similar allergen uptake occurred in an ex vivo human skin model.

NativeSkin® models (Genoskin, France) consist of ex vivo human skin collected after plastic surgery, which maintain viability for 7 days. Viaskin® patches loaded with fluorescein-tagged peanut protein extract (Viaskin-PPE-AF647) or Viaskin placebo were applied to freshly harvested skin inserts from 2 donors for 12 and 24 hrs. Colocalization of PPE-AF647 and LCs (stained with anti-CD207 and anti-CD1a antibodies) was determined on skin cross sections by classical fluorescent microscopy and in situ on epidermal sheet layers by confocal microscopy.

The dried PPE loaded on the backing of the Viaskin patch solubilized within 12 hrs due to transepidermal water loss. PPE-AF647 was present in the epidermis as well as colocalized with LCs. This was confirmed on detached epidermal sheets. After 24 hrs, a significant increase of PPE-AF647 was colocalized with LCs for both donors. For donor #1, 8% of the LCs were colocalized with PPE-AF647, while for Donor #2, 22% of cells were positive. Observations of LCs in contact with PPE-AF647 suggest that there are 2 phases in the interaction: at 12 hrs, the allergen appears to be associated with the surface membrane and at 24 hrs appears to be internalized in LCs. Other analyses are under investigations (ie. mRNA expression of cytokines in the tissue).

In conclusion, NativeSkin® model allows us to confirm the role of LCs in the antigen uptake and processing after Viaskin application on human skin.

P8) Effect of chronic stress in a mouse model of contact hypersensitivity

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Chronic stress is known to have a suppressive effect on the immune system via the secretion of glucocorticoids mediated by the hypothalamus-pituitary-adrenal (HPA) axis. Interestingly, chronic stress is known to increase the susceptibility and disease progression of inflammatory skin diseases in humans. We studied inflammatory skin reactions in a well-established mouse model of contact dermatitis termed contact hypersensitivity (CHS) using the established contact allergens DNTB (2,4-dinitrothiocyanobenzene) and DNFB (2,4-dinitrofluorobenzene). DNTB is a weak contact allergen that does not trigger allergic reactions in non-allergic individuals, but instead has been shown to induce tolerance to the strong contact allergen DNFB. Tolerance induction in this model is facilitated by Langerhans cells (LC), which migrate from the epidermis to draining lymph nodes (dLNs), where they activate regulatory T cells and lead to the inactivation of specific cytotoxic CD8+ T lymphocytes. We have shown that tolerance induction in response to the weak contact sensitizer is strongly reduced in stressed mice. Furthermore we were able to prove that the reduced tolerance induction is accompanied by significantly impaired migration of LCs from the epidermis to dLNs following application of DNTB. We could further show that impaired migration of LCs in stressed mice is due to a reduced activation of LCs in the sensitized skin. Collectively our results suggest that chronic stress alters the reactivity towards contact allergens leading to a higher susceptibility to establish CHS reactions even towards weak contact allergens.

P9) Hypoxia inducible factor-1 alpha downregulates Langerhans cell functions in a murine irritant dermatitis model

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Hypoxia inducible factor-1 alpha (HIF1-aα) regulates oxygen homeostasis through the control of erythropoietin and the expressions of multiple genes involved in a variety of biological processes, including cell proliferation, adhesion, and migration. Cells rapidly respond to hypoxic and inflammatory environments by upregulating gene expression under control of the transcription factor HIF-1aα. Dendritic cells (DCs) in the skin are important sentinels of the cutaneous immune system. In vitro studies have indicated that HIF-1aα plays an important role for differentiation and maturation of DCs. However, in vivo functions of HIF-1aα of cutaneous DCs in response to the inflammatory stimuli remain unclear.

To this end, we newly generated Langerin positive cell specific HIF-1aα knockout (KO) mice and evaluated in vivo functions of HIF-1aα. Langerin-specific HIF-1aα KO mice showed exacerbation of ear swelling in response to croton oil-induced irritant contact dermatitis (ICD) compare to wild type (WT) mice. In addition, the expression levels of costimulatory molecules, such as CD86, on Langerhans cells (LCs) were significantly up-regulated in KO mice, whereas those on other skin DC subsets in KO mice did not show significant differences compare to those in WT mice. These results suggest that HIF-1aα regulates the inflammation of murine ICD by modulating the maturation status of LCs. Since it is known that HIF-1aα is induced by hypoxia, we cultured epidermal cell suspensions of the mouse ear under hypoxic or normoxic conditions. In line with in vivo findings, we found that LC maturation induced by lipopolysaccharide stimulation was impaired under the hypoxic condition when compared to the normoxic condition. Taken together, HIF-1aα downregulates LC functions under certain physiological conditions, such as irritation stimuli and hypoxia.

P10) Histone H3K4 trimethylation determines human Langerhans cell transcriptional programmes.

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Langerhans cells (LCs) are professional antigen presenting cells. However, their ability to produce pro-inflammatory mediators, such as cytokines, chemokines and matrix proteinases, is profoundly limited. We sought to elucidate the molecular networks underpinning this critical difference between LCs and other dendritic cell populations.

Whole transcriptome analysis confirmed the key role of interferon regulatory factors (IRFs) as controllers of the human LC response to epidermal cytokines. In silico simulations of our Petri net based IRF gene regulatory network (IRF-GRN) parametrised with human LC transcription factor expression values, correctly predicted the expression pattern of the 31 out of 47 network output genes (64%), including genes associated with antigen presentation (HLA A,B,C, CIITA, HLA-DR), immunoproteasome (PSME1, PSME2, PSMB10), and endocytosis (Caveolin). Dependence of human LC function on the IRF-GRN behaviour was further confirmed in functional in vitro assays, demonstrating reduction of LC ability to induce CD8 T cell by epidermal cytokine signalling ($p<0.01$). To determine chromatin methylation state we performed ChIP-seq analysis of histone H3K4 trimethylation (H3K4Me3, n = 3, ± TNFα) in human LCs. 291 out of 812 immune genes (Immunome collection) were poised for transcription in unstimulated LCs (n=3, Bowtie2 alignment to hg19, MACS, FDR p<10xE-5). While genes involved in receptor binding, and cytokine receptor activity leading to IRF activation are poised for transcription (FDR p<2x10E-29), trimethylation pattern for chemokine and pro-inflammatory cytokine genes in LC was low or absent in both unstimulated, and TNFα exposed LCs. In contrast, H3K4Me3 was strongly enriched in genes involved in antigen processing, presentation and T lymphocyte stimulation (FDR p < 1x10E-29).

Our analyses implicate, that while IRF-GRN is conserved in human LCs, specific H3Me4Me3 may determine responsiveness of LCs to stimulation. This pattern may reflect a different origin of LCs, or may be acquired during adaptation to the specific requirements of the epidermis.

P11) Langerhans cells in hypospadias: is the skin immune system compromised in patients with this congenital malformation?

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INTRODUCTION. Hypospadias are not uncommon, affecting 1 in 300 men. Langerhans cells, the dendritic cells of the epidermis, play a major role in immune responses (HPV, HIV). The epidermis shares an ectodermal origin with the urethra. Therefore, skin – often considered “dysplastic” – has also shown to be involved in this congenital malformation of the penis. The skin immune system, however, has never been investigated in these patients.

PATIENTS AND METHODS. Epidermal sheets were prepared from skin samples of 18 patients with hypospadias (10 distal, 8 proximal) and 10 patients with normal healthy foreskin (circumcision) by means of ammoniumthiocyanate. They were stained with anti-HLA-DR FITC and anti-CD207/Langerin A594 antibodies. By conventional immunofluorescence microscopy the immunophenotype of epidermal Langerhans cells, their frequency and their morphology was assessed in 12 high power fields of at least one representative sheet per patient.

RESULTS. The mean frequency of epidermal HLA-DR/CD207 positive cells accounted to 860,5 ± 66,74 square millimeters in patients with hypospadias as compared to 888,3 ± 92,59 in patients with normal penile development (p=0.8089, t-test). There was no significant difference in Langerhans cell frequency between distal and proximal hypospadias (856,5 ± 82,26, n=13, distal vs. 869,1 ± 124,8, n=6 proximal, p=0.9331). The morphology of the cells appeared normal in both groups.

CONCLUSION. Despite meticulous investigation we were not able to demonstrate gross differences in morphology or frequency of epidermal Langerhans cells in the foreskin of hypospadias patients compared to patients with normally developed foreskin. This suggests that patients with hypospadias are not different from patients with normal penile development considering their specific skin immunity, at least considering the epidermal compartment. Dendritic cell subset composition of the dermis remains to be studied.

P12) Laser-assisted skin immunisation to target dendritic cells in human skin

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Skin dendritic cells (DC) are antigen presenting immune cells which induce immune responses against cutaneous infection and tumours. Due to their localisation in the skin, they are also able to recognise cancer cells developing in the skin and to start an immune response against tumours. The immunotherapeutic approach called \"epicutaneous immunisation\" aims at loading DC subtypes with tumour antigens in situ. To improve this vaccination modality we loaded skin DC with fusion antibodies directed against DC surface molecules, such as the lectin receptors DEC-205 (CD205) and Langerin (CD207) that are essential for antigen incorporation. We hypothesised that laser poration of the skin may substantially improve epicutaneous immunisation. An infrared laser (P.L.E.A.S.E.® Laser System, Pantec Biosolutions, Liechtenstein) creates defined and adjustable micropores in the skin by excitation of water molecules. These micropores should allow macromolecules to diffuse into the skin, and therefore enable and facilitate the transcutaneous application of molecules with high molecular weight, like (fusion) antibodies. Human skin samples were laser-treated ex vivo to determine the optimal parameters for delivery of antibodies into epidermis and dermis. DC targeting by antibodies against Langerin and DEC-205 was evaluated. We were able to induce pores of definable depths. Laser-induced thermal cell damage seemed negligible since no increased apoptotic signals were found in the surroundings of the pores. Both Langerhans cells and dermal DC could be targeted via laser-\"holed\" skin by anti-DEC-205 and anti-Langerin antibodies, respectively. However, the DC targeting efficiency after conventional intradermal injection was higher as compared to laser treatment. Ongoing work is attempting to figure out conditions for better targeting and, eventually, investigate the benefit of co-applied adjuvants and the immune-stimulatory capacity of antigen-targeted DC in laser treated skin.

P13) Regulation of murine skin and gut anti-microbial peptide responses by dendritic cells

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The gastrointestinal tract and the skin are tissues permanently exposed to a wide range of microorganisms, including resident microbial flora and potential pathogenic microbes and have, therefore, developed a multitude of effective strategies, such as anti-microbial peptides, to contain translocation and invasion of bacteria and initiate wound healing.

In Diphtheria Toxin treated Clec4a4-DTR mice which lack cDC2, we have observed a strong epithelial anti-microbial peptide response (e.g. Reg3β, Reg3γ, S100A8 and S100A9) via innate SCART-2+Vγ4+ IL-17 secreting γδ+ T cells (γδT17 cells). The phenotype was exclusively restricted to the colon and absent in the small intestine. Since the same γδT17 cells are present in the dermis we profiled epidermal keratinocyte mRNA upon intradermal LPS challenge and compared WT to Clec4a4-DTR mice. Remarkably, high expression levels of keratinocyte antimicrobial proteins, such as S100A8 and S100A9, were detected in Clec4a4-DTR mice when compared to control WT mice in response to LPS. Possible mechanisms will be presented and discussed.

P14) Human antigen presenting cells and keratinocytes internalize extracellular vesicles released from the skin commensal yeast Malassezia sympodialis

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Malassezia is the dominant commensal fungi in the human skin mycobiome but is also associated with common skin disorders including atopic eczema (AE). More than 50 % of AE-patients have specific IgE and T-cell reactivity towards Malassezia sympodialis, which is one of the most frequently isolated species from both AE patients and healthy individuals. We have found that Malassezia releases nanosized exosome-like vesicles, designated MalaEx, which carry allergens and can induce allergic inflammatory cytokine responses in PBMC from AE patients. Recently, we detected small RNAs in MalaEx and interestingly, bioinformatics analyses indicated that MalaEx have an RNAi-independent route for biogenesis. By comparing protein content of MalaEx with the whole yeast cells using iTRAQ based quantitative proteomics we have identified 2439 proteins in whole yeast cells and of those 110 proteins to be enriched in MalaEx, among those two of the major M. sympodialis allergens, Mala s 1 and s 7. Gene ontology analysis identified proteins involved in translation, metabolic processes and oxidoreductase complex and activity among the MalaEx enriched proteins. In functional experiments we co-cultured Vybrant DiI labeled MalaEx to primary human keratinocytes or monocytes to investigate possible interactions. Confocal laser scanning microscopy demonstrated an uptake of MalaEx by monocytes and binding to keratinocytes after 2 h. After 16 h both monocytes and keratinocytes had internalized MalaEx within the cytosol. At 4ºC no internalization was observed suggesting that an active mechanism is involved in the uptake seen at 37ºC. Thus, our results support an involvement of MalaEx in host-microbe interactions, by uptake into host cells, and by the spreading of allergens, thereby contributing to the allergic inflammation. By understanding the role of MalaEx in the sensitization and maintenance phases of AE, novel prevention strategies and potential therapeutic targets may arise.

P15) Towards human Langerhans cell specific immunomodulation by Langerin targeting nanoparticles

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Transcutaneous immunomodulation using nanoparticle-based antigen delivery systems is an area of extensive research. Previous studies have shown the potential to manipulate the body's own defense mechanisms. Particularly, epidermal Langerhans cells have gained attraction when antibody-based delivery systems have proven that these cells are effective cytotoxic T cell activators. To overcome the drawbacks of antibody-based approaches such as antigenicity, production and scalability, small molecule-mediated targeting would remarkably improve the delivery process. To this end, we decorated liposomes with small molecules targeting the human Langerin receptor to selectively address Langerhans cells. We show specific binding to human Langerin using recombinant expressed C-type lectin receptors and in several Langerin expressing cell lines. Binding and internalization kinetics were studied and toxicity assays performed. Moreover, encapsulated antigens in liposomes were delivered into human Langerin expressing cells and liposomal internalization and routing was tracked with endosomal markers. Additionally, we were able to show specific uptake of the targeted liposomes into human Langerhans cells of epidermal cell suspensions. This new approach will help reveal the function of Langerhans cells in the context of immunity and tolerance as well as paving the way towards new immunotherapeutic applications.

P16) Deciphering the myeloid cell compartment in transplantable melanoma mouse models

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Transplantable mouse models are a useful tool to test therapies against melanoma. In order to reliably investigate immunotherapies, the immune infiltrate into the tumors needs to be analyzed and characterized properly. Defining myeloid cells has been challenging since the expression of markers highly depends on the activation status of the cells and the tumor microenvironment. So far, no unique surface markers are described to be expressed only on one myeloid cell type. However, the transcription factor Zbtb46 was reported to be expressed by conventional dendritic cells but not by macrophages.

We used multicolor flow cytometry to analyze the expression of various myeloid markers in two subcutaneous transplantable melanoma mouse models: B16.OVA and D4M (BRAFV600E; PTEN-/-). We found that the myeloid cell infiltrate was very diverse. Besides monocytes and myeloid-derived suppressor cells, we found dendritic cells and a CD64+ cell population. To confirm the gating strategy to differentiate between dendritic cells and macrophages we used Zbtb46. Interestingly, Zbtb46 seemed to be expressed by the CD64+ population which was positive for the macrophage-markers MerTK and F4/80. Reliably defining the myeloid cell types requires a further extensive functional and transcriptional analysis.

P17) Keratinocytes derived TGFβ is not required for immune homeostasis in mouse skin

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TGFβ is essentiaKl the epidermal retention resident memory T cells and Langerhans cells, but the source of TGFβ that is important for these functions is unknown. Since the majority of cells in epidermis are keratinocytes (KC), we hypothesized that TGFβ from KC might be important for skin homeostasis and the maintenance of skin immune cells. To examine this, we generated K14CreERT2TGFβfl YFP mice which enables tamoxifen inducible specifically ablation of TGFβ in KC. Systemic application of tamoxifen was lethal likely due to esophageal inflammation. Topical application of 4-hydroxytamoxifen (4-OHT) once a day for 2 days was well tolerated. We confirmed the deletion of TGFβ in bulge, isthumus and interfollicular keratinocytes based on YFP expression and TGFβ mRNA 28 days post 4-OHT treatment. Mice with KC specific deletion of TGFβ developed acanthosis without observable skin inflammation, implicating a TGFβ autocrine/paracrine suppression of KC proliferation. We also found that ablation of TGFβ in KC has no effect on the numbers of epidermal-resident Langerhans cell, αβ T cells and Innate lymphoid. A modest decrease in DETC was observed. In dermis, we did not find any alteration in numbers of DC subsets, αβ T cells or dermal γδ T cells. Thus, under homeostatic conditions deletion of TGFβ in KC enhances epidermal acanthosis but has limited effect on cutaneous residence of most immune cells.

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