12th INTERNATIONAL WORKSHOP ON LANGERHANS CELLS

November 3 – 6, 2011
Innsbruck, Tyrol, Austria

Program & Abstracts

Ladies and gentlemen,
friends and colleagues,
Langerhans researchers all over the world,

Langerhans cell research has „boomed“ in the past few years. It has become an important part of research into dendritic cells in general. This was mainly due to the development of exiting new experimental models. They reinvigorated interest in Langerhans cells with regard to both fundamental and clinical immunology. The relationship of Langerhans cells with the other members of the dendritic cell family is becoming more and more clear. Langerhans cells have also become attractive as targets for immunotherapies of inflammatory and infectious skin diseases and cancers. The past three Langerhans Cell Workshops in Madeira in 2005 and 2009 and in Berne in 2007 have impressively documented this development.

This year we decided to bring the Langerhans Cell Workshop to the “Heart of the Alps”, to the Tyrol in Austria. Again, we wish to make this Workshop an event that allows ample interactions between advanced and young researchers but also between researchers and industry. Important collaborations were sparked off at the previous workshops and useful networks were established. We have done our best to ensure that LC2011 will again be a very communicative and fruitful scientific meeting.

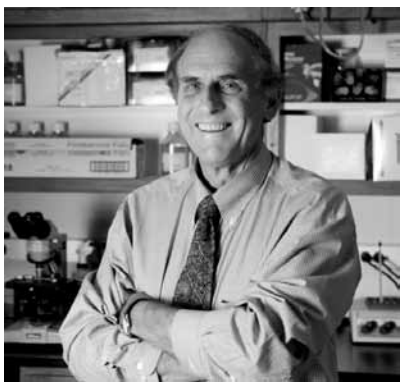
We cannot offer historical ties of Paul Langerhans with Austria, as it was the case in Madeira. Yet, Austria offers much in terms of culture and tourism – and we have been strong in Langerhans cell research starting some 110 years after Paul Langerhans. Langerhans Cell Workshops have always given fresh impetus to the immunological field of dendritic cells. We would very much like to see the field keep up this momentum and gather even more. Therefore, we cordially invite you to actively participate in this 12th International Workshop on Langerhans Cells here in Innsbruck, Austria. We appreciate that industry takes part and interacts with basic research, and we thank all sponsors for their support without which this Workshop would not be possible.

Welcome to Innsbruck!

We dedicate this Workshop to our late mentor and friend and winner of the 2011 Nobel Prize for Medicine,

Ralph M. Steinman

He always promoted and stimulated the Langerhans Cell Workshops.



Niki Romani, Patrizia Stoitzner, Adelheid Elbe-Bürger & Georg Stingl
Innsbruck & Vienna, November 2011

COMMITTEES

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2005 Funchal, Madeira, Portugal
2007 Berne, Switzerland
2009 Funchal, Madeira, Portugal

Participation fees

	early bird period (payment received by August 29, 2011)	payment after August 29, 2011
Registration fee	390,- Euro	490,- Euro
Residents, Post-Docs	290,- Euro	390,- Euro
Students	190,- Euro	290,- Euro

Congress venue

Ursulinensäle am Marktplatz
Innrain 7
6020 Innsbruck, Austria
Telefon: 0043 (0)512 57 03 89

Registration hours

During the whole conference the registration desk in the foyer will be open 30 minutes before the daily program starts and will close 15 minutes after the end of the daily program. Please contact us for any question you may have on the meeting.

Congress Organization

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	Thursday, November 3, 2011	Friday, November 4, 2011	Saturday, November 5, 2011	Sunday, November 6, 2011
08:30		Registration	Registration	Registration
09:00		**Session I**	**Session III**	**Session V**
10:30		Coffee break	Coffee break	Coffee break
11:00		**Session I, continued**	**Session III, continued**	**Session V, continued**
12:30		Lunch & **Poster viewing**	Lunch & **Poster viewing**	**Invitations Concluding Remarks**
14:00			**Session IV**	
14:30		**Session II**		
15:30			Coffee break	
16:00		Coffee break		
16:30	**Registration**	**Session II, continued**	**Session IV, continued**	
19:00	**Introductory Plenary**	Poster Party	Social Evening	
20:30	Welcome Reception			

Chair: Niki Romani

19.00 *Niki Romani* – Welcome and Opening Remarks

19.15 *Ethan Shevach, NIAID, NIH, Bethesda, MD*
A Dendritic Cell Centric View of T Regulatory Function

19.45 *Dmitry Gabrilovich, Moffit Cancer Center, Tampa, FL*
Mechanism of dendritic cell dysfunction in cancer

20.30 Welcome Reception

SESSION I - ONTOGENY & ORGANISATION OF THE DC / LC SYSTEM

Chairs: Adelheid Elbe-Bürger & Björn Clausen

09.00 *Shalin Naik, Netherlands Cancer Institute, Amsterdam, The Netherlands*
Dendritic cell progenitor output at the single cell level, *in vivo*

Fréderic Geissmann, King's College, London, UK
Two separate myeloid systems: origin of Langerhans cells and tissue macrophages

Florent Ginhoux, Singapore Immunology Network (SIgN), Singapore
The embryonic origin of Langerhans cells: a tale of two sites

10.30 Coffee break

Chairs: Christine Heufler & Matthew Collin

11.00 *Herbert Strobl, Medical University of Vienna, Vienna, Austria*
Transcription factor control of Langerhans cell differentiation

11.20 Selected talks

Bianca Blom
CD34+ hematopoetic progenitor cells reside in the dermal layers of the human fetal skin and are able to differentiate into mature Langerhans cells

Kristin Seré
Development of two distinct types of Langerhans cells in steady state and inflammation

Thomas Bauer
The anti-inflammatory phagocytosis receptor Axl is constitutively upregulated by LC in response to epidermal TGF-beta

Florian Sparber
Ablation of the adaptor molecule p14 under the control of the CD11c promotor impairs the homeostasis of the murine Langerhans cell network

Naomi McGovern
Human langerin+ DC are independent of epithelial Langerhans cells; a novel perspective on Langerhans Cell Histiocytosis

Elodie Segura
Characterisation of resident and migratory dendritic cells in human lymph nodes

Nathalie Eissing
Characterization of dendritic cells in human lymphoid organs

Muzlifah Haniffa
CD141+ human non-lymphoid tissue dendritic cells cross-present exogenous antigens and are homologous to murine CD103+ dendritic cells

12.50 Lunch & Poster viewing

SESSION II - MIGRATION & MATURATION

Chairs: Mark Udey & Herbert Strobl

14:30 *Michael Sixt, Institute of Science and Technology (IST), Vienna, Austria*
Chemotactic guidance of dendritic cells in the skin

Reinhold Förster, Medizinische Hochschule, Hannover, Germany
Homing of lymph derived cells to lymph node

Eynav Klechevsky, Washington University, St.Louis, Missouri, USA
Human Langerhans Cells: mechanism and implications in vaccine science

16.00 Coffee break

Chairs: Florent Ginhoux & Martin Zenke

16.30 Selected talks

Thomas Hieronymus
The scatter factor receptor/Met trosinase kinase is essential for dendritic cell migration in skin immunity

Akiharu Kubo
Langerin-positive Langerhans cells but not Langerin-negative inflammatory dendritic epidermal cells penetrate epidermal tight junctions barriers in atopic dermatitis

Egawa Gyohei
Intercellular space of keratinocytes is a migratory pathway for Langerhans cells

Maria Becker
LC-selective deletion of EpCAM (CD326) attenuates Langerhans cell motility and migration *in vivo* and enhances contact hypersensitivity reactions

Sandrine Dubrac
The pregnane X receptor (PXR) controls Langerhans cell migration via CCR7

Aleh Bobr
Autocrine TGF-beta acts as regulator for maturation and migration for Langerhans cells

Lim Clarice Xiu Fang
Expression of TROP2 reveals differences between human epidermal Langerhans cells and other CD207+ dendritic cell subsets

Clare Bennett
Langerhans cells regulate cutaneous injury by licensing CD8 effector cells recruited to the skin

17.50 *Keisuke Nagao, Keio University, Tokyo, Japan*
Antigen capture through tight junctions by Langerhans cells induce pre-emptive immunity in experimental staphylococcal scalded skin syndrome

following Poster Party (with food, drinks & poster discussion)

SESSION III - LANGERHANS CELLS IN INFECTIONS

Chairs: Sandrine Dubrac & Thomas Schwarz

09.00 *Dan Kaplan, University of Minnesota, Minneapolis, MN*
Control of adaptive responses by skin-resident DC subsets

Teunis Geijtenbeek, Academic Medical Center, University of Amsterdam, The Netherlands
C-type lectin Langerin in immunity & infections

Marcel Teunissen, Academic Medical Center, University of Amsterdam, The Netherlands
Langerhans cells support tolerance to the bacterial skin flora

10.30 Coffee break

Chairs: Clare Bennett & Teunis Geijtenbeek

11.00 Selected talks

Angelika Stöcklinger
Depending on the antigen, Langerin+ dendritic cells enhance or suppress gene gun-induced immune responses

Esther De Jong
Virus selectively primes human Langerhans cells for CD70 expression promoting CD8+T cell responses

Yonatan Ganor
The neuropeptide calcitonin gene-related peptide (CGRP) inhibits HIV-1 transfer from Langerhans cells to T cells

Botond Igyarto
Skin-resident murine dendritic cell subsets promote distinct and opposing antigen-specific T helper cell responses

Alexis Boyd
Relatively quiescent response of human Langerhans cells to filarial parasites suggest a method of immune evasion used by filariae and other skin-transiting helminths

Linda van den Berg
Molecular mechanisms of antigen-transfer between human Langerhans cells and dendritic cells

Marija Zaric
Direct transcutaneous targeting of antigen to the specific skin dendritic cell subsets for improved vaccine efficacy

Esther von Stebut
Langerhans cells suppress the anti-Leishmania response via induction of regulatory T cells

12.20 Lunch and Poster viewing

SESSION IV - LANGERHANS CELLS IN TOLERANCE AND IMMUNITY

Chairs: Paul Bergstresser & Daniel Kaplan

14.00 *Sandrine Henri, CIML, Université de la Méditerranée, Marseille, France*
Dermal dendritic cells: from phenotype to function, XCR1 as a universal marker of cross-presenting dendritic cells

Juliana Idoyaga, Rockefeller University, New York
Targeting steady state dendritic cells using anti-Langerin mAbs coupled with a self-antigen promote de novo generation of Foxp3+ regulatory T cells

Björn Clausen, Erasmus University, Rotterdam, The Netherlands
Skin dendritic cell subsets: Functional specialization and molecular control *in vivo*

15.30 Coffee break

Chairs: Esther de Jong & Georg Stingl

16.10 Selected talks

Astrid van Beelen
Targeting antigen to DC-SIGN using glycan-modified liposomes elicits activation of both CD4+ and CD8+ T cells

Vincent Flacher
Induction of tolerance or immunity after langerin targeting reveals intrinsic properties of langerin+ dendritic cells

Diana Dudziak
Cross presentation of CD11c+CD8- dendritic cells under inflammatory conditions

Cynthia Fehres
Targeting of epidermal Langerhans cells to establish anti-tumor immunity

Mercedes Gomez de Aguero
Langerhans cells protect from allergic contact dermatitis in mice by tolerizing CD8+T cells and activating FoxP3+ regulatory T cells

Celine Leroy
The role of Langerhans cells in T cell priming

Christian Lehmann
Antigen targeting of Fc-receptors induces strong Tcell responses *in vivo*

Karin Loser
Increased anti-tumoral immunity and reduced skin- carcinogenesis in K14-RANKL transgenic mice

Max Schnurr
ISCOM vaccines activate the NLRP3 inflammasome and target antigen to dendritic cells *in vivo* for effective cross-presentation

17.40 *Patrizia Stoitzner, Innsbruck Medical University, Innsbruck, Austria*
Langerhans cells in tumor immunity

19.00 Social Evening

SESSION V - LANGERHANS CELLS IN DISEASE AND THERAPY

sponsored by 

Chairs: Marcel Teunissen & Martin Thurnher

09.00 *Frank Nestle, St. John's Institute of Dermatology, King's College, London, UK*
Harnessing dendritic cells in inflammatory skin diseases

Matthew Collin, Newcastle University, Newcastle upon Tyne, UK
Human dendritic cell deficiency

SangKon Oh, Baylor Institute for Immunology Research, Dallas, TX, USA
Harnessing human vaginal dendritic cell subsets for developing vaccines

10.20 Selected talks

Elisabeth Glitzner
The role of dendritic cells in psoriasis

Patrick Brunner
CD11c+DC and CD163+macrophages are the main source of TNF-alpha in plaque-type psoriasis

10.40 Coffee break

Chairs: Sandrine Henri & Keisuke Nagao

11.10 *Cheong Cheolho*
CD103+ Langerin+Aortic dendritic cells protect from Atherosclerosis

Maud Plantinga
Langerin+ dendritic cells in the lung and their role in a HDM-induced allergic-airway inflammation

Verena Martinz
Effects of epidermal barrier disruption on vitamin D3- induced atopic dermatitis-like inflammation in mice

Kenji Kabashima
Langerhans cells play a key role in the development of atopic dermatitis via TSLP receptor signaling

Ernst Kriehuber
NOTCH signalling is constitutively active in Langerhans cell histiocytosis (LCH) and confers key LCH features on dendritic cells

12.00 *James W. Young, Memorial Sloan Kettering Cancer Center, New York*
Human Langerhans-cells: An opportunity to break tolerance against self-differentiation tumor antigens

12.30 *Yong-Soo Bae:* Invitation to DC2012 in Daegu, Korea

12.40 *Marcel Teunissen:* Invitation to LC2013 in Amsterdam

12.45 Concluding Remarks

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SCIENTIFIC PROGRAM

Ethan Shevach, NIAID, NIH, Bethesda, MD

A Dendritic Cell Centric View of T Regulatory Function

19.00 – 20.15 ***INVITED SPEAKER***

Dmitry Gabrilovich, Moffit Cancer Center, Tampa, FL

Mechanism of dendritic cell dysfunction in cancer

Shalin Naik, Netherlands Cancer Institute, Amsterdam, The Netherlands

Dendritic cell progenitor output at the single cell level, *in vivo*

09.00 – 10.30 ***INVITED SPEAKER***

Fréderic Geissmann, King's College, London, UK

Two separate myeloid systems: origin of Langerhans cells and tissue macrophages

Florent Ginhoux, Singapore Immunology Network (SIgN), Singapore

The embryonic origin of Langerhans cells: a tale of two sites

09.00 – 11.20 ***INVITED SPEAKER***

Herbert Strobl, Medical University of Vienna, Vienna, Austria

Transcription factor control of Langerhans cell differentiation

CD34+ hematopoietic progenitor cells reside in the dermal layer of the human fetal skin and are able to differentiate into mature Langerhans cells

Mark Vondenhoff[1], Martha Hernandez[2], Arie Voordouw[1], Kees Weijer[1], Bianca Blom[1]
[1] Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [2] Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Langerhans cells (LCs) are a distinct subset of dendritic cells (DCs) that reside in the epidermis of the skin where they form a contiguous network and act as sentinels for immunity. To fully take advantage of the functional properties of LCs, it is of interest to understand how LCs develop from their progenitor cells. It is incompletely resolved however, particularly during human ontogeny, what is the exact nature of LC precursor cells that travel from the hematopoietic organs via the blood to the skin. Based on the assumption that human progenitor cells express CD34 on their cell surface, and CD34+ cells with skin homing properties, which express the cutaneous lymphocyte antigen (CLA), are present in adult peripheral blood, we investigated the presence of such progenitor cells in the human fetus. We observed that CD34+CLA+ hematopoietic progenitor cells resided not only in fetal bone marrow and fetal liver, but in addition also in fetal blood. Moreover, in the fetal skin CD34+ hematopoietic cells were present in the dermis, whereas they were absent in the epidermis. We also analyzed whether fetal skin resident CD34+ cells had LC precursor activity. For this, we isolated different hematopoietic subsets from fetal skin and cultured them *in vitro* in LC supporting conditions, including TGF-beta. The most potent ability to differentiate into mature LCs, as indicated by the expression CD1a and CD207 (Langerin), was contained within a subset of the CD34+ progenitor cells. Hence, we conclude that the dermal layer of the human fetal skin contains CD34+ hematopoietic cells with potential to differentiate into mature LCs, which may be imperative to shape the epidermal LC network.

Development of Two Distinct Types of Langerhans Cells in Steady State and Inflammation

Kristin Seré[1,2], Jea-Hyun Baek[1,2], Julia Ober-Blöbaum[1,2], Gerhard Müller-Newen[3], Frank Tacke[4], Yoshifumi Yokota[5], Martin Zenke[1,2], and Thomas Hieronymus[1,2]

[1] Institute for Biomedical Engineering, Department of Cell Biology, Medical Faculty, RWTH Aachen University, Aachen, Germany

[2] Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany

[3] Institute for Biochemistry and Molecular Biology, Medical Faculty, RWTH Aachen University, Aachen, Germany

[4] Medical Clinic III, Medical Faculty, RWTH Aachen University, Aachen, Germany

[5] Division of Molecular Genetics, Department of Biochemistry and Bioinformative Sciences, Faculty of Medical Sciences, University of Fukui, Fukui, Japan

Langerhans cells (LCs), the contingent of dendritic cells (DCs) in skin epidermis, possess an exceptional life cycle and developmental origin. Here we investigated LC development during inflammation and steady state. We demonstrate the existence of two types of LCs, short-term and long-term LCs. We further identify a distinct role for the transcription regulator Id2 (inhibitor of differentiation/DNA binding 2) in their development. We show that Gr-1high monocytes are precursors for short-term LCs under inflammatory conditions. This process does not require Id2. Conversely, adult bone marrow contains LC precursors, which give rise to long-term LCs. Id2-/- bone marrow cells fail to generate long-term LCs, thus their development is Id2-dependent. Short-term and long-term LCs also differ phenotypically. Moreover, transplantation of Id2+/+ and Id2-/- bone marrow into NOD-scid IL-2Rgnull mice revealed that LC reconstitution after UV light exposure occurs in two waves: an initial fast wave of Gr-1high monocyte-derived short-term LCs is followed by a second wave of steady state precursor-derived long-term LCs. Taken together, our data provide evidence for the existence of two types of LCs, short-term and long-term LCs that develop through different pathways in inflammation and steady state, respectively.

The anti inflammatory phagocytosis receptor Axl is constitutively upregulated by Langerhans cells in response to epidermal TGF-β1

Thomas Bauer, Jennifer Jurkin, Anna Zagorska, Nighat Yasmin, René Koeffel, Bernhard Gesslbauer, Greg Lemke, Herbert Strobl
1. Institute of Immunology, Medical University Vienna, Austria
2. Salk Institute for Biological Studies, La Jolla, CA, United States

Tyro-3, Axl and Mer (TAM) receptor tyrosine kinases represent an anti-inflammatory phagocytosis system, which is upregulated on dendritic cells (DC) during inflammation. These receptors use their ligands protein S and Gas6 for recognizing phosphatidylserine on apoptotic cells. Activation leads to a block in pro-inflammatory cytokine production, therefore implicating this system in silent phagocytosis and resolution of inflammation. We here identified Axl to be induced by TGF-β1 during Langerhans cell (LC) commitment of human CD34+ myeloid progenitor cells, monocytes and mouse bone marrow. Axl surface expression was specific for human LCs, since it could not be detected in other DC subsets and macrophages. We further demonstrate that Axl and Gas6 are expressed in the suprabasal keratinocyte layers of human skin, co-localizing with TGF-β1. Axl activation and Mer upregulation was observed in *ex vivo* human skin explant cultures during Nickel induced inflammation. Antibody mediated inhibition of Axl during DC generation led to an increased proinflammatory cytokine response upon activation. TAM KO mice exhibited reduced LC numbers, and this effect preceded the development of lupus like disease and associated skin inflammation developing in older mice. Therefore, TGF-β1 controls the steady-state expression of Axl in DCs. Constitutive Axl expression by epidermal LCs may play a key role during silent phagocytosis and resolution of inflammation, previously known to be largely dependent on TGF-β.

Ablation of the adaptor molecule p14 under the control of the CD11c promotor impairs the homeostasis of the murine Langerhans cell network

Florian Sparber, Nicole Amberg, Julia Scheffler, Martin Hermann, Boris Reizis, Maria Sibilia, Lukas A. Huber, Patrizia Stoitzner, Nikolaus Romani
Department of Dermatology, Innsbruck Medical University, Innsbruck, Institute for Cancer Research, Medical University of Vienna, Vienna, Division of Cell Biology, Biocenter, Innsbruck, KMT Laboratory, Innsbruck Medical University, Innsbruck, Columbia University Medical Center, New York, NY, USA

Aim: Dendritic cells (DC) are important regulators of immunity and tolerance. To fulfil their antigen presenting capacity, DC need to process and distribute incorporated antigen via endosomal sorting to distinct cellular compartments so that they can present it to effector T cells. The extracellular signaling-regulated kinase (ERK) cascade is involved in endosomal sorting processes. Hence, we investigated the role of the adaptor molecule p14, an essential part of the ERK cascade, in the context of DC function.
Methods: We generated a DC specific knock out mouse model by Cre-CD11c-mediated ablation of p14. Phenotypical analysis of the DC populations was carried out by flow cytometry as well as with immunofluorescence microscopy of epidermal sheets and cryostat sections.
Results: We noted greatly diminished numbers within the fraction of migrating DC in the skin-draining lymph nodes of both Langerhans cells and langerin+ CD103+ dermal DC. The reduced number of skin DC, especially epidermal Langerhans cells was further confirmed *in situ* by quantitative and qualitative analysis of the skin. Investigating the ontogeny of Langerhans cells by analysing the skin of newborn mice, revealed, that Langerhans cells are capable of populating the epidermis within 3 days after birth. However, the maintainance and homeostasis of the network is affected in p14 knock-out mice as indicated by a constant loss of Langerhans cells starting within one week after birth. The responsible mechanisms are being studied. Preliminary evidence suggests increased apoptotic activity.
Conclusion: In summary, our observations identify p14 as an important molecule regulating the homeostasis of the Langerhans cell network.

Human Langerin+ Dendritic cells are independent of epithelial Langerhans Cells; a Novel Perspective on Langerhans Cell Histiocytosis

N McGovern[1], V Bigley[1,2], C Allen[3], M Haniffa[1,2], X Nong Wang[1], R Dickinson[1], S Pagan[1], K Windebank[2], F Ginhoux[4], K McClain[3], M Merad5, M Collin[1,2]

[1] Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK
[2] Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK
[3] Department of Pediatrics, Texas Children's Cancer Center, Baylor College of Medicine, Houston, Texas, USA
[4] Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Immunos, Singapore
[5] Department of Gene and Cell Medicine, Mount Sinai School of Medicine, New York, USA

Since its identification on Langerhans cells (LC) over a decade ago, Langerin (Lang), a C-type lectin, has been thought to be specific to these cells. However, in mice, langerin expression has recently been demonstrated on specific dendritic cells (DC); CD103+ peripheral tissue migratory dendritic cells and CD8a+ lymphoid resident DC. Murine Lang+DC subsets differ from LC phenotypically, anatomically and homeostatically and show independent growth factor requirements. LC are dependent on M-CSF and TGFb, while Lang+ dermal DC require Flt3 signalling. Examining human tissues by flow cytometry and fluorescence microscopy, we show that Langerin-expressing DC are found in healthy human dermis, lung and liver. Similarly to murine Lang+DC, these cells differ phenotypically from LC in their surface antigen expression, intracellular langerin distribution and TLR profile. They are also distinct in their anatomical location, repopulation kinetics following stem cell transplantation, and their self-renewal capacity in the recently described human dendritic cell deficiencies. That langerin expression is not specific to human LC has direct relevance to the pathophysiology of the rare but potentially fatal disease Langerhans Cell Histiocytosis (LCH). LCH lesions are characterised by the accumulation of Lang+ cells (LCH cells) admixed with lymphocytes, eosinophils, neutrophils and macrophages. They occur in single or multiple organs including bone, skin, pituitary and central nervous system, liver, lung and gastrointestinal tract. Due to their langerin expression, LCH cells have been presumed to originate from LC but demonstration of the more promiscuous nature of langerin expression on DC and the accumulation of LCH cells in non-epithelial tissues raises the question of the true origin of these cells. Examining lesional material, we show that the phenotype of LCH cells, by mRNA and surface antigen expression, is at least as close to that of Langerin-expressing DC as LC. In addition to the inflammatory cellular infiltrate, LCH lesions have been shown to contain high levels of TNFa, GM-CSF and TGFb. In keeping with the hypothesis that LCH cells may originate from resident or recruited DC, independent of LC, we show that langerin expression can be induced *in vitro* on peripheral blood CD11c+DC in 12-18hrs in the presence of an inflammatory cellular milieu or specific cytokine combinations, including GM-CSF and TNFa or TGFb. This is in contrast to the 10-14 day *in vitro* culture period traditionally employed to differentiate Lang+ cells from monocytes or CD34+ progenitors. These findings suggest that langerin expression may be triggered by the inflammatory components of the lesions and crucially changes the emphasis of LCH research and consequently the search for effective treatments.

Characterisation of resident and migratory dendritic cells in human lymph nodes

Elodie Segura[1,2], Xavier Sastre[3], Sebastian Amigorena[1,2]
[1] INSERM U932, 26 rue d'Ulm, 75005 Paris, France
[2] Institut Curie, Section Recherche, 26 rue d'Ulm, 75005 Paris, France
[3] Institut Curie, Département de Biologie des Tumeurs, 26 rue d'Ulm, 75005 Paris, France

Dendritic cells (DC) are professional antigen presenting cells. In mouse, DC can be divided into lymphoid organ-resident DC and tissue-derived DC that migrate into the lymph nodes. In humans, different subsets of DC have been identified in the blood, spleen and skin, however whether populations of lymphoid organ-resident and migratory tissue-derived DC also exist in human lymphoid organs remains unknown. We have analyzed DC populations in human lymph nodes and identified three populations of migratory DC, that are present only in skin-draining lymph nodes and correspond to the DC subsets identified in the skin, and two populations of resident DC, that are present in all lymph nodes analyzed as well as in spleen and tonsil. Resident DC subsets showed similar properties in terms of T cell activation and polarisation, whereas skin-derived DC subsets displayed functional differences. In addition, we provide evidence of a precursor-progeny relationship between blood DC and lymphoid organ-resident DC. These results have important implications for the use of DC in therapeutic strategies.

Characterization of Dendritic Cells in human lymphoid organs

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Dendritic Cells (DCs) play an important role as antigen presenting cells in the immune system. In peripheral tissues immature DCs continually recirculate as sentinels and search for invading pathogens. After encounter of antigen in the presence of microbial products maturation of the DCs is induced by pattern-recognition-receptors. Costimulatory molecules become upregulated while the endocytotic capacity of mature DCs is drastically diminished. In addition, mature DCs release proinflammatory cytokines to attract other inflammatory cells, and present antigens to T cells. In contrast to thoroughly investigated murine tissue DCs, human DC-subpopulations are far less studied. This is mainly based on the poor availability of human organ material. Here, we provide data on human lymphoid tissue DC-subpopulations. First, we developed a protocol to efficiently purify leukocytes from limited patient material. Second, in order to deepen the understanding of human DC-subpopulations and their potentially distinct functionalities, we compared the expression of numerous cell surface markers responsible for migration, antigen uptake or costimulation by extensive Multicolor-FACS-analyses of various lymphoid organs. Third, high resolution confocal-immunofluorescence-analyses were performed to identify DC-subset localization in lymphoid tissues in the steady state. Genearray analyses will provide additional evidence. With this study we aim to identify uniquely expressed, tissue- and subset-restricted surface molecules which can be utilized for future antibody mediated antigen targeting in humans.
This study (D.D.) is supported by DC-Thera, the German Research Foundation (SFB643-TPA7 and DU548/2-1). D.D. is a fellow of the ‘Förderkolleg’ of the Bavarian Academy of Sciences.

CD141+ human non-lymphoid tissue dendritic cells cross-present exogenous antigen and are homologous to murine CD103+ dendritic cells

Muzlifah Haniffa[1,2], Amanda Shin[2], Venetia Bigley[1], Naomi McGovern[1], Xiao-nong Wang[1], Pearline Teo[2], Danelle Sung[2], Frano Gonzales Marinarich[2], Benoit Malleret[2], Peter See2, Anis Larbi[2], Helen Zhou[2]
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Dendritic cells (DCs) are critical in the induction and regulation of immune responses. DCs are heterogeneous implicating functional specializations that are starting to be unraveled. We previously described two distinct DC subsets in the human dermis and murine non-lymphoid tissues (1, 2). The similarities between human and murine interstitial DC network has been implied but never rigorously explored. We undertook a comprehensive analysis of the human non-lymphoid tissue antigen presenting cell compartment to identify homologues between humans and mice.
Our dissection of the human dermis, lung and liver by multi-parameter flow cytometry shows the presence of three myeloid DC subsets; CD1c+, CD14+ and a previously undescribed subset expressing CD141. Human blood and non-lymphoid tissue CD141+ DCs express CLEC9A, FLT3, CD11c and are negative for CX3CR1 and CD11b. CD141+ dermal DCs migrate spontaneously from skin explant cultures. Migration was selectively enhanced in the presence of the chemokine XCL1. Microarray and RQ-PCR analyses revealed equivalence between human interstitial CD141+ DCs with murine interstitial CD103+ and splenic CD8+ DCs.
Human dermal CD141+ DCs were superior to CD1c+ and CD14+ DCs in activating alloreactive T cell proliferation. All three interstitial DC subsets were capable of internalizing exogenous soluble Hepatitis B surface antigen (HBsAg) but CD141+ DCs were vastly superior at cross-presenting HBsAg to activate HLA-A2*01 s183-91 restricted CD8+ T cell clones.
Blood CD141+ DCs were not in active DNA replication but up to 1.5% of dermal CD141+ DCs were in S/G2/M phase. Blood CD141+ DCs are related to interstitial CD141+ DCs by the acquisition of a number of surface markers suggesting that interstitial CD141+ DCs are likely to derive from circulating blood precursors.
Our findings describe a human interstitial DC subset homologous to murine CD103+ DCs, with potent cross-presenting capacity that can be potentially targeted for anti-viral and anti-tumour responses.

1. Haniffa et al. J Exp Med 2009; 206 (2): 371-385.
2. Ginhoux et al. J Exp Med 2009; 206 (13):3115-3130.

Michael Sixt, Institute of Science and Technology (IST), Vienna, Austria

Chemotactic guidance of dendritic cells in the skin

14.30 – 16.00 ***INVITED SPEAKER***

Reinhold Förster, Medizinische Hochschule, Hannover, Germany

Homing of lymph derived cells to lymph node

Eynav Klechevsky, Washington University, St.Louis, Missouri, USA

Human Langerhans Cells: mechanism and implications in vaccine science

The scatter factor receptor/Met tyrosine kinase is essential for dendritic cell migration in skin immunity

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The migratory properties of dendritic cells (DCs) are crucial for their immune function. Langerhans cells (LCs) have been shown to express functional scatter factor (SF) receptor/Met and SF can induce their emigration from epidermis. Met is a receptor tyrosine kinase and the receptor for SF, also known as hepatocyte growth factor (HGF). Met signaling exhibits mitogenic, morphogenic, and mitogenic activity in various cells, e. g. during embryonic development, wound healing, and in tumorigenesis. In this work, a detailed analysis of Met function in LCs and dermal DCs (dDCs) is presented. The role of Met signaling under steady state and inflammatory conditions was addressed by employing a conditional Met KO mouse model (Mx-cre/Met fl/fl). *In vivo*, a distinct pattern of Met surface expression was found in different DC subpopulations, including LCs and dDCs in peripheral and lymphoid organs in steady state. Activation of LCs/dDCs upon inflammation efficiently induced expression of Met. Dermal DCs showed higher Met expression than LCs and faster migration kinetics towards draining lymph nodes. Genetic or pharmacological deficiency of Met signaling severely impaired motility of LCs/dDCs resulting in failed mobilization of LCs/dDCs from skin and thus in diminished contact hypersensitivity reactions. The regulation of matrix metalloproteinase activity by Met signaling was identified as one potential mechanism. Taken together, the data of this work demonstrate an essential function of Met for DC mobilization contributing to skin immunity and suggest new ways for treatment of autoimmune skin diseases.

Langerin-positive Langerhans cells but not Langerin-negative inflammatory dendritic epidermal cells penetrate epidermal tight junction barriers in atopic dermatitis

Akiharu Kubo[1,2], Kazue Yoshida[1], Mariko Yokouchi[1,3], Hiroshi Kawasaki[1], Tamotsu Ebihara[1], Keisuke Nagao[1], Masayuki Amagai[1]
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The epidermal barrier function has three elements: the stratum corneum (SC) and tight junctions (TJs) form physical barriers, and the epidermal dendritic cells (DCs) form immunological barriers. SC barrier dysfunction is regarded as a predisposing factor for atopic dermatitis (AD), however, it is not well documented what happens to TJs and DCs under the impaired SC barrier. In this study, we clarified the behavior of epidermal DCs especially in their dynamic relationship with TJs in AD patients. We observed TJs and DCs concomitantly via 3D-visualization of whole mounted epidermis. We found that the dendrites of activated Langerhans cells (LCs) penetrated epidermal TJs in human as well as mice. The number of LCs in lesional and nonlesional AD skin was significantly reduced than that of healthy skin. Nonetheless, the number of LCs that penetrated TJs was dramatically increased in lesional AD skin. In the AD epidermis, there appear langerin-negative DCs called inflammatory dendritic epidermal cells (IDECs). Surprisingly, IDECs never penetrated TJs even when surrounding LCs vigorously penetrated TJs. To our knowledge, this is the first report to demonstrate behavioral difference between LCs and IDECs. In AD, both of LCs and IDECs expressed high-affinity receptors for IgE (FceRI). Interestingly, langerin but not FceRI accumulated to the TJ-penetrated dendrite tips of LCs, suggesting that IgE-dependent antigen uptake by LCs and IDECs occurs mostly under TJ barriers after antigens penetrate both SC and TJ barriers. These findings will provide new insights of immunological significance of epidermal DCs in the pathophysiology of AD.

Intercellular space of keratinocytes is a migratory pathway of Langerhans cells

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The epidermis is composed of keratinocytes that bind tightly each other. This feature contrasts to the property of the dermis where cellular density is low and filled with extracellular matrix. Therefore, it remains a question how Langerhans cells migrate within such a “packed” epidermis. Here, we examined the motility of Langerhans cells in the epidermis by means of two-photon microscopy. First, we found that cellular membrane of keratinocytes was labeled with a fluorescent lectin. Using this dye, the positional relationship of Langerhans cells and keratinocytes were easily determined in the living tissues. We have revealed that there exist 1-2 nm gaps between adjacent keratinocytes *in vivo*. Langerhans cells lie in the gaps and elongate their dendrites into the gaps. They were almost static in the steady state, however, they actively moved within the gaps when the skin inflammation was induced. When they migrated, the elongated dendrites preceded the cell body and a ballooning of the tip of dendrites was observed. Intriguingly, alpha-beta T cells also elongated dendrites in to the gaps when they penetrated into the epidermis. Our study has demonstrated that the dendritic morphology of Langerhans cells is confined by the location where they exist. The cells residing in the gaps of keratinocytes exhibit a dendritic morphology regardless of cell types. Moreover, the gaps between keratinocytes may provide a migratory pathway for immune cells. It is essential for the immune cells in the epidermis to migrate in a non-tissue destructive manner.

Langerhans Cell-selective Deletion of EpCAM (CD326) Attenuates Langerhans Cell Motility and Migration *In vivo* and Enhances Contact Hypersensitivity Reactions

Maria R. Becker[1], Tim Lämmermann[2], Xu Feng[1], Botond Z. Igyarto[3], Daniel H. Kaplan[3], Lino Tessarollo[4], Ronald N. Germain[2], and Mark C. Udey[1]

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After activation, Langerhans cells (LC) migrate from epidermis to regional lymph nodes where they influence the magnitude and quality of immune responses initiated in response to epicutaneously-applied antigens. LC migration that occurs in the absence of skin perturbation may contribute to peripheral tolerance. Modulation of adhesion of LC to keratinocytes is likely to be central to regulation of LC migration. LC are distinct from other dendritic cells in that they express high levels of EpCAM (CD326), a cell surface protein that is characteristic of some epithelia and many carcinomas, and that has been implicated in intercellular adhesion and metastasis. Because EpCAM knockout mice die en utero, we generated mice with EpCAM-deficient LC (LC/EpCAM cKO mice) to determine functional consequences of EpCAM expression by LC. LC/EpCAM cKO mouse epidermis contained two-fold increased numbers of LC that expressed normal levels of MHC Class II and costimulatory molecules, and exhibited normal T cell stimulatory activity *in vitro*. Studies of skin explants revealed that migration of LC/EpCAM cKO LC was inhibited, while chemotaxis of dissociated LC was not. The ability of contact allergen-stimulated EpCAM-deficient LC to exit epidermis *in vivo* was delayed, and fewer hapten-labeled LC accumulated in regional lymph nodes. Attenuated LC migration in LC/EpCAM cKO mice caused enhanced contact hypersensitivity responses equivalent to those seen in LC-deficient mice. Intravital microscopy revealed dramatically reduced LC translocation and dendrite motility (dSEARCH) *in vivo* in contact allergen-treated LC/EpCAM cKO mice. Our results suggest that LC EpCAM facilitates LC disengagement from keratinocytes, and promotes LC emigration from skin. These studies also validate the concept that LC trafficking from skin to draining lymph nodes is essential for normal LC function.

The pregnane X receptor (PXR) controls Langerhans cell migration via CCR7

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Department of Dermatology, Epidermal Biology laboratory, 6020 Innsbruck, Austria

The pregnane X receptor (PXR) is a ligand-activated transcription factor regulating genes central to drug and hormone metabolism in the liver. We here show that PXR is highly expressed in different subsets of mouse and human immature DC and is down-regulated in mature DC including Langerhans cells (LC). PXR activation down-regulates CCR7 expression at the cell surface of mouse LC without affecting expression of other maturation markers such as CD40, CD86 and CXCR4 *in vitro*. Similarly, transgenic overexpression of the huPXR also called SXR (Tg-SXR) in LC decreases expression of CCR7, mimicking effects of pharmacological activation of PXR. Interestingly, transgenic co-expression of CYP3A4 (Tg-SXR/CYP), a well-known PXR downstream gene, further decreased CCR7 expression in LC, suggesting an involvement of CYP3A4 in this regulation. In contrast, treatment of cells with A-792611, a novel potent and specific antagonist of PXR, up-regulates CCR7 expression at the cell surface of mouse and human LC. *In vivo*, PXR deficiency increases percentages of LC while transgenic expression of huPXR decreases percentages of LC in skin draining lymph nodes of mice after skin sensitization with a contact allergen. Transgenic overexpression of both SXR and CYP3A4 in LC further increases the percentages of LC in skin draining lymph nodes of sensitized mice. Furthermore, langerin+ cells loose PXR while migrating into skin tumors and PXR expression is lowered in intra-tumoral CCR7+ cells in mice. All together these results demonstrate that PXR controls LC migration via CCR7 with relevance to tumoral context.

Autocrine TGFβ acts as regulator of maturation and migration for Langerhans cells

Aleh Bobr[1], Botond Z. Igyarto[1], Krystal M. Haley[1], Ming O. Li[2], Richard A. Flavell[3] and Daniel H. Kaplan[1]
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Langerhans cells (LC) are skin resident dendritic cells located in the epidermis. We have demonstrated using mice with a LC-specific ablation of TGFβ (Langerin-Cre x TGFβ-flox) that LC ontogeny requires autocrine TGFβ *in vivo*. Since these mice have a constitutive absence of LC, the effect of TGFβ on adult LC could not be evaluated. We have now developed Langerin Cre-ERT2 mice that allow for tamoxifen-inducible gene excision. Using ROSA26.YFP Cre reporter mice, we confirmed that Cre-ERT2 expression is specific for LC and highly efficient. We next generated Langerin Cre-ERT2 x TGFβ-flox mice. Tamoxifen-inducible excision of TGFβ led to increased expression of costimulatory markers on LC followed by migration to the regional lymph node. At the same time levels of costimulatory markers expression and levels of cytokines secreted by LC in lymph nodes were same as in control mice, which indicated that observed migration is reflective of steady state migration of LC. We observed an identical phenotype in Langerin Cre-ERT2 x TGFβRII-flox mice. Therefore, we conclude that LC derived TGFβ acts directly on LC in an autocrine/paracrine fashion and serves as epidermal retention factors that prevents LC maturation. We also showed that an interruption of TGFβ signaling is an essential step during LC migration in response to inflammation. This may be an important mechanism preventing inappropriate immune responses against skin commensal microorganisms.

Expression of TROP2 reveals differences between human epidermal Langerhans cells and other CD207+ DC subsets

G. Eisenwort, C.X.F. Lim, J. Jurkin, N. Yasmin, T. Bauer, B. Gesslbauer, and H. Strobl
Institute for Immunology, Medical University Vienna

TGF-β1-dependent Langerhans cells (LCs) are the dendritic cells of the epidermis, the outermost layer of the skin. They have been classically identified by the expression of the C-type lectin receptor Langerin (CD207). Recently, other CD207+ dendritic cell subsets. That are not dependent on TGF-β1, have been defined in mice, whereas data in the human system are lacking. The objective of this study was to identify CD207+ human dendritic cell subsets and analyze their relation to epidermal LCs with regard to the expression of TGF-β1-dependent marker molecules. Using gene profiling we searched for such novel markers. We found that the surface molecule TROP2 (TACSTD2) is strongly and rapidly induced during TGF-b1-dependent LC commitment of human CD34+ hematopoietic progenitor cells. TROP2 is conserved between mouse and human and shares substantial amino acid identity with EpCAM, an established marker for murine epidermal LCs. Neither TROP2 nor EpCAM expression has to our knowledge been analyzed in human DC subsets. We here show that all human epidermal LCs are TROP2+EpCAM+; furthermore, human dermis lacks CD207+EpCAM- or CD207+TROP2- DCs, i.e. equivalents of murine dermal CD207+ DCs; moreover, pulmonary CD207+ cells are TROP2-EpCAM-. Finally, while EpCAM was broadly expressed by pulmonary and intestinal epithelial cells as well as by bone marrow erythroid progenitor cells, these cells lacked TROP2. We conclude that CD207+ cells in human lung are distinct from epidermal LCs. Additionally we here identified TROP2 as a highly specific and TGF-β1-dependent marker for human epidermal LCs.

Langerhans Cells Regulate Cutaneous Injury by Licensing CD8 Effector Cells Recruited to the Skin

Clare L. Bennett, Farnaz Fallah-Arani, Thomas Conlan, Celine Trouillet, Hugh Goold, Laurent Chorro, Barry Flutter, Terry K. Means, Frédéric Geissmann and Ronjon Chakraverty
Transplantation Immunology Group, Department of Haematology, Division of Cancer Studies, University College London, Centre for Molecular and Cellular Biology of Inflammation, Division of Immunobiology, Infection and Inflammatory Diseases, King's College London
Center for Immunology and Inflammatory Diseases, Harvard Medical School, Massachusetts General Hospital

Langerhans cells (LC) are a distinct population of dendritic cells (DC) that form a contiguous network in the epidermis of the skin. Although LC possess many of the properties of highly proficient DC, recent studies have indicated that they are not necessary to initiate cutaneous immunity. In this study, we employed a tractable model of cutaneous graft-versus-host disease (GVHD), induced by topical application of a Toll-like receptor agonist, to explore the role of LC in the development of tissue injury. By adapting this model to permit inducible and selective depletion of host LC, we found that GVHD was significantly reduced when LC were absent. However, LC were not required either for CD8 T-cell activation within the draining lymph node or subsequent homing of effector cells to the epidermis. Instead, we found that LC were necessary for inducing transcription of IFN-γ and other key effector molecules by donor CD8 cells in the epidermis, indicating that they license CD8 cells to induce epithelial injury. These data demonstrate a novel regulatory role for epidermal LC during the effector phase of an inflammatory immune response in the skin.

Keisuke Nagao, Keio University, Tokyo, Japan

Antigen capture through tight junctions by Langerhans cells induce pre-emptive immunity in experimental staphylococcal scalded skin syndrome

Dan Kaplan, University of Minnesota, Minneapolis, MN

Control of adaptive responses by skin-resident DC subsets

09.00 – 10.30 ***INVITED SPEAKER***

Teunis Geijtenbeek, Academic Medical Center, University of Amsterdam, The Netherlands

C-type lectin Langerin in immunity & infections

Marcel Teunissen, Academic Medical Center, University of Amsterdam, The Netherlands

Langerhans cells support tolerance to the bacterial skin flora

Depending on the Antigen, Langerin+ DC Enhance or Suppress Gene Gun-induced Immune Responses

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In previous studies we found that the absence of langerin+ DC led to a total breakdown of CD8+ T cell responses and IgG1 isotype antibodies in LangDTR mice immunized against E.coli beta-galactosidase (b-Gal). Here we show that this effect was totally reversed when we used a gene gun vaccine encoding chicken ovalbumin (OVA). LangDTR mice, when depleted of all langerin+ DC, responded with strongly enhanced CTL activity, cytokine secretion by CD8+ and CD4+ T cells and OVA-specific IgG1 and IgE antibody titers. These opposite findings were not simply due to b-Gal and OVA being cytosolic or secreted proteins, respectively, because the lost of CTL and IgG1 that was typical for b-Gal was not observed with the non-secreted variant of OVA. In fact, the response against this gene gun vaccine was completely unaffected by the absence of langerin+ DC and, hence, represents yet another type of antigen. From these observations we conclude that langerin+ DC are able to, either, induce or suppress, at least some specialized, immune functions against skin-borne antigens. Moreover, some as yet undefined features of the antigen itself seem to be critical in this decision-making.

Virus selectively primes human Langerhans cells for CD70 expression promoting CD8+ T cell responses

Angelic M.G. van der Aar[1,2], Rosa de Groot[1], Marta Sanchez-Hernandez[2], Esther W.M. Taanman[1], Rene. A. W. van Lier[3], Marcel B.M. Teunissen[2], Martien L. Kapsenberg[1,2] and Esther C. de Jong[1,2]
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[3] Sanquin Research, Amsterdam, The Netherlands

The two outermost compartments of skin are populated by different antigen-presenting dendritic cell types. Epidermal Langerhans cells (LCs) are evolutionary adapted to the continuous presence of harmless skin commensals by the selective lack of cell surface TLRs that sense bacteria. Here we analyze the ability of LCs and dermal dendritic cells (DDCs) to respond to virus infection. Live virus and intracellular TLR3-agonist dsRNA commit LCs more effectively to stimulate naïve CD8+ T cell-expansion and their differentiation into effector cells that express IFN-γ, Granzyme B and are cytotoxic. This potent CD8+ T cell-promoting capacity of LCs is causally related to virus-induced, high levels of CD70 expression, but not to IL-12 production. These data suggest a remarkable specialization of LCs in the induction of pathogen-class specific adaptive immunity. Whereas LCs ignore bacteria they are superior to DDCs to initiate effective CD70-mediated CD8+ T cells in response to virus stimulation.

The neuropeptide calcitonin gene-related peptide (CGRP) inhibits HIV-1 transfer from Langerhans to T-cells

Yonatan Ganor[1,2,3], Anne-Sophie Drillet-Dangeard[1,2,3], Daniela Tudor[1,2,3], Lucia Lopalco4, Morgane Bomsel[1,2,3]
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Background: Productive infection of Langerans cells (LCs) with HIV-1 is inefficient. Yet, LCs transfer HIV-1 to T-cells, resulting in massive viral replication. Stratified epithelia and secondary lymphoid organs (where LCs reside or migrate to, respectively) are highly innervated by neuropeptidergic neurons. Among the secreted neuropeptides is calcitonin gene-related peptide (CGRP), a potent vasodilator that additionally exerts profound immunosuppressive effects on LC functions (e.g. antigen presentation, cytokine secretion). Whether CGRP can also limit HIV-1 transfer/capture by LCs was never investigated.
Methods: Monocyte- and MUTZ3-derived LCs were pre-treated with CGRP, exposed to HIV-1, and incubated with CD4+ T-cells. A week later, viral replication was measured in the co-culture supernatants by p24 ELISA.
Results: CGRP inhibited HIV-1 transfer from LCs to T-cells in a time- and dose-dependent manner, with maximal inhibition of 80% following 24h LCs pre-treatment with 100nM CGRP (i.e. similar to its concentration in neuronal tissues). Such inhibition was mediated by the CGRP receptor I (CGRPRI), as it was completely blocked by the CGRPRI antagonist CGRP8-37. RT-PCR confirmed the expression of the CGRPRI multi-components calcitonin-recpetor-like-receptor and receptor-activity-modifying-proteins 1/2. CGRP-mediated HIV-1 transfer inhibition was associated with: 1) reduced HIV-1 capture by LCs; 2) decreased formation of LC-T-cell conjugates; 3) altered secretion of several cytokines and CCR5-binding chemokines. Finally, compared to healthy controls, CGRP plasma levels in HIV-1-positive patients were significantly lower, which might have rendered them more susceptible to HIV-1.
Conclusions: These results reveal a completely novel mechanism by which a neuro-endocrine factor restricts LC-mediated HIV-1 transmission. Hence, boosting CGRP-LC interactions might turn out useful as an innovative anti-HIV-1 clinical strategy.

Skin-Resident Murine Dendritic Cell Subsets Promote Distinct and Opposing Antigen-Specific T Helper Cell Responses

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Skin-resident dendritic cells (DCs) are well positioned to encounter cutaneous pathogens and are required for the initiation of adaptive immune responses. There are at least three subsets of skin DC — Langerhans cells (LC), Langerin+ dermal DCs (dDCs), and classic dDCs. Whether these subsets have distinct or redundant function *in vivo* is poorly understood. Using a Candida albicans skin infection model and targeting antigen directly to LC, we have shown that direct presentation of antigen by LC is necessary and sufficient for the generation of antigen-specific T helper-17 (Th17) cells but not for the generation of cytotoxic lymphocytes (CTLs). In contrast, Langerin+ dDCs are required for the generation of antigen specific CTL and Th1 cells. Langerin+ dDCs also inhibited the ability of LCs and classic DCs to promote Th17 cell responses. We have also found when antigen presentation is restricted *in vivo* to either LC or Langerin+ dDC, the generation of Th1, Th2, and Th17 subsets depends on the adjuvant used but still differs between the two DC subsets. These results demonstrate that skin-resident DC subsets promote distinct and opposing antigen-specific responses.

Relatively quiescent response of human Langerhans cells to filarial parasites suggest a method of immune evasion used by filariae and other skin-transiting helminths

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From an immunologic perspective, lymphatic filariasis is one of the most complex infections of humans. The infection is initiated by a mosquito-derived, third-stage larvae (L3) deposited on the skin and transiting through the epidermis containing Langerhans cells (LC) and keratinocytes (KC) among other cells. The earliest interaction between the L3 and the LC likely conditions the priming of the immune system to the parasite. Thus, understanding the interaction between the L3 and the antigen presenting cells (APC) in the skin (e.g. LC) becomes crucial. Previously, by using ex-vivo epidermal skin explants exposed to live L3 of Brugia malayi - one of the extracellular parasites responsible for lymphatic filariasis in humans -- we showed that L3 exposure led to a decrease in the function of LCs in terms of antigen presentation and T cell activation. In subsequent studies of ex-vivo epidermal skin explants exposed to L3, the production of IL-18 protein and of mRNA for caspase 1, CD207 (Langerin), and IL-18 binding protein (BP) was induced by the L3. Since caspase 1 is central to the inflammasome and the production of proinflammatory responses, we further investigated the potential involvement of inflammasomes as a mechanism of the innate function of L3-exposed LCs. We generated human LCs (Langerin+, E-cadherin+, CD1a+) *in vitro*, exposed them to L3, LPS, or media and assessed their expression of critical cell surface markers, pro-inflammatory cytokines and the genes involved in inflammasome activation. In contrast to a known inflammasome activator, LPS, L3s only induced minimal up-regulation of surface expressed CD14, CD86 and CD83 with no changes in surfaced expressed CD207, E-cadherin, CD80, CD40 and HLA-DR. No significant changes in mRNA expression of the inflammasome-associated genes NLRPs, NLPR1, NLRC4, AIM2, ASC and IL-18 were seen in the LC response to L3, although there was a small L3-induced increased (but not statistically significant) expression of IL-18BP and caspase 1. L3 failed to induce the production of the cytokines IL-1β, IL-6, IL-8, IL-18, IL-18BP, IL-33 and IFN-γ from *in vitro* LCs, nor did the L3 alter the LC response to LPS or Poly I:C. This quiescent response of LC L3 may be one of the first immune evasion strategies used by the filarial worm. The apparent discrepancy between L3 exposed skin explants and the *in vitro* generated LCs might be explained by the presence of KC in the human skin explant model. Studies designed to be more physiologic (co-culturing foreskin-derived KC with the *in vitro* generated LC) are underway and should help elucidate the nature of the early response to the infective stage of the filarial parasites.

Molecular mechanisms of antigen transfer between human Langerhans cells and dendritic cells

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Human epidermal Langerhans cells (LCs) initiate adaptive T cell responses after infection. However, we have shown that LCs are not able to cross-present measles virus (MV)-derived antigens to virus-specific CD8+ T cells. Notably, co-culturing of MV-treated LCs with dendritic cells (DCs) allowed transfer of MV-derived antigens to DCs and, remarkably, cross-presentation by DCs to T cells. Therefore LCs transfer antigens to DCs to facilitate antigen presentation. Nothing is known about the interactions of LCs with DCs. Here we have investigated the molecular mechanisms underlying LC-DC interactions and antigen transfer. LCs efficiently cluster with DCs, and clustering increased over time, with a maximum at 90 minutes. Clustering was observed with both immature and mature LCs, so independent of their maturation status. Using neutralizing antibodies and specific inhibitors, we have identified the adhesion receptors involved in LC-DC clustering. Inhibition of the receptors by inhibitors prevented clustering of the cells and might affect antigen transfer. In conclusion, we have shown that LCs and DCs are distinct APC subsets that cluster and cooperate in inducing adaptive immune responses. The interaction between LCs and DCs via specific adhesion receptors might be involved in cross presentation and subsequent T cell activation. Manipulation of these adhesion receptors could be used to develop novel vaccination strategies to target LCs and enhance or decline T cell responses.

Direct transcutaneous targeting of antigen to the specific skin dendritic cell subsets for improved vaccine efficacy

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Dendritic cells (DC), key professional antigen presenting cells, play a pivotal role in linking the innate and adaptive immunity. Recently, several studies highlighted the complexity and functional specialization of the cutaneous DC network, showing that at least five subsets of DC coexist in homeostatic murine skin and that these DC subsets may exhibit specific immune functions. Therefore, due to the high number of DC present in the epidermis and dermis, the skin represents an optimum site for vaccine delivery. For the first time dissolvable polymeric microneedle (MN) arrays laden with ovalbumin (OVA) encapsulated nanoparticles (NP) were explored to target skin DC subsets to promote enhanced immune responses. We demonstrated that following the efficient internalization of fluorescent-labeled NP, DC upregulated maturation markers expression as efficiently as LPS induced activation. Furthermore, we observed that OVA loaded NP efficiently stimulated bone marrow derived DC to induce OVA specific CD4+ and CD8+ T cell proliferation *in vitro*. Following application of MN laden with rhodamine encapsulated NP, we confirmed efficient skin delivery of NP *in situ* and demonstrated that skin DC were able to readily uptake and successfully deliver fluorescent NP to cutaneous draining lymph nodes. Finally, we confirmed that following the application of MN loaded with OVA NP, ex-vivo purified DC from cutaneous draining LN induced proliferation of OVA specific, IFN-gamma producing effector CD8+ T cells. Therefore, direct targeting of antigen to the specific skin DC subsets will help us understand the precise contribution of particular skin DC subsets during antigen specific immune responses.

Langerhans cells suppress the anti-Leishmania response via induction of regulatory T cells

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Defense mechanisms against pathogens are orchestrated by different skin-derived dendritic cell (DC) subsets, including epidermal Langerhans cells (LC) and dermal DC (dDC). Here, we analyzed the role of LC in murine experimental leishmaniasis by their inducible ablation in vivo using the Langerin-DTR mouse model. Upon physiologically relevant low-dose infection with L. major (1,000 parasites), mice selectively depleted of LC developed significantly smaller ear lesions that contained a lower parasite burden, which correlated with reduced numbers of lesional CD4+/Foxp3+ regulatory T cells (Treg) as compared to control mice. These findings were confirmed in lethally irradiated Langerin-DTR mice reconstituted with WT C57BL/6 bone marrow (BM). Due to their radioresistancy, epidermal LC remain of Langerin-DTR (recipient) origin, whereas all other DC subtypes are radiosensitive and replaced by donor BM-derived cells. Again, DT-induced selective depletion of LC led to significantly smaller ear lesions. In contrast, specific ablation of Langerin+ dDC in reciprocal Langerin-DTR (donor) into WT (recipient) BM chimeras had no beneficial effect on disease development. To investigate the suppressive capacity of LC in more detail, DC were isolated from lymph nodes of Langerin-DTR mice either treated with DT (lacking Langerin+ DC) or PBS (including Langerin+ DC). Subsequently, both DC preparations were pulsed with soluble Leishmania antigen and co-cultured with CD4+ CD25neg T cells. Intriguingly, induction of CD4+ CD25+ Foxp3+ T cells was significantly increased when the antigen presenting DC comprised Langerin+ DC (LC) as compared to those lacking Langerin+ DC. In conclusion, we demonstrate that LC drive the induction of Leishmania-specific Treg and thus, vaccination strategies should aim to circumvent targeting LC.

14.00 – 15.30 ***INVITED SPEAKER***

Sandrine Henri, CIML, Université de la Méditerranée, Marseille, France

Dermal dendritic cells: from phenotype to function, XCR1 as a universal marker of cross-presenting dendritic cells

Juliana Idoyaga, Rockefeller University, New York

Targeting steady state dendritic cells using anti-Langerin mAbs coupled with a self-antigen promote de novo generation of Foxp3+ regulatory T cells

14.00 – 15.30 ***INVITED SPEAKER***

Björn Clausen, Erasmus University, Rotterdam, The Netherlands

Skin dendritic cell subsets: Functional specialization and molecular control *in vivo*

Targeting antigen to DC-SIGN using glycan-modified liposomes elicits activation of both CD4+ and CD8+ T-cells

Astrid J van Beelen, Wendy WJ Unger, Medha Joshi, Sven C Bruijns, Marleen Verstege, Martino Ambrosini, Hakan Kalay, Erik Hooijberg, Tanja D de Gruijl, Gert Storm and Yvette van Kooyk
Departments of Molecular Cell Biology and Immunology, Pathology and Medical Oncology, VU University Medical Center, Department of Pharmaceutics, Utrecht University of Pharmaceutical Sciences (UIPS), Utrecht University, The Netherlands

Cancer immunotherapy requires potent tumor-specific CD8+ and CD4+ T-cell responses, initiated by dendritic cells (DCs). Our aim is to develop a therapy that targets DCs *in vivo* in human skin using glycosylated, tumor antigen-loaded liposomes. The use of different glycans allows directing the liposomes to specific DC subsets, such as dermal DCs or Langerhans cells. Since DC-SIGN is a potent uptake receptor, expressed on DCs in human skin, liposomes were modified with DC-SIGN-binding glycans Lewis (Le)B or LeX. First, these glycosylated liposomes were tested in *in vitro* setting on monocyte-derived DCs (moDCs) and murine BMDCs. Glycan modification of liposomes resulted in increased binding and internalization by hSIGN Tg BMDC and subsequently led to a 100-fold more efficient presentation of the encapsulated antigens to CD8+ and CD4+ T-cells than using control liposomes or soluble antigen. Similarly, human moDCs showed increased uptake of liposomes when modified with LeX and this was blocked in the presence of anti-DC-SIGN antibodies or EGTA, demonstrating a crucial role for DC-SIGN. Analogous to the murine data, targeting human moDC by Melanoma antigen MART-1-encapsulated liposomes coated with LeX led to an enhanced antigen presentation to a MART-1-specific CD8+ T cell clone and to enhanced numbers of MART-1 tetramer-specific, IFN-γ producing effector CD8+ T cells in an autologous primary response. Finally, injection of glycosylated liposomes in human skin led to their enhanced uptake by emigrated DCs. Our data demonstrate the potency of a glycoliposome-based vaccine targeting DC-SIGN in inducing activation of both CD4+ and CD8+ T-cells. This may be a crucial step towards development of an anti-cancer vaccine that targets skin DCs *in situ*.

Induction of tolerance or immunity after langerin targeting reveals intrinsic properties of langerin+ dendritic cells

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Antigens targeted to C-type lectin receptors expressed by dendritic cells (DCs) are efficiently internalized and presented to CD4+ and CD8+ T cells. When deposited in the skin, antigen-antibody (Ab) conjugates are captured by epidermal Langerhans cells (LCs) and dermal DCs. Part of the conjugates also flows through the lymph into skin-draining lymph nodes (LN), where they target emigrated skin DCs and LN-resident CD8+ DCs. We showed previously that LCs isolated after intradermal targeting by ovalbumin (OVA)-coupled anti-langerin Ab did not trigger *in vitro* proliferation of transgenic OVA-specific T cells. In C57BL/6 (B6) mice, intradermal targeting with anti-langerin/OVA conjugates induced endogenous cytotoxic T cell (CTL) responses only in the presence of TLR3 agonist poly(I:C) and anti-CD40 Ab (pIC+aCD40), but not TLR7 agonist imiquimod. This is in contrast to DEC-205 targeting, which is immunogenic with both adjuvants. In line with this, a combination of anti-langerin/OVA and imiquimod (but not the conjugates alone) opposed CTL responses induced by a subsequent challenge with anti-DEC-205/OVA + pIC. This impaired OVA-specific responses against circulating target cells and melanoma. Tolerance was associated with sub-optimal differentiation and lack of memory of OVA-specific CD8+ T cells. Finally, langerin targeting with pIC+aCD40 was ineffective in bone marrow chimera mice where langerin was expressed only by LCs, but not dermal DCs. Interestingly, imiquimod demonstrated efficient adjuvancy for langerin targeting in [B6 x BALB/c] F1 mice, where CD8+ DCs express langerin, as opposed to B6 mice. Altogether, our results demonstrate that the choice of adjuvant in lectin targeting strategies is of critical importance in the outcome of immune responses. In addition, we provide insights into the intrinsic properties of langerin+ DC subsets, showing that CD8+ DCs, langerin+ dermal DCs, but not LCs, cross-present antigen internalized through langerin *in vivo*. Current investigations now aim at better defining the sequence of events leading to tolerance.

Cross presentation of CD11c+CD8- DC under inflammatory conditions

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Dendritic cells are very important initiators of immune responses. Several subpopulations have been described. Recently, we could show that antigen targeting to either CD11c+CD8- or CD11c+CD8+ DCs induces different T cell responses in C57BL/6 mice *in vivo*. For *in vivo* antigen targeting we are using antibodies against DCIR2 (33D1) or DEC205 which are endocytic C-type lectin receptors expressed on CD11c+CD8- or CD11c+CD8+ DCs, respectively. These antibodies are genetically modified and carry the model antigen Ovalbumin. Due to a mutation in the Fc part, the antibodies are unable to bind to Fc receptors. When we inject these antibodies under steady state conditions we are able to induce a CD4 T cell response, when DCIR2-Ova is targeting to CD11c+CD8- DCs and a prominent CD8 T cell response, when we are targeting DEC205-Ova to CD11c+CD8+ DCs. Interestingly, we now found that when we inject DEC205-Ova under inflammatory conditions we can induce a strong CD8 T cell response by CD11c+CD8- DCs in the *in vitro* culture. Moreover, we not only see a strong upregulation of costimulatory molecules and migration of the DCs into the T cell area in the murine spleen, but also an upregulation of DEC205 on CD11c+CD8- DCs. However, the expression of DCIR2 was not influenced. Our data suggest that under inflammatory conditions DEC205 targeting is inducing cross presentation of antigen by CD11c+CD8- DCs.

Targeting of epidermal langerhans cells to establish anti-tumor immunity

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Targeted delivery of tumor antigens to dendritic cells (DCs) using nanoparticles holds great potential for cancer immunotherapy. Human skin contains several subsets of DCs, like the CD14+ dermal DCs, CD1a+ dermal DCs and CD1ahigh epidermal langerhans cells (LCs). We have shown that each DC subset expresses a distinct pattern of surface receptors, like C-type lectin receptors (CLRs) and toll-like receptors (TLRs). Differences in CLR expression provide a valuable tool to specifically target single subsets of these DCs, since most CLRs have endocytic capacities and therefore are able to take up antigens. The CLR langerin is expressed on LCs present in human epidermis and LCs have previously been described to be able to process and present antigens to T cells through MHC class I and II pathways. Therefore, LCs are likely candidates to exploit in an anticancer therapy. The aim of this study is to determine whether we can specifically target LCs through langerin with liposomes loaded with melanoma antigens in order to raise an antigen-specific T cell response. Here, we show that both langerin-transfected cell lines as well as primary LCs isolated from human skin can be specifically targeted using anti-langerin antibodies or langerin-binding glycans. *In situ* targeting of DCs in the dermis resulted in an enhanced uptake of anti-langerin coated liposomes by CD1ahigh LCs. These findings demonstrate the potency of targeting liposomes to LCs for anti-tumor vaccination strategies.

Langerhans cells protect from allergic contact dermatitis in mice by tolerizing CD8+ T cells and activating Foxp3+ regulatory T cells

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Allergic contact dermatitis is the most frequent occupational disease in industrialized countries and is caused by CD8+ T-cell-mediated contact hypersensitivity (CHS) reactions to weak haptens. Despite the myriad of potentially allergenic substances that can penetrate the skin, sensitization is relatively rare and immune tolerance often induced by as yet poorly understood mechanisms. Here we show, using the innocuous chemical 2,4-dinitrothiocyanobenzene (DNTB), that cutaneous immune tolerance critically depends on epidermal Langerhans cells (LC) that capture DNTB and migrate to lymph nodes for direct presentation to CD8+ T cells. Depletion and adoptive transfer experiments revealed that LC conferred protection from development of CHS by a mechanism involving both anergy and deletion of allergen-specific CD8+ T cells and activation of ICOS+CD4+Foxp3+ regulatory T cells. These data highlight the unique tolerogenic potential of LC in protection from skin allergy.

The role of Langerhans cells in T cell priming

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Langerhans cells (LCs) are positioned in the skin to encounter pathogens and their role in immunity seems to depend on the route of immunization as well as on antigen formulation [1,2]. To address more precisely the contribution of LCs in T cell priming, we employed Rac1(N17)-mice, which were previously shown to have defects in uptake of exogenous Ag and subsequent cross-presentation. In order to restrict this transgene-mediated deficiency to LCs, we generated mixed bone marrow chimeras (wt > Rac(N17); Rac(N17) > wt) and immunized them intradermally with particulate antigen. Here, LCs support priming of $CD8^+$ and $CD4^+$ T cells, as presence of the dominant negative transgene diminished T cell expansion substantially. We further investigated the role of LCs in the epicutaneous priming route, by targeting LCs with very low amounts of antigen. Under these conditions, $CD4^+$ T cell priming can be observed, while LCs do not prime $CD8^+$ T cells. In conclusion, LCs can enhance T cell priming by other skin DCs, but cannot prime themselves $CD8^+$ T cells.

[1] Kaplan D. H. In vivo function of Langerhans cells and dermal dendritic cells. Trends in Immunol. 31(12): 446-51, 2010.
[2] Romani N, et al. Langerhans cells and more: langerin-expressing dendritic cells subsets in the skin. Immunol Rev. 234:120-41, 2010.

Antigen targeting of Fc-receptors induces strong T cell responses *in vivo*

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Dendritic cells are very important antigen presenting cells (APC) and responsible for the initial induction of effective immune responses but also for the maintenance of peripheral T cell tolerance. We recently found that different DC subpopulations are able to induce different T cell responses after targeting antigens to the endocytosis receptors DEC205 and DCIR2 (33D1) *in vivo*. For understanding how immune responses can be controlled, we are studying the role of activating and inhibitory antigen uptake receptors. Beside C-type lectin receptors, Fc-receptors are highly efficient endocytosis receptors expressed on APCs. Fc-receptors control immune responses via activating (ITAM) and inhibitory (ITIM) motifs. We found that Fc-receptors were differentially expressed on the main DC-subpopulations as CD11c+CD8+ DCs express FcγRIIB (CD32), FcγRIII (CD16) and in low level FcγRIV; whereas CD11c+CD8- only express FcγRIV; and pDCs are positive for FcγRIIB (CD32), and FcγRIII (CD16). To study the role of activating and inhibitory motifs in the immune system, we have cloned the variable regions of a variety of anti-Fc-receptor-antibodies (Ly17.2-FcγRIIB, 2.4G2-FcγRII/III, 9E9-FcγRIV) in frame to a non-Fc-receptor-binding murine IgG1 constant region. For the immunological readout we genetically engineered the Ovalbumin model antigen into the C-terminal region. Here, we show that targeting antigens via recombinant Ovalbumin carrying Fc-receptor-antibodies to different DC-subpopulations and Fc-receptors induces different T cell responses *in vitro* and *in vivo* regarding the type and the subpopulation that was targeted.

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Increased anti-tumoral immunity and reduced skin-carcinogenesis in K14-RANKL transgenic mice

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The development of skin cancer is controlled by the immune system and innate as well as adaptive immune responses are crucially involved in the regulation of tumor growth. In a transgenic mouse model (K14-RANKL tg) over-expression of RANK ligand (RANKL, CD254) resulted in the peripheral expansion of regulatory T cells (Tregs) via interaction with RANK-RANKL activated Langerhans cells (LC). Since Tregs are potent suppressors of anti-tumoral immunity, we investigated the relevance of RANKL signaling during carcinogenesis. Surprisingly, in a two-stage chemo-carcinogenesis study K14-RANKL tg mice developed significantly fewer skin tumors compared to wildtype controls although tg mice exhibited increased numbers of functional Treg in secondary lymphoid organs after DMBA/TPA treatment. As demonstrated by immunohistology, tumors of wildtype mice showed a high degree of dysplasia whereas tumors of K14-RANKL tg mice presented as small papillomas with less invasive cells. Interestingly, in tumor draining lymph nodes from K14-RANKL tg mice the total numbers of CD8+ T cells were up-regulated compared to wildtype mice and additionally, these CD8+ T cells expressed higher levels of activation and cytotoxic markers such as CD43, granzyme B, Fas ligand, or the activating CD94 receptor (NKG2B/D). Moreover, gene expression analysis revealed elevated mRNA levels of perforin, IFN-gamma as well as RUNX3 and Eomesodermin (Eomes), both transcription factors associated with cytotoxic T lymphocyte (CTL) function. Since during skin carcinogenesis cutaneous antigen presenting LC migrate from the epidermis to regional lymph nodes and induce the differentiation and activation of CD8+ anti-tumoral effector T cells and since RANK-RANKL signaling has been shown to modulate LC phenotype, we analyzed the numbers and function of LC in tumor draining lymph nodes. Indeed, LC from tumor-bearing K14-RANKL tg mice showed an up-regulated expression of activation markers like CD80 and CD86, as wel as an increased IL-12 secretion. Furthermore, these LC exhibited a prolonged life-span and higher viability suggesting an increased T cell stimulatory capacity. To scrutinize this hypothesis we performed mixed lymphocyte reactions and could demonstrate up-regulated proliferation in CD8+ T cells upon co-culture with LC from tumor-draining lymph nodes of K14-RANKL tg mice compared to LC from wildtype controls most likely explaining the elevated numbers of CTL in regional lymph nodes from tg mice. Taken together, our data indicate that RANK-RANKL signaling seems to play an important role for the differentiation and activation of CTL via up-regulating the viability and modulating the phenotype of cutaneous antigen presenting LC thus, favoring rejection of tumors.

ISCOM vaccines activate the NLRP3 inflammasome and target antigen to dendritic cells *in vivo* for effective cross-presentation

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Cancer vaccines aim to induce CTL responses against tumors. Challenges for vaccine design are Ag targeting to DCs *in vivo*, facilitating cross-presentation and conditioning the microenvironment for Th1 type immune responses. Here we report that ISCOM vaccines, which consist of ISCOMATRIX adjuvant and protein Ag, meet these challenges. Subcutaneous injection of an ISCOM vaccine in C57BL/6 mice lead to a substantial influx and activation of innate and adaptive immune effector cells in draining lymph nodes. Cytokine arrays revealed high levels of IL-1b and IL-6. Interestingly, IL-1b production was dependnet on NLRP3 and ASC, thus identifying ISCOM vaccines as potent acivators of the NLRP3 inflammasome. Moreover, an OVA/ISCOM vaccine was efficiently taken up by CD 8+ DCs and induced their maturation and IL-12 production. Cross-presentation of OVA/ISCOM vaccine *in vivo* was mediated by CD8a+ DCs, whereas Langerin+ DCs were dispensable. The vaccine induced a high frequency of OVA-specific CTL capable of tumor cell killing in different tumor models. Thus, ISCOM vaccines combine potent immune activation with Ag delivery to DCs *in vivo*.

16.10 – 18.00 ***INVITED SPEAKER***

Patrizia Stoitzner, Innsbruck Medical University, Innsbruck, Austria

Langerhans cells in tumor immunity

Frank Nestle, St. John's Institute of Dermatology, King's College, London, UK

Harnessing dendritic cells in inflammatory skin diseases

09.00 – 10.20 ***INVITED SPEAKER***

Matthew Collin, Newcastle University, Newcastle upon Tyne, UK

Human dendritic cell deficiency

SangKon Oh, Baylor Institute for Immunology Research, Dallas, TX, USA

Harnessing human vaginal dendritic cell subsets for developing vaccines

The Role of Dendritic Cells in Psoriasis

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Psoriasis is a frequent, chronic, debilitating autoinflammatory disease of the skin. In this condition, the skin is affected by disorganization of the epidermis, resulting in loss of stratification as well as thickening and dedifferentiation of the corneal layer. Based on these observations an intrinsic defect of the keratinocyte layer has been proposed to be the primary event in disease initiation. Within psoriatic lesions, Langerhans cell (LC) numbers are often reduced compared to noninvolved skin, whereas plasmacytoid dendritic cell (pDC) numbers are increased. Recently, it was reported that mice harboring an inducible deletion of the AP-1 transcription factors c-jun and junB in the basal layer of the epidermis (jun/junBΔep) develop a psoriasis-like phenotype resembling human disease. Upon initiation of disease in jun/junBΔep mice, LC number in the epidermis was increased, however, as lesions progressed, LC numbers decreased. To study LC function in psoriasis, we crossed jun/junBΔep mice to Langerin-DTREGFP mice that can be inducibly depleted of Langerin+ cells by application of diphtheria toxin (DT). With this technique, we were able to deplete Langerin+ cells efficiently and consistently over an extended time period. Additionally, we were able to selectively deplete Langerhans cells and Langerin+ dermal DC, respectively by using bone marrow chimeric mice. The advent of another DT-based depletion strategy using BDCA2-DTR mice enables us to furthermore selectively deplete pDC from jun/junBΔep mice. These studies will provide important insights into the function of LC and pDC in psoriasis.

CD11c+ dendritic cells and CD163+ macrophages are the main source of TNF-alpha in plaque-type psoriasis

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The spectrum of tumor necrosis factor (TNF)-alpha-producing cells in psoriasis is not clearly defined. The elucidation of this question should allow us to better understand the mode of action, efficacy and, perhaps, also the risks of an anti-psoriatic treatment with TNF-alpha-antagonists. Using conventional immunofluorescence methods, we were not able to reproducibly detect TNF-alpha in sections of lesional psoriatic skin, but by the application of a tyramide amplification system, which allows the detection of even trace amounts of antigen, we obtained reproducible and firm stainings. TNF-alpha was exclusively found on dermal leukocytes co-expressing CD11c and HLA-DR and, to a lesser extent, CD163. This marker profile is consistent with that of mDCs and macrophages. Consistently, we found corresponding populations of TNF-alpha-producing mDCs and monocytes in PBMCs of psoriatic patients but not of healthy individuals. TNF-alpha+ mDCs of the peripheral blood were of the 6sulfo LacNAc (slan) rather than the BDCA-1 or BDCA-3 subtype, and their number closely correlated with disease activity. In addition, the total amount of slanDCs was increased in psoriatic patients compared to healthy controls with a concomitant decrease of BDCA-1+ DCs. Isolated slanDCs produced high amounts of TNF-alpha, IL-1beta, IL-6 and IL-23 upon stimulation with LPS or peptidoglycan. We could not find detectable levels of these cytokines in BDCA-1+ DCs. However, they produced high amounts of IL-10. Our data strongly suggest that certain myeloid cells (slanDCs, monocytes/macrophages) are the main source of TNF-alpha and other proinflammatory molecules in chronic plaque-type psoriasis and serve as a key target of TNF-alpha-antagonists.

CD103+Langerin+ Aortic Dendritic Cells Protect Atherosclerosis

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Early events in atherosclerosis occur in the aortic intima and involve monocytes that become macrophages. We looked for these cells in the steady state adult mouse aorta and surprisingly, we found a dominance of dendritic cells (DCs) in the intima. In contrast to aortic adventitial macrophages,CD11c+MHCII+ DCs were poorly phagocytic but immune stimulatory. DCs were of two types primarily: classical Flt3/Flt3L dependent, CD103+Langerin+CD11b-DCs and M-CSF dependent,CD14+CD11b+DC-SIGN+ monocyte-derived DCs. Both types expanded during atherosclerosis. By crossing Flt3-/- to Ldlr-/- atherosclerosis-prone mice, a selective and marked deficiency of classical CD103+ aortic DCs developed and was associated with exacerbated atherosclerosis without alterations in blood lipids. Concomitantly, the Flt3-/-Ldlr-/- mice had less Foxp3+ Treg and increased inflammatory cytokine mRNA in the aorta. Therefore, functional DCs rather than monocytes are dominant in normal aortic intima and in contrast to macrophages, at least CD103+ classical DCs are associated with atherosclerosis protection.

Langerin+ Dendritic cells in the lung and their role in a HDM-induced allergic airway inflammation.

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In the lung, cDCs can be discriminated by their CD11b expression, of which the CD11b- DC population expresses CD103 and co-expresses langerin. So far, the function of lung DC subsets has been studied in the context of viral infection, but little is known about their function in the context of allergic diseases. Here, we tried to elucidate the role of the different lung DC subsets in a HMD-induced allergic airway inflammation. We found that both subsets could capture fluorescent HDM administered in the airways. We also found that langerin-DTR mice administered with a low dose of HDM failed to get sensitized to the allergen. However, when langerin-DTR mice were injected with diphtheria toxin (DT) at the time of HDM administration , they developed a severe allergic inflammation in the lung, accompanied with substantial production of Th2 cytokines. To test the role of the langerin+ DC subset in Th2 sensitization, both CD11b+ and langerin+ DCs were sorted from the mediastinal lymph node (MLN) of HDM-injected mice. These cells were adoptively transferred intratracheally into naive recipients, which were rechallenged with HDM allergens a week later. Strikingly, with only very few dendritic cells adoptively transferred to recipient mice, only CD11b+ DCs were able to induce sensitization to HDM, as seen by an eosinophilic influx to the lung, and the Th2 cytokine profile in the supernatant of re-stimulated MLN cells. Langerin+ DCs were not able to sensitize the recipient mice to HDM. All together, these results suggest a regulating role for the langerin+ DC subset in lung allergic responses. These data will help to better understand the role of the different subsets in the lung and their contribution to allergic airway inflammation.

Effects of epidermal barrier disruption on vitamin D3-induced atopic dermatitis-like inflammation in mice

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Topical vitamin D3 (VitD3) induces an atopic dermatitis (AD)-like inflammation by upregulating the expression of thymic stromal lymphopoietin (TSLP) in keratinocytes and requires the presence of epidermal Langerhans cells (LC). According to the hypothesis that TSLP-induced skin inflammation is aggravated by skin barrier impairment, Balb/c mice were topically treated with VitD3 after disruption of the epidermal skin barrier by tape stripping. Tape stripping prior to topical VitD3 worsened AD-like skin inflammation, including hyperkeratosis, epidermal hyperplasia and inflammatory infiltrates, when compared to controls treated with VitD3 only. In contrast, epidermal barrier disruption did not further enhance levels of plasma IgE and TSLP expression by keratinocytes, induced by topical VitD3. Furthermore, tape stripping combined with topical VitD3 induced greater cell numbers in skin draining lymph nodes, when compared to control mice treated with VitD3 alone. However, the VitD3-induced increase in numbers of activated CD4+ T cells, CD4+ CD25+ FOXP3+ regulatory T cells and emigrated dendritic cells (DC) in skin draining lymph nodes was not significantly further enhanced by epidermal barrier disruption. These results suggest that abnormal immune reactivity and impaired skin barrier function synergize in eliciting local AD-like symptoms.

Langerhans cells play a key role in the development of atopic dermatitis via TSLP receptor signaling

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Dendritic cells (DCs) play an important role in the initiation of acquired immune responses. In the skin, there exist at least two populations of DCs, epidermal Langerhans cells (LCs) and dermal DCs. It has been reported that cutaneous DCs mediate the pathogenesis of atopic dermatitis (AD), but it remain unknown which subset is directly involved. Using Langerin-diphtheria toxin receptor (DTR) mice, we demonstrated that specific Langerhans cells (LCs) depletion showed much milder clinical manifestations together with lower serum IgE levels in a topical OVA application-induced AD-like mouse model. In addition, LC depletion impaired OVA-specific T cell proliferation and IL-4 production in the draining lymph nodes. Thymic stromal lymphopoietin (TSLP) is known to be highly expressed in the keratinocytes of the AD skin lesions and influences on DCs to induce Th2 responses. However, it remains unknown how TSLP induces the establishment of AD. In this study, we used newly generated LC-specific TSLP receptor-deficient mice using bone-marrow chimeric technique and applied these mice to the OVA-induced AD-like mouse model. We found that LC-specific TSLP receptor-deficient mice showed much milder clinical and histological findings together with lower IL-4 and IgE levels than control mice. Taken together, here we showed for that first time that epidermal LCs are the responsible cutaneous DC subsets which mediate the development of AD directly through TSLP-TSLP receptor signaling.

NOTCH signaling is constitutively active in Langerhans cell histiocytosis and confers key LCH features on dendritic cells.

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Langerhans cell histiocytosis (LCH) is an enigmatic disease defined by the accumulation of eponomous Langerhans-cell-like cells. Here we demonstrate that LCH cells exhibit a unique and distinct transcription profile that clearly separates them from autochthonous DC, i.e. LC, MDC and PDC. Molecular analysis revealed that LCH cells selectively expressed Notch ligand Jagged 2 (JAG2) and were the only DCs that coexpressed both Notch ligand and receptor. In line, activated Notch was detected in LCH but not control biopsies. Furthermore, we show that Jagged 2 signalling induces key LCH markers CD1a and Langerin on MoDCs independent from IL4, suggesting a role of Notch signalling in LCH ontogenesis. Interestingly, JAG2 also strongly induced matrix-metalloproteinases (MMPs) 1, 9 and 12 in MoDCs, which are highly expressed in LCH lesions and mediate massive tissue destruction and remodelling. The MMP induction by Jagged 2 was selective for DCs and not recapitulated in monocytes. Together these findings strongly suggest that Jagged2 mediated Notch activation confers phenotypic and functional aspects of LCH to DCs. Thus, interference with Notch signalling may prove an attractive target to combat this disease.

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P01 The Adaptor Protein Bam32 in Human Dendritic Cells Participates in the Regulation of MHC Class I-Induced CD8+ T Cell Activation

*Daniela Ortner, Daniela Grabher, Martin Hermann, Elisabeth Kremmer, Susanne Hofer and Christine Heufler**

The B lymphocyte adaptor molecule of 32 kDa (Bam32) is strongly induced during the maturation of dendritic cells (DC). Most known functions of Bam32 are related to the signaling of the B cell receptor for Ag. Because DC do not express receptors specific for Ag we aim at characterizing the role of Bam32 in human monocyte-derived DC in this study. Our results show that binding of allogeneic T cells to mature DC causes an accumulation of Bam32 on the contact sites and that this translocation is mimicked by Ab-mediated engagement of MHC class I. Silencing of Bam32 in mature monocyte-derived DC results in enhanced proliferation of CD8+ T cells using an Ag-specific T cell proliferation assay. Further studies identify galectin-1 as an intracellular binding partner of Bam32. Regulating immune responses via regulatory T cell modulation is one of many immunological activities attributed to galectin-1. Therefore we assayed mixed leukocyte reactions for regulatory T cell expansion and found fewer regulatory T cells in reactions stimulated with DC silenced for Bam32 compared to reactions stimulated with DC treated with a nontarget control. Based on our findings we propose a role for Bam32 in the signaling of MHC class I molecules in professional Ag-presenting DC for the regulation of CD8+ T cell activation. It is distinct from that of MHC class I recognized by CD8+ T cells leading to T cell death. Thus, our data indicate a novel level of T cell regulation that may be of biological relevance.

P02 Langerin-Cre knock-in mice: A novel tool to achieve conditional gene targeting in Langerin+ DC beyond LC

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Originally, expression of the C-type lectin Langerin was considered to be specific for Langerhans cells (LC). In the meantime, Langerin-expressing DC populations that are distinct from LC have been identified in lymphoid and non-lymphoid tissues. To date, the role of these Langerin+ DC in regulating immunity and tolerance is largely unknown. Moreover, the molecular cues governing Langerin+ DC homeostasis and function remain elusive. To this aim, we have generated Langerin-Cre knock-in mice that drive robust and specific DNA recombination in LC and other Langerin+ tissue DC. Breeding of these mice to a red fluorescent protein (RFP) Cre-reporter strain revealed a complete overlap of Langerin-Cre/RFP expression and MHCII in epidermal LC. Moreover, all CD103+ dermal DC were RFP-positive. In contrast, only about 30% of the CD103+ CD11bneg DC in lung tissue expressed Langerin-Cre/RFP, which suggested the existence of two distinct CD103+ pulmonary DC subsets. This was confirmed in diphtheria toxin-treated Langerin-DTR mice, which lacked all Langerin+ DC, but retained about 70% of the CD103+ DC in the lungs. In agreement with the requirement of TGF-β signaling during LC ontogeny, crossing Langerin-Cre to mice homozygous for a loxP-flanked TGF-β receptor 1 allele (LDC-TβR1KO) resulted in a permanent loss of LC. Similar to LC-depleted Langerin-DTR mice, induction of contact hypersensitivity (CHS) in LDC-TβR1KO mice elicited diminished ear swelling as compared to controls. Therefore, this novel LC-deficient mouse model provides further evidence against a regulatory function of LC in CHS.

P03 A three-dimensional full-thickness skin model fulfills all criteria to test the differentiation potential of Langerhans cell precursor candidates

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Previous work in mice and men suggests that undifferentiated leukocytes colonize embryonic epidermis. These precursors eventually acquire the cell surface marker profile of Langerhans cells (LCs), depending either on the epidermal microenvironment or their lineage. Despite numerous studies, the exact phenotype of LC precursors is not known. In embryonic human epidermis, we occasionally observed CD45+ leukocytes which are negative for CD1a, CD1c, HLA-DR, CD36, CD14, and CD207. We have used a three-dimensional full-thickness skin model to compare the integration and differentiation potential of defined LC precursor candidates and, thus, to recapitulate the *in vivo* situation. Using immunohistochemistry and ELISA we found that our skin equivalents produce TGF-ß1 and GM-CSF, key cytokines required for the development of LCs. Subsequently, we tested the differentiation capacity of potential LC precursors phenotypically resembling those observed *in vivo* during ontogeny. CD34+ hematopoietic stem cells were differentiated using GM-CSF, Flt3L, SCF, and TNF-alpha for seven days. FACS-sorted, CD207- populations (CD14+CD1a-, CD14-CD1a+, CD14-CD1a- cells) were then co-cultured with primary keratinocytes to allow the integration of the precursor cells in the newly formed epidermis of the skin model. Dendritic, CD207+ cells were detectable in all epidermal fractions already after eight days with a frequency of 188.5 vs. 116 vs. 32.1 cells/mm2 (n=2) in the CD14-CD1a+, CD14-CD1a- and CD14+CD1a- fraction, respectively. Taken together, we found that CD14-CD1a+ progenitor cells possess a much higher differentiation capacity than CD14+CD1a- progenitor cells.

P04 VDR beta-catenin and TGF-beta1 signaling co-operate in inducing differentiation and epithelial gene expression by Langerhans dendritic cells

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Epithelial Langerhans dendritic cells (LCs) are unique among dendritic cell (DC) family members in that their differentiation depends on transforming growth factor-beta1 (TGF-b1). Within the skin, TGF-b1 is constantly produced by epithelial cells of the epidermis (Keratinocytes, KC) as well as by Langerhans cells (LCs) themselves and is required for LC differentiation as well as the expression of epithelial cell junction components E-cadherin and Claudin-1 by LCs. Moreover, E-cadherin and TGF-b1 have been implicated in LC's tolerogenic activity. Because LCs form junctional contacts within epithelial tissues, we studied whether TGF-b1 signaling may induce the expression of various junctional molecules in LC precursors. Indeed our data suggest that multiple epithelial genes are expressed during LC differentiation in the presence of TGF-b1. We investigated the transcriptional regulation of epithelial genes during LC differentiation. The transcription factors vitamin D receptor (VDR) and beta-catenin are co-induced by TGF-b1 during LC differentiation induction. Since both beta-catenin or VDR signaling have independently been implicated in DC-mediated tolerance induction and both synergize in regulating epithelial genes in carcinoma cells, we further studied their role in LC differentiation. We found that activation of beta-catenin or ectopic expression of VDR signaling enhances LC differentiation and increase epithelial gene expression by LCs. The VDR ligand 1,25 vitamin D3 (VD3) enhances the co-expression of TGF-b1, beta-catenin and VDR in LC precursors indicating a positive-regulatory circuitry established by these molecules. Since VDR and beta-catenin physically interact and VD3 mediates beta-catenin binding to VDR resulting in VDR target gene induction, we investigated whether VDR requires beta-catenin for enhancing LC differentiation. A VDR mutant lacking beta-catenin interaction domain (VDR.deltaAF2) indeed failed to promote LC differentiation, and VD3 synergized with beta-catenin in promoting LC differentiation and E-cadherin induction. We could confirm functional interactions of both factors by using TCF4/beta-catenin and TCF4/beta-catenin/VDR reporter gene assays. Therefore, VDR-beta-catenin and TGF-b1 signaling synergism positively regulates LC differentiation as well as epithelial gene expression by LCs. This gene circuit may both induce LC differentiation and LC's tolerogenic capacity.

P05 Conditional Gene Ablation of the MAP Kinase Adapter Protein p14 in Dendritic Cells induces a Myeloid Proliferative Disorder

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Dendritic cells are key players of the immune system and link innate to adaptive immune responses. Their major task is the uptake and processing of pathogens and subsequent presentation of antigens to T cells. These processes strongly depend on endosomal/lysosomal trafficking. Conditional gene disruption of the adapter protein p14 in mice demonstrates that the late endosomal p14/MP1-MEK1 signaling complex is required to control endosomal traffic and tissue homeostasis (Teis et al., J Cell Biol, 2006). To address the molecular function of p14 in dendritic cells, we generated a conditional knock out mouse model, which allows the specific deletion of p14 in CD11c expressing cells. The effects were analyzed in tissue (histological methods, FACS, ELISA) and primary cell culture (Western Blot). The knock out mice were viable but developed a severe pathological phenotype resembling a myeloid proliferative disorder (MPD) at the age of two to three months. The most obvious morphological symptoms included enlarged lymph nodes and splenomegaly. The structural morphology of these organs was disarranged and massive leukocyte infiltrates were observed, which could further be identified as dendritic cells. Additionally, the mice developed infiltrates of monocytes and activated dendritic cells in skin and liver. These infiltrates were also surrounded by single T cells being known as the direct interaction partners of activated dendritic cells. The bone marrow of the CD11c-p14 knock out mice was hyperplastic, accompanied by an increase of hematopoietic stem cells. Furthermore a MPD characteristic shift from the granulocytic towards the monocytic/dendritic cell lineage, an increase in the T helper cell population and a decrease of the erythrocyte progenitors were observed. In the serum of the CD11c-p14 knock out mice at the age of 1 to 6 months, Flt3-ligand, a specific cytokine inducing conventional dendritic cell differentiation, was significantly elevated. Additionally, its receptor Flt3 showed an increased surface localization on splenic dendritic cells. Similar observations were made in p14 depleted keratinocytes where the degradation of the EGF receptor was severely disturbed leading to an accumulation on the plasma membrane (Teis D. et al., 2006, JCB). The accumulation of the receptor on the cell surface and the enhanced availability of its ligand resulted in an increased downstream signaling of Flt3 shown by the phosphorylation of AKT and the mTOR target p70 S6 kinase 1. This pathway downstream of the Flt3 receptor is known to be crucial for dendritic cell differentiation (Sathaliyawala T. et al., 2010, Immunity). Finally we can conclude that p14 deletion in dendritic cells severely affects their tissue homeostasis and leads to a MPD.

P06 Toll-like receptor ligand-induced migration: comparisons of *in vivo* Langerhans' cells and the XS106 skin dendritic cell line

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Langerhans' cells (LC) are a subset of dendritic cells (DC) found in the epidermis. Expression of chemokine receptors on the surface of LC allows their migration to the lymph nodes, where they can initiate an immune response. Migration of XS106 cells, a DC cell line isolated from murine epidermis, was analysed using a Transwell migration assay. Migration of LC following intradermal injection of BALB/c strain mice with various toll-like receptor (TLR) ligands was assessed in epidermal sheets by immunofluorescence staining for MHC class II. Unstimulated XS106 cells migrated towards CXCL12, but not to CCL19, chemokines which cause migration of LC to the dermis and lymph nodes, respectively. XS106 also migrated towards supernatants from the skin-derived fibroblast cell line NS47. A CXCR4 inhibitor (AMD3100) blocked CXCL12-dependent migration, but did not inhibit the migration induced by the NS47 supernatant. After 24h stimulation with the TLR4 ligand lipopolysaccharide (LPS), migration to CXCL12 and to CCL19 was down regulated and increased, respectively. Stimulation with the TLR ligands peptidoglycan and flagellin also decreased migration towards CXCL12, whereas migration after stimulation with CpG and R-848 remained at baseline levels. In contrast, all of the TLR ligands investigated stimulated the *in vivo* migration of LC. These data suggest that migration of LC *in vivo* after encounter with CpG or R-848 may be secondary to interaction of the TLR ligands with other cells, likely keratinocytes. Further work will be conducted to determine whether *ex vivo* LC can respond to CpG and R-848 *in vitro* in the absence of keratinocytes.

P07 The requirement of branched actin networks for dendritic cell physiology

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Major events in dendritic cell (DC) physiology like antigen uptake, migration to the draining lymph node and stimulation of naïve T cells depend on the coordinated remodeling of the actin cytoskeleton. Actin networks are established by both crosslinked filamentous actin bundels and branched lattice-like structures. Their dynamics rely on the constant turnover of actin filaments by cycles of polymerization and depolymerisation. How geometry and dynamics of actin filaments affect dendritic cell function hasn't been assessed yet. Therefore we aim to describe the necessity and importance of branched actin networks on DC morphology, migration and interaction with T cells. Whereas de novo assembly (nucleation) of actin filaments is mediated by several actin nucleators the only known "actin brancher" is the Arp2/3 complex, whose activity is dependent on the association with nucleation promoting factors (NPFs). One of the major NPFs is the multimeric WAVE complex, which functions downstream of Rac-GTPase signaling. Its assembly was shown to be essential for lamellipodia formation and the absence of each single component of the complex results in the destabilization of the whole complex. A specific subunit of the hematopoietic WAVE complex is the HEM1 protein. We characterize the consequences of WAVE complex ablation, and the resulting loss of branched actin filaments, on DC maturation and the abovementioned DC functions. Lack of hem1 in bone-marrow derived DC cultures does not affect DC differentiation and maturation but results in pronounced morphological alterations. Surprisingly, WAVE complex deficient DCs are able to migrate *in vitro* and *in vivo*, however, with decreased velocity and a modified migratiory behaviour. Similarly, hem1-/- DCs are able to prime naïve T cells with the same strength as wildtype DCs but the frequency of DC-T cell interactions seems to be reduced. Taken together, these results indicate a general importance of branched actin networks in DC physiology but also show that actin remodeling doesn't exclusively rely on branching but can be at least partially compensated by linear actin nucleation and elongation mechanisms.

P08 An immobilized chemokine gradient guides dendritic cell migration to afferent lymphatics

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During their steady-state migration between peripheral tissues and lymphoid organs, dendritic cells (DCs) encounter different types of barriers that they have to cross. One example is the interstitium of the skin dermis, where DCs have to crawl through arrays of fibrillar collagen bundles and penetrate the basement membrane (BM) of lymphatic vessels in order to gain access to a draining lymph node. Using an *ex vivo* mouse ear crawl-in assay that allowed the visualization of DC migration in this three-dimensional environment in real time, it was shown that DCs neither require integrin mediated adhesion nor pericellular proteolysis to reach and enter lymphatics, but rather navigate there in a chemokine receptor 7 (CCR7)-dependent manner through coordinated shape changes and cytoskeletal flow. Although the chemokine signal is the decisive cue in this system, it is not understood how chemokines are distributed within the interstitium and how chemokines guide the directional migration of DCs towards and into lymphatic vessels. CCL21, the heparan sulfate-anchoring ligand of CCR7, is produced by lymphatic endothelial cells from where it might distribute and form an immobilized gradient. CCL19, the second ligand of CCR7, is expressed by DCs and largely distributes in the soluble form as it has lower affinity towards extracellular matrix. We find that DCs approach lymphatic vessels in a directed manner and provide evidence that they follow a graded pattern of extracellularly anchored CCL21. Interstitial CCL21 is visible over a relatively short distance emanating from the lymphatic vessels. When this gradient is disturbed by addition of exogenous CCL21 or enzymatic removal of endogenous CCL21, DCs no longer find their way into the lymphatic vessels.

P09 The applicative impact of hapten DNCB and zeolite microparticles on the LC/dDC migration and bone marrow hemopoiesis

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High accessibility of skin and the presence of immunocompetent cells in the epidermis have lately drawn the attention of the developers of new generation vaccines. At the same time, sweeping use of biologically active and immunogenic ingredients in cosmetics and hygienic products can produce allergic reactions in consumers of such products. We studied the effect of the epicutaneous application of contact allergen DNCB and zeolite mineral particles water suspension (ZMC, particle size ≤ 100 μm) on the leukocytes emigration intensity from skin and on the clonogenicity of Balb/c mice bone marrow cells (BMC). Skin applications of mice TNF-α and saline were used as positive and negative controls. Applications were carried out on the dorsal surface of both ears of Balb/c mice. Leukocytes emigration was determined after single impact by Larsen's method in skin-patch 24 h culture. In migratory leukocytes populations (without any enrichment) leukocytes expressing CD11c and CD86 were detected using flow cytometry methods. BMC were explored after triple impact: BMCs were grown in methylcellulose medium supplemented with murine growth factors. Hematopoietic colonies (BFU-E + CFU-E, CFU-GM and CFU-GEMM) were counted on day 14 . RESULTS: Applications of rmTNF-α in the dose 500 ng/mouse, ZMC or 1% DNCB stimulate leukocyte emigration from mice skin (210%, 283% and 372% as compared with control). CD11c/CD86-positive cells account for 46.5%, 36.8% and 16.4% of migratory cells, respectively. Epicutaneous applications of ZMC, TNF-α and DNCB influence the formation of different types of colonies in mice BM: TNF reduces erythroid colonies, DNCB increases CFU-GM-colonies and early mixed-colonies, but reduces erythroid colonies; ZMC increases all types of BM colonies. CONCLUSIONS: These observations led us to speculate about a possible influence of skin applications of simple chemicals and natural mineral microparticles on the induction of bone marrow hemopoiesis; maturation status of migrating skin DC and local changes of proinflammatory cytokines, may be, manage the bone marrow.

P10 Evidence for functional cross-talk between TLR2 and the high affinity receptor for IgE (FcεRI) on Langerhans cells.

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Atopic dermatitis (AD) is a severe chronic skin disease, with increasing incidence. Patients suffering from early onset AD have a higher risk of developing asthma and allergic rhinitis. We and others have previously shown that in contrast to epidermal Langerhans cells (LC) from healthy donors, LC from AD patients express a trimeric form (alpha gamma2) of the high affinity receptor for IgE, FcepsilonRI. It is well known that the skin of AD patients is highly colonized by Staphylococcus aureus (SA). Thus we hypothezised that innate receptors for SA may be involved in the pathology of AD by impacting on the functional behaviour of epidermal LC and could show that TLR2 is upregulated on LC in a subgroup of AD patients. To study the impact of TLR2 on FcepsilonRI, we established a model of immature LC generated from CD34+ hematopoietic stem cells expressing both receptors. While there is similar surface-expression of TLR2 on CD34LC from different donors, FcepsilonRI is expressed heterogeneously. We could show that treatment of CD34LC with the TLR2 ligand Pam3Cys induces LC maturation. Though no early internalization of either TLR2 or FcepsilonRI was observed and TLR2 surface expression is little affected after 24 h, surface FcεRI is downregulated rather on mRNA level, as we could pinpoint by quantitative PCR. Additionally we could show that mRNA of both components of the trimeric FcepsilonRI receptor (alpha gamma2) is reduced in CD34LC after stimulation with Pam3Cys. Taken together, we show a direct impact of TLR2 stimulation on the expression profile of FcepsilonRI on LC. This interaction may contribute to the pathophysiological role of LC in AD.

P11 Vitamin D induces regulatory T cells by modulating Langerhans cells

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Painting of haptens onto ultraviolet radiation (UVR)-exposed skin does not result in sensitization but induces regulatory T cells (Treg). Emigration of UVR-damaged but still viable Langerhans cells (LC) from the epidermis into the regional lymph nodes is essential for the induction of Treg. Topical application of 1α,25-Dihydroxyvitamin D3 (VD3) was recently found to induce Treg as well. To study whether VD3 similarly to UVR induces the emigration of damaged LC from the epidermis into the lymph nodes, C57/B6 mice were painted with VD3 on the ears and backs for four days. On day 5 mice were sensitized with dinitrofluorobencene, 48 hours later ears and the regional lymph nodes were obtained. Immunofluorescence analysis of ear sheets using an anti-MHC class II antibody revealed upon VD3 treatment a dramatically reduced number of LC which were also altered in their morphology. FACS analysis of cells suspensions from the regional lymph nodes revealed an increased number of cells staining both for Langerin and annexin V, indicating that VD3 damages LC ultimately undergoing apoptosis in the lymph nodes. *In vitro* incubation of LC with VD3 resulted in downregulation of MHC class II and B7-2 expression. Together this suggests that VD3 damages LC which downregulate surface molecules relevant for antigen presentation and finally undergo apoptosis. This implies that VD3 might induce Treg in a similar fashion like UVR.

P12 The Role of SWAP-70 in Tolerogenic Maturation of Murine Dendritic Cells

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Dendritic cells (DCs) are known to be the most efficient antigen presenting cells and act as a key regulator of the immune system. In contrast to the signaling pathways in DCs that lead to T cell activation during inflammation, relatively little is known about the signals leading to tolerance. Recent observations suggest an important role of β-catenin and TGF-β in tolerogenic functions of DCs(1). Murine SWAP-70 is expressed in hematopoietic cells including DCs obtained from bone marrow precursors grown in the presence of GM-CSF (BMDCs). SWAP-70 also controls surface localization of peptide-loaded MHCII on BMDCs(2) and interacts with F-actin, Rac and RhoA-GTP. Unlike wild-type (wt) BMDCs, Swap-70-/- BMDCs show constitutively active RhoA2. Recently, it was also found that SWAP-70 regulates S1P-induced migration in BMDCs through RhoA activation(3). Experiments showed that Swap-70-/- BMDCs exhibit an increased percentage of spontaneously activated cells at day 11 of culture as depicted by upregulation of surface CD86, CD80, B7-DC, MHCII and CCR7 as well as downregulation of E-cadherin molecules, compared to wt BMDCs. This *in vitro* phenotype is independent of GM-CSF source or concentration during the culture. Spontaneously activated Swap-70-/- BMDCs are able to activate OVA-specific OTI CD8+ T cells *in vitro* which has been reported to be a feature of tolerogenic DCs(4). Consistent with a recent publication by Brian Vander Lugt et al.(1), TGF-β incubation during culture abrogates the spontaneous activation of Swap-70-/- BMDCs as well as of wt cells. Mixed cultures of wt and Swap-70-/- BMDCs result in increase and decrease spontaneous activation respectively, when compare to single cultures. This suggests that a soluble factor or cell-cell contact might play a role in the increased spontaneous activation observed in Swap-70-/- BMDCs. These results indicate that SWAP-70 plays a role in regulating tolerogenic activation of DCs. Whether this control is through TGF-β and β-catenin signaling remains to be investigated. Our Swap-70-/- ko mouse model might be a potent tool to study the signals that lead to tolerogenic activation of DCs.

P13 Langerhans cells suppress the immune response to a commensal bacteria

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Epithelial surfaces form interfaces to the environment and serve as a barrier to protect the organism from physical damage, dehydration and infection. These barriers, such as the skin, are colonized with commensal microorganisms, which contribute to protection and health of the organism. Langerhans cells reside in the epidermis where they are ideally positioned to sample the external milieu and may regulate the immune response to commensal skin flora. To test this hypothesis, we cultured bacteria from ears swabs of wild type and mice with a constitutive absence of Langerhans cell (i.e. huLangerin-DTA). The number of colony forming units was similar in both lines. Surprisingly, based on analysis of 16S rRNA sequences, we found that the vast majority of the bacterial isolated belonged to a single species, the gram positive actino-bacterium Rothia nasimurium. The ubiquity of this species in all animals allowed us to examine the immune response to a naturally occurring skin commensal bacteria. Footpad challenge with heat killed R.nasimurium without prior immunization resulted in considerable footpad swelling in WT mice. In contrast, challenge with the non-commensal bacteria, S.aureus, P. aeroginosa and S.pyogenes did not result in appreciable footpad swelling. Interestingly, footpad challenge with R.nasimurium was significantly increased in Langerin-DTA mice but was unaffected in huLangerin-DTR mice in which LC are ablated shortly before challenge. These data suggest that in the steady-state, the immune system is able to recognize and respond to skin commensal organisms. Furthermore, the constitutive presence of Langerhans cells is required to limit the extent of priming but does not affect the efferent immune response.

P14 1,25VitaminD3 differentially targets epidermal and dermal skin dendritic cells for the induction of distinct types of regulatory T cells

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The vitamin D metabolite 1,25(OH)2D3 (VitD3) is a potent immunosuppressive drug and, among others, is used for topical treatment of psoriasis. A proposed mechanism of VitD3-mediated suppression is priming of dendritic cells (DCs) to induce regulatory T (Treg) cells. Currently, there is confusion about the phenotype of VitD3-induced Treg cells and the DC-derived molecules driving their development. We investigated Treg cell induction after VitD3 priming of 2 distinct skin DC subsets: Langerhans cells (LCs) and dermal dendritic cells (DDCs). LCs and DDCs primed with VitD3 were cocultured with allogeneic naive T cells. The phenotype and function of the DCs and induced T cells were analyzed. Both VitD3-primed DC subtypes induced T cells with regulatory activity. Unexpectedly, whereas the Treg cell populations generated by VitD3-primed LCs were CD25(hi)CD127(lo) forkhead box protein 3 (Foxp3)-positive cells, which meet the criteria of classical inducible Treg cells, the T cells developing in response to VitD3-primed DDCs were Foxp3(-) T(R)1 cells expressing IL-10. Inhibition experiments revealed that LC-derived TGF-β is a key factor in the induction of Foxp3(+) Treg cells, whereas DDC-derived IL-10 is important for the induction of IL-10(+) T(R)1 cells. Thus we report the novel finding that distinct but closely related DC subsets are differentially programmed by VitD3 to support development of either TGF-β-dependent Foxp3(+) Treg cells or IL-10-dependent IL-10(+) Treg cells.

P15 Epigenetic regulation of dendritic cell differentiation and function by oxidized phospholipids

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Dendritic cells (DCs) are the key cell type in the regulation of an adaptive immune response. Under inflammatory conditions monocytes can give rise to immunostimulatory DCs, depending on microenvironmental stimuli. Here we show that oxidized phospholipids (Ox-Pls), which are generated during inflammatory reactions, dysregulate the differentiation of DCs. DCs generated in the presence of Ox-Pls up-regulated the typical DC marker DC-SIGN but did not express CD1a, CD1b, and CD1c. These DCs generated in the presence of Ox-Pls had a substantially diminished T cell–stimulating capacity after stimulation with Toll-like receptor ligands. Toll-like receptor ligand–induced production of interleukin-12 also was strongly diminished, whereas induction of CD83 was not altered. In addition, we found that Ox-Pls strongly inhibit inflammatory stimuli-induced phosphorylation of histone H3, a key step of interleukin-12 production, yet leaving activation of nuclear factor-kB unaltered. Taken together, Ox-Pls present during differentiation yielded DCs with a reduced capacity to become immunostimulatory mature DCs. Furthermore, the presence of Ox-Pls blocked histone modifications required for full activation of DCs. Therefore, inflammation-derived Ox-Pls control DC functions in part by epigenetic mechanisms.

P16 Human three-dimensional full-thickness skin models for Langerhans cell research

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Studies regarding Langerhans cells (LCs), the immature dendritic cells of the epidermis, are somehow hampered by the difficult cultivation *in vitro*. Here we show a new three-dimensional full thickness skin model consisting of primary human keratinocytes and fibroblasts and integrated Langerhans-like cells. Langerhans-like cells were derived from MUTZ-3 cells, a CD34+ human acute myeloid leukemia cell line. This cell line can be differentiated into Langerhans-like cells (MUTZ-3-LCs) in the presence of a cytokine cocktail including GM-CSF, TGF-β1 and TNF-α. As differentiation markers served the expression of langerin, CD1a, CCR6 and the intracellular presence of Birbeck granules. For the integration of MUTZ-3-LCs into a three-dimensional full thickness skin model a mixture of keratinocytes and MUTZ-3-LCs were seeded on top of a fibroblast-containing collagen matrix (Henkel AG & Co. KGaA, Düsseldorf) and cultured for 24 hours. Subsequently, the models were lifted up to the air liquid interface. Histological evaluation featured a fully stratified epidermis with all characteristic epidermal strata. Langerin-positive cells were detected suprabasally within the epidermis indicating that keratinocytes and/or fibroblasts provide environmental conditions for long-time maintenance of MUTZ-3-LCs. These skin models provide a relevant research tool to study LC biology *in vitro*. Particularly, the interactions between LCs and other skin cells and their contribution in cutaneous immune responses can be investigated.

P17 Langerin+ Dendritic Cells play a role in susceptibility to Squamous Cell Carcinoma

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Squamous Cell Carcinoma is one of the most frequent types of non-melanoma skin cancer. Since tumor incidence is increased in immunocompromised individuals we are interested in investigating the role of skin resident and tumor infiltrating denritic cells in immunosurveillance of skin tumors. Tumors resembling Squamous Cell Carcinoma were induced by a two-stage carcinogenesis model in Langerin-DTR mice. Tumor onset and tumor numbers were evaluated and excised tumors were analysed for tumor-infiltrating cell populations by FACS. Initial experiments showed that tumors contained elevated numbers of Dendritic Cells, myeloid-derived suppressor cells and regulatory T cells compared to tumor-free skin. Longterm depletion of Langerin+ Dendritic Cells during the two-stage carcinogenesis revealed that mice depleted of Langerin+ cells developed tumors earlier and in higher numbers compared to control Langerin-DTR mice. These observations suggest a critical effect of Langerin+ Dendritic Cells on tumor induction and early responses to those tumors. Future experiments will examine the impact of tumor-infiltrating cells on the cytokine milieu and antigen-presenting capability of tumor-resident Dendritic Cells. The expected findings will be key to enlighten the role of skin Dendritic cells in tumor induction.

P18 Langerhans cells in psoriasis

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Psoriasis is a chronic inflammatory skin disorder, in which a vigorous infiltration of T cells, neutrophils and dendritic cells (DCs) occurs into the skin. DCs is a heterogeneous cell population that exhibit a wide complexity in inflamed skin, yet with little known about their role in psoriasis. DCs residing in healthy epidermis, known as Langerhans cells (LCs), migrate to regional lymph nodes upon antigen uptake. The exact role of LCs in infection and inflammation is not known despite excessive studies on these cells in mice and human. Impaired epidermal LC mobilization has been shown in non-lesional skin from psoriasis patients and we propose that they might have a local immune-modulatory role in the inflamed skin. Human LCs are characterized by expression of the C lectin Langerin, CD1a, EpCAM and HLA-DR. We have found that Langerin expressing DCs are present in dermis of psoriasis patients. Electron microscopy will be used to detect the LC-specific intracytoplasmic organelle, known as Birbeck granule, within dermal Langerin expressing cells in order to prove that these cells are true LCs originating from the epidermis. In order to investigate if LCs act as local immune modulators in the skin of psoriasis patients, skin biopsies are collected from lesional and non-lesional areas of untreated patients with different phenotypes, as well as from healthy subjects. Epidermal cells are separated from underlying dermis and DC phenotyping is performed using FACS and immunofluorescence. To further assess the inflammatory environment, RNA profiles will be analyzed in FACS sorted LCs using realtime-PCR.

P19 Characterisation of Imiquimod effects in the skin dependent on the presence of Langerin+ cells

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Imiquimod (IMQ) is a small molecule immune response modifier that has become a model for successful immunological treatment of cancer. Topical treatment of basal cell carcinoma with IMQ leads to tumor regression accompanied by a strong immune cell infiltrate in almost all patients treated. Langerhans cells (LC) seem to be part of this immune response since they show an activated morphology and increased migration to skin draining lymph nodes in response to IMQ treatment. To better characterize the contribution of LC to the effects of IMQ we analyzed the infiltrates into the skin of IMQ treated Langerin-DTR mice. Macroscopically the skin reacted similarly and no difference could be observed in epidermal thickness whether langerin+ cells were present or not. However, an increased infiltrate of hematopoietic cells into the skin could be observed after 7 days of IMQ treatment. In the absence of langerin+ cells we detected slightly increased numbers of CD8a+ but also of CD11b+ dendritic cells. Whereas the percentage of CD4+ T-cells is increased in IMQ treated C57BL/6 mice they are reduced in the skin of Langerin-DTR mice. Additionally, Gr1+CD11b+CD115+ monocytes, which are recruited to IMQ treated skin, are increased in the absence of Langerin+ cells. Mast cells, which have previously been shown to be critical for the activation and emigration of LC, were increased to the same extent but were found more frequently degranulated in the absence of LC. We have previously shown that topical IMQ treatment reduces the tumor growth rate of M3 melanoma cells in DBA/2 mice. A similar effect can be observed in the B16F10 melanoma model. When tumor bearing mice were depleted of Langerin+ cells before IMQ treatment we found a reduced infiltrate of CD4+/MHCII+ and CD8a+/MHCII+ cells. However the effect of IMQ on tumor growth was not affected by these alterations in the dendritic cell infiltrate.

P20 Targeting Leishmania major antigens to Dendritic cells *in vivo* induces protective Th1 CD4+ T cells

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The study of the protozoan parasite Leishmania major in mice has provided significant contributions to the current understanding of T cell differentiation *in vivo*. In this infection model, a dominant Th1 response is associated with healing in B6 mice, while a predominant Th2 response is associated with disease progression in Balb/c mice. T-cell responses are initiated by DCs, the immunological sentinels of the immune system, which function as critical regulators of anti-Leishmania immune responses. Therefore DCs are attractive targets for immunotherapeutic approaches. In particular, skin dendritic cells that interact with parasites present at the lesion site during the cutaneous form of the infection are considered important regulators in the initiation phase of immune responses to Leishmania *in vivo*. In line with the overarching theme of our laboratory in dendritic cell biology, particularly as it pertains to vaccine science, this project focuses on the engineering of different antigens from L. major into monoclonal antibodies that target to specific receptors on dendritic cells. We have used these fusion antibodies to investigate how DCs control T cell mediated immune responses and provide protection against cutaneous leishmaniasis. Preliminary results indicate that anti-receptor fusion mAb conjugated to clinically relevant L. major antigens, administered together with Poly IC as an adjuvant, increase the frequency of IFNg+ IL-2+ TNFα+ CD4+ T cells in Balb/c mice when compared with soluble non-targeted protein. Experimental infection of immunized mice also demonstrated significant protection against L. major infection accompanied by reduced parasite loads at the infected site and draining lymph nodes. This model provides an opportunity to study DC-targeting vaccination as a novel therapeutic approach in cutaneous leishmaniasis, an increasing public health problem with no currently available vaccine.

P21 Role of the Endothelin System in Adult Pulmonary Langerhans´Cell Histiocytosis and migration of murine LCs.

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Endothelin (ET) receptor blockers have been administered in patients with pulmonary Langerhans´ cell histiocytosis (PLCH) and concomitant pulmonary hypertension. The effects of these drugs on key cells of PLCH have yet to be explored. Our aim was to analyse the expression of ET receptor A and B in PLCH and investigate their functional significance *in vitro*. ETAR and ETBR expression was studied in 25 formalin/paraffin-embedded PLCH biopsies. For *in vitro* analysis, the murine LC-like cell line XS52 and freshly prepared murine LCs were used. Target expression was determined by RT-PCR, cell viability was analysed by trypan blue exclusion test and colorimetric assay, ET-1 expression by enzyme-linked immunosorbent assays and cell migration assays were performed in 48-well Boyden chambers for XS52 cells and by murine epidermal explant cultures. The immunohistochemistry on the biopsies revealed the expression of both, ETAR and ETBR in PLCH. *In vitro*, the expression of the ET system was proven in murine LCs (ETAR+, ETBR+) and in XS52 cells (ETAR+). Furthermore, treating XS52 cells with the dual ET receptor blocker bosentan revealed impaired cell viability. Regarding cell migration bosentan and the selective ETAR antagonist BQ123 were capable of inhibiting ET-1 dependent migration of XS52 cells. The number of migratory LCs from murine epidermal skin explants after 48 hours of co-incubation with ET-1 was comparable to the one after CCL21 stimulation. Treatment with the dual ET receptor antagonist bosentan reduced the chemotactic potency of ET-1 in LCs. To conclude, this study proved not only the unique expression patterns of the endothelin system in PLCH and in murine LCs but also revealed the antiproliferative and antimigratory properties of ET receptor blockers *in vitro* apart from showing for the first time the involvement of ET-1 in Langerhans cell migration.

P22 Topical vaccination with functionalized nanoparticles

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In addition to target disease, population, intended immune response and antigen, targeting strategies and vaccine formulations need to be addressed in vaccine development. To date nearly all vaccines are injected, a painful procedure, especially for children, that bears the risk of propagating infections when needles are re-used. Since the latter is often the case, especially in developing countries, development of needle-free applications is being priori-tised. Aiming at vaccines for induction of cell-mediated immunity (CMI) and based on knowl-edge and extensive works integrating basic immunology and skin physiology we are develop-ing approaches and models for topical vaccines. The skin is the largest immune organ and directly in contact with the environment. It has an unbroken network of Langerhans and der-mal dendritic cells that can both sense danger and induce immune responses, and control and silence immune responses to avoid chronic inflamma-tion. The skin is also an active transport organ for controlled inside-out and outside-in transport of chemically diverse compounds and formulations. We have established that hair follicles are the main import routes into the skin and that particle-coupled compounds are imported much more efficiently than soluble compounds. Integrating these findings with our definition of the essential constituents of vaccines for induction of CMI – an-tigens/epitopes for effector and for helper T cells, and agonists for pattern recognition receptors (PRR) - we are developing skin targeting vaccines. These model nanoparticle vaccines combine the immunological principles of CMI induction with targeting principles for delivery to and activation of dendritic and Langerhans cells in the skin for topical non-invasive vaccination. * Collaborative project supported by the Nanobiotechnology Funding Initiative of the Federal Ministry of Science and Education (BMBF), Project Coordinator Physical Technologies, VDI Technologiezentrum GmbH; FKZ 13N9196

P23 Human skin dendritic cells can be targeted in situ by antibodies against lectin receptors.

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Dendritic cells are pivotal for the induction of primary immune responses, and hence interesting targets to induce immune responses against tumors. They express various lectin receptors which are essential for antigen incorporation. Langerhans cells, the dendritic cells of the epidermis, express the C-type lectins Langerin/CD207 and DEC-205/CD205, wheras dermal dendritic cells just express DEC-205. We are interested in augmenting uptake and presentation of antigen to improve immunotherapy with dendritic cells. This can be achieved by targeting antigens with the help of antibodies to lectin receptors on the surface of dendritic cells. To test the feasibility of this approach, we incubated human skin biopsies on medium containing antibodies against Langerin and DEC-205 for 24h to allow them to diffuse into the tissue. Alternatively, antibodies were injected intradermally into skin biopsies. We either analyzed skin tissue directly after 24h incubation or cultured skin explants for several days to enable dendritic cells to emigrate from skin explants. Immunofluorescence stainings of skin sections demonstrated that Langerin targeted exclusively Langerhans cells in the epidermis, whereas DEC-205 mainly stained dermal dendritic cells and only weakly Langerhans cells *in situ*. Migratory dendritic cells showed a similiar staining pattern except that DEC-205 targeting was more efficient on activated Langerhans cells. This indicates that Langerhans cells need some activation stimulus to upregulate DEC-205 for being optimally targeted by antibodies against DEC-205. In summary we conclude, that antigen conjugated to Langerin will mainly be loaded onto immature Langerhans cells *in situ*, in contrast DEC-205 can be used to target antigen to a broader range of activated dendritic cells in epidermis and dermis.

P24 Characterization of anti-tumoral immune responses in a mouse model of spontaneous melanoma

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When a tumor develops in the skin, immune effector cells infiltrate the affected tissue. However, subsequent immune responses fail to control tumor growth. The reason for this tolerance is largely unknown, and could involve skin DCs. The transgenic mouse strain EPv shows abnormal proliferation of melanocytes, resulting in spontaneous and progressive development of large tumors with age. This allows monitoring of immune status of these mice throughout tumorigenesis. We have extensively analyzed cell populations in skin-draining lymph nodes as well as immune infiltrates within the tumor mass. Within lymph nodes draining tumors in EPv mice, proportions of CD4+ and CD8+ T cell were unaffected. However, there was a striking increase in natural regulatory T cells (Treg) among CD4+ T cells. Total NKT cells were also expanded, but had a lower expression of the activation marker CD69. Conversely, NK cells displayed a more activated phenotype. We also found less Langerin+ dermal Dendritic cells (DC) and Langerhans cells in tumor-bearing mice, while the proportion of Langerin- dermal DC increased. Immunohistochemistry revealed tumor-infiltrating CD45+ immune cells. Most infiltrating T cells were CD4+ rather than CD8+, and included Treg. FACS analysis of established tumors also revealed infiltrating NK cells and plasmacytoid DC. Langerhans cells were depleted from skin with melanoma, while the Langerin- dermal DC population was enlarged. All tumors contained a prominent population of GR1 high CD11b+ CD11c- cells, which could be myeloid-derived suppressor cells (MDSC). Quantitative PCR analyses of tumor lysate showed no significant difference in production of TGF-beta and IL-10 between healthy and tumor-bearing skin. Finally, we observed that transgenic CD8+ T cells specific for the melanoma antigen gp100 proliferate upon transfer into tumor-bearing, but do not undergo differentiation into memory cells. Overall, despite the presence of activated effector cells and efficient presentation of tumor antigens, melanoma-bearing EPv mice do not mount efficient cytotoxic immune responses against tumors, possibly due to the tolerogenic influence of MDSC and Treg.

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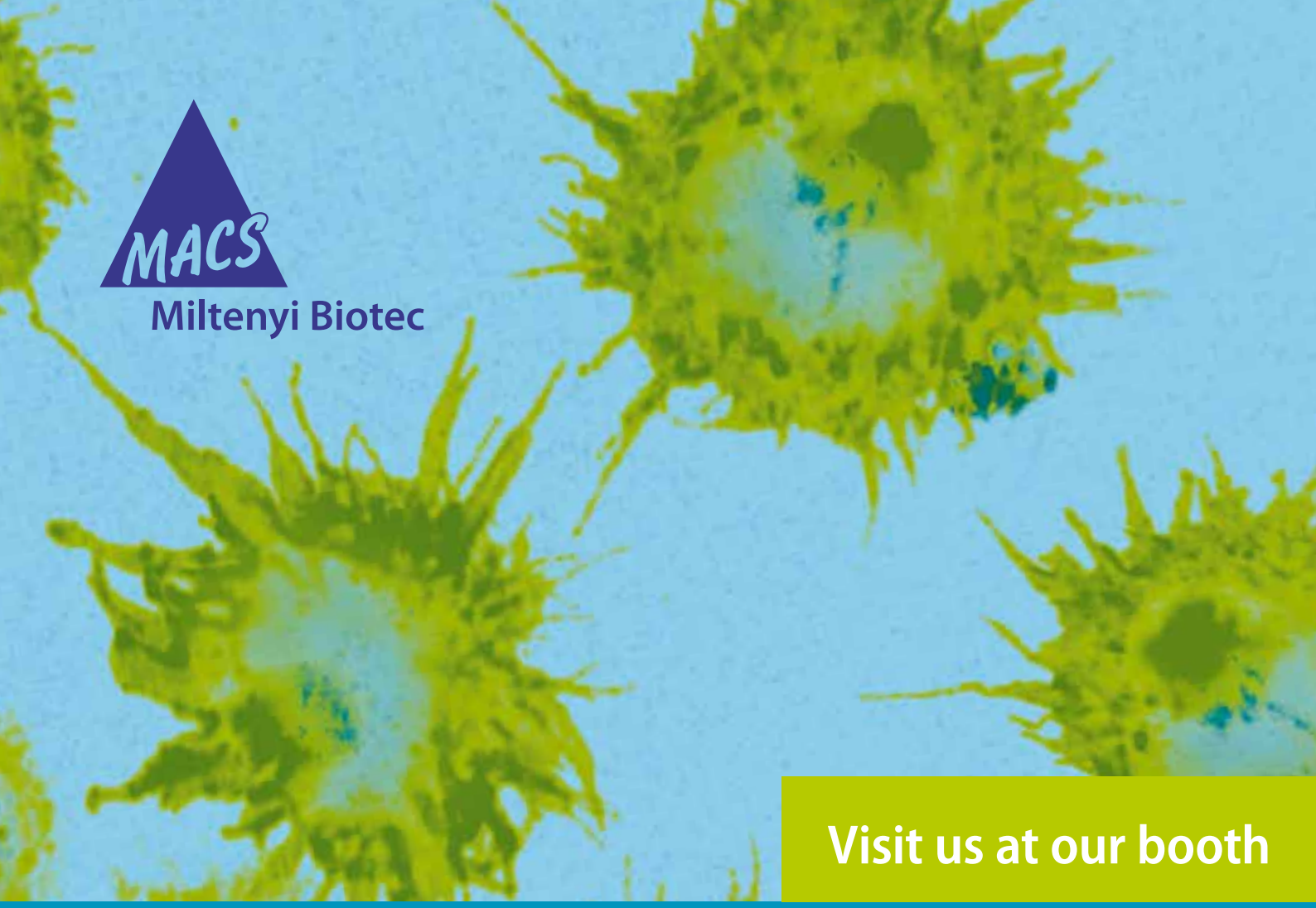

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References:
1. van Tendeloo, V.F. *et al.* (2010) Proc. Natl. Acad. Sci. USA 107: 13824–29.
2. Tel, J. *et al.* (2010) DC 2010: Forum on Vaccine Science, S05–003.

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