16th Meeting

of the

European Epidermal Barrier Research Network (E2BRN)

27 and 28 September, 2018

Hotel Mercure Golf de Seilh

(Toulouse, France)

Local Organising Committee: Hélène Duplan, Michel Simon

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Program

7 invited conferences, 16 shorter oral presentations (O1-O16) selected from abstracts and 25 posters (P1-P25)

Thursday, September 27

09:00-12:00 Bus shuttle transfer Toulouse-Blagnac airport / Hotel
Registration

12:30-14:00 Buffet lunch

14:00-15:00 Registration and poster installation

15:00-15:15 Welcome introduction: **Hélène DUPLAN, Michel SIMON and Johanna BRANDNER.**

15:15-18:00 **Session 1 – Chairman: Johanna BRANDNER and Dusko ILIC**

15:15-15:45 **Nicolas GAUDENZIO (Toulouse, France):** *Guanine nucleotide exchange factor RABGEF1 regulates keratinocyte-intrinsic signaling to maintain skin homeostasis.*

15:45-16:15 **Hugh BYRNE (Dublin, Ireland)**
Biomedical Applications of Vibrational Spectroscopy – Disease Diagnostics and Beyond – Interest for the skin.

16:15-16:45 *coffee break*

16:45-17:00 Janina TROTHE (Frankfurt am Main, Germany): *Contribution of the volume-regulated anion channel subunit LRRC8A to cell volume regulation and differentiation in human keratinocytes (O1).*

17:00-17:15 Clare ROGERSON (London, UK)
Keratinocyte nuclear degradation requires AKT1-dependent lamin phosphorylation and the formation of lamin-containing cytoplasmic vesicles (O10).

17:15-17:30 Christiane BIERKAMP (Toulouse, France)
Epidermal differentiation and barrier defects in mice lacking microtubule-organizing protein ninein (O11).

17:30-17:45 Corinne LEPRINCE (Toulouse, France)
The actin based myosin 5b motor is crucial for lamellar body trafficking in the epidermis (O9).

17:45-18:00 Julia LACHNER (Vienna, Austria)
Identification of evolutionary ancient and lineage-specific epidermal differentiation genes in organotypic models of chicken skin (O15).

19:30-21:00 *Dinner*

21:00-22:30 **Poster Viewing**

Friday, September 28

09:00 – 12:00 **Session 2** - **Chairman: Yves POUMAY and Ellen van den BOGAARDT**

09:00-09:30 **Carien NIESSEN (Cologne, Germany)**
Biomechanical signaling in the making, maintaining and breaking of the epidermal barrier.

09:30-10:00 **Patrick ZEEUWEN (Nijmegen, The Netherlands)**: *Cystatin M/E and its target proteases: their role in skin biology and pathology.*

10:00-10:30 **Roger SANDHOFF (Heidelberg, Germany):**
Skin barrier ceramide metabolism in health and disease.

10:30 – 11:00 *coffee break*

11:00-11:15 Sandrine DUBRAC (Innsbruck, Austria)
PPAR-alpha deficiency drives altered bacterial skin colonization and abnormal eicosanoid skin composition (O7).

11:15-11:30 Hanna NIEHUES (Nijmegen, The Netherlands)
STAT1 gain-of-function compromises skin host defense in the context of interferon-γ signaling (O2).

11:30-11:45 Giel TANGHE (Gent, Belgium)
Ripk4 maintains epidermal homeostasis and prevents skin cancer by suppressing mitogenic signaling (O6).

11:45-12:00 Mark DONOVAN (Aulnay, France)
Characterization of the Stratum Corneum Heparanome (O12).

12:15 – 13:30 *Lunch*

13:30- 14:30 **Poster Viewing**

14:30-17:45 **Session 3 - Chairman: Corinne LEPRINCE and Giel TANGHE**

14:30-15:00: **Sanja KEZIC (Amsterdam, The Netherlands):** *Deficiencies in filaggrin expression and processing impair corneocyte surface texture and stiffness in mice.*

15:00-15:30: **Saadia KERDINE-RÖMER (Paris, France):** *Control of mechanism of DC's cell death induced by chemical sensitizers*.

15:30-15:45 Gijs RIKKEN (Nijmegen, The Netherlands) *The triple action of leflunomide: a novel therapeutic mechanism of action in the treatment of inflammatory skin diseases (O13).*

15:45-16:00 Aline RIGAL (Châtenay-Malabry, France) *Characterization of the barrier function on skin "very" aging using a multi-parametric approach: biometric measurements, in vivo Raman spectroscopy, HTGC/MS and LC/HRMS (O5).*

16:00-16:15 Jérome LAMARTINE (Lyon, France) *Chronological aging impacts abundance, function and microRNA content of extracellular vesicles produced by human epidermal keratinocytes (O16).*

16:15-16:30 Céline EVRARD (Namur, Belgium) *Diverse problems in keratinocyte culture impede epidermal barrier studies: sharing failures to improve success rate (O14).*

16:30-17:00 *coffee break*

17:00-17:15 Emeline PAGES (Toulouse, France)
NativeSkin®, an immunocompetent human skin model to study antigen uptake and processing by Langerhans cells (O4).

17:15-17:30 Mei HUANG (London, UK)
Developing an in vitro 3D model of X-linked recessive ichthyosis using CRISPR-Cas9 editing of the STS gene (O8).

17:30-17:45 Céline LANCELOT (Isnes, Belgium)
Effective in vitro 3D skin model to study epidermal response to Th-17 driven inflammation (O3).

17:45-18:00 **Announcement of Poster prices**

18:00-18:30 **E2BRN network meeting**

19:30-02:00 **Gala Dinner and more**

Saturday, September 29

09:00 Departure to Toulouse-Blagnac Airport (bus shuttle)

Toulouse E2BRN meeting is supported by

And organized by

Abstracts:

O1- Contribution of the volume-regulated anion channel subunit LRRC8A to cell volume regulation and differentiation in human keratinocytes

Janina Trothe[1], Isabella Schindler[1], Victoria Lang[2], Vanessa Scalise[2], Torsten Ertongur-Fauth[1], Claudia Buerger[2]
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The barrier function of the human epidermis is constantly challenged by environmental osmotic fluctuations and keratinocytes can become a direct target of osmotic stress. Hypotonic stress triggers cell swelling, which is counteracted by a compensatory mechanism called regulatory volume decrease (RVD) involving volume-regulated anion channels (VRACs). Recently, LRRC8A (Leucine-rich repeat-containing protein 8A) has been identified as the essential VRAC component in different mammalian cell types. We analyzed the function of LRRC8A during hypotonic stress response of human keratinocytes and asked whether it may be additionally involved in cell volume changes that are occurring during epidermal differentiation.
LRRC8A-deficient keratinocytes were generated using the CRISPR-Cas9 technology. These cells showed greatly reduced VRAC activity and impaired RVD suggesting that LRRC8A is involved during hypotonic stress response of human keratinocytes. Immunohistochemical staining of human skin indicated that LRRC8A is preferentially expressed in basal and suprabasal epidermal layers, which declines in further differentiated layers. This expression pattern was also observed in vitro when keratinocytes were driven into differentiation by Ca2+ switch or post-confluent growth. Moreover, RNA and protein expression pattern of LRRC8A seemed to differ between isolated epidermal stem cells, transient amplifying keratinocytes and post-mitotic differentiated cells. Interestingly, LRRC8A knockout keratinocytes showed a delayed expression of differentiation markers as well as reduced proliferation rates.
Our findings suggest that LRRC8A not only mediates cell volume regulation during hypotonic stress response but is also an integral part of epidermal proliferation and differentiation. We hypothesize that LRRC8A contributes to the switch from proliferation to differentiation when cells leave the basal layer to contribute to epidermal stratification and cornification. Since disorders related to impaired epidermal barrier function can be aggravated by osmotic stress our findings provide a starting point to evaluate LRRC8A as a novel target to control detrimental osmotic effects.

O2- *STAT1 gain-of-function compromises skin host defense in the context of interferon-γ signaling*.

Hanna Niehues
Radboudumc Nijmegen, The Netherlands

Introduction: Defective mucosal and skin host defense mechanisms are the hallmarks of the primary immunodeficiency chronic mucocutaneous candidiasis (CMC). We previously reported that heterozygous mutations in the signal transducer and activator of transcription 1 (*STAT1*) gene are responsible for autosomal dominant CMC. Moreover, we demonstrated that gain-of-function (GOF) mutations of *STAT1* lead to its hyperphosphorylation and subsequent impairment of Th17 responses, finally resulting in a severe mucocutaneous *Candida albicans* infection. Although CMC manifests itself at the level of epithelia (skin and oral mucosa), research has so far been limited to the study of immune cells. Using genetically defined epidermal cells, either wild type or carrying STAT1 GOF mutations, we investigated their response to proinflammatory cytokines, with respect to skin barrier and host defense gene expression.
Methods and Materials: We generated 3D epidermal equivalents from keratinocytes of healthy controls and CMC patients (STAT1 GOF), and stimulated these with IL-17, IL-22 or IFNγ. The cellular responses were evaluated by immunohistochemistry.

Results: Stimulation by IFNγ, but not by Th17 cytokines, caused abnormal epidermal morphology and a strongly reduced expression of Late Cornified Envelope 3 (LCE3) proteins. We found that, in addition to their known antibacterial activity, LCE3 proteins had antifungal activity against *Candida albicans*.
Discussion: This study demonstrates that epithelia of patients with a STAT1 GOF mutation have a functional defect that becomes apparent when immune cell-derived IFNγ is present. This results in structural abnormality of the epidermis and compromises the innate anti-*Candida* activity of the tissue.

O3- Effective in vitro 3D skin model to study epidermal response to Th-17 driven inflammation

Céline Lancelot, Coralie Bastin, Kassandra Watillon, Michel Salmon
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Introduction: The Th-17 axis demonstrates a growing importance in inflammatory skin diseases. In addition to their well-known involvement in psoriasis, Th-17 cytokines are also implicated in other skin diseases such as atopic dermatitis especially in pediatric patients or from Asian origin. In the search for specific strategies to improve skin resilience towards inflammation, we developed a 3D in vitro human reconstructed epidermis (RHE) model that efficiently reproduces T-cell mediated inflammatory conditions like psoriasis or eczema driven by Th17 cytokines.
Methods and materials: RHE were exposed to IL-17 and TNF-α for 48H. The IKK inhibitor PS1145 was used as a benchmark and incubated along with cytokines. After cytokines exposure, the RHE morphology was assessed. The expression of the Th-17 driven inflammation biomarkers i.e., IL-19, IL-23, DEFB4 and S100A7 was evaluated using specific TaqMan assays. Culture supernatants were used for ELISA quantification of IL-19 and IL-23.
Results: Our so called RHE-Th17 model showed hyperplasia and thickening of the stratum corneum. Data demonstrated an increase of psoriasis biomarkers at the mRNA levels, including IL-19, IL-23, DEFB4 and S100A7, as well as, IL-19 and IL-23 levels in RHE supernatants. In order to use this model for in vitro testing and screening, we tested if the RHE-Th17 model can respond to inhibition of the IL-17 pathway though the IKK inhibitor, PS1145. RHE exposure to PS1145 partially restored the stratum corneum thickening, the basal expression of the 4 Th-17 driven inflammation markers, as well as the level of IL-19 and IL-23 in the culture supernatants.
Discussion: We demonstrate the relevance of the RHE-Th17 in vitro skin inflammatory model based on Th-17 cytokines stimulation. This model recapitulates psoriasis, as well as some AD-subtypes features and expresses disease specific biomarkers. The relevance of the model as a screening tool for therapeutic compounds has been validated using the IKK inhibitor PS1145.

O4- NativeSkin®, an immunocompetent human skin model to study antigen uptake and processing by Langerhans cells.

E Pages[1], L Mondoulet[2], E Braun[1], V Dhelft[2], C Dupont[3], H Sampson[4], P Descargues[1]
[1]Genoskin SAS, France; [2]DBV Technologies, France; [3]Hôpital Necker, France; [4]DBV Technologies, USA.

Epicutaneous immunotherapy (EPIT) relies on the principle that the allergen is taken up by Langerhans cells (LCs) and transported to regional lymph nodes. DBV Technologies demonstrated in allergen sensitized mice that allergens applied on intact skin during EPIT induced tolerance. NativeSkin® is an *ex vivo* human skin model which can be kept in culture 7 days while maintaining normal viability and responsiveness of various immune cells. In this study, we demonstrated the ability of this model to react to topically administered allergens, and to allow the study of EPIT mechanism of action.
Viaskin® patches loaded with fluorescein-tagged peanut protein extract (PPE) or PBS were applied onto NativeSkin from 2 donors for 12 and 24 hrs. Co-localization of PPE-AF647 and LCs (stained with anti-CD207 and anti-CD1a) was observed on skin cross-sections and in situ on epidermal sheets by confocal microscopy.

PPE-loaded on Viaskin patches solubilized within 12 hrs due to transepidermal water loss and reached the epidermis. After 24 hrs, a significant increase in PPE co-localizing with LCs was measured. Observation of LCs in contact with PPE-AF647 suggest that there are 2 phases in the process: at 12 hrs, the allergen associates with the surface membrane and at 24 hrs it is internalized in LCs which seemed to be losing their dendritic morphology.
LCs present in NativeSkin (1) interact with the antigen, (2) internalize it, and (3) lose their dendritic shape, which is the first step of subsequent migration to the lymph node. Thus, NativeSkin contains functional LCs and is relevant to study antigen uptake and processing.

O5- Characterization of the barrier function on skin "very" aging using a multi-parametric approach: biometric measurements, in vivo Raman spectroscopy, HTGC/MS and LC/HRMS

Aline Rigal[1], Rime Michael-Jubeli[1], Armelle Bigouret[2], Alex Nkengne[2], Arlette Baillet-Guffroy[1], Ali Tfayli[1]
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Because of its social impact, skin aging is one of the most common dermatologic concerns in modern society. Moreover, due to the increase in life expectancy, an increasing part of the population looks for new creams and procedures that can improve the skin appearance. Despite this, almost no information is available on the skin of people over the age of 70 in the literature. Clinical manifestations of skin aging like wrinkles and slackness are related to underlying complex molecular phenomena in the different layers of the skin. These phenomena can nowadays be better characterized with recent techniques, giving a better understanding of the very skin aging.
Information were collected from the forehead of 22 young volunteers (18-24 yo) and 18 elderly volunteers (70-75 yo). Biometric measurements were obtained by usual methods (TEWL, corneometry, sebumetry, skin pH, cutometer). Raman spectra and in-depth profiles were collected in vivo. This technique provides information on the organization of the lipids of the Stratum Corneum (SC) and their barrier function, and on water content and mobility. To obtain relevant information about molecular composition of high complex lipid mixtures, i/ skin Surface Lipids (SSLs) were analyzed in one run keeping the lipid structures intact, using High Temperature Gas Chromatography coupled with Mass Spectrometry (HTGC/MS). ii/ SC lipids were analyzed by one run allowing to characterize more than 2500 compounds in their intact state, using Liquid Chromatography coupled with High-Resolution Mass Spectrometry (LC/HRMS).
The combinations of all these information lead to a multi-parametric investigation which better characterize the very skin aging. Important modifications on the structure of the SC lipids, and on the epidermis lipids composition (SSLs and SC lipids) can be observed for elderly. Moreover, our results show a good association between biometric parameters and in vivo Raman descriptors.

O6- Ripk4 maintains epidermal homeostasis and prevents skin cancer by suppressing mitogenic signaling

Giel Tanghe[1,2], Corinne Urwyler-Rösselet[1,2,5], Michael Devos[1,2], Barbara Gilbert[1,2], Kelly Lemeire[2], Cédric Blanpain[3,4], Peter Vandenabeele[1,2], Wim Declercq[1,2]
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The skin is a fast renewing organ with the continuous commitment of proliferative progenitor keratinocytes into a terminal differentiation program forming the stratified epidermis. Previous studies demonstrated that RIPK4, a serine/threonine kinase, is crucial for proper barrier formation during epidermal development. In in vitro proliferating keratinocyte cultures RIPK4 is continuously activated. Keratinocyte-specific RIPK4 ablation causes cleft palate, epithelial fusion, aberrant differentiation and a defective barrier leading to perinatal death. Here, we addressed the homeostatic functions of RIPK4 in adult mouse skin.
Tamoxifen-inducible K14-CreER-driven RIPK4 deletion in adult mouse epidermis caused significant hyperplasia due to the expansion of proliferative basal keratinocytes marked by high p63 expression. Although epidermal keratinocytes eventually commit to differentiation, the barrier is dysfunctional witnessed by increased transepidermal water loss and which coincides with local immune infiltration. Using ex vivo cultures of primary keratinocytes we found that RIPK4 enables cell cycle exit by suppressing p63 expression in a keratinocyte autonomous manner. Additionally, RIPK4 down-regulates EGFR expression and its downstream ERK and STAT3 mitogenic signaling pathways in a kinase-dependent manner in mouse and human keratinocytes. Loss of RIPK4 led to spontaneous tumor formation of the keratoacanthoma type, with reduced latency by additional deletion of tumor suppressor p53. Furthermore, RIPK4 serves as a brake on tumor growth driven by an oncogenic $Kras^{G12D}$ knockin transgene.
Together, our work demonstrates that RIPK4 fulfills a central role in maintaining the homeostatic balance between keratinocyte proliferation and differentiation by suppressing p63 expression and EGFR signaling and providing a tumor suppressive function in clinically relevant genetic squamous skin cancer models.

O7- PPAR-alpha deficiency drives altered bacterial skin colonization and abnormal eicosanoid skin composition.

Stefan Blunder[1], Ralph Rühl[2], Dorothea Orth-Höller[3], Matthias Schmuth[1], Sandrine Dubrac[1]
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Introduction***:*** PPARα is a transcription factor that serves as a lipid sensor and that contributes to skin homeostasis. Because PPARα is down-regulated in the skin of atopic dermatitis patients, investigations into its functions might provide further understanding to disease pathogenesis.
Methods and Materials***:*** We studied the skin microbiome and the immune system of PPARα deficient mice and littermate controls. Moreover, we also determined the composition of epidermal eicosanoids, leukotrienes and prostaglandins.
Results: We found that, in the steady state, PPARα deficient mice exhibit a discreet alteration of epidermal barrier function with a moderate increased TEWL and a normal skin surface pH. PPARα deficiency alters the composition of skin microflora and leads to changes in the innate and adaptive skin immune system. We observed a decreased production of IL-17A by γ/δ but not α/β resident T-lymphocytes despite an increased proportions of all dendritic cells subsets (cDC1 and cDC2) in the skin of PPARα deficient mice when compared to littermate controls, suggesting functional impairment. Moreover, the proportions of innate immune cells such as neutrophils and macrophages (M1 and M2) are similar in both mouse groups. Nevertheless, the reduced expression of the activation marker CD11b by neutrophils suggests an impaired function. Moreover, we determined an important alteration of epidermal eicosanoids, leukotrienes and prostaglandins in PPARα deficient mice when compared to littermate controls.
Discussion***:*** Thus, down-regulation of PPARα in atopic skin might contribute to modifications of skin microenvironment not only by impairing the skin immune system and altering the skin lipid composition, but also by modifying the skin microbiome.

O8 - Developing an in vitro 3D model of X-linked recessive ichthyosis using CRISPR-Cas9 editing of the STS gene

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X-linked recessive ichthyosis (XLRI) is a genetic skin disease caused by a deletion or point mutation in the steroid sulfatase (STS) gene that affects 1 in 3000 boys. Loss of STS prevents the conversion of cholesterol sulphate to cholesterol resulting in an accumulation of cholesterol sulphate and a decrease of cholesterol levels in the epidermis. This leads to abnormal permeability of the skin barrier and a delay in desquamation resulting in skin hyperkeratosis and barrier dysfunction. The aim of this study is to generate a 3D model of XLRI and further investigate the pathomechanism of this disease.
The CRISPR-Cas9 gene editing system was used to generate 3 different deletions of the STS gene in N/TERT keratinocytes. After confirmation of the mutation by Sanger sequencing, western blot and qPCR, we further investigated cell proliferation, migration and steroid sulfatase activity. The STS knockout cell lines were used to generate 3D organotypic models to recapitulate the XLRI epidermis phenotype. To characterise the XLRI 3D model, we performed Haematoxylin and Eosin staining, immunostaining of differentiation markers and Nile Red staining. Three different STS knockout cell lines were successfully generated. The deletion of STS was confirmed by western blotting and qPCR, at the protein and mRNA level, respectively. Cell proliferation, migration and sulfatase activity were significantly reduced in CR-KOs when compared to wild type cells (CR-WT). An increase in epidermal thickness and altered lipids were observed in the 3D model. In addition, we found a decrease in expression of keratin 10 and early onset of expression of involucrin in the 3D model, similar to RXLI patient epidermis.
In summary, we have generated an in vitro 3D model that simulates the typical epidermal phenotype of RXLI and this model can be used in explore the pathomechanism of XLRI and test potential therapeutic compounds for XLRI treatment.

O9 - The actin based myosin 5b motor is crucial for lamellar body trafficking in the epidermis

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Most of the barrier function of the skin is ensured by the outermost layer of the epidermis, the stratum corneum, composed of flattened, anucleated corneocytes surrounded by a lipid-enriched lamellar matrix. Many components present in the intercorneocyte matrix are delivered by the underlying granular keratinocytes, through a secretion process dependent on lysosome-related organelles, the lamellar bodies. In a recent study, we have shown that lamellar body biogenesis is controlled by Rab11a, a Rab-family GTPase regulating vesicular trafficking between a recycling compartment and the TGN (Reynier et al., J. Invest. Dermatol. 2016). One of Rab11a effectors is the myosin 5b (Myo5b), an actin based molecular motor, well described in a series of other epithelia. Our aim was to test whether Myo5b could be involved in lamellar body biogenesis and/or trafficking, downstream of Rab11a, in granular keratinocytes.
In the epidermis, Myo5b distribution was shown to be dependent on Rab11a and Myo5b colocalized with a number of lamellar body markers, such as corneodesmosin. Drug treatments inducing actin depolymerization were able to modify the intracytoplasmic distribution of corneodesmosin in granular keratinocytes. In a 3D reconstructed human epidermis, MYO5B silencing by RNA interference induced defects in epidermal permeability and impaired the structure of the stratum corneum with disappearance of intercorneocyte spaces and reduction of the lipid content quantified by chromatography. Concomitantly, the intracytoplasmic pool of lamellar bodies in granular keratinocytes and some intercellular junctional proteins such as claudin 1 were decreased.

Taken together, our results suggest that the actin based molecular motor Myo5b is an essential component of the lamellar body trafficking pathway in granular keratinocytes. We hypothesize that Myo5b plays a role first, in lamellar body docking on a cortical actin scaffold and second, in lamellar body trafficking during maturation steps along the secretion pathway.

O10 - Keratinocyte nuclear degradation requires AKT1-dependent lamin phosphorylation and the formation of lamin-containing cytoplasmic vesicles

Clare Rogerson, Ryan FL O'Shaughnessy
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Introduction: Keratinocyte cornification and epidermal barrier formation are tightly controlled processes which require complete degradation of intracellular organelles, including removal of keratinocyte nuclei. We have shown that degradation of the nuclear lamina, a network of Lamin proteins beneath the nuclear envelope, essential for nuclear integrity, is required for this nuclear removal. Specifically, phosphorylation of Lamin A/C by AKT1, a protein kinase, is required for nuclear removal. However, the mechanisms of complete nuclear removal, nuclear lamina breakdown and how these processes are controlled have yet to be defined.
Methods and Materials: Post-confluent cultures of rat epidermal keratinocytes (REKs) undergo spontaneous and complete differentiation, developing into enucleate keratinocytes, allowing visualisation and perturbation of the differentiation process in vitro. By introducing labelled Lamin A/C constructs into REK cultures we can also follow the differentiation process in real time.
Results: Following nuclear degradation with labelled Lamin A/C constructs we have been able to define, for the first time, the duration of this process in differentiating keratinocytes. We have also identified the formation of Lamin A/C containing structures at the nuclear lamina and dispersal of Lamin A/C vesicular structures throughout the cytoplasm in suprabasal keratinocytes. AKT1 coincides with phosphorylated Lamin A/C both at the nucleus and at dispersed vesicular structures and inhibiting Akt1 or introducing non-phosphorylatable Lamin A/C constructs prevents nuclear degradation. Dispersed Lamin A/C containing vesicles were not coincident with the nuclear protein, Histone 2B, or a fluorescent tag targeted to the nucleus.
Discussion: Our results suggest AKT1 phosphorylation of Lamin A/C may control dispersal of Lamin A/C cytoplasmic vesicles as part of keratinocyte terminal differentiation. These cytoplasmic vesicles interestingly do not disrupt nuclear integrity but may indicate an important intermediate for nuclear lamina degradation prior to nuclear removal.

O11 - Epidermal differentiation and barrier defects in mice lacking microtubule-organizing protein ninein

Nicolas Lecland, Chiung-Yueh Hsu, Cécile Chemin, Andreas Merdes, and Christiane Bierkamp
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Introduction: The microtubule cytoskeleton fulfills essential cellular roles, from the assembly of the spindle during mitosis to the directed transport of different cargoes during interphase. Its organization is dynamic: in the developing epidermis, basal progenitor cells develop radial microtubules from the centrally located microtubule organizing center, the centrosome, while in suprabasal, differentiating keratinocytes, microtubules are found at the cell cortex, colocalizing with desmosomal junctions and microtubule anchoring proteins. The microtubule-organizing protein ninein relocates from the centrosome in progenitor cells to the cellular cortex in differentiating keratinocytes, preceding microtubule reorganization. Because the molecular mechanisms and developmental roles of this reorganization are poorly understood, we investigated whether ninein is essential for microtubule reorganization, and whether any interference during epidermal development has consequences for cell differentiation and tissue formation.
Methods and materials: We analyzed microtubule organization and intercellular junctions upon onset of differentiation, in ninein-depleted mouse progenitor epidermal keratinocytes (MPEK).

Moreover, we generated ninein-knockout mice using Cre-recombinase: an epidermis-specific and a constitutive ninein knockout, in which we studied the developmental consequences of ninein-loss on epidermal morphology and homeostasis.
Results: First, ninein-deficient cells in culture and progenitor cells in vivo displayed mitotic spindle misorientation, associated with reduced pools of progenitor and differentiating cells, and a thinner skin. Second, both differentiating keratinocytes in culture and suprabasal cells in vivo displayed abnormal organization of cortical microtubules, associated with reduced numbers of suprabasal desmosomes and a delay in epidermal barrier formation.
Discussion: In the epidermis, ninein plays two roles, one at the spindle poles required for the orientation of the mitotic spindle, and one at the cortex, enabling the recruitment of additional microtubule binding proteins and the organization of cortical microtubules. We believe that the latter has direct consequences on microtubule-dependent transport, influencing the formation of desmosomes and lamellar bodies, and thus contributing to barrier function.

O12 - Characterization of the Stratum Corneum Heparanome

Mark Donovan[1], Rawad Abdayem[1], Jerry Turnbull[2], Anne Potter[1], Dominique Bernard[1]
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N –linked glycans and the heparan sulfate (HS) are the major forms of protein glycosylation in the epidermis. However, until recently there has been a paucity of knowledge of these glycans in the stratum corneum. Our electron microscopic and AFM studies characterized the distribution of N-glycans and heparan sulfate on corneocytes in different levels of the stratum corneum (SC). The objective of this study was to further characterize the SC heparanome.
The presence of the heparan sulfate proteoglycan syndecan 1 in the SC was evidenced by ELISA, western blot and EM studies suggesting that heparan is tethered to this proteoglycan on the surface of corneocytes. In order to gain insights into HS function, heparin affinity chromatography and MS/MS proteomic analysis of soluble SC protein extracts were performed and showed that the antimicrobial peptide RNase7 and transglutaminase 3 are high affinity SC heparan binding proteins. Disaccharide analysis of HS purified from SC showed that it has a highly sulfated and unusual chemistry. In order to better understand the optimal HS chemistry that is required to stimulate transglutaminase activity, we examined the effect of a heparan library of similar chemistry to endogenous HS but of different chain length. The data showed that long chain highly sulfated isoforms significantly activated transglutaminase activity while shorter chain length isoforms did not.
Taken together our data suggests heparan sulfate is likely to have an important role to play in corneocyte envelope maturation, SC innate defense and barrier function.

O13 - The triple action of leflunomide: a novel therapeutic mechanism of action in the treatment of inflammatory skin diseases.

Gijs Rikken, Joost Schalkwijk, Ellen H. van den Bogaard
Department of Dermatology, Radboud Institute for Molecular Life Sciences (RIMLS), Radboud University Medical Center (Radboudumc)

Introduction: Leflunomide, a FDA approved drug for the treatment of rheumatoid arthritis (RA). The active metabolite of leflunomide, teriflunomide, inhibits the synthesis of pyrimidine, leading to a reduction of lymphocytes in RA patients which is considered the main therapeutic mechanism. Leflunomide treatment is reported for atopic dermatitis (AD) and psoriasis in case studies and has recently been described to be an agonist of the aryl hydrocarbon receptor (AHR). Targeting the AHR, like in coal tar treatment, alleviates the symptoms of chronic inflammatory skin diseases like AD. We therefore postulate that leflunomide-mediated AHR activation has therapeutic potential in AD and psoriasis.
Methods and Materials: Primary human keratinocyte monolayer cultures and organotypic epidermal equivalents for normal and AD skin were used to evaluate the effects of leflunomide and teriflunomide on epidermal morphology, keratinocyte differentiation and proliferation,

inflammatory responses, AHR activation and cell toxicity. Proliferation rates of peripheral blood mononuclear cells (PBMCs) were also determined after exposure to leflunomide and teriflunomide.
Results: AHR activation by leflunomide (10 µM) in keratinocytes resulted in induced epidermal differentiation. Furthermore, leflunomide counteracted the IL-4-mediated effects in a 3D skin model for AD and downregulated keratinocyte and PBMC proliferation. Cell toxicity was only observed at concentrations of 100 µM and higher. Strongly reduced keratinocyte and PBMC proliferation was also observed for teriflunomide in an AHR-independent manner.
Discussion: We propose that leflunomide may be a good candidate drug for treatment of hyperproliferative inflammatory skin diseases due to its triple action by 1) reducing lymphocyte and keratinocyte proliferation, 2) interference with the IL-4 mediated JAK/STAT pathway and 3) AHR-mediated epidermal differentiation. The reported excellent skin permeation of leflunomide and its metabolism to teriflunomide in keratinocytes, suggests that leflunomide could be administrated as a topical drug for local treatment of mild to moderate skin inflammation.

O14 – Diverse problems in keratinocyte culture impede epidermal barrier studies: sharing failures to improve success rate

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Research on human epidermal barrier relies on keratinocytes cultures. Whereas each published culture protocol is claimed being successful, eventual problems may arise during regular practice, but all of them as well as clues to bring solutions remain poorly advertised. Here, weird but confirmed data from epidermal cultures are described. Reconstructed human epidermis (RHE), regularly produced since 2003 on polycarbonate filters using primary cultured keratinocytes, suddenly exhibited unusual low TEER (trans-epithelial electrical resistance) values, revealing a drop in barrier efficacy since November 2017. Moreover, abnormal weakened barriers were observed during infection of RHE by keratinolytic fungi. Defects in the cornified layer were also observed during the same period in RHE made of hTERT-keratinocytes. After checking cells strains for absence of bacteria and mycoplasma and culture incubators and hoods for calibration and accuracy, we investigated whether problems could originate from either different donors, polycarbonate filters, unknown or undisclosed alterations in culture medium. RHE were produced using cells from several patients and using different batches of medium and polycarbonate inserts before analyzing tissue histology and barrier efficacy. Variable results altogether indicate that the encountered problems could originate from a change in medium performances. In support to this hypothesis, primary keratinocytes isolated by skin trypsinization displayed rates of clonogenicity which vary with the culture medium used. Such data indicate that publication of failing experiments can warn other scientists working with same materials, especially when secrecy is kept on composition. Experiments usually considered as wasted could thereby be turned into useful information.

O15 – Identification of evolutionary ancient and lineage-specific epidermal differentiation genes in organotypic models of chicken skin

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The skin barrier to the environment is formed by keratinocytes under the control of a specific set of epidermal differentiation genes. Comparative genomics has revealed that several mammalian epidermal differentiation genes have homologs in reptiles and birds, but comprehensive information about keratinocyte differentiation in a non-mammalian species has remained elusive.

Here, we established a culture protocol for chicken keratinocytes that allows, to the best of our knowledge, for the first time to model 3-dimensional differentiation of non-mammalian skin cells in vitro. By transcriptomic analysis of non-differentiated and fully differentiated keratinocytes of the chicken, we identified genes that are upregulated in their expression during skin barrier formation. The inducible genes encode both proteins specific for avian skin, such as corneous beta-proteins (beta-keratins) and the antimicrobial protein gallinacin-14, and homologs of human skin barrier-associated proteins, such as ABCA12 and loricrin. Likewise, homologs of AQP3, a gap junction protein, and SDCBP2, a protein implicated in papilloma virus infection, were abundantly expressed in differentiated chicken keratinocytes. Furthermore, homologs of human keratinocyte differentiation proteins of as-yet-unknown functions, such as POF1B, annexin 8, spectrin beta (SPTBN2), a LY6 domain protein (LY6EL), and TMEM45, were induced in chicken skin equivalents.
We conclude that the comparative analysis of keratinocytes from phylogenetically diverse species under defined in vitro differentiation conditions helps to identify conserved and therefore evolutionary ancient epidermal cornification genes.

O16 – Chronological aging impacts abundance, function and microRNA content of extracellular vesicles produced by human epidermal keratinocytes

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Introduction: The intercellular dialog is crucial to ensure the homeostasis and the normal function of the skin. This dialog implies direct cell-cell interactions but also exchange of messengers molecules directly secreted by cells or transported by extracellular vesicles (EVs). EVs carry complex cargo, including messenger RNAs, microRNAs, LncRNAs, DNA fragments and proteins. The present study was therefore conducted to elucidate whether the characteristics and functions of EVs released from keratinocytes can be modulated during aging process.
Materials and Methods: EVs were purified from conditioned medium of primary keratinocytes isolated from infant or aged adults skin, cultured at early or late passage. EVs size and abundance were determined using a Malvern Zetasizers. Their functional impact was investigated by treating monolayer cultures of keratinocytes and reconstructed epidermis, and by local injection in C57BL6 mice suffering a back wound. The EVs microRNA content was explored by smallRNAseq on an Ion Torrent Proton sequencer.
Results: We observed that aging did not impact the EVs size distribution whereas the relative number of EVs released from aged keratinocytes was significantly increased. We also observed that EVs from aged keratinocytes were able to reduce the proliferation of young keratinocytes, to impact their organogenesis properties in a reconstructed epidermis model and to slow down the early steps of skin wound healing in mice, three features observed in aged epidermis. We then investigated the microRNA content of EVs from young and old keratinocytes: we identified several microRNAs with a modified abundance in EVs from old cells including miR-30a, a microRNA that we recently characterized as a key regulator of barrier function in human epidermis.
Conclusion: this work reveals that intercellular communication mediated by EVs is modulated during aging process in keratinocytes and might be involved in the functional defects observed in aged skin. We identified several microRNAs transported by EVs that could be the mediators of this “aging message” in the epidermis. Further experiments are in progress to validate the role of these microRNAs in the epidermal barrier dysfunction observed in aged skin.

P1 - Ex vivo human skin model to study efficiency of dermo-cosmetic compounds on re-epithelialization

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Introduction: Re-epithelialization begins by the proliferation and migration of stem and differentiated skin cells, and extracellular matrix components. Deregulation of one or more wound healing phases can cause pathological healing, such as keloid scars, or chronic wounds. To develop new ingredients able to facilitate superficial re-epithelialization, dermo-cosmetic industry uses different in vitro and ex vivo models before clinical tests. currently the most suitable model is skin explant because of structure and immune defense's preservation.
The purpose of this study is to report an experimental model based on stripped skin explant to mimic a superficial injury to evaluate the superficial re-epithelialization, in order to test dermo-cosmetics compounds.
Materials and methods: A piece of abdominoplasty is stripped or not (control) with a specific adhesive. After we created a biopsy which is include in a nourishing matrix disposed in an insert (Nativeskin©). The evolution of reparation is followed at different days by immunostaining on skin paraffin section. To evaluate the repair treatment on model, we treated a stripped explant with a commercial wound healing product.
Results: Measures of stratum corneun thickness on stripped explant show a natural increase between the first and 7th day. At 7th day, we observe a better regeneration of stratum corneum with the wound healing treatment. The quantification of mean fluorescence intensity of loricrin, filaggrin and decorin shows a decrease on stripped explant compared to skin control. Over time, these markers increase with the re-epithelialization. With the wound healing treatment, we quantified a significant increase of filaggrin compared to stripped skin. Ki67 staining shows an increase of proliferation on stripped skin from 0 to 3th day. But at 7th day, the proliferation rate is important only with the wound healing treatment.
Discussion: These results have shown a natural re-epithelialization of the skin model. We observed also a better regeneration with a wound healing treatment and demonstrate that this model can be used to test efficiency of dermo-cosmetic ingredients.

P2 - Impact of cigarette smoke on different skin models

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Introduction: The human skin, and especially the upper layer of the epidermis, plays the role of a barrier, and is also one of the first and major targets of air pollutants. Epidemiological and in vitro studies have shown that cigarette smoke had multiple negative effects on skin inducing premature aging. Our aim is to develop tools to study the consequences of skin exposure to cigarette smoke.
Material and Methods: For this work, we set up two systems. The first method consists of creating cigarette smoke extracts by bubbling cigarette smoke in cell culture media and then by depositing it on cell models. This technique provides an evaluation of the cytotoxicity of pollutants contained in the smoke on primary skin cells. The second system is based on skin biopsies enclosed in a chamber saturated in cigarette smoke. This both methods allow the study of protein expression's consequences of cigarette smoke applied on the skin and the study in genomic level using genomic chip that can quantify ninety-five genes involved in the skin physiology.
Results: Exposition of cells with cigarette smoke extract induced a cytotoxicity in a cigarette dose-dependent manner. Exposure of explant to tobacco leads to the deterioration of the epidermal layers with the appearance of pyknotic nuclei, significant degradation of the dermo-epidermal junction and an increase of cells positive for γ-H2AX after cigarette smoke exposure of skin biopsies. At the genomic level, preliminary results indicate that the cigarette smoke

causes significant changes in the expression of genes implicated in different function, especially in barrier function.
Conclusions: Additional markers must be studied to confirm cigarette smoke effect on skin models. The systems are now proposed for the identification and development of new compounds able to reduce the harmful effects of tobacco smoke on the skin.

P3 - Inhibition of deimination with Cl-amidine alters cornification and keratinocyte autophagic flux.

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Introduction: Deimination is a posttranslational modification catalyzed by a family of enzymes called peptidylarginine deiminases (PADs). The exact role of protein deimination remains to be fully described, although it has been associated with numerous pathophysiological processes and diseases. Three PADs are expressed in the epidermis and skin appendages. Our aim is to study their importance in the epidermis homeostasis.
Methods: Three-dimensional reconstructed human epidermis were treated for two days with increased concentrations (from 0 to 800 µM) of Cl-amidine, a specific pan-PAD inhibitor. The efficacy of Cl-amidine treatment was analyzed by western blotting with antibodies specific for deiminated proteins, its effect on the morphology of the epidermis by transmission electron microscopy, and its consequence on protein expression by RT-qPCR and immunochemistry.
Results: Treatments with Cl-amidine inhibited deimination in a dose-dependent manner, without any cytotoxic effect or decrease in cell proliferation. At 800 µM, Cl-amidine was shown to decrease deimination by half, to reduce the number of corneocyte layers, and to increase the number of transitional cells. In granular keratinocytes, mitochondria and nucleus morphology was altered, and heterogeneous cytoplasmic vesicles were observed. LC3-II, a marker of autophagic vesicles, and sestrin-2, an inhibitor of mTOR signaling pathway, were up-regulated after Cl-amidine treatment, as well as mRNAs encoding sestrin-2, LC3B, ATG5 and ATF4. Inversely, treatments of reconstructed human epidermis with rapamycin, a known inducer of autophagy, increased deimination as shown by immunodetection of total protein extracts. This supports the hypothesis of a link between PAD activity and autophagy flux in the epidermis.
Conclusion: These results demonstrated that Cl-amidine treatments slow down cornification through modulation of the autophagic flux in the granular layer. This suggests that PADs and deimination play a key role in the constitutive autophagy process that occurs at the transition between granular and cornified layers.

P4 - Tolerance induction towards a neo-antigen expressed in skin grafts

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Epidermolysis Bullosa (EB) represents a group of genodermatoses caused by mutations in anchoring proteins of the skin leading to skin fragility and blisters formation. Replacing defective genes by ex-vivo gene therapy and subsequent transplantation of corrected skin grafts is a promising treatment approach. As this therapy introduces the wild-type version of the mutated protein in the transplant, it bears the risk of eliciting immune responses against this neo-antigen, which can lead to graft destruction. Therefore, patients without residual protein expression (i.e. who carry null-mutations) are currently excluded from these clinical trials. We thus aim to elucidate the immune responses leading to skin graft rejection with the goal to develop an immune tolerance treatment to protect neo-antigen-expressing skin grafts.

To this end, we developed two mouse models that mimic ex-vivo gene therapy in patients with null mutations. In these models, we graft syngeneic skin expressing a neo-antigen (either ovalbumin or human type XVII collagen/hBPAG2). These skin grafts were rejected around two to three weeks after the transplantation. The onset of rejection was correlated with increased CD4+ T-cell numbers in the skin grafts, associated with an elevated release of Th1 associated cytokines. We hypothesized that the induction and maintenance of suppressive regulatory T-cells (Treg) in the skin would prolong graft survival. We developed a protocol to generate neo-antigen-specific peripheral Treg (pTreg) in vivo using IL-2/anti-IL-2 complex therapy combined with rapamycin co-applied with the neo-antigen. The percentage of Treg was significantly increased and resulted in prolonged graft survival to up to 55 days after transplantation.
These findings underline the immunogenicity of neo-antigen expressing skin grafts. We further found that antigen-specific pTreg could prolong graft survival in our mouse model. In future research we will further determine the mechanisms of graft rejection and the role of pTreg in long-term graft acceptance.

P5 – K14-Cre-mediated deletion of the autophagy factor Atg7 reveals essential roles of autophagy in Merkel cells, sweat glands and enamel epithelium

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Autophagy contributes to the turnover of proteins and to the removal of damaged organelles in eukaryotic cells. The characteristic consequence of impaired autophagy is the accumulation of protein aggregates containing filaments of sequestosome 1 (Sqstm1)/p62, which is a prototypical autophagy substrate. The relevance of autophagy for the homeostasis of the different cell types of the skin has remained incompletely understood.
Here, we deleted the essential autophagy gene Atg7 by the Cre-loxP system in keratin K14-expressing epithelial cells and their progeny in the mouse. Sqstm1/p62 was immunolabelled on tissue sections to detect aberrations of protein turnover in specific cell types. The suppression of autophagy was compatible with epithelial keratinization in the interfollicular epidermis where p62 was not significantly increased in the absence of Atg7. By contrast, p62 showed massive accumulation in Merkel cells and secretory cells of the sweat glands of Atg7f/f K14-Cre mice, indicating that in epithelial cells undergoing a differentiation-associated switch from K5/K14 to K8/K18 expression, autophagy is required for normal protein homeostasis. Ameloblasts which form the enamel of teeth also differentiate from K14-positive precursors and express K8/K18. In Atg7f/f K14-Cre mice, the enamel epithelium showed an aberrant accumulation of p62. Impaired secretion of iron from Atg7f/f K14-Cre ameloblasts led to change in the color of incisors from normal yellow to white.
In summary, these results suggest that K8/K18-positive cells of the skin and of the enamel epithelium require autophagy for the control of p62 and normal homeostasis whereas interfollicular epidermal keratinocytes do not require Atg7-dependent autophagy for the control of p62.

P6 – An atypical case of Netherton syndrome with mild severity and no hair dysplasia in a Caucasian patient harbouring novel compound heterozygous variants in *SPINK5*

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Netherton syndrome (NS) is a syndromic ichthyosis due to mutations in *SPINK5* encoding LEKTI. When absent or non-functional, LEKTI is no longer able to play its role as serine protease inhibitor, resulting in premature desquamation, impaired epidermal barrier and inflammation. Affected individuals consequently present with skin inflammation, desquamative lesions, atopic manifestations and specific hair shaft abnormality (trichorrexis invaginata). We report here an atypical case of NS.
An 18 years old Caucasian woman presented mild congenital ichthyosiform erythroderma without hair dysplasia neither familial history nor consanguinity. Next generation sequencing analysis of genomic DNA revealed two compound heterozygous mutations in *SPINK5* (NM_001127698): c.750_751insG in exon 9 and c.1342C>T in exon 15. The first variant predicted a frameshift with a premature termination codon (*p.Arg252fs11**). The second mutation was a missense (*p.Arg448Trp*) reported in the Exome Aggregation Consortium database with a very low occurrence and no homozygosity, but no associated skin diseases reported in the literature. Detection of LEKTI by immunoblot performed on epidermal proteins revealed abnormal LEKTI profile consistent with altered maturation process of the protein and no immunoreactive band at a molecular weight corresponding to the predicted truncated mutant. This suggested that only the missense variant was expressed and responsible for the mild phenotype. Functional analysis of these mutations is ongoing using primary keratinocyte cultures established from the patient's skin. More than 80 *SPINK5* mutations have been reported in NS among which only two are missense and associated with mild NS. A few other cases of mild NS seem related to splice-site mutations with residual LEKTI expression.
This new case of mild NS reported here enlarges *SPINK5* mutation spectrum and contributes to a better understanding of the relationship between genotype and phenotype. In case of congenital ichthyosiform erythroderma, even if hair dysplasia is lacking, clinicians must think about diagnosis of NS.

P7 - Lipid defects in chronic dermatoses: development of a 3D model of reconstructed human epidermis to evaluate the efficacy of Ceramide-Replacement Therapy.

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The uppermost layer of epidermis, the *stratum corneum*, mainly provides the so-called barrier functions that protect the body against external aggressions and prevent the loss of water and electrolytes. A fundamental element for the integrity of this barrier is the presence of ω-O-acylceramides. Those epidermis-specific ceramides are indeed mandatory for *stratum corneum* lipid structures organization and function. Altered ω-O-acylceramide metabolism is observed in common inflammatory dermatoses such as atopic dermatitis or psoriasis. It is linked to the pathophysiology of some rare genodermatoses as well, such as autosomal recessive congenital ichthyosis (ARCI), especially those caused by mutations in *PNPLA1*. Topical application of ω-O-acylceramides could therefore constitute an original therapeutic strategy for restoring the epidermal barrier in these patients.
We developed and characterized a 3D model of reconstructed human epidermis to evaluate this type of treatment. This model relies on our recent work showing that PNPLA1 is essential for ω-O-acylceramide synthesis. *PNPLA1* knockdown by RNA interference (shRNA) in this organotypic model reduces ω-O-acylceramide production by about 40%. As expected, disturbance of ω-O-acylceramide metabolism disrupts *stratum corneum* lipid organization and significantly alters epidermal permeability.
This model is therefore a relevant tool for testing the efficacy of ω-O-acylceramide replacement therapy to restore the lipid balance of the stratum corneum and improve the epidermal "barrier".

P8 - Assessing skin barrier parameter modulation and recovery, in a sensitive skin model exposed to dryness.

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Background: The epidermis is sensitive to different stimuli and keratinocytes are at the forefront of the sensory system. For about 40% of the population, under the influence of environmental factors (cold, hot, dryness, pollution, wind, chemicals ...), this sensitivity is exacerbated and causes intolerance and unpleasant sensations. The complete physiological mechanism is still unknown even if the nervous system and some neuromodulators are suspected. Activation of PAR-2 receptors and the serine protease cascade involvement are described, leading to skin barrier weakening and activation of inflammation explaining the unpleasant sensations.
Methods: As cold weather, wind and air-conditioned atmospheres could explain a large part of the uncomfortable sensation origin, we decided to use a skin reconstructed model to mimic the sensitive skin exposed to environmental stresses. We chose to submit the model to a dehydration stress after conditioning the skin with histamine, reproducing a mast cell degranulation situation after neurogenic inflammation (PAR-2 activation).
Results: Using immunolabelling of Claudin 1, permeability of the fluorescent dye Lucifer Yellow through the stratum corneum and the TEER measurement, we tried and optimized the model conditions to obtain the impairment of the barrier integrity of the model. It was also accompanied by a defect of TRPV4 expression by immunolabelling. Optimised conditions were used to test the prevention capability of a formulation dedicated to sensitive skin. All these parameters could be restored by topical application of the formulation, previous the dehydration stress, demonstrating its efficacy in protecting sensitive skin from barrier failure and water homeostasis caused by environmental factors.
Conclusion: To conclude, we have developed a model that could mimic a sensitive skin exposed to environmental stress and help to measure effectiveness of topical products on reconstructed skin exposed to dryness, especially on barrier integrity recovery and which allows other adaptations.

P9 - Development of a full-thickness skin model to mimic the fragile skin of the elderly: skin care protection with rhealba® oat plantlet extract

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Background**:** The proportion of adults over 60 years of age is rapidly increasing, and age-related changes in skin integrity and barrier function make skin of older adults more “fragile” and more susceptible to develop skin pathologies such as xerosis, pruritus, dermatitis, and infections. The development of specific skin care products for the elderly that can play a key role in improving their quality of life, might help them to age better. The aim of this study was to develop a full-thickness skin equivalent model that would present characteristics of the fragile skin of elderly that could be used for topical product evaluation.
Methods**:** we submitted a full-thickness skin equivalent to different chronic ultraviolet A (UVA) exposure conditions and investigated the effect on the different compartments of the skin using laser scanning confocal microscopy (LSM) and transmission electron microscopy: dermis, dermo-epidermal junction (DEJ) and epidermis.
Results: We confirmed in our model that chronic UVA exposure induced an important disruption of the elastic network (fibrillin and elastin), and a loss of density in the dermis. In response to UVA, the DEJ was also disorganized and presented a loss of homogeneity in the lamina but also a decrease in the anchoring fibers. We then observed a defect in cell junctions such as the tight junction (claudin 1) and desmosomal markers (desmoglein 1 and desmocollin 1), suggesting a

major skin barrier alteration, as observed in the fragile skin of the elderly. We then investigated the improvement of the skin fragility in our model by evaluating the benefits of a formula, containing Rhealba® Oat Plantlet Extract. We observed that formula restored the elastic system, improved the DEJ cohesion, and restored claudin 1, desmosomal marker expression and preserved the ultrastructure of desmosomes.
Conclusion**:** We developed a full-thickness skin model that mimics the fragile skin of the elderly and used it to demonstrate that a dermo-cosmetic product containing Rhealba® Oat Plantlet Extract could afford an efficient skin care improvement.

P10 - Development and validation of a RHE model with impaired barrier function: application to medical devices biocompatibility assessment

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Medical devices (MD) composed of substances or combinations of substances belong to a large heterogeneity of products intended by the manufacturer to be used, alone or in combination, for specific human medical purposes (e.g. diagnosis, prevention, treatment or alleviation of disease) and which does not achieve their principal intended action by pharmacological, immunological or metabolic mechanical means. New Medical Device Regulation EU n. 2017/745 (MDR) indicates that MD that come into contact with injured skin are classified in IIb risk class consequently the assessment of their biocompatibility plays a key role in device classification. By using a commercially available and standardized reconstructed human epidermis, the objective of this study was to recapitulate the biological target of MD intended to be applied on injured skin.
The lesional model with impaired barrier function has been induced by a mechanical abrasion targeting the epidermal physical barrier: SC and tight junctions associated to granular layer without involving deeper epidermal structures. A multiple endpoint analysis (MEA) approach has been adopted by quantifying the following parameters in negative and positive controls (lesional RHE in presence /absence of reference irritant) and on a series of raw materials after 24h exposure.

- Trans-Epithelial-Electrical-Resistance (TEER)
- Caffeine permeability assay to identify a different kinetics
- Cell viability by MTT test at 24h
- Transepidermal Water Loss (TEWL) by Tewitro® TW 24 monitored during 24h in a separate RHE series.
- Histo-morphological and immune-histochemical analysis: H&E, Biotin staining, filaggrin-IF and Claudin-1-IF.

The results have shown that the lesional RHE is characterized by an increased permeability to caffeine during the first 3h, a stable impairment of TJs as measured by biotin assay and Claudine-1- IHC and associated to significant morphological modifications of the tissue architecture, TEER reduction and TEWL increase during the experiment, no modification of cellular viability as per MTT.

P11 – Targeted Disruption of the Hornerin Gene in Mice Causes Hair Loss

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Skin barrier homeostasis is essential for human survival. Impaired epidermal barrier function often leads to serious skin diseases such as atopic dermatitis (AD). Loss-of-function mutations in the filaggrin (FLG) gene, encoding an epidermal scaffold protein, were uncovered to be major predisposing factors for AD. Hornerin (HRNR), a profilaggrin-like protein, is a component of the

epidermal cornified cell envelopes and plays a role as antimicrobial materials in the skin. However, its biological function remains unclear.
To understand the underlying mechanisms, we established the hrnr knockout mouse model by using the Cas9/CRISPR technique. The fluorescent protein gene (Tdtomato) with a terminator T (WPRE) was inserted into the exon 3, which led to loss expression of the functional domains of HRNR. To obtain the F0 generation allophenic mice, the F0 generation microinjected oosperm were implanted into surrogate mice uterus. The F1 stable genetic generation mice were obtained by mating and breeding. Ten new-born hrnr-KO homozygote mice showed hair loss in the back and the hip.
Our results indicate the hornerin gene might play an important role in skin homeostasis and normal hair growth.

P12 - Genome-wide analyses provide key insights into AHR signaling skin barrier biology and disease

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Introduction: Despite the longstanding clinical use and efficacy of coal tar in dermatological practice, the molecular mechanism was unknown until the discovery that coal tar activates the aryl hydrocarbon receptor (AHR).
Materials and methods/Results: The identification of the AHR-dependent therapeutic effects of coal tar led us to fractionate coal tar in order to identify its active ingredients, to study the therapeutic activity of novel AHR ligands for atopic dermatitis (AD) and to investigate the downstream effects of AHR signaling in keratinocytes. The fractionation of coal tar resulted in 144 single fractions that were pooled to 11 fractions based on GC-MS data. Coal tar odor was restricted to the colorless fractions 1-5, while fractions 6-11 were fluorescent yellow to orange and having an unpleasant chemical odor. AHR activity and induction of skin barrier proteins was restricted to fractions 6-11, containing apolar compounds with a less favorable safety profile (potential mutagens). ChIP-sequencing (t=30 min) and RNA-sequencing analysis of the early and late responses of AHR signaling in keratinocytes after coal tar exposure (t=2 hours, t=24 hours) indicate that the AHR interacts with other transcription factors and signaling pathways to regulate the expression of genes involved in the cell cycle, keratinocyte differentiation and host defense responses.
Discussion: Our data indicate that components that cause the typical coal tar odor are dispensable for its therapeutic effect. However, they maybe indispensible for providing a counterbalance to other pharmacologically active and potentially toxic chemicals in coal tar. The genome-wide analysis of signaling events provide key insights into the regulation of skin homeostasis by the AHR and are key for the identification of novel therapeutic targets for the treatment of skin barrier-related diseases.

P13 – Tight Junctions: Indispensable Gatekeepers in Skin

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Tight junctions (TJs) are contact sites found in epithelial and endothelial cells that regulate the permeation of molecules and ions through the space between cells. In essence, they decide what comes in and out of our body and various tissues, and hence are critical to the physiology of the body. The dysregulation of the barrier properties of TJs can lead to pathologies such as inflammation, skin allergies, and metastasis. The main structural and functional component of TJs are claudins, a family of transmembrane proteins. Claudins regulate the leakage of molecules between cells by forming barriers involving their extracellular domains. Claudin 1 is specific to the skin being indispensable in ensuring that its effectiveness as one of the tightest barriers in the body. The definitive molecular organisation of the claudins in their native environment (embedded in cell membranes) is currently inaccessible by experiment.
Here, we address this problem by employing molecular dynamics simulations of the extracellular domains of claudin 1, with a view to elucidate the architecture and barrier properties of TJs. The simulations yield structural information, dynamics and also thermodynamic quantities characterising the system.
Our results reveal that within the same lipid bilayer (cis-interaction) the extracellular domains of the claudin 1 interact with each other in a non-specific way, while between opposing lipid bilayers (trans-interaction) the non-conserved regions of the loops dictate their interaction. The overall morphology of the resulting claudin strands agrees with electron micrographs, where TJs appear as a network of cross-linked intramembranous particles.
A better understanding of the molecular organisation of the TJ strands linked back to the structure could potentially lead to the development of new strategies for enhancing transdermal drug delivery, treatment of skin disease such as atopic dermatitis, and other TJ-linked diseases.

P14 - Clinical phenotypes correlating with trans-epidermal water loss (TEWL) and skin hydration in East London Bangladeshi eczema patients – preliminary findings from the Tower Hamlets Eczema Assessment (THEA)

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Introduction: The filaggrin (FLG) gene represents the major AE risk gene in the Bangladeshi population in London, who have severe AE. Filaggrin pays a significant role in barrier formation and skin hydration via the production of natural moisturising factors. In the THEA study, we are performing detailed clinical phenotyping and genotyping in Bangladeshi children and young adults with AE.
Methods & Materials: Data were collected from Paediatric/Adult Dermatology and Allergy clinics in a large teaching hospital. Patients were examined and eczema morphology and presence or absence of hyperlinear palms (HLP), soles (HLS) and lips (HLL), keratosis pilaris, xerosis and ichthyosis vulgaris (IV) were recorded. Eczema severity and effect on quality of life was assessed. Barrier function (units = g/h/m2) and skin hydration (SH; units – AU) were measured in triplicate using Courage and Khazaka's (Köln, Germany) Tewameter® TM 300 and Cornemeter® CM 825 on non-lesional volar forearm skin.
Results: We collected data on 62 participants with a mean age of 11.3 years (1.2-30.9). No significant difference in TEWL/SH were noted in those with HLP (n=60), HLS (n=48) or IV (n=42). Significant reduction in skin hydration was noted in those with HLL (n=52) – 33.9 vs 23.2 (p=0.001) and clinical xerosis (n=33) – 28.0 vs 22.1 (p=0.01). A higher TEWL (p=<0.0001) and lower SH (p=0.005) were significantly correlated with increased eczema severity measured by

EASI score. Finally there was a trend of reduction in TEWL of 17.4 vs 14.1 (p=0.06) in those with a history of hospital admission with eczema herpeticum, independent of eczema severity.
Discussion: We present preliminary data that link specific clinical signs with an impaired barrier in Bangladeshi AE patients in the UK. AE severity is linked with high TEWL and reduced skin hydration values.

P15 – Derivation and characterization of induced pluripotent stem cell lines from atopic dermatitis patients heterozygous for filaggrin

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Introduction: Atopic dermatitis (AD) or eczema is an incurable, non-contagious, extensive inflammatory and extremely pruritic chronical skin disease with a high prevalence worldwide. Loss-of-function mutations in exon 3 of the filaggrin gene (FLG) are associated with eczema etiopathology. Currently available in vitro models of AD are relatively simplistic and cannot be used to correlate extent of the symptoms and genetic polymorphism of AD patients. Thus, there is a need to develop more sophisticated and more relevant in vitro platforms to evaluate new drug candidates for the treatment of AD.
Methods and materials: Epidermal keratinocytes from three patients carrying different heterozygous mutations in FLG were reprogrammed into induced pluripotent stem cell (iPSC) using non-integrating Sendai virus-based vectors. The entire process of derivation and expansion of AD-iPSC lines were performed under xeno-free culture conditions.
Results: We have generated iPSC lines from AD heterozygous patients, carrying the three the most frequent European mutations in the FLG gene (p.R501X, c.2282del4, p.R2447X). Characterization included molecular karyotyping, mutation screening using restriction enzyme digestion and Sanger sequencing, while pluripotency and differentiation potential were confirmed by expression of associated markers in vitro and by in vivo teratoma assay. Furthermore, we demonstrated that AD-iPSC lines with the FLG mutations can be differentiated into epidermal keratinocytes.
Discussion: Library of iPSC lines with the most common variants in the FLG gene can be efficiently used to construct highly specific in vitro 3D human epidermal equivalents (HEE) for drug discovery towards novel personalized medicine in atopic dermatitis.

P16 – Evolution of newborn skin surface lipids from birth until six months

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After life in utero and birth the skin is submitted to an important process of adaptation to a relatively dry gaseous environment. Skin surface lipids (SSLs) contribute actively to the protection of the skin barrier. Our objective was to study the evolution of each lipid compound during the postnatal period.
SSLs were collected from six newborns a few days after birth until the age of six months. 70 samples were analyzed using high-temperature gas chromatography coupled to mass spectrometry (HT-GC/MS). The use of separative techniques coupled to mass spectrometry for the analysis of samples containing complex mixtures of lipids, generates a large volume of data

which renders the results interpretation very difficult. We propose a new approach to handle the raw data, a Clustering-based preprocessing method (CB-PPM), to achieve; 1-volume reduction of data provided by each chromatogram without loss of information, 2- alignment of time retention shift between different runs, 3 - clustering mass spectra of the same molecule in one qualitative group, 4- Integration of all data into a single matrix to be explored by chemometric tools.
Using this approach, we have demonstrated an increase of cholesterol esterification with epidermal fatty acids (C20 to C25) with age. Once it is synthesized, cholesterol may be esterified with a fatty acid; a decrease in production of sebaceous free fatty acids after the age of 1 month could promote cholesterol esterification by epidermal fatty acids.
The decrease in sebaceous lipids and the increase of epidermal lipids with age may be a compensatory phenomenon related to an adjustment phase after birth. This epidermis participation in SSLs at molecular level in the first period of life has not been shown yet. This result could be very interesting for the development and the improvement of product destined for the protection of infant skin

P17 - Ceramide biosynthesis characterization on 2D keratinocyte cultures: a multimodal approach

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Stratum corneum lipids play a key role in the barrier function of the skin. This is due to the complex composition and organization of lipid classes and subclasses. Stratum corneum lipid biosynthesis is initiated at the beginning of keratinocytes differentiation. During this process, evolution of simpler lipids biosynthesis towards more complex lipids is observed. At the terminal differentiation stage, ceramides, fatty acids and cholesterol are synthesized to fill the intercellular region of stratum corneum and to surround the corneocytes. These molecules are known to be responsible of the barrier function in the outermost layers of the skin. Lipids classes are divided into subclasses which differ by their structural microheterogeneity. Lipids biosynthesis at the molecular level during keratinocytes differentiation have not fully been investigated.
In this study, keratinocytes HaCaT cells were de-differentiated and then submitted to a high calcium medium to stimulate the differentiation in vitro and to reproduce the lipid profile of epidermis. To investigate lipids biosynthesis, comprehensive lipid profiling was performed with High-performance liquid chromatography coupled to high resolution mass spectrometry (HPLC/MS) and the evolution of the spatial subcellular lipid organization and distribution was explored by Raman hyperspectral imaging. Five stages were compared: after 2h, 3, 6, 9 and 13 days of differentiation in high calcium medium.
In this study, some keratinocyte morphological evolutions due to differentiation have been observed. HPLC/MS allowed us to demonstrate the lipid profile evolution. In fact, several ceramide sub-classes have been detected at differentiated keratinocyte level. In addition, Raman microspectroscopy was useful to highlight lipid signal modification by calculating the ratio of bands: lipid / protein, saturated / unsaturated lipids.
Thus, our multimodal approach represents an efficient methodology for studying epidermal lipids at the cellular and molecular levels.

P18 – The kinase RIPK1 protects from detrimental ZBP1 activation to maintain skin homeostasis

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Receptor interacting protein kinase 1 (RIPK1), which has kinase-dependent and kinase-independent functions, has been shown to be required to maintain epidermal homeostasis. Epidermis-specific RIPK1 deficient mice ($RIPK1^{EKO}$) mice are born with the expected Medelian ratio, but after 3 weeks of age, $RIPK1^{EKO}$ mice spontaneously develop severe skin lesions. This phenotype can be rescued by additional ablation of necroptotic signaling molecules such as RIPK3 and MLKL (mixed lineage kinase domain like pseudokinase). It has been genetically shown that RIPK3 gets activated in a ZBP1-dependent way. ZBP1 (Z-DNA binding protein 1) is cytoplasmic nucleic acid sensor mainly known for its function in detecting viruses thereby leading to a protective host innate immune response. We investigated whether the nucleic acid sensing capacity was involved ZBP1-dependent RIPK3 activation in $RIPK1^{EKO}$ mice.
Therefore we crossed $RIPK1^{EKO}$ mice with $ZBP1^{mZα1mZα2}$ mice containing inactivating mutations in the ZBP1 nucleic acid sensing domains (Zα1Zα2). $RIPK1^{EKO}$;$ZBP1^{mZα1mZα2}$ double knock-out mice were completely rescued from developing skin lesions, suggesting that sensing of endogenous nucleic acids by ZBP1 can contribute to skin inflammation. Interestingly, histological analysis indicated that both apoptotic and necroptotic keratinocyte cell is stopped in $RIPK1^{EKO}$;$ZBP1^{mZα1mZα2}$ mice, while in $RIPK1^{EKO}$;RIPK3 or $RIPK1^{EKO}$;$MLKL^{EKO}$ double deficient mice only necroptotic cell death was rescued, which was sufficient to protect from skin lesion development. We also developed dox-inducible keratinocyte cell lines expressing ZBP1 or $ZBP1^{mZα1mZα2}$. Overexpression of ZBP1 wild-type induced cell death, while similar levels of $ZBP1^{mZα1mZα2}$ did not, again suggesting that ZBP1 can sense endogenous danger signals present in certain cell types.
Taken together, our data indicate that ZBP1 not only senses foreign danger signals, but also react to endogenous triggers. It will be interesting to investigate whether ZBP1 could be involved in certain inflammatory skin diseases.

P19 – *Malassezia restricta*-mediated scalp surface lipoperoxidation

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Introduction: Squalene (SQ) is a major component of sebum, and squalene monohydroperoxide (SQOOH), derived from SQ, is an early biomarker of lipoperoxidation. Increased content of SQOOH on dandruff scalp surface have recently been described and suggested as a new etiological dandruff factor. The yeast *Malassezia restricta*, more abundant on dandruff than on dandruff-free scalps, has been hypothesized as a possible source of this elevated content of SQOOH. The objectives of this *in vitro* program were i) to test whether *M. restricta* does induce squalene peroxidation and ii) to explore the deleterious effects of SQOOH on epidermal barrier function.
Materials & Methods: In a first study, *M. rectricta* (CBS 7877) was cultured in modified Dixon medium (mDixon) supplemented with SQ 2% - SQ was added during the exponential phase of yeast growth. SQOOH was quantified by LC/MS in culture supernatant at different time points following the SQ addition. In a second study, SQOOH was topically applied during 24h at physiological concentration on a reconstructed human epidermis (RHE) model. The vehicle consisted in mDixon supplemented with SQ 2% diluted in sterile water. Triplicates of RHE were used for each condition. The effect of this application on the RHE integrity was followed by MTT cellular viability and Lucifer Yellow permeability assays, and compared to those induced by the vehicle alone. Morphology of RHEs was also observed after haematoxylin-eosin staining.

Results: SQOOH content in the *M. restricta* culture supernatant continuously increased over time after the addition of SQ in the culture medium (not detected at t = 0h, 236 ng/ml at t = 24h). In the same time, SQ content continuously decreased (from 1.33 µg/ml at t0 to 0.52 µg/ml at t24). The dramatic increase of SQOOH content and decrease of SQ content did not occur in control conditions without *M. restricta* or SQ addition. On the other hand, SQOOH solution topically applied onto RHEs decreased cell viability compared to the vehicle, and increased Lucifer Yellow diffusion into the RHEs. The morphology of RHEs was also different from the controls (disorganization of keratinocyte layers) when the SQOOH solution was applied.
Conclusions: Previous *in vivo* studies have shown elevated quantities of *M. restricta* with SQOOH content on dandruff scalp surface. Here, we clearly show that *M. restricta* is able to induce the production of SQOOH and exerts deleterious effects on epidermal barrier function. These new *in vitro* studies go further in suggesting a new paradigm in dandruff origin where *M. restricta* would induce the lipoperoxidation of some sebaceous lipids leading to molecular entities with adverse effects on barrier function.

P20 - Gene expression analysis and phylogenetic profiling of keratin K24

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Keratins are the main cytoskeletal proteins of epithelial cells. Most of the 54 keratin genes in the human genome are known to be expressed in a cell type- and tissue-specific manner, but some of the keratin genes have remained incompletely characterized. Here we determined the expression pattern of K24 which was previously proposed to be a cell differentiation marker in the epidermis.
Screening of publicly available microarray data showed that K24 is expressed at highest levels in the human cornea and other non-cornifying stratified epithelia. RT-PCR and western blot analysis confirmed high expression levels of K24 in the cornea but not in the epidermis. By immunofluorescence with a new antibody, K24 was detected in the suprabasal layers of the corneal epithelium. Comparative genomics revealed loss of the *Krt24* gene in fully aquatic mammals in which many epithelia have adapted to the special environment. By contrast, *Krt24* was conserved in the blind mole rat despite evolutionary degeneration of the corneal epithelium.
These data suggest that K24 contributes to the cytoskeleton of the corneal epithelium but also other epithelia. Further studies will determine the expression pattern of K24 in diseases affecting these epithelia.

P21 - An *in vitro* model for evaluating the bioavailability of topically applied compounds on damaged skin: application to sunscreen analysis.

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Background: The stratum corneum (SC) is the most important skin barrier against exogenous physical and chemical effects in addition to protecting against dehydration. Penetration of chemicals through the SC is generally considered to be the key step that limits percutaneous absorption. Information is lacking on dermal penetration of topically applied formulations on in vitro skin models, under conditions where the SC is damaged. Therefore, we have developed a standardized *in vitro* barrier disrupted skin model using tape stripping.
Methods: Different tape-stripping conditions were evaluated using histology, TEWL, infrared densitometry, and caffeine absorption. Human skin and porcine skin were used to develop the in vitro model. Indeed, due to the limited access of human skin explants, animal skin is frequently

used. The most relevant surrogate animal model for human skin is pig skin, as recommended by the SCCS opinion and the OECD test guidelines.
Results: The effects of tape stripping were comparable using pig and human skin. The data obtained by the measurement of different parameters (skin morphological analysis, NIR densitometry, TEWL, caffeine diffusion) permits to highlight the best conditions for disrupting the skin barrier of both models selected: human and pig skin and standardized them. The condition selected was 20 tape strips using application duration of 15 seconds. Optimised conditions were used to test the effect of SC damage and UV irradiation on the absorption of an UV filter combination present in a sunscreen. The bioavailability of the filters was extremely low regardless of the extent of skin damage, suggesting bioavailability would not be increased if the consumer applies the sunscreen to sun-damaged skin.
Conclusion: This standardised *in vitro* methodology using pig or human skin for damaged skin will add valuable information for the safety assessment of topically applied products.

P22 – Stabilization of Microtubules Restores Barrier Function after Cytokine-induced Defects in Reconstructed Human Epidermis

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Introduction: A variety of human skin disorders is characterized by defects in the epidermal barrier, leading to dehydration, itchiness, and rashes. Previously published literature suggests that microtubule stabilization at the cortex of differentiating keratinocytes is necessary for the formation of the epidermal barrier. We tested whether stabilization of microtubules with paclitaxel or epothilone B can repair barrier defects that were experimentally induced in three-dimensional culture models of epidermis.
Methods and Materials: We established two models of defective epidermis in vitro, using three-dimensional cultures of primary human keratinocytes on filter supports: immature reconstructed human epidermis (RHE), and RHE that was compromised by treatment with inflammatory cytokines, the latter mimicking defects seen in atopic dermatitis.
Results: Both paclitaxel and epothilone B promoted keratinocyte differentiation, accumulation of junctional proteins at the cell cortex, and the early appearance of lamellar bodies in immature RHE, whereas destabilization of microtubules by nocodazole had the reverse effect. Moreover, stabilization of microtubules rescued the barrier after cytokine treatment. The rescued barrier function correlated with the restoration of filaggrin and loricrin protein levels, the cortical accumulation of junctional proteins (E-cadherin, beta-atenin, and claudin-1), and with the secretion of lamellar bodies.
Discussion: Our data suggest that the microtubule network is important for the formation of the epidermis, and that stabilization of microtubules promotes barrier formation. Microtubule stabilization may support regeneration of damaged skin, by restoring or improving the barrier.

P23 - in vitro 3D models sharing atopic dermatitis characteristics

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The aim of this study was to establish robust 3D *in vitro* reconstructed epidermis (RE) mimicking the main alterations observed in the course of atopic dermatitis (AD). For this purpose, normal human primary keratinocytes were used to generate RE. Resulting 3D models were submitted to different conditions (inflammatory cocktail (compromised RE), solution of sodium lauryl sulfate (SLS)) to mimic biological pathways involved in atopic dermatitis.

Transcriptomic analyses followed by real-time quantitative PCR identified 19 genes in the compromised model similarly modulated in atopic skin. At the morphological level, the compromised RE treated by SLS expressed significantly less claudin-1, filaggrin and loricrin mRNAs and showed an accumulation in Lucifer yellow penetration. The SLS-treated compromised RE also presented an elevated carbonic anhydrase II expression, a feature of moisture loss. Finally, the secretion of TSLP and IL-8, two inflammatory cytokines synthesized in the course of AD, was increased in this 3D model. On the whole, the present treatment of RE induced characteristic alterations suggesting reduced epidermal cohesion and differentiation, a defect in the skin barrier function with loss of hydration and an increased Th2 inflammatory response.
Conclusions: these *in vitro* 3D RE models developed in this study present the essential alterations of atopic dermatitis and should be a very valuable tool to screen new molecules likely to improve this serious skin condition and test their efficacy.

P24 – The role of the gram positive anaerobic cocci Finegoldia magna in the human skin microbiome

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Introduction: We have recently reported that the absence of epidermal filaggrin expression affects the composition of the microbiota present on the skin and that filaggrin-deficient skin harbors a lower abundance of gram-positive anaerobic cocci (GPAC). Lower levels of filaggrin expression are related to atopic dermatitis (AD) skin and we therefore aimed to uncover a potential role of the most abundant GPAC in the human skin microbiome, Finegoldia magna, in AD pathophysiology by the analysis of its interaction with immune cells and keratinocytes.
Methods and Materials: The effect of F.magna and other skin commensals/pathogens on the cytokine production of peripheral blood mononuclear cells (PBMC) was determined. Gene expression was analyzed of human primary keratinocyte monolayers stimulated with heat-killed bacteria and bacterial/PBMC culture supernatant. Organotypic human epidermal equivalents were used to investigate the host-microbe interaction in a biologically relevant model system.
Results: F.magna differentially induced TNF-α, IL-1β and IL-10 secretion by PBMCs as compared to other skin commensals or S.aureus strains. In addition, F.magna and the culture supernatant of PBMCs treated with F.magna strongly induced the expression of host defense-related genes (e.g. DEFB4) in monolayer keratinocytes after 24 hours of co-culture. We were able to topically apply F.magna on epidermal skin equivalents and maintain bacterial survival during the co-culture period. Exposure to F.magna resulted in induced gene and protein levels of various antimicrobial proteins (e.g. hDB2, SKALP, LL37) in a dose and time dependent manner.
Discussion: Our data suggest that F.magna can act as an ‘alarm signal’ by inducing a strong host defense response in keratinocytes and immune cells. We therefore postulate that early production of keratinocyte-derived antimicrobial proteins induced by F.magna may protect against the colonization of pathogens like the AD-associated S.aureus.

P25 - Unripe citrus unshiu extract benefit to fight against atopic symptoms induced by indoor pollution

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Pollution is a major concern nowadays. Nonetheless environmental pollution has been shown to have critical effects on health and particularly on the skin, recent data suggest that home environmental factors may present a relevant impact. Indeed, people spend most of their time in their homes, schools, and public buildings, consequently concerns regarding indoor air pollution are rising. Many sources of indoor pollutants have been identified including furniture, tobacco smoke, air conditioners, construction materials, cloths, carpets, wall paints and human body itself. Pollutants include sulfur oxide compounds, formaldehydes, nitrogen oxide compounds, carbon monoxide, nitrogen dioxide, particulate matter, metals, smog and microbiological contaminants. Several studies involving children, in Europe and Asia have shown that indoor home remodeling activities (floor covering, wallpapering, new furniture) were associated with the development of Atopic Dermatitis (AD). AD prevalence and severity was also correlated to indoor polluted environments, but the causal relationship is not clear. AD is a chronic inflammatory allergic disease characterized by a T-lymphocyte activation leading to the production of Th2 type cytokines (IL-4, IL-5, IL-9, IL-13), eosinophils accumulation, IgE synthesis and mast cells activation [5]. AD is also associated with irritation, and itching sensation (nerve cells and mast cells involvement), confirming the central role of neuro-immune communication.
In this paper, we present the first *in vitro* and *ex vivo* experimental models demonstrating the clear link between some indoor pollutants and the resulting IL-4 production, which is an important factor triggering AD. Moreover, we address the downstream characteristic cascade of event occurring in the allergic response with eosinophil recruitment and mast cell activation, using specific or innovative models. Besides, we developed a well characterized plant extract from unripe Citrus Unshiu fruit (CUE) able to modulate immune response in allergic condition induced by pollutants.

P26 - Presence of very long-chain 3-ketodihydrosphingosine in both healthy and KDSR patients' skin

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Mutations in the KDSR gene, which is responsible for the encoding of 3-ketodihydrosphingosine reductase (KDSR), the second enzyme in the sphingolipid (SL) biosynthetic pathway, lead to recessive Mendelian disorders of keratinization characterized by a thick, scaly skin on the face and genitals and a thick reddish skin on soles and palms. It is assumed that defects in KDSR function are connected with an increased accumulation of its substrate – 3-ketodihydrosphingosine (KDS) and a subsequent decrease in SL products in such patients.
In the present work using UPLC-MS2, we described that besides the KDSR patients with significantly increased KDS, the KDS can also be found in the healthy subjects and the chain lengths of the KDS are longer than previously expected – the most commonly found KDS in healthy human skin reaches 24-26 carbons. To confirm this, the standard of C24-KDS was chemically synthesized. The synthesis started from 1-bromononadecane, its chain was prolonged using lithium acetylide to henicos-1-yne and was connected to the Garner aldehyde. Following reduction of a triple bond, oxidation of 3-OH group and deprotection provided final C24-KDS in moderate yield. The standard was then compared with the biological samples and the presence of very long-chain KDS was proved by the identical retention time and MS2-fragmentation characteristics at different collision energies.

Quantitative analysis of stratum corneum from the KDSR patient revealed not only highly increased levels of KDS as compared to controls, but also a shift of the base pattern to shorter chain length, which was also observed for sphinganine. A similar trend was observed also for ceramides (nonsubstituted, omega hydroxylated and omega esterified). Since serine palmitoyl-CoA transferase, the first enzyme in the SL biosynthesis, is responsible for the resulting chain length, we speculate, that KDSR might also have a regulatory effect on its substrate specificity.