European Epidermal Barrier Research Network

We proudly welcome you to our 1st virtual E²BRN live event

22. September 2021
Remote to Amsterdam
as Satellite Meeting of ESDR 2021

Co-organizers:
Julia Lachner
Yves Poumay

Thanks to great support:
Johanna Brandner
Leopold Eckhart

Meeting program:

13 :00-13 :10 *Get together and Welcome (program overview)*

Chairs: Julia Lachner (Vienna) and Ryan O'Shaughnessy (London)

13:10-13:40 1st Keynote speaker: **Esther Hoste**, Unit for Cellular and Molecular Pathophysiology, VIB-UGent Center for Inflammation Research, Ghent (BE)
"Linear deubiquitination is crucial for skin homeostasis by controlling keratinocyte death and stem cell identity"

13:40-13:50 Abstract #1: **AJ Hughes** (London UK): Increased BMP signaling correlates with increased eczema severity

13:50-14:00 Abstract #2: **P Jancalkova** (Hradec Králové CZ): The role of the low-temperature lipid phase transition in human skin permeability barrier

14:00-14:10 Abstract #3: **F Pardow** (Nijmegen NL) Regulation of keratinocyte differentiation through aryl hydrocarbon receptor signaling involves the transient activation of TFAP2A

14:10-14:20 Abstract #4: **G Rikken** (Nijmegen NL): Technical advance: bacterial colonization of 3D organotypic skin models for long term host-microbe interactions and microbiome intervention studies

14:20-14:40 *Break and Refresher*

Chairs: Katerina Vavrova (Hradec Kralove) and Michel Simon (Toulouse)

14:40-14:50 Abstract #5: **J Smits** (Nijmegen NL): Filaggrin knock-out in keratinocytes indicates a functional role in skin barrier formation

14:50-15:00 Abstract #6: **A Progneaux** (Namur BE) IL-2R□/IL-4R□ receptor induced in keratinocytes by IL-4 and IL-13 in atopic dermatitis can alter the epidermal barrier

15:00-15:10 Abstract #7: **E Clement** (Toulouse FR) Evaluation of an anti-inflammatory dendrimer to topically treat psoriasis

15:10-15:20 Abstract #8: **E Panoutsopoulou** (Hradec Králové CZ): Cutaneous imiquimod delivery efficiently assisted by phospholipid-based microemulsions

15:20-15:30 Abstract #9: **I M Dijkhoff** (Fribourg CH) The Effects of Antioxidant Trans-Resveratrol on an Atopic Eczema Epidermal Model *in vitro*

15:30-15:40 Abstract #10: **C Jacques-Jamin** (Toulouse FR) Effect of barrier disruption and inflammation on skin lipids

15:40-16:00 *Break and Refresher*

Chairs: Ellen van den Bogaard (Nijmegen) and Yves Poumay (Namur)

16:00-16:10 Abstract #9: **D Wotherspoon** (London UK): Single cell RNA seq revealed altered cytoskeletal dynamics and the formation of cytoplasmic RNA-Lamin A bodies during keratinocyte terminal differentiation

16:10-16:20 Abstract #10: **I Sagrafena** (Hradec Králové CZ): Spontaneous formation of long periodicity phase in model membranes from human stratum corneum lipids

16:20-16:30 Abstract #11: **H Niehues** (Nijmegen NL): Identification of keratinocyte mitogens: implications for hyperproliferation in psoriasis and atopic dermatitis

16:30-17:00 2nd Keynote speaker: **Nicolas Fortunel**, CEA, Genomics and Radiobiology of Keratinopoiesis Laboratory-Paris-Saclay University (FR)
"Exploring the epidermal barrier at the 'stem' level"

17:00-17:10 *Voting for the best Poster*

17:10-17:30 *General discussion. Bye-bye & end of the Meeting*

Refreshers

For our REFRESHERS we planed something new and unconventional.
We are looking forward to everybody who wants to join -

FIRST COME FIRST SERVE PRINCIPLE

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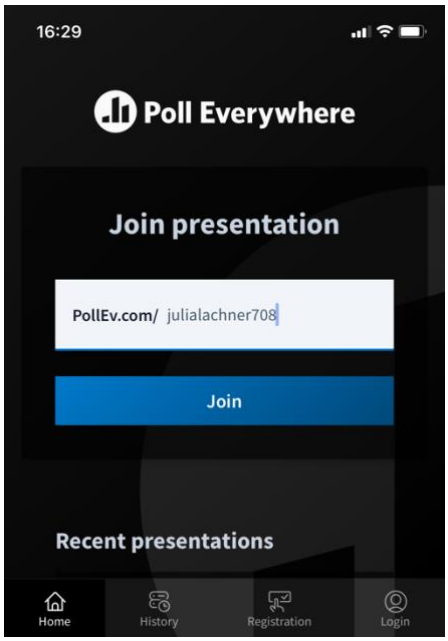

We are asking you to do this before the meeting, so we aren't losing a lot of time. If you have questions please connect with: julia.lachner@hotmail.com

Abstracts

P01 Abstract #1

Increased BMP signalling correlates with increased eczema severity

AJ Hughes, EA O'Toole, RFL O'Shaughnessy
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Filaggrin (FLG) is a key cornified envelope and epidermal barrier protein. *FLG* mutations are the most strongly implicated genetic risk factor for atopic eczema (AE). FLG is part of a gene complex, called the epidermal differentiation complex (EDC), the genes of which contribute to various aspects of epidermal barrier function. A number of EDC and Keratin genes were downregulated and components of the bone morphogenetic protein (BMP) signalling pathway upregulated in FLG siRNA knockdown keratinocytes

FLG siRNA knockdown caused significant reduction in FLG, Keratin 1, Keratin 10, Loricrin, Repetin and SPRR3 protein and RNA expression and an increase in phospho-SMAD (p-SMAD) 1/5 signalling, a marker of active BMP signalling. Western blots of tape strip samples taken from AE patients who were clinically phenotyped and genotyped for FLG mutation status, showed no correlation of EDC, Keratins or SMAD proteins with FLG expression but a statistically significant increase in p-SMAD 1/5 signalling in FLG compound heterozygote patients and a statistically significant correlation between EASI score and p-SMAD 1/5 expression in non-lesional skin.

Other studies show that SMAD signalling is increased in AE and that BMP2 signalling can control FLG expression. In these studies, increasing BMP2 expression led to impaired epidermal FLG, Loricrin and Keratin expression. As our data suggests that p-SMAD 1/5 is more highly expressed in AE with a pronounced genetic FLG deficiency, we propose a positive feedback model of barrier impairment in AE in which reduced FLG expression increases BMP2 signalling, consequently reducing EDC and keratin expression, which further impairs the epidermal barrier.

P02 Abstract #2

The role of the low-temperature lipid phase transition in human skin permeability barrier

Pavla Jancalkova, Monika Kopecna, Katerina Vavrova

Skin Barrier Research Group, Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

Stratum corneum (SC) lipids in healthy skin of mammals form vital barrier against water loss from the body and reversely against entrance of harmful substances from the environment. It has been described that these barrier lipids undergo a transition from a very tight orthorhombic to a slightly looser hexagonal arrangement at approximately 38°C, which is only a shade above the physiological temperature. The possible physiological purpose of this lipid transition is unknown. We proposed a *heat-shock lipids* hypothesis: loosening of the lipid arrangement may allow increased water loss, thus enhancing the cooling of the organism at elevated temperatures. The aim of this work was to characterize the human SC permeability for water and model permeants around this lipid transition.

Permeabilities of an *ex vivo* human SC for water, indomethacin as a model permeant and fluorescent labelled inulin as a model macromolecule were studied at 10 – 50°C (or 26 – 50°C for model permeants).

Water loss increased with temperature; however, Arrhenius plot did not show any change in activation energy. In contrast, the activation energy for indomethacin permeability decreased above 40°C. The permeation experiments with a model macromolecule also gave promising results.

Thus, the phase transition of SC lipids just above the physiological temperature seems not to be important for water loss but more likely the loosening of tight lipid arrangement above the temperature of transition opens the barrier for translocation of larger compounds, such as signalling or defensive molecules, in response to heat stress or inflammation.

P03 Abstract #3

Regulation of keratinocyte differentiation through aryl hydrocarbon receptor signaling involves the transient activation of TFAP2A

Felicitas Pardow[1] , Jos P.H. Smits[1]†, Jieqiong Qu[2]† , Diana Rodijk-Olthuis[1] , Ivonne M.J.J. van Vlijmen-Willems[1] , Simon J. van Heeringen[2] , Huiqing Zhou[2]† , Ellen H. van den Bogaard[1]†

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† These authors contributed equally

Terminal differentiation of epidermal keratinocytes is essential for skin barrier development and tightly coordinated by a complex network of interacting transcription factors. Previous work identified the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor and environmental sensor, to orchestrate epidermal differentiation through transient activation of putative downstream transcription factors, amongst others TFAP2A. For proof-of-concept studies on a presumed AHR-TFAP2A axis that is co-opted in epidermal differentiation, we utilized siRNA-mediated TFAP2A knockdown in primary keratinocytes and CRISPR-Cas9 induced TFAP2AKO in N/TERT keratinocytes combined with transcriptomic analysis and organotypic modeling of the epidermis. Keratinocyte-specific ablation of TFAP2A negatively impacted epidermal development resulting in disorganized stratification, aberrant differentiation and reduced barrier function (by transepithelial resistance) in organotypic TFAP2AKO epidermis. Furthermore, TFAP2A deficiency impeded the AHR ligand-mediated induction of terminal differentiation gene expression (e.g. HRNR, DSC1, S100A8, MMP1). We conclude that TFAP2A is an essential transcription factor for human keratinocyte differentiation and epidermal development and that AHR regulates epidermal differentiation through transient activation of a specific panel of key transcription factors, as herein illustrated by TFAP2A. Identification of this AHR-TFAP2A axis provides further insights into the complex regulatory network driving epidermal differentiation in response to environmental cues and prompts new avenues for the treatment of barrier dysfunction related diseases by targeting AHR’s partners in crime.

P04 Abstract #4

Technical advance: bacterial colonization of 3D organotypic skin models for long-term host-microbe interactions and microbiome intervention studies

G. Rikken[1], L. Meesters[1], I.M.J.J. van Vlijmen-Willems[1], D. Rodijk-Olthuis[1], P.A.M. Jansen[1], P. Oláh[2], B. Homey[2], H. Niehues[1], P.L.J.M. Zeeuwen[1], E.H. van den Bogaard[1]

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Studies on the human skin microbiome have taken a flight after the initiation of the human microbiome project. Following descriptive studies on skin microbiota in health and disease, mechanistic studies on the interplay between skin and microbes are on the rise and experimental models are in great demand. We here present a technical advance for the microbial colonization of organotypic skin models ensuring a standardized application area onto the stratum corneum, a homogenous distribution of bacteria, and importantly preventing infection of the basolateral culture medium even during prolonged co-culture up to two weeks. This model allows ample opportunities for studying host-microbe interactions and intervention strategies. Multi-parameter end-point analysis from one single organotypic culture permits the collection of samples for colony forming unit count, bacterial profiling, host gene and protein expression analysis, and microscopic imaging. With this methodology we identified differential host responses to various skin commensals and found striking differences in epidermal responses towards clinical isolates of Staphylococcus aureus from atopic dermatitis patients. The herein presented methodology using glass cylinders is easily transferable to a wide variety of organotypic skin models of different diameters and cellular components and can be used in every cell culture facility considering the various sizes and commercial availability. Our study may therefore kick start the highly needed 2 mechanistic studies into the intricate host-microbe interactions contributing to skin health and disease, providing a platform for intervention studies using pre-, pro- or antibiotics.

P05 Abstract #5

Filaggrin knock-out in keratinocytes indicates a functional role in skin barrier formation

Jos P.H. Smits[1], Noa J.M. van den Brink[1], Luca D. Meesters[1,2], Ivonne M.J.J. van Vlijmen-Willems[1], Céline Evrard[3], Yves Poumay[3], Michel van Geel[4,5,6], Wiljan J.A.J. Hendriks[7], Patrick L.J.M. Zeeuwen[1], Ellen H. van den Bogaard[1]

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Skin barrier function is the result of orchestrated terminal differentiation of keratinocytes, forming the lipidsurrounded hydrophobic cornified envelope of the stratum corneum. CRISPR-Cas9 has the potential to meticulously dissect the functional and structural components of the stratum corneum by precisely editing any gene of interest. We here illustrate the complementary possibilities of introducing CRISPR-Cas9 machinery in immortalized N/TERT keratinocytes to generate Filaggrin knockout (ΔFLG) isogenic cell lines and organotypic human ΔFLG epidermal equivalents (ΔFLG-HEE). FLG deficiency was accompanied by (partial) loss of other structural and functional proteins, such as involucrin, hornerin, and transglutaminases. Consequently, transepithelial electrical resistance (TEER) indicated a decreased barrier function in ΔFLG-HEEs. Homology directed repair of the ΔFLG clonal lines reinstated FLG protein expression and the concomitant expression of the aforementioned epidermal differentiation proteins in FLG-restored HEEs. The phenotypical consequences of FLG deficiency in cell lines with an identical genetic background and in absence of predicted CRISPR-Cas9 off-target effects indicate a functional role for FLG – not only in epidermal barrier function – but also in epidermal barrier development which provides new insights into the disease pathogenesis of atopic dermatitis and ichthyosis vulgaris.

P06 Abstract #6

IL-2Rγ/IL-4Rα receptor induced in keratinocytes by interleukins 4 and 13 in atopic dermatitis can alter the epidermal barrier.

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Interleukins (IL)-4 and IL-13, released in atopic dermatitis (AD), activate the IL-4Rα/IL-13Rα1 receptor, modifying the epidermal phenotype and barrier. In addition, IL-4 is able to activate the IL-2Rγ/IL-4Rα receptor normally expressed only by hematopoietic cells. However, a microarray analysis reported expression of IL-2Rγ by keratinocytes in reconstructed human epidermis (RHE) exposed to IL-4, IL-13 and IL-25. Thisstudy investigated occurrence/function of IL-2Rγ/IL-4Rα receptor in keratinocytes and its potential involvement in AD pathophysiology. IL-2Rγ mRNA is indeed detected in lesional AD biopsies using in situ hybridization. In vitro, exposure of RHE to IL-4 and IL-13 induces expression of IL2Rγ at the mRNA level, both in RHE made of primary keratinocytes or reconstructed using immortalised N/TERT keratinocytes. Located on chromosome X, IL13RA1 and IL2RG genes have been individually inactivated using CRISPR/Cas9 in N/TERT cells to generate deficient RHE. Characterization of IL13RA1-/0 RHE indicates that IL-4Rα/IL-13Rα1 receptor activation is required to induce IL-2Rγ expression. On the other hand, IL2RG-/0 RHE were analysed to investigate IL-2Rγ potential function in keratinocytes. Reduced transepithelial electrical resistance is typical of RHE exposed to IL-4 and IL-13, revealing altered epidermal barrier. This susceptibility appears absent from IL2RG-/0 RHE, suggesting involvement of IL-2Rγ/IL4Rα to alter the epidermal barrier.

P07 Abstract #7

Evaluation of an anti-inflammatory dendrimer to topically treat psoriasis

Emily Clement[1], Ranime Jebbawi[1,2], Abdelouahd Oukhrib[2,3], Hélène Labie[2], Séverine Fruchon[1], Cedric-Olivier Turrin[3], Muriel Blanzat[2], Rémy Poupot[1], Michel Simon[1]

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Psoriasis is an auto-immune disease resulting from a chronic and exaggerated inflammation of the skin and hyperproliferation of keratinocytes. Conventional topical treatments for this disease, such as antiinflammatory drugs, present low efficiency, and systemic administration of synthetic drugs or biologic immunomodulators can present severe side effects and/or are highly expensive. So, there is an unmet need to develop new drugs that could provide sustainable therapeutic effects. In this study, we evaluated the potential efficacy of anti-inflammatory dendrimersfor the topical treatment of psoriasis. Dendrimers are hyperbranched and perfectly defined macromolecules of nanometer size, constituted of branches grafted on a central core. IMD-006, a phosphorus-based dendrimer capped with azabisphosphonate groups, has strong immuno-modulatory effects towards different immune cell types. We tested the effects of IMD-006 in two psoriasis models: the imiquimod-induced murine model and a reconstructed human epidermis (RHE) model, in which cocktails of pro-inflammatory cytokines are used to induce psoriasis-associated changes. IMD-006 presented dose-dependent therapeutic efficacies, significantly reducing lesions and histopathological changes associated with psoriasis. Moreover, we show that IMD-006 is rapidly taken up by keratinocytes in 2D culture, decreasing their proliferation and increasing their differentiation. In keratinocytes in 2D culture, IMD006 associated with mitochondria, increased mitochondrial ROS production and ultimately lysosomal degradation of these organelles. Therefore, the anti-psoriatic effect of dendrimers is, at least in part, the result of a direct effect on keratinocytes. Our results demonstrate that anti-inflammatory dendrimers are good candidates for the topical treatment of psoriasis with a broad effect on multiple cell types involved in the development and progression of the disease.

P08 Abstract #8

Cutaneous imiquimod delivery efficiently assisted by phospholipidbased microemulsions

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The topical application of imiquimod (IMQ), an immune response modifier, is employed clinically in the treatment of cutaneous infections and cancer and experimentally as a psoriasis-inducing model. Yet, drawbacks concerning the compound's solubility and skin permeation profile are limiting the development of effective formulations and skin delivery. The aim of this work was to develop IMQ-loaded microemulsions (MEs) based on phospholipids and oleic acid to improve IMQ penetration into the epidermis. A pseudoternary phase diagram was constructed and selected microemulsions were further characterized and studied for their ability to deliver the active substance into and through human skin ex vivo. The superiority of the microemulsions over the commercially available product was evidenced. ME1 with 1% IMQ (9 nm droplet size and Bingham rheology) provided a similar amount of IMQ to the human epidermis in vitro as the commercial product, which contains 5 times higher IMQ concentration. After applying ME1 on human skin for 8 hours (25 µl/cm2 dose), IMQ was not detected in the receptor phase, indicating a lower risk of systemic absorption than the established product. Infrared spectroscopy data suggest the possible penetration of the microemulsion's components into the stratum corneum at a molecular level, along with less ordered and less tightly packed lipids. The ME1-induced barrier disruption was restored within less than 5 hours after the preparation was removed, as detected by transdermal water loss. In conclusion, the findings of this study indicate that ME based on phospholipids and oleic acid may be a promising alternative for topical IMQ administration.

P09 Abstract #9

The Effects of Antioxidant Trans-Resveratrol on an Atopic Eczema Epidermal Model *in vitro*

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that is highly expressed in all skin cells. Alongside its role as a sensor for xenobiotic chemicals, it plays a role in maintaining homeostasis through epidermal differentiation. Its expression has been shown to be upregulated in lesional skin of atopic eczema patients. This chronic recurring inflammatory skin disorder has a complex pathogenesis and is associated with multifactorial interactions between a genetically-driven skin barrier defect, T_H2-driven immune dysregulation, and environmental risk factors. To investigate the role of AHR in atopic eczema, a reconstituted human epidermal model consisting of healthy keratinocytes stimulated with T_H2 cytokines (IL-4 and IL-13) and TNF-α to mimic the conditions of atopic eczema. This resulted in morphological changes, upregulation of pro-inflammatory cytokines, upregulation of AHR expression and its target genes, and downregulation of barrier-related protein markers, similar to what has been reported in the epidermis of patients with atopic eczema. Following that, the antioxidant trans-resveratrol was used to systemically treat the atopic eczema model. As a control, 6-formylindolo[3,2-b]carbazole was used to activate AHR. Trans-resveratrol could partly rescue the induced mRNA expression of metabolizing enzymes of the cytochrome P450 family, in a similar fashion as in the control. Furthermore, the inflammatory phenotype was decreased by systemic treatment with trans-resveratrol. Interestingly, trans-resveratrol induced the mRNA expression of occludin. This study will give insight into the role of AHR in an atopic eczema skin model and the protective effects of trans-resveratrol.

P10 Abstract #10

Effect of barrier disruption and inflammation on skin lipids

C. Jacques-Jamin[1], C. Mias[1], I. Cerruti[1], C. Dejean[1], O. Vantroeyen[1], C. Filaquier[2], F. Carballido[2], S. Bessou-Touya[1] & H. Duplan[1]

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Skin is characterized by active lipid metabolism and lipids play crucial roles for structural integrity and functionality. Lipid metabolism can be modified by skin barrier perturbation and inflammation leading to reduction of relative ceramides content and a shorter mean lipid chain length inducing barrier dysfunction. The aim of this study was to investigate the impact of barrier alteration and inflammation on the expression of lipids enzymes and lipids levels. Ex vivo by using human skin model (3 donors) exposed to a solution of SDS was evaluated with different formulations to balance stress. Proteins enzyme expression (ALOX12B and EVOL1) was performed by immunofluorescence with confocal microscopy. Ceramides, free fatty acids and cholesterol of stratum corneum were analysed on HPTLC. MALDI-HRMS imaging was performed to evaluate penetration of the ingredients of formulations into the skin. SDS Stress modified expression of ALOX12B and EVOL1 but also the synthesis of epidermal lipids in particular ceramides ratio. Similar results were described in the literature after inflammatory stress in vitro or in pathology like atopic dermatitis and psoriasis. Lipidic ingredients of formulations penetrate through the skin and help to restores lipid metabolic disorders due to SDS stress. Disruption of skin barrier function induced inflammation that impact lipid metabolism and lipids ratio that create a vicious circle for the disruption of barrier function that can be avoid with cosmetics formulations.

P11 Abstract #11

Single cell RNA seq revealed altered cytoskeletal dynamics and the formation of cytoplasmic RNA-Lamin A bodies during keratinocyte terminal differentiation

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Keratinocyte cornification and epidermal barrier formation are tightly controlled processes, requiring degradation of organelles, including the nucleus. However, the underlying molecular mechanisms are not well defined. Post-confluent cultures of rat epidermal keratinocytes (REKs) undergo spontaneous and complete differentiation, allowing visualisation and perturbation of the differentiation process *in vitro*. Using this model, we have shown that the actomyosin network and the non-muscle Myosin IIa - Myh9 are required for key processes involved in nuclear degradation, comprising Akt1-mediated phosphorylation of Lamin A and the dispersal of phosphorylated Lamin A into the cytoplasmic bodies followed by rapid nuclear shrinkage. Based on these finding, we hypothesised that specific changes in actomyosin dynamics and the actin cytoskeleton are required for Lamin dispersal and nuclear shrinkage during epidermal terminal differentiation.

We have designed a single-cell transcriptomics pipeline to better analyse these 'sub-optimal', dying/cornifying cells, and high-throughput confocal imaging to analyse the actin cytoskeleton and related markers during nuclear degradation. We identified changes in expression of actin remodelling genes that coincided with nuclear degradation processes and altered distribution of the actin cytoskeleton in terminally differentiating keratinocytes. We also identified widespread up-regulation of ribosomal protein mRNAs, that correlated with the formation cytoplasmic RNA bodies associating with phosphorylated lamin A. Therefore, actin dynamics, increased translation, and increased cytoplasmic RNA are all potentially involved, and may serve as signposts for key stages in nuclear degradation and end-stage terminal differentiation in keratinocytes.

P12 Abstract #12

Spontaneous formation of long periodicity phase in model membranes from human stratum corneum lipids

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The stratum corneum (SC) is the outermost layer of the skin, and it is responsible for the firstline protection of the human body. SC is characterized by corneocytes embedded in a lipidic matrix with an equimolar mixture of cholesterol, ceramides, and free fatty acids. Model lipid membranes have been considered an effective tool to reproduce and study healthy and diseased skin states in vitro. To achieve a multilamellar arrangement of the lipidic mixture in model membranes in vitro, the process of high-temperature annealing was considered essential, even if this arrangement is present at skin temperature in vivo. This study is focused on the effects of various annealing in the preparation of model membranes. Human-isolated SC lipids were used to prepare model lipid membranes, which were annealed at different temperatures (from room temperature to 90 °C) in the presence or absence of water vapor. X-ray diffraction data confirmed the formation of a long periodicity phase and a cholesterol phase in all the samples. A short periodicity phase was visible only when the annealing was performed at temperatures of 70 °C or higher, in the presence of water vapor, or at 80 °C or higher, without the presence of water vapor. Furthermore, Fourier-Transform Infrared spectroscopy showed a slightly increasing trend of ordered all-trans solid-state of the lipids and a tighter lipid chain packing at higher annealing temperatures. The model permeabilities decreased when they were annealed over the main phase transition. Thus, our data suggest that the long periodicity phase, an epidermal-specific lipid arrangement essential for the barrier function, spontaneously forms in isolated human SC lipids, without the need for high-temperature annealing. Although the SC lipids have a high affinity for this arrangement, our permeability studies suggest that forming a correct barrier requires another factor(s), represented in vitro by the annealing step.

P13 Abstract #13

Identification of keratinocyte mitogens: implications for hyperproliferation in psoriasis and atopic dermatitis

H. Niehues[1] , G. Rikken[1] , I.M.J.J. van Vlijmen-Willems[1] , D. Rodijk-Olthuis[1] , P.E.J. van Erp[1], P.L.J.M. Zeeuwen[1] , J. Schalkwijk[1] , E.H. van den Bogaard[1]

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Psoriasis and atopic dermatitis (AD) are chronic inflammatory skin diseases characterized by keratinocyte hyperproliferation and epidermal acanthosis (hyperplasia). The milieu of diseaseassociated cytokines and soluble factors is considered a mitogenic factor, however, pinpointing the exact mitogens in this complex microenvironment is challenging. We employed organotypic human epidermal equivalents (HEEs), faithfully mimicking native epidermal proliferation and stratification, to evaluate the proliferative effects of a broad panel of (literature-based) potential mitogens. The keratinocyte growth factor molecule (KGF), the Thelper 2 (Th2) cytokines interleukin-4 (IL-4) and IL-13 and the psoriasis-associated cytokine IL-17A caused acanthosis by hyperplasia through a doubling in the number of proliferating keratinocytes. In contrast, IFN-γ lowered proliferation, while IL-6, IL-20, IL-22 and oncostatin M (OSM), induced acanthosis not by hyperproliferation but by hypertrophy. The Th2-cytokine mediated hyperproliferation was JAK/STAT3 dependent, while IL-17A and KGF induced MEK/ERK-dependent proliferation. This discovery that key regulators in AD and psoriasis are direct keratinocyte mitogens not only adds evidence to their crucial role in the pathophysiological processes but also highlights an additional therapeutic pillar for the mode 2 of action of targeting biologicals (e.g. dupilumab) or small molecule drugs (e.g. tofacitinib) by the normalization of keratinocyte turnover within the epidermal compartment.

Appendix

E-mails of chairs and speakers:
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