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Regulation of retinoid-mediated signaling in the skin and its implication for skin homeostasis in mice

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The examination takes place at the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, at 11 AM on February 14, 2014.

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The PhD Defense takes place at the Lecture Hall of Building A, Department of Internal Medicine, Faculty of Medicine,University of Debrecen at 1 PM on February 14, 2014.

INTRODUCTION

Retinoids, retinoid metabolism, and retinoid function in skin

Vitamin A (VA) is an essential component of the human diet which can not be synthesized within the human body. The term vitamin A (VA) designates all compounds that possess qualitatively the biological activity of the VA-alcohol retinol, such as retinal, retinyl esters and various pro-VA carotenoids. Furthermore, the term retinoids designates all natural and synthetic compounds exhibiting similar structure and/or effectiveness like retinol and therefore also includes retinoic acids (RA). Active metabolites of VA, such as RA and retinal, play important roles in several physiological processes such as vision, epithelial surface maintenance, immune competence, reproduction, and development.

Retinoids are primarily obtained via the diet as retinyl esters from foods of animal origin, or they can be generated from plant-derived pro-VA carotenoids like β-carotene. Upon ingestion, retinyl esters are hydrolyzed by pancreatic and intestinal enzymes into free retinol which is then, together with pro-VA carotenoids, taken up by enterocytes. Within enterocytes, centric or eccentric cleavage of pro-VA carotenoids is catalyzed by the enzymes beta-carotene oxygenase 1 and 2 to generate retinal which is subsequently reduced to retinol. Following, the available retinol is bound to the cellular retinol binding protein 2 (Crbp2) which promotes retinol re-esterification by the enzymes lecithin:retinol acyl transferase (Lrat) and to a smaller extent by diacylglycerol acyltransferase 1 (Dgat). In fact, Lrat mRNA expression is increased in response to high RA levels which is thought to be a feedback mechanism. Subsequently to esterification, retinyl esters together with further lipids are incorporated into chylomicrons which are secreted into the lymph for transport into the blood stream. Chylomicron triacylglyceride hydrolysis results in the formation of chylomicron remnants which are then taken up by hepatocytes or extra-hepatic target cells. Within the liver, retinyl esters are in turn hydrolyzed, the resulting retinol is bound to retinol binding protein 1 (Rbp1) and transferred to perisinusoidal stellate cells where dietary retinoids are stored as retinyl esters. Hepatic stellate cells are the main storage site of VA in the human organism containing 50-80% of the body's total retinol as retinyl esters. In order to maintain a steady plasma retinol concentration of 1-2 µM, retinol can be mobilized in hepatic stellate cells, associated to Rbp4 and is then secreted into the general circulation. In the blood stream, this complex associates with another protein, namely transthyretin, which reduces glomerular filtration of retinol. Rbp-retinol complexes are thought to be taken up by target cells via the transmembrane-spanning receptor

encoded by the Stra6 gene. Within peripheral target tissues such as skin, retinol can be stored upon Lrat-catalyzed esterification or it is converted into its bioactive metabolite all-*trans* retinoic acid (ATRA) via a two-step oxidation process. Firstly, all-*trans* retinol is oxidized by alcohol/retinol dehydrogenases and/or short-chain dehydrogenases/reductases to generate all-*trans* retinal. By means of three different aldehyde dehydrogenases 1A (Aldh1a1, 2, and 3) all-*trans* retinal is further oxidized to ATRA, the major biologically active VA metabolite.

ATRA is then bound by the cellular RA binding protein 2 (Crabp2) and shuttled to nuclear hormone receptors (NR) to exert its biological activity via gene expression regulation. Interestingly, Crabp2 itself is a direct target of ATRA and its expression increased upon high RA levels. However, RA levels are tightly controlled in skin cells and other tissues resulting in its degradation to more polar and less active metabolites by cytochrome P450 enzymes such as Cyp26a1, Cyp26b1, and Cyp2s1 in case of excess. Another cellular RA binding protein, Crabp1 is believed to promote this degradation pathway via RA transport towards Cyp enzymes. Therefore, the steady-state-system of intracellular retinoid concentrations appears to be regulated by complex feedback mechanisms which involve several of the above mentioned enzymes and retinoid binding proteins. Moreover, also topically applied retinoids are partly absorbed by skin cells via slow diffusion through epidermis and dermis. Depending on the kind of retinoid applied, the compound can be further metabolized in the skin into various derivatives which can potentially influence the expression of several genes in the skin.

Retinoids are essential for skin physiology through their role in the regulation of several aspects of skin cell proliferation, differentiation, apoptosis, epidermal barrier function, and immune regulation. Noticeably, alterations of retinoid metabolism and signaling were found in the skin of patients with various skin diseases, such as psoriasis, ichthyosis, and atopic dermatitis (AD). Thereby, it is unclear whether these alterations are the trigger or if they are consequence of these skin diseases.

Epidermal barrier – Retinoid function in skin barrier maintenance is mainly based on the regulation of epidermal permeability, epidermal differentiation, sebum secretion, and apoptosis. Furthermore, disturbance of retinoid receptor-mediated signaling (especially via RXR) in the skin was shown to result in various epidermal alterations such as disturbed keratinocyte proliferation, alopecia, and induction of AD in different mouse models.

Immune system – Retinoids are important modulators of immune function and immune response. It has been shown recently that VA can modify cell numbers of immune competent cells, as well as cytokine and chemokine levels, and antibody responses. Interestingly, RAR- and RXR-mediated pathways have been found to be involved in Th2 development and

Th1/Th2 balance. Thereby, RA is possibly able to directly modify pro- and anti-inflammatory immune responses. Moreover, a role of retinoids in the modification of the immune phenotype of atopic skin diseases such as AD has been shown previously.

Gene expression regulation by nuclear hormone receptors and retinoids

Retinoids exert the vast majority of their functions in organs and tissues, such as the skin, via NR-mediated gene expression regulation. NRs comprise a superfamily of DNA binding, ligand-dependent transcription factors like RAR α, β, and γ, RXR α, β, and γ, or peroxisome proliferator-activated receptors (PPARα, β/δ, and γ). These molecules are present in the cytoplasm or nucleus and play a crucial role in development, homeostasis, and other biological processes in mammals. NRs feature a specific structure consisting of a N-terminal extension which is highly variable in length and sequence, a well-conserved central DNA-binding domain containing two zinc-fingers as structural DNA-binding motif, a hinge region which influences intracellular trafficking and subcellular distribution of the NR, the large C-terminal ligand-binding domain (LBD) with moderately conserved sequence, and a variable C-terminal extension. The variable sequences allow the discrimination between different NR subfamilies and further between various subtypes within these families.

NRs are sequence specific transcription modulators which regulate the gene expression in a ligand-dependent manner. This implies the plasma membrane penetration of lipophilic ligands, e.g. retinoids, followed by their delivery to the NR and ligation to the LBD. Upon ligation, RARs and PPARs form heterodimers with RXRs and conformational changes take place. This enables the dimer to bind with high affinity to a specific hormone response element. This structure is a specific hexanucleotide half-element in the DNA which is arranged in a particular motif, such as inverted or direct repeats, with spacing between these half-sites. It is usually located close to the core promoter, the region where the transcription process starts. These elements are referred to as RA-responsive element in the case of RARs and as PPAR-responsive element for PPARs, respectively. Ensuing, in cooperation with several co-regulating proteins the gene expression of respective target genes is modulated.

The RAR was discovered in 1987 and up-to-date 3 subtypes, RAR α, β, and γ, were identified. These receptors belong to the same type 2 class as the PPARs α, β/δ, and γ within the NR superfamily and therefore always act as heterodimers with either RXR α, β, or γ. The activated (i.e. liganded) RAR-RXR regulates the expression of multiple genes in skin and

various other tissues while its transcriptional activity is dependent on the RAR-activating ligand. The transport protein Crabp2 is responsible for the delivery of RAR agonists to this NR. On the other hand, the heterodimer is believed to function as a transcriptional silencer in the absence of appropriate ligands. The most abundant RAR and RXR subtypes in the skin are RXRα and RARγ, followed by lower quantities of RARα. Since retinoid receptors exhibit tissue and cell type-specific distribution patterns, functional specificity of each subtype is suggested. Moreover, RAR and RXR subtypes differ in ligand specificity and/or affinity, therefore, it can be assumed that their contribution to gene expression patterns in tissues such as skin differs, depending on quantitative receptor distribution, on the nature and level of co-regulators, as well as on available retinoid receptor-selective agonists and antagonists.

Unsaturated fatty acids and eicosanoids are applicable ligands for PPARα, β/δ, and γ. The most abundant PPAR subtype in the skin is PPARβ/δ. PPAR-mediated pathways are important in skin physiology because they are involved in epidermal barrier recovery, keratinocyte differentiation, and lipid synthesis. For example, overexpression of PPARδ in the epidermis causes a psoriasis-like skin disease featuring hyperproliferation of keratinocytes, dendritic cell accumulation, and endothelial activation.

The retinoid ATRA is the well known endogenous ligand of RARs which is able to induce or decrease the expression of various genes. Interestingly, a cross-talk exists between RAR and PPARδ pathways. Indeed, besides RARs, also PPARδ can be activated by ATRA, depending on the ratio of their specific ligand transport proteins. Crabp2 initiates RAR signaling, whereas Fabp5 promotes PPARδ-mediated signaling upon ATRA-binding. Moreover, PPARδ activation has been reported at high ATRA concentrations suggesting that tissue levels of ATRA can determine which NR pathways are up-regulated. However, these findings are still controversially discussed in the literature.

The skin

The skin of mice and man consists of epidermis, dermis, and subcutis and represents the essential physical barrier between an organism and its surrounding environment which may contain pathogens, allergens, chemicals, etc. Therefore, a functioning epidermal barrier is the precondition to avoid percutaneous penetration and to prevent the development of cutaneous disorders. The epidermis is mainly composed of keratinocytes. Additionally, several structural proteins, enzymes, and lipids are involved in assembly and maintenance of the epidermal

barrier. During terminal differentiation, keratinocytes move towards the stratum corneum and transform into flattened and anucleated corneocytes. Their plasma membrane is replaced by an insoluble protein layer, the cornified envelope, and the cells are locked together by corneodesmosomes. The cornified envelope is predominantly composed of structural proteins such as loricrin (Lor), involucrin (Ivl), and filaggrin (Flg) which are cross-linked by transglutaminase 1 (Tgm1). Its function is to act as a scaffold for the attachment of lipids from the surrounding lipid lamellae matrix. This matrix consists of cholesterol, cholesterol esters, free fatty acids, and ceramides, and it helps to prevent water loss and the penetration of water soluble substances. Several enzymes, such as 3-Hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2), β-Gluco-cerebrosidase (Gba), and UDP-glucose ceramide glucosyltransferase (Ugcg), are involved in the generation of these matrix components.

Furthermore, corneocytes are continuously shed from the epidermal surface and replaced by new keratinocytes. This desquamation is regulated by proteases (e.g. kallikrein-related peptidase 5 and 7) which break down the extracellular corneodesmosomal adhesion proteins, as well as by protease inhibitors like Kazal-type 5 serine protease inhibitor LEKTI.

Atopic dermatitis

The allergic skin disease AD is the most common inflammatory skin condition, predominantly affecting infants and children and it is characterized by a disturbed epidermal permeability barrier, eczematous lesions, epidermal hyperproliferation, skin dryness, and a Th2-type immune response. Interestingly, alterations of retinoid metabolism and signaling were recently found in the skin of AD patients. Furthermore, it has been shown that retinoids are able to modify the immune phenotype of AD as well as further atopic diseases. Notably, various studies reported an “outside-inside-outside” pathogenic mechanism of AD while the exact disease pathogenesis is not yet fully elucidated.

The aim of this work was to determine how topically applied agonists or antagonists selective for RARs or RXRs influence retinoid metabolism and signaling in mouse skin, as well as epidermal barrier homeostasis and skin-based immune regulation relevant for skin disorders such as AD. Moreover, it was of interest whether the induction of allergic immune responses by systemic or combined systemic and topical treatments with ovalbumin (OVA) is able to modify retinoid metabolism and retinoid-mediated signaling in the skin of mice and its correlation to receptor specific agonist or antagonist treatments of mice.

MATERIALS AND METHODS

Mice

For retinoid applications, 8-12 weeks old female C57BL/6 mice and for OVA treatments, 8-10 weeks old female BALB/c mice were housed within the animal facility of the University of Debrecen, Hungary. Mice were maintained in single cages on standard animal chow and water *ad libitum*. All experimental procedures were approved by the Committee of Animal Research of the University of Debrecen, Hungary (Approval number: 25/2006 DEMÁB).

Topical treatment with retinoid receptor specific agonists and antagonists

Mice (n=6-8/group) were anesthetized and shaved on dorsal skin sites. Retinoid receptor specific agonists and antagonists were applied topically each other day in 25 µl acetone (vehicle/control; Merck) per treatment for two weeks. According to previous studies by other groups following amounts of agonists and antagonist were applied per treatment: ATRA, BMS753, and BMS189961, each 40 nmol; LG268, BMS614, UVI2041, BMS493, and UVI3003, each 100 nmol. On day 14, four hours after the last treatment, mice were sacrificed, sera and full thickness skin biopsies were collected from equal body sites, skin specimens were shock frozen in liquid nitrogen and all samples were kept at -80 °C until analyses.

Retinoid receptor specific agonists and antagonists

ATRA was a gift from BASF and the synthetic RXR activator LG268 was kindly provided by Ligand Pharmaceuticals). All other synthetic agonists and antagonists were synthesized by Prof. Ángel de Lera (Vigo, Spain) according to the following protocols. Agonists selective for RARα (BMS753) and RARγ (BMS189961) were produced as described in the original patents. The RARγ-selective antagonist (UVI2041) was prepared by the condensation of the ester 15 derived from chalcone 14 with hydroxylamine followed by hydrolysis. The RAR pan-antagonist/inverse agonist (BMS493) and the RXR pan-antagonist (UVI3003) were synthesized according to reported procedures. The RARα-specific antagonist (BMS614) was made following the patented procedure developed at BMS. The purity of synthesized compounds was determined to be >95% by HPLC after crystallization. We have confirmed that these retinoids are stable when stored as solids or in solution at -78 °C and during the time frame of biological experiments.

Sensitization with ovalbumin

Sensitization of mice (n=8/group) was performed by repetitive systemic administration of OVA and allergen-induced dermatitis based on a model previously reported. Briefly, mice were sensitized at days 47, 60 and 67 with 10 µg OVA intraperitoneally (i.p.) (Sigma-Aldrich) adsorbed to 1.5 mg aluminum hydroxide ($Al(OH)_3$) (Thermoscientific) or with phosphate-buffered saline (PBS; control). For combined treatment, mice were sensitized i.p. on days 1, 14 and 21 with 10 µg OVA adsorbed to 1.5 mg $Al(OH)_3$. This was followed by topical application of 100 µg OVA adsorbed to 1.5 mg $Al(OH)_3$ in 100 µl PBS (weekly dose) onto shaved dorsal skin, divided into four applications of 25 µl every other day of one week. Epicutaneous (e.c.) treatment was repeated for a total exposure of three weeks separated by two-week intervals. Three days after the last treatment (day 70) mice were sacrificed, skin and serum samples were collected and kept at -80 °C until analyses.

Quantitative real-time reverse transcription polymerase chain reaction

RNA was isolated using Tri® reagent according to the manufacturer's protocol and was transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies) according to the manufacturer's instructions. qRT-PCR was performed in triplicates (n=5-6 mice/group) using pre-designed TaqMan® Gene Expression Assays or FAM-TAMRA assays. TaqMan® Low Density Array (TLDA) cards were used for the OVA mouse model with duplicate determinations using TaqMan® Gene Expression Master Mix (all Applied Biosystems). All gene expression analyses were conducted on an ABI Prism 7900. Relative quantification of mRNA expression was achieved using the comparative C_T method and values were normalized to cyclophilin A mRNA. The Sequence Detector Software version 2.1 was used for data analysis.

Histological analysis

Frozen skin specimens were sectioned (4 µm) and stained with hematoxylin and eosin (H&E) (retinoid model, n=3/group). For the OVA model, skin samples were fixed overnight with 4% paraformaldehyde at 4 °C and embedded in paraffin. 5 µm sections were stained with H&E or Giemsa (n=8/group).

Immunohistochemical analysis

Frozen 5 µm skin sections (n=5/group) were fixed in acetone, blocked with mouse seroblock FcR block (AbD Serotec) or 10% goat serum (NGS; Vector Laboratories) and

incubated with FITC rat anti-mouse CD3 molecular complex (17A2); biotin rat anti-mouse CD8a (53-6.7); purified hamster anti-mouse CD11c (HL3; all BD Biosciences - Pharmingen); or purified rat anti-mouse CD4 (GK1.5; BioLegend). Antibody binding was detected using biotinylated goat anti-rat Ig for anti-CD4 (Amersham Biosciences UK limited) and biotin mouse anti-hamster IgG cocktail for anti-CD11c (BD Biosciences - Pharmingen), followed by incubation with Alexa Fluor 594-linked streptavidin (Invitrogen) for anti-CD4, anti-CD11c, and anti-CD8. Skin sections stained for CD11c were counterstained with FITC-linked rat anti-mouse I-A/I-E (BD Biosciences - Pharmingen) to identify MHC class II-positive cells. Staining of paraffin-embedded skin sections with rabbit Fabp5 polyclonal antibody (1:50; ProteinTech) was performed following the manufacturer's directions using antigen retrieval buffer (0.1 M sodium citrate, 0.1 M citric acid) and blocking with 5% donkey serum. Biotinylated donkey anti-rabbit Ig (Amersham Biosciences UK limited) and Alexa Fluor 594-linked Streptavidin were applied for detection of antibody binding. Nuclei were visualized with DAPI and all sections were mounted with Vectashield Mounting Medium (Vector Laboratories). $CD3^+$, $CD4^+$, $CD8^+$, MHC-class II^+ and $CD11c^+$ cells were counted under an Olympus BX60 epifluorescence microscope using 40x objective lenses and a calibrated grid (six fields per section).

Total IgE levels in serum

Sera of OVA-sensitized mice (n=8/group) were collected at day 70 and kept at -80 °C until analysis. Plasma IgE concentration was measured by using the mouse ELISA kit from BD-Pharmingen.

Determination of Fabp5 protein in skin

Fabp5 protein levels were determined in protein lysates prepared from whole mouse skin (n=2/group). Skin samples were lysed in RIPA lysis buffer in the presence of protease inhibitor (Pierce). Lysates were separated by 4-12% SDS-PAGE and then transferred to a nitrocellulose membrane (Invitrogen). The membrane was incubated in blocking solution (Pierce) for 30-60 minutes at room temperature. Subsequently, the membrane was incubated with the Fabp5 antibody (1:500, ProteinTech) diluted in blocking buffer overnight at 4 °C. Endogenous proteins were detected with Alexa A680-conjugated anti-rabbit secondary antibody (1:10.000, Invitrogen). Blots were scanned with a LI-COR Biosciences analyzer. Anti-β-actin (Sigma) was used as loading control.

Cytokine levels in serum

Levels of TSLP, IL-4 and IL-12 (p70) were determined in serum (n=4/group) using Quantikine Mouse Immunoassays (R&D Systems). Assay sensitivity was 2.63 pg/mL for TSLP, <2 pg/mL for IL-4, and <2.5 pg/mL for IL-12.

High performance liquid chromatography mass spectrometry – mass spectrometry (HPLC MS-MS) analysis

Concentrations of ATRA and retinol were determined in mouse skin samples (n≥5 mice or n=3 mice for the retinoid or OVA model, respectively) by our previously described HPLC MS-MS method. The HPLC system consisted of a Waters 2695XE separation module (Waters), a diode-array detector (model 996, Waters, Hungary) and an MS-MS detector (Micromass Quattro Ultima Pt, Waters). In summary, 100 mg of skin biopsy (if samples were below 100 mg, water was added up to the used standard weight: 100 mg) were diluted with a threefold volume of isopropanol, tissues were minced by scissors, vortexed for 10 seconds, put in an ultra sonic bath for 5 minutes, shaken for 6 minutes and centrifuged at 13000 rpm in a Heraeus BIOFUGE Fresco at 4 °C. After centrifugation, the supernatants were dried in an Eppendorf concentrator 5301 (Eppendorf) at 30 °C. The dried extracts were resuspended with 60 µL of methanol, vortexed, shaken, diluted with 40 µL of 60 mM aqueous ammonium acetate solution and transferred into the autosampler for subsequent analysis.

Statistical Analysis

Data are indicated as mean ± standard error of mean (SEM). In case of topical retinoid treatment, statistical analysis of qRT-PCR data was performed using one-way ANOVA followed by Dunett's post-test. Statistical analysis of the OVA model was performed using one-way ANOVA followed by Tukey correction. Significance of HPLC MS-MS results was determined using Student's *t*-test. Differences were considered significant at $p<0.05$.

RESULTS

Retinoid signaling in the skin after topical treatment with various retinoid receptor agonists or antagonists

Initially, we investigated the expression pattern of genes involved in retinoid metabolism, retinoid transport, and retinoid signaling in murine skin upon treatment with various retinoid receptor selective agonists or antagonists.

RARα and RARγ differentially regulate retinoid-mediated signaling in mouse skin

Since both, RARα and RARγ are expressed in skin we were interested in the effect of topically applied RAR subtype selective agonists on retinoid metabolism. Interestingly, we found that the synthetic RARα agonist down-regulated the expression of all genes with a role in retinoid metabolism that is RA synthesis, retinoid receptors, and target genes.

Only mRNA levels of the lipid transporter Fabp5 and an enzyme involved in retinal synthesis (Rdh16) were significantly increased by the agonist. In contrast, the synthetic agonist for RARγ and the natural RAR agonist ATRA induced the expression of nearly all retinoid target genes in the skin of mice, e.g. Cyp26a1, Cyp26b1 (both degradation enzymes), Rbp1, Crabp1, Hbegf, and Krt4 as a marker for retinoid activity. We were also interested in the effect of a synthetic RXR agonist on skin retinoid metabolism. Topical application of this agonist induced the expression of some retinoid target genes (Cyp26a1, Cyp26b1, Rbp4, Crabp1, Krt4), while the treatment did not affect or slightly decrease the expression of other targets (Crabp2, Fabp5, Rbp1, Hbegf). Moreover, repetitive treatment with the RARγ-selective agonist showed no significant effect on retinal and RA synthesis enzymes and retinoid receptor gene expression in the skin. However, the endogenous RAR ligand ATRA and the RXR agonist markedly increased mRNA levels of Aldh1a2 and ATRA further induced Rara and Rxra gene expression, while it decreased Aldh1a3 expression in the skin.

RAR and RXR antagonists decrease the expression of genes involved in retinoid signaling in mouse skin

Topical application of antagonists for RARα or RARγ resulted in non-significantly reduced or unaltered expression of several genes involved in retinoid signaling in the skin.

However, some genes seemed to be slightly induced by both antagonists, such as Bco2, Rbp4, Aldh1a1, Rara, Rarg, and some target genes like Cyp26a1, Cyp26b1, and Krt4. In contrast, antagonists for RAR and RXR decreased the expression of nearly all of these genes below detection limit. Only mRNA levels of Bco2, Rdh16, Rbp4, and Fabp5 were found to be elevated by the antagonists. Most surprisingly, this expression pattern strongly resembled to that which we observed in mice treated with the RARα agonist.

ATRA levels in the skin of topically treated mice

ATRA levels in the skin were found to be differentially affected depending on the applied receptor-selective agonist or antagonist. Concentrations of ATRA were significantly decreased in the skin of mice treated with the synthetic RARα agonist and non-significantly by the RARγ agonist. Furthermore, treatments with antagonists for RARγ, RARs, or RXRs resulted in elevated ATRA levels, while only the RARα antagonist induced a significant increase. As expected, we found ATRA levels markedly elevated upon treatment with this RAR agonist itself. Noticeably, however, was the pronounced elevation of ATRA in mouse skin after application of the synthetic RXR agonist which is in accordance with a significantly elevated gene expression of the RA synthesis enzyme Aldh1a2.

Impact of retinoid receptor agonists and antagonists on skin and immune homeostasis

RAR-RXR signaling pathways are well known to be involved in the modulation of immune responses as well as skin physiology. Furthermore, we recently found disturbed retinoid signaling in skin of AD patients, indicating the involvement of retinoid signaling in the disease's pathogenesis. Therefore, we aimed to investigate the impact of topically applied retinoid receptor specific agonists and antagonists on murine skin and immune homeostasis.

RAR-RXR signaling pathways induce epidermal hyperproliferation

After two weeks treatment, obvious signs of dryness (scales) could be observed in some groups compared to control mice. Control animals were treated with acetone (vehicle) and their skin appeared normal without scales at the end of two weeks. Similar observations were made in the group treated with the RARα agonist showing only a very few scattered white scales on the back skin. In contrast, application of synthetic agonists for RXR or RARγ and

the natural RAR ligand ATRA resulted in visibly dry and scaly skin. Compared to rather mild effects induced by the RXR agonist we could detect small scales already after the third treatment with the synthetic RARγ agonist. During the following days, number and size of scales increased and the skin appeared red and slightly shiny compared to control mice. Application of ATRA showed the strongest effects resulting in apparently very dry skin with big white scales already shortly after initiating the treatment. Skin of these mice also seemed shiny. Skin regions treated with receptor antagonists appeared mostly normal at day 14. A few small scales could be observed only after application of the RARα and RXR antagonists.

In order to verify these visual impressions we also performed histological analysis. In accordance, epidermal thickness seemed comparable to control mice in all treatment groups except for mice treated with the synthetic RXR agonist, RARγ agonist, or ATRA. Epidermal thickness was markedly increased in all three groups but appeared stronger in mice treated with the RARγ agonist and was most pronounced in ATRA-treated mice. Additionally, the epidermal surface seemed scaly after application of the synthetic RARγ agonist and ATRA.

RAR-RXR signaling pathways modify epidermal barrier homeostasis

We next investigated the expression of genes with significant functions in epidermal barrier homeostasis. Application of the synthetic RARγ agonist and ATRA both induced genes involved in skin barrier function (Abca12, Flg, Lor, Spink5, Krt16, Hbegf). On the other hand, mRNA levels of genes implicated in ceramide metabolism (Acer1, Gba, Ugcg) or cholesterol synthesis (Hmgcs2) were mainly decreased or unaffected by the treatment. Compared to RARγ ligand application, expression of these genes was markedly down-regulated (in several cases below detection limit) when mice were treated with the synthetic RARα agonist. Noticeably, the same expression profile was observed after application of RAR or RXR antagonists. Treatment with the RXR agonist and RARα- and RARγ-specific antagonists resulted in inconsistent gene expression patterns with an increase of some genes (Spink5, Flg, Klk7) and decrease of other genes (Abca12, Krt16, Ugcg) involved in epidermal barrier function. Krt6b expression was below the limit of detection in all groups.

RAR-RXR signaling pathways modify skin based immune responses

Next we investigated whether topical application of receptor selective retinoids is sufficient to alter the expression of genes implicated in the immune response in skin, such as

the Th2 cell attracting chemokines Ccl11, Ccl17, Ccl22, Ccl24, Ccr3, and the inflammatory marker Krt17. The synthetic RXR activator exerted only a slight effect on their gene expression in skin, while levels of chemokines and Krt17 were markedly decreased in response to the RARα agonist (except for Ccl17). Once more, this result strongly resembled to those found after application of RAR or RXR antagonists. Topical treatment with the synthetic RARγ agonist and ATRA as well as the RARγ antagonist decreased mRNA levels of Ccl11 and Ccl24 but induced Ccl17 and partly Ccl22. This was the opposite in mouse skin treated with the RARα antagonist. Moreover, the chemokine receptor Ccr3 was below detection level in all groups. Expression of Krt17 was increased only in response to the RARγ agonist or RARα antagonist while it was decreased or unaltered in all other groups.

Mouse model of allergen-induced dermatitis

Several parameters involved in immune response pathways as wells as skin homeostasis, were investigated in response to OVA treatment in order to verify the induction of an allergen-induced dermatitis.

Systemic sensitization with OVA induces mild allergen-induced dermatitis when compared to additional topical OVA applications

BALB/c mice were systemically sensitized with OVA in addition or not to topical sensitization onto shaved back skin and compared with PBS-injected mice (controls). Sensitization with OVA induced mild but statistically significant focal hyperplasia with a two-fold or three-fold increase in epidermal thickness, respectively. Histological analysis further revealed scaly skin in both OVA-sensitized groups.

Interestingly, repeated systemic sensitization with OVA did not alter total serum IgE levels compared to control mice. However, combined systemic and topical sensitization leading to allergen-induced dermatitis resulted in significantly increased total serum IgE when compared to controls and systemically sensitized mice.

Furthermore, inflammatory cells infiltrating the skin were characterized. Numbers of mast cells were significantly elevated in the dermis of both OVA-sensitized groups, while the increase in macrophages, dermal dendritic cells, and $CD4^+$ lymphocytes reached statistical significance only in allergen-induced dermatitis. Sensitization with OVA induced elevated numbers of $CD3^+$ lymphocytes in dermis and epidermis. Only few $CD8^+$ lymphocytes could

be detected in skin sections of OVA-sensitized mice. Eosinophils could only be found in the dermis of OVA-sensitized mice and predominantly in allergen-induced dermatitis. In the epidermis, several Langerhans cells exhibiting activated morphology could be observed in OVA-sensitized mice. Altogether, numbers of mast cells, macrophages and dermal dendritic cells were significantly higher only in allergen-induced dermatitis.

Systemic sensitization with OVA triggers IL-4 serum levels

Levels of the cytokines IL-4, TSLP and IL-12 (IL-12p70) were determined in the sera of OVA-sensitized mice. IL-4 but not TSLP was significantly increased in both OVA-treated groups. Serum levels of IL-12, a Th1-type cytokine, remained unaltered in all groups.

Expression of Th1- and Th2-type immune response genes is only altered in allergen-induced dermatitis

Expression of genes relevant for Th1- or Th2-type immune responses was analyzed in the skin of mice by qRT-PCR and TLDA. Gene expression levels of the inflammation-associated Krt17 were moderately increased in the skin of OVA-sensitized mice. In contrast, expression of Th1 related genes including interferon γ and Il12a and of Th2 associated genes including Il4, Il10, Ccl11, and its corresponding chemokine receptor 3 (Ccr3) was significantly elevated only in the skin of mice with allergen-induced dermatitis.

Systemic sensitization with OVA is sufficient to modify the expression of genes involved in epidermal barrier homeostasis

mRNA levels of several genes involved in epidermal barrier formation and maintenance, such as keratinocyte differentiation markers Ivl and Lor, matrix metalloproteinase 9, and Spink5 were significantly decreased in the skin of OVA-treated mice. Notably, expression levels of all tested genes, except for psoriasin, were lower in the OVA-treated mice. Indeed, psoriasin expression was induced in mice treated with OVA.

In contrast to the protein compartment, genes related to the epidermal lipid compartment were mainly up-regulated in mouse skin after OVA challenges. In fact, mRNA expression levels of Hmgcs2, involved in cholesterol synthesis, of serine palmitoyltransferase 2, Ugcg, both catalyzing the synthesis of ceramides and glycosyl-ceramides, and of alkaline

ceramidase 1, which is responsible for ceramide degradation, were significantly elevated in the skin of mice with allergen-induced dermatitis and/or solely systemic OVA sensitization. Noticeably, only expression of Abca12, which is responsible for lipid loading into lamellar bodies, was decreased in OVA-treated groups.

Retinoid metabolism and signaling in allergen-induced dermatitis

By means of our mouse model we aimed to investigate whether repeated systemic and combined systemic and topical sensitizations with OVA are able to induce changes in retinoid metabolism and retinoid-mediated signaling on the gene expression level in the skin.

ATRA levels are increased in allergen-induced dermatitis

Interestingly, we found significantly elevated concentrations of ATRA only in the skin of mice with allergen-induced dermatitis. Furthermore, retinol levels in mouse skin remained unchanged in all treatment groups.

Retinoid metabolism is increased in allergen-induced dermatitis

After sensitization, the expression of Sdr16c5, responsible for the oxidation of retinol to retinal, was induced compared to controls while expression of Rdh10 remained unchanged. In contrast, expression of enzymes responsible for the conversion of retinal to the bioactive retinoid ATRA (Aldh1a1, 1a2 and 1a3) was significantly increased only in allergen-induced dermatitis and corresponding to ATRA levels in the skin. Effects of ATRA are mediated by different retinoid receptors in the skin. In parallel to the determined ATRA skin content, elevated mRNA levels of RARγ and RXRα, both the most abundant retinoid receptors in skin, were evidenced only in allergen-induced dermatitis.

Retinoid-mediated signaling is increased in allergen-induced dermatitis

We found the expression of genes encoding RA degradation enzymes, Cyp26a1, and Cyp26b1 increased in allergen-induced dermatitis. Expression of proteins involved in retinoid transport (Rbp1, Crabp2) and metabolism (Lrat) was similarly increased in the skin of mice treated with OVA, regardless of further topical sensitization with OVA. In contrast,

expression of RAR target genes not involved in retinoid signaling (Krt4, Rarres2, Tgm2) was not significantly altered. Notably, the ratio of Fabp5 vs. Crabp2 expression, both delivering ATRA to their cognate NR, was significantly increased in allergen-induced dermatitis.

Gene targets involved in PPARδ pathways in skin are mainly up regulated in allergen-induced dermatitis

Systemic or systemic plus topical sensitization of mice with OVA led to reduced Ppard gene expression compared to controls and this decrease was somewhat more pronounced in mice systemically sensitized only. In contrast, mRNA expression of Fabp5 which delivers ligands to PPARδ, was increased after OVA sensitization.

Moreover, Krt6b, Krt16, Hbegf, and Hmgcs2, all of which known to be induced upon PPARδ activation and involved in epidermal barrier homeostasis, showed significantly elevated gene expression levels in skin after systemic and topical sensitization. Only the PPARδ target gene Abca12, responsible for epidermal barrier formation and maintenance, showed decreased mRNA levels in both OVA groups.

Systemic sensitization with OVA increases Fabp5 protein levels

Because Fabp5 gene expression was induced in the skin after repeated systemic OVA sensitization we also assessed levels of Fabp5 protein in murine skin. Levels of Fabp5 protein as measured by Western Blots, increased in the skin of mice sensitized with OVA compared to controls. However, highest Fabp5 protein levels were detected in whole skin of mice systemically treated with OVA. In order to determine the localization of Fabp5 across the skin, we performed immunohistochemical analysis. We found intense staining for Fabp5 in the thickened epidermis and around hair follicles of mice treated with OVA.

DISCUSSION

Retinoid function in the skin

Retinoids, such as ATRA are important modulators of epithelial surface maintenance and immune competence. Retinoids mediate their functions mainly via gene expression regulation through NRs such as RARs and RXRs. Up to now it is unknown whether all retinoid receptor subtypes present in the skin contribute equally to retinoid metabolism and signaling and, thereby, to skin physiology or whether differences may exist. Notably, alterations of retinoid metabolism and signaling were found in the skin of patients with various skin diseases such as AD. However, it is still unclear whether these alterations are the trigger or if they are consequence of these skin diseases.

In order to determine the effect of selective retinoid-mediated signaling in the skin on retinoid metabolism, epidermal barrier homeostasis, and immune regulation mice were treated topically with various retinoid receptor specific agonists or antagonists. The main finding is the strong difference between the positive retinoid-mediated signaling via RARγ pathways in contrast to the negative signaling via RARα. Furthermore, using an OVA-induced allergic dermatitis mouse model, the present work demonstrates that the immune response in allergen-induced dermatitis is associated with increased retinoid signaling and RA concentrations in murine skin. Moreover, signaling via PPARδ-mediated pathways, mostly through Fabp5 up-regulation, is mainly enhanced in allergen-induced dermatitis. Thus, retinoid-mediated signaling is involved in the pathogenesis and/or maintenance of allergic dermatitis and possibly further atopic skin diseases but the exact pathway has yet to be determined.

Retinoid signaling in the skin is oppositely regulated by RARα and RARγ

Topical treatment with retinoid receptor specific agonists affected the expression of all genes involved in retinoid-mediated signaling in the skin in general oppositely to NR antagonists. Likewise, target genes were mainly induced after treatment with ATRA or the synthetic RARγ agonist as previously reported. Moreover, both agonists induced very similar gene expression patterns and given the fact that RARγ is the predominant RAR subtype in the skin it is indicated that ATRA mediates its activity in skin through RARγ rather than RARα.

Most interesting however, was a consequent down-regulation of gene expression by the synthetic RARα agonist which is in line with reduced ATRA levels in mouse skin, possibly due to decreased ATRA synthesis via Aldh enzymes. Only Fabp5 and Rdh16 expression was

increased in response to the agonist. This expression pattern strongly resembled to that in response to RAR or RXR antagonists while both antagonists further seemed to induce Bco2 and Rbp4 expression. The proteins encoded by those genes are implicated in retinoid metabolism and transport. Thus, it seems plausible that ATRA or different retinoid derivatives, like oxo-retinoids or still unknown endogenous RAR ligands, could be generated upon retinoid receptor antagonism and shuttled to NRs different from RARs, as it was already proposed for Fabp5-mediated ATRA-induced PPARδ activation.

Retinoid-mediated signaling is induced in allergen-induced dermatitis

Corresponding to topical RARγ agonist treatment, several RAR target genes as well as genes involved in RA synthesis, degradation, transport, and esterification were induced in allergen-induced dermatitis indicating the involvement of this NR in the skin disease. In contrast, the expression of RAR targets which are not implicated in retinoid signaling or which are rather related to epidermal differentiation remained unaltered or reduced. These data indicate that potentially increased ATRA synthesis via Aldh1a enzymes and elevated ATRA levels in mouse skin, as observed in allergen-induced dermatitis, might not result in an overall increase of RAR-mediated signaling. In fact, these data further suggest the involvement of additional RA-mediated signaling pathways in murine skin.

Alternative retinoid-mediated signaling pathways in the skin

The involvement of other NRs than RARs in retinoid-mediated signaling is not unlikely as also NR4A1/NUR77 and RXR were shown to form heterodimers which respond to RXR activators *in vivo* and *in vitro*. Such heterodimers might participate in retinoid signaling especially when RARs are antagonized. Moreover, Volakakis et al. demonstrated that NR4A1/NUR77 can induce the expression of Fabp5 in HEK293 cells which potentially enhances RA-mediated PPARδ signaling. Indeed, it has previously been shown that ATRA can activate PPARδ when the ratio of the lipid transporters Fabp5 vs. Crabp2 is high within cells such as keratinocytes. Interestingly, we found Nr4a1/Nur77 and Ppard expression in the skin of NR ligand-treated mice significantly decreased or below detection limit in response to those ligands which markedly induced Fabp5 expression, namely the RARα agonist, RAR and RXR antagonists. This may be indicative of (late) negative feedback regulations on the gene expression level in response to induced Fabp5 expression.

Whether Fabp5-mediated PPARδ signaling and/or a novel, as yet undetermined retinoid(s) might mediate such an alternative retinoid pathway in the skin is currently unknown. In fact, mRNA levels of ATRA-synthesizing enzymes (Aldhs) following RAR and RXR antagonist application were not in accord with elevated ATRA levels in the skin of those mice. This suggests that ATRA synthesis upon antagonist treatment may be mediated by other enzymes such as Bco2, Rdh16, RBP4, and/or other pathways, from precursors present in the skin and/or via transporter-mediated pathways delivering retinoids to the skin.

ATRA-induced PPARδ-mediated signaling in allergen-induced dermatitis

Moreover, the increased Fabp5 vs. Crabp2 ratio in the skin of mice with allergen-induced dermatitis further suggests favored ATRA signaling through PPARδ under disease conditions. This pathway may significantly contribute to the specific gene expression patterns observed in this mouse model. Indeed, PPARδ signaling and several of its target genes were previously found increased in psoriasis and lesional AD skin and Romanowska et al. further showed the induction of an inflammatory skin disease similar to human psoriasis in PPARδ-overexpressing mice. Interestingly, in our model of allergen-induced dermatitis we observed an increased expression of several target genes involved in PPARδ signaling. This further indicates the involvement of PPARδ signaling pathways in allergen-induced dermatitis.

Retinoid receptor subtypes have distinct roles in mouse skin

Furthermore, our observations on retinoid receptor-selective signaling indicate different roles of RXR-, RARα-, and RARγ-mediated signaling pathways in the skin. These data suggest that induction of RARα signaling might result in the suppression of RARγ-mediated pathways in murine skin. Considering the induced RARα gene expression after topical ATRA treatment, this appears to be an efficient physiological switch to different retinoid-mediated signaling pathways. So far, it is unknown how RARα mediates its suppressive action on RARγ signaling. High RARα expression was found in inflammatory cells infiltrating the skin in several dermatoses, however, in normal skin its expression level is fairly low compared to RARγ. Thus it seems unlikely that a competition between both receptors for RXRα as heterodimer partner could be the explanation. Instead, RARα apparently regulates the expression of different sets of genes, possibly also in different skin cell types than does RARγ and might also induce the transcription of co-repressor molecules upon activation.

RXR-mediated epidermal hyperproliferation

Another well established effect of RAR-activation in skin is epidermal hyperproliferation. This was found induced by ATRA and the synthetic RARγ agonist and also in the allergen-induced dermatitis model. Furthermore, hyperproliferation was supported by an induced expression of regulators of desquamation such as Spink5, Klk5, and Klk7 in RAR agonist-treated skin. Moreover, elevated mRNA levels of Hbegf and Krt16, which have already been related previously to induced keratinocyte proliferation, further confirmed the outcome.

Surprising however, was the induction of epidermal proliferation by the synthetic RXR agonist since no such observation was reported in a previous study using another synthetic RXR agonist. Retinoid effects in the skin are most likely mediated by RARγ-RXR heterodimers while their transcriptional activity is dependent on the RAR-activating ligand. Upon treatment with the RXR agonist we observed increased Aldh1a2 gene expression and elevated ATRA levels in the skin. This indicates an induced ATRA synthesis which might account for the mild epidermal hyperproliferation, most probably mediated by the RAR partner. However, another RXR heterodimer partner, PPARδ, was previously found to be implicated in the regulation of keratinocyte hyperproliferation. Compared to RAR-RXR, this heterodimer is permissive which means the ligand of PPAR or RXR, respectively, is sufficient to activate transcription of respective target genes. This might suggest alternative pathways to be involved in RXR agonist-induced hyperproliferation.

Retinoid-mediated signaling is one piece of the epidermal barrier puzzle

Additionally, retinoid receptor ligand application affected various other processes in the skin as indicated by altered expression levels of genes involved in epidermal barrier homeostasis, such as Abca12, Flg, and Lor, and of genes with roles in lipid barrier formation and ceramide metabolism, e.g. Hmgcs2, Ugcg, Gba, Acer1. Consistently, such retinoid-mediated effects have already been reported in epidermal keratinocytes. These results suggest that retinoid-mediated signaling is required for normal barrier homeostasis and that retinoid-induced dysregulation may be a predisposing factor for skin diseases such as allergen-induced dermatitis. Thereby, both antagonism and induction of RAR- and/or RXR-mediated signaling in skin appear to disturb barrier homeostasis.

Furthermore, also allergen-induced dermatitis led to altered expression of genes responsible for epidermal barrier formation and/or maintenance. However, this pattern was

rather different from that observed after NR ligand application (e.g. Abca12, Lor, Ivl mainly down-regulated in allergic dermatitis but mainly up-regulated by topical ATRA and RARγ agonist). This data indicates the involvement of additional, not RARγ-RXR-mediated signaling pathways in epidermal barrier disturbance under disease conditions.

Allergen-induced immune response in the skin is associated with increased RA signaling

It is well established that retinoids play important roles in the immune system, especially in Th2-type cell differentiation. Interestingly, the expression of various chemokines which are preferentially attracting Th2-type lymphocytes during inflammatory processes was differently altered by the applied retinoid receptor ligands. However, undetectable mRNA levels of the corresponding chemokine receptor which is expressed by infiltrating immune competent cells suggests the absence of inflammatory cells in the skin upon retinoid treatments.

The opposite was true for the applied mouse model of allergen-induced dermatitis where high numbers of infiltrating dermal macrophages, dendritic cells, and mast cells were found in the skin. Moreover, a mixed Th1- and Th2-type immune response was found, indicating that high RA levels in the skin might directly impact on systemic and local immune responses. In contrast, mice systemically treated with OVA exhibited only a partial phenotype with lower inflammatory infiltrates and cytokine expression. Interestingly, highest levels of immune response-related gene expression, inflammatory cell infiltrates, and serum cytokines correlated with increased RA synthesizing enzymes and ATRA levels in inflamed skin.

Increased ATRA levels in the skin of OVA-sensitized mice might reflect the induced expression of RA synthesizing enzymes. Besides resident skin cells, infiltrating immune cells might be a source of ATRA in sensitized skin. For example, human basophils which have been shown to infiltrate AD skin were found to express Aldh1a2 enzyme and to produce RA upon activation with IL-3 in an *ex vivo* model. However, identification of specific cell types producing RA in inflamed skin is currently not feasible due to problems in acquiring sufficiently large numbers of highly purified cells from the skin.

Inside-out pathogenesis in allergic skin disease

Notably, one further major outcome of the present work is to demonstrate that systemic OVA sensitization of mice per se is sufficient to induce partial skin immune responses and an impairment of expression of key genes involved in skin homeostasis and barrier function.

Previous studies and reviews reported an “outside-inside-outside” pathogenic mechanism of AD. In contrast, our data support an “inside-out” mechanism significantly contributing to the development of overt skin inflammation. Moreover, OVA-induced alterations are accompanied by partially altered retinoid signaling, suggesting a causative relationship.

Retinoids act on various pathways in skin with implication for allergic skin disease

In summary, this study lets us emphasize that there must be yet unidentified alternative retinoid signaling pathways or a broader range of endogenous retinoids present in skin for selective RARα, RARγ, or RXR activation.

Moreover, our data indicate that unbalanced retinoid signaling in the skin mediated by RARα, RARγ, and/or RXR signaling pathways as well as potential unidentified pathways, affect epidermal barrier homeostasis and skin-based immune responses in mice. This retinoid dysregulation may play a central role in various skin diseases as it is also indicated in our mouse model of allergen-induced dermatitis which is associated with increased retinoid signaling and elevated ATRA levels in the skin. Because expression of genes involved in RA metabolism is increased, whereas expression of RAR target genes involved in other pathways such as epidermal differentiation remains largely unchanged, allergen-induced dermatitis might additionally redirect intracellular retinoid flux and metabolism. Moreover, PPARδ gene targets are mainly induced indicating that RAR-mediated signaling and certain pathways/ molecules involved in PPARδ signaling are altered in allergic dermatitis skin. Furthermore, systemic sensitization with an allergen is sufficient to modify the expression of genes central to epidermal homeostasis suggesting an “inside-out” effect of allergen in allergic skin disease pathogenesis possibly by increasing allergen penetration through the skin. Whether disturbed retinoid metabolism and retinoid-mediated signaling are symptoms or potential initiators of atopic sensitization still remains to be elucidated.

The role of mouse models for human allergic skin disorders

Mouse models of allergic skin diseases are of great importance to understand the pathomechanism of these disorders and to target possible therapies. However, the pathology of skin inflammation in mice and human is not entirely the same. Therefore, data obtained within animal studies provide indispensable scientific knowledge but demand further verification using human samples such as skin explants as well as human studies with allergic skin diseases before the results could be translated into treatment strategies in the clinic.

SUMMARY

Endogenous retinoids like all-*trans* retinoic acid (ATRA) play important roles in skin physiology and immune-modulatory events in the skin via nuclear hormone receptor-mediated signaling through RARs and/or RXRs. Moreover, it has been shown recently that ATRA can activate another nuclear receptor involved in skin homeostasis, namely PPARδ, depending on specific transport proteins: Fabp5 initiates PPARδ signaling whereas Crabp2 promotes signaling via RAR. Notably, alterations in retinoid metabolism, signaling, and concentrations have been found in various skin diseases such as atopic dermatitis. Thereby, it remains unclear whether these changes are symptoms or the trigger of such diseases.

The aim of this study was to determine how topically applied agonists or antagonists selective for RARs or RXRs and how the induction of an allergic immune response by systemic or combined systemic and topical treatment with ovalbumin influence retinoid metabolism and signaling in mouse skin. Of further interest were nuclear receptor ligand treatment effects on epidermal barrier homeostasis and skin-based immune regulation relevant for skin disorders such as atopic dermatitis, as well as their correlation to the allergen-induced dermatitis mouse model.

Our data indicate that RARα and RARγ subtypes possess different roles in mouse skin and may be of relevance for the auto-regulation of endogenous retinoid signaling in the skin. Moreover, dysregulated retinoid signaling mediated by RXR, RARα and/or RARγ as well as potential unidentified pathways may promote skin-based inflammation and disturbance of epidermal barrier properties. This is further supported by elevated ATRA levels and mainly increased signaling mediated by RAR or PPARδ in allergen-induced dermatitis skin. Furthermore, systemic sensitization with an allergen is sufficient to modify the expression of genes central to epidermal homeostasis suggesting an “inside-out” effect of allergen in allergic skin disease pathogenesis possibly by increasing allergen penetration through the skin. In summary, disturbed retinoid metabolism and retinoid-mediated signaling in the skin may contribute to the development and/or maintenance of allergic skin diseases. Whether these alterations are symptoms or potential initiators of atopic sensitization still remains to be elucidated.

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List of publications related to the dissertation

1. **Gericke, J.**, Ittensohn, J., Mihály, J., Dubrac, S., Rühl, R.: Allergen-induced dermatitis causes alterations in cutaneous retinoid-mediated signaling in mice.
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2. **Gericke, J.**, Ittensohn, J., Mihály, J., Álvarez, S., Álvarez, R., Törőcsik, D., de Lera, Á.R., Rühl, R.: Regulation of Retinoid-Mediated Signaling Involved in Skin Homeostasis by RAR and RXR Agonists/Antagonists in Mouse Skin.
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List of other publications

3. Mihály, J., **Gericke, J.**, Törőcsik, D., Gáspár, K., Szegedi, A., Rühl, R.: Reduced lipoxygenase and cyclooxygenase mediated signaling in PBMC of atopic dermatitis patients.
Prostaglandins Other Lipid Mediat. Article in press, 2013.
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4. Mihály, J., **Gericke, J.**, Aydemir, G., Weiss, K., Carlsen, H., Blomhoff, R., Garcia, J., Ralph, R.: Reduced retinoid signaling in the skin after systemic retinoid-X receptor ligand treatment in mice with potential relevance for skin disorders.
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