

A new era for translational atopic dermatitis research and management

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Introducing a new era for atopic dermatitis patients, doctors, and researchers

Detailed analyses on atopic dermatitis (AD) were performed and are ongoing in regard to the genetic basis of AD, the inflammatory signatures and immune players, risk factors and triggers, comorbidities, AD-associated pruritus, barrier dysfunction and the so called dysbiosis with severe alterations in the cutaneous microbiome (1-5). With the very recent approval of the first biologic treatment in AD (Dupilumab), we are facing a new era for affected patients and the medical and scientific community. These circumstances will be accelerating AD research as new resources will be allocated, scientists and doctors attracted to the field and this will altogether further foster

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development of new AD treatments. This new era for AD includes a novel awareness for its pathogenesis and possible druggable targets, for how microbes and infections trigger, modulate or even ameliorate AD inflammation, how pruritus develops and is effectively targeted, and how AD can be better stratified to choose the best treatment for the individual patient. Along with these personalized diagnostic procedures, identifying and describing comorbidities and whether these will be treated by a given therapy of AD inflammation are cutting edge fields of investigation. To this end, the current Focus Theme Issue of *Experimental Dermatology* that we have the honor of guest-editing combines new original research manuscripts with invited theoretical articles into a multi-faceted survey that highlights key recent developments in translationally relevant AD research, thus servicing the current demand for insights into and authoritative overviews of this exciting new era of AD research.

New insights into AD pathogenesis: The microbiome, its sensing, and consequences

Characterization of the human cutaneous microbiome using molecular biology techniques helped us to much better understand the complexity and regulation of the human microbiome (6, 7). Under healthy steady state conditions, the individual microbiome, despite its variations in regard to localization, seems to be rather stable (8).

In contrast, the breakdown of the diversity of the cutaneous microbiome represents a hallmark finding in AD (9). One new frontier is to dissect the skin microbiome more deeply for the non-bacterial kingdoms, the virome and the mycobiome. For the mycobiome in AD, new insights are presented in this special issue (10).

Skin swab samples were collected from the antecubital fossa from Korean patients with AD (AD duration > 10 years) and healthy controls following standardization of no bathing or moisturizing for 24 hours. Polymerase chain reaction was used for amplification followed by sequencing and taxonomic assignment. Basidiomycota was the most abundant fungal phyla in both groups and when analyzed at the class, order and family level, Exobasidiomycetes, Malasseziales and Malasseziaceae represented the highest abundance. The genus *Malassezia* was most common accounting for 53.84% in AD and 80.44% in healthy controls. Diversity analyses were limited, based on the high interpersonal

variations present, however and in contrast to the bacterial microbiome, AD samples tended to be more diverse in regard to fungal communities. Given that *Malassezia*, for example, induces cutaneous immune responses including type I sensitizations in AD patients, further analyses are needed to associate fungal communities on human skin with specific skin diseases and different disease states and to identify determinants of fungal compositions under homeostatic conditions (10).

The loss of species diversity within the bacterial communities in AD was associated with a dominant prevalence of *Staphylococcus aureus* (9). Among the mechanisms relevant for AD, innate immune signals, e.g. triggering TLR2, were shown to promote AD inflammation (11) or its chronification (12). In some studies, expansion of *S. epidermidis* was found in addition to that of *S. aureus* (9). Based on the assumption that *S. epidermidis* may be less pro-inflammatory or exert even beneficial functions to preserve homeostasis (13, 14) functional consequences of innate immune sensing of lipoteichoic acid (LTA) from *S. aureus* or *S. epidermidis* were investigated. Both LTA preparations activated dendritic cells (DC) via TLR2 and induced DC maturation. However, immune profiles differed with LTA from *S. epidermidis* always inducing IL-10 balanced immune profiles and LTA from *S. aureus* inducing dominantly pro-inflammatory cytokines (15). T cells pre-dominantly produced IFN- γ and IL-17 when the DC used for priming were exposed to *S. aureus* LTA whereas in those primed with DC activated by LTA from *S. epidermidis* IFN- γ and IL-17 production was balanced by IL-10. Mimicking the situation in AD, concerted activation of DC by IL-4 and LTA was used as previously described (12, 16), demonstrating that the observed differences were even more pronounced. These findings demonstrate that ligands of microbial recognition receptors such as TLR2 may contribute to immune balance and resolution of inflammation by allowing opposing immune profiles to develop (15). A limitation of this study is that the functional analyses were performed in murine DC utilizing also DC from knock-out mice.

Miers et al. present data showing how human DC can be analyzed *ex vivo* to gain insights into the state of inflammation, the cellular response and possibly also into the efficacy of treatments (17).

Different types of DC are believed to play an important role in the pathogenesis of AD (1-4). Miers et al. present work where they used induced suction blisters on atopy patch test lesions induced by house dust mite antigens. These were used to monitor Langerhans cells and inflammatory dendritic

epidermal cells (IDECs). They defined a patient to be responsive to the atopy patch test based on IDEC density to also allow a better representation of these cells in their analyses (n=6). As intervention, 8 day pre-treatment with emollients significantly reduced influx of IDECs and Langerhans cells in parts remained immature. This pilot study indicates that emollient application may interfere with human AD inflammation and would allow also more insights when used also in clinical trials with targeted therapies in AD (17).

New models for AD: Investigating the pathogenesis and monitoring therapeutic interventions

Investigating organotypic cultures is of importance to link therapeutic or preventive procedures in animal models to human biology and an important step before human *in vivo* studies are performed. Based on the 'inside out signaling' established in AD, epidermis is exposed to type 2 immune cytokines such as IL-4 and IL-13 and produces the type 2 immune cytokine, IL-25. De Vuyst et al. (18) used reconstructed human epidermis (RHE) and exposed RHE to a mixture of these three cytokines to mimic the clinical setting in AD. RHE was in addition treated with methyl- β -cyclodextrin (M β CD). M β CD extracts cholesterol from plasma membranes thereby disrupting lipid microdomains without affecting barrier function and morphology. Thus, RHE was exposed to M β CD for two hours and then for 48 hours to the cytokine mix.

Among others and as expected, the Th2 cytokines downregulated filaggrin and loricrin expression as in AD skin. Carbonic anhydrase II and neural epidermal growth factor-like 2 are upregulated in AD and upon RHE exposure to type 2 cytokines. As in AD, hyaluronan synthase 3 was found to be upregulated by type 2 cytokines, and co-exposure to M β CD2 enhanced this. Most importantly, TSLP, i.e. the epithelial cytokine effective upstream and inducing the Th2 pathway, remained highly upregulated in RHE exposed to type 2 cytokines and M β CD2. Thus, this model may be used as a screening tool to identify compounds that prevent or treat the epidermal consequences of the dysregulated cytokine milieu that is associated with AD AD (18).

Various pre-clinical models for AD-like symptoms and inflammation are used for research, each of them with certain limitations, but also the advantage of mechanistic analyses and 'easy-to-evaluate' preclinical interventional approaches (19). Most of the pre-clinical models are established in mice, but interesting canine and rat models exist. In one rat model (20), AD-like symptoms such as dermatitis and scratching behavior are induced by the neonatal injection of capsaicin. Three weeks thereafter, skin signs and symptoms start to develop. Analyses of this model now revealed that proteolytic processing of pro-filaggrin is altered by capsaicin. Especially matriptase degradation was induced, which also attenuated pro-filaggrin cleavage preventing appropriate filaggrin processing. These data indicate that other proteins may also be regulated in a similar way by the capsaicin-induced release of neuropeptides from sensory nerve fibers and/or by the stimulation of capsaicin-sensitive receptors such as TRPV1 (e.g. on keratinocytes and mast cells) in post-natal rat skin. Further in-depth characterization of this rat model for certain aspects of AD pathogenesis may thus allow one to assess both, the regulation of intracutaneous proteolysis and its consequences for AD pathogenesis and the efficacy of compounds regulating the epidermal and barrier functioning in AD (20).

Based on recent insights into the pathogenesis of AD, compounds expected to display anti-inflammatory activity should be evaluated for their efficacy and mode of action. To this end, astaxanthin, a carotenoid and natural xanthophyll, was evaluated in the treatment and prevention of a chronic irritant atopic dermatitis-like inflammation in mice (21). A mouse strain susceptible to dry skin and irritant dermatitis is the Hos:HR-1 mouse. Using the phthalic anhydride (PA) model, three groups of mice were investigated: control group with 5 % PA solution on the dorsal and ear skin three times a week for four weeks, one group with the same treatment and 1 mg/ml astaxanthin three hours after PA and another group with 2 mg/ml astaxanthin three hours after PA at the application. The authors show that the histologically assessed changes of PA dermatitis are attenuated especially by high dose-astaxanthin. With regard to the inflammatory signature, upregulation of iNOS and COX2 and NF- κ B activity as well as increased TNF- α , IL-1 β , and IL-6 release and the induction of IgE all were attenuated. As these pathways are also upregulated in AD, astaxanthin is a novel candidate agent in the treatment of AD, with both the mouse model and this lead agent serving as a standard with which the results of future investigations in this area can be compared (21).

In vivo monitoring of AD and the search for 'good' AD biomarkers

Sweat contributes to the functioning of the cutaneous barrier and shapes the cutaneous microbiome. Impairment of sweating along with reduced levels of dermcidin, an anti-microbial peptide specific for sweat, has been reported in atopics (22, 23), but the available data on this remain controversial. Thus, new methods to approach and measure 'sweating', sweat glands and ducts to assess the contribution of 'sweat' to AD pathogenesis are important and are expected to evolve so that stratification of AD patients into 'sweaters' and sweating-impaired subgroups can be accomplished.

The impression mold technique (IMT), which uses silicone material that is spread onto sweating human skin, is one such method (24). After hardening, sweat droplets become visible in the silicone matrix and can be quantified and their mean diameter can be assessed. Shimeda-Komatsu et al. demonstrate that the number of sweat droplets under baseline condition is reduced in the skin of AD patients, while the diameter of droplets, however, is increased (24). These differences can still be detected after application of a thermal stimulus that promotes sweating. In addition, dermcidin was used as a marker for sweat and sweat distribution and the authors found a leakage of dermcidin into the tissue surrounding the sweat ducts in AD, supporting their theory of sweat-induced inflammation. All in all, sweating defects observed in AD seem to be due to the dysfunctioning of sweat gland and/or ducts, and monitoring the ability to sweat in AD patients may be of additional value, also when evaluating new therapeutic options (24).

So-called omics-technologies allow to gather large amounts of data derived from healthy and inflamed skin in AD patients. Therefore, inflammatory signatures (mRNA level) and the whole profile of proteins present in the skin (proteomics) were characterized. Since these approaches are mostly based on taking biopsies and thus are limited with regard to dynamic monitoring, *in vivo* confocal Raman microspectroscopy measurements allows to detect the composition and confirmation of lipids, the lipid-to-protein ratio, the secondary structure of proteins along with the water content non-invasively and intravitaly. In this context, Verzeaux et al. (25) present two-component analysis revealing a distinct clustering of healthy and atopic skin. Amongst other findings, the authors

identified an altered lipid-to-protein ratio in AD, a ratio shown to be important for barrier function of the skin, altered secondary structures of proteins, important for correct functioning, and reduced total and bound water contents revealing a decrease in functional water in the stratum corneum of AD individuals. Thus this new technique could be used to monitor the effects of topical and even systemic treatments in AD patients and allows to assess reconstitution of barrier functions in the stratum corneum (25).

These analyses aim to assess also the dynamics of stratum corneum biology and could well be combined with the pilot study assessing human DC subsets *ex vivo* following suction blister induction, as both present interesting 'cutaneous' biomarkers for AD that are subject to modulation by treatments (17). Next to serum biomarkers and analyses of biopsies, 'cutaneous' biomarkers may allow to more comprehensively assess AD biology. Analyzing fractions found in peripheral blood is increasingly referred to as "liquid biopsies". For AD, CCL17 (TARC), CCL22 (MDC), and CCL18 (PARC) are used also in clinical trials to monitor the severity of AD inflammation as these have been shown to be involved in AD pathogenesis e.g. for the recruitment of Th2 cells into the skin (26, 27).

In addition, micro-RNAs (miRNAs) have increasingly been addressed as regulators also for inflammatory skin diseases. Chen et al. analyzed human blood plasma samples for miRNAs by high-throughput sequencing and used this method to compare AD patients with healthy controls. They detected miR-151a to be differentially expressed and found that miR-151a directly targets the β 2-subunit of the IL-12 receptor (IL-12RB2). Functional analysis using Jurkat cells demonstrated that miR-151a indeed suppresses IL-12RB2 consequently also suppressing the production of IL-2, IFN- γ and IL-12. This work adds a new micro-RNA to the list of possible biomarkers in AD, and further investigation of this biomarker also in interventional studies is warranted, as are further mechanistic studies (28).

Targeting pruritus in AD for new treatments

AD is characterized by chronic T helper (Th)2 cell-mediated eczematous skin with skin barrier dysfunction and pruritus, which may lead to other allergic disorders, consisting of the “atopic march” (5, 29-33). The impaired barrier function associated with the itch-scratch cycle further augments this vicious cycle (34-37). Pruritus is induced by a variety of pruritogens and mediated by cutaneous pruriceptive primary sensory nerves (C-fibers), which transmit the pruritic signal via afferent fibers to the spinal cord. One of possible pruritogens is substance P, an endogenous neuropeptide known to mediate intracellular signaling, mainly through G-protein coupled receptor-linked NK-1R. In this FTI, Choi et al. describe the unexpected finding that topically applied substance P significantly alleviated AD-like clinical symptoms in 2, 4, 6-trinitrochlorobenzene (TNCB)-induced dermatitis in NC/Nga mice, along with reduced infiltration of mast cells and CD3-positive T cells in AD-like skin lesions (38).

It has been demonstrated that interleukin (IL)-31, which is produced mainly by Th2 cells, is a potent pruritogenic cytokine. In this FTI, the roles of IL-31 and IL-31RA on human and murine AD especially in terms of pruritus are reviewed in detail (39). In addition, induction of pruritus by IL-31 was confirmed in *Cynomolgus* monkey (40). AD is known to be initiated by aero-allergens, such as house dust mite (HDM), but it remains unclear how aero-allergens affect pruritus. Szegedi et al. found increased frequencies of HDM-reactive IL-31-producing T cells in AD blood when compared with those in normal blood, suggesting that allergen-specific T cells predispose AD patients to pruritus (41). Moreover, Yamada et al. have found that HDM induced biphasic increase in the number of scratching behavior in NC/Nga mice. An increase in the content of mast cell-derived mediators in the lesional skin was observed in the early phase, and consistently, H1 receptor antagonist and mast cell stabilizer reduced the scratching behavior in the early phase but not that in the later phase. On the other hand, the content of various endogenous pruritogens such as IL-31 was increased in both early and late phases (1 and 24 hours) after HDM application in lesional skin (42). These experimental observations may link the recent clinical findings and trials targeting interleukin-31 receptor A in AD (43-45).

AD endotypes and comorbidities for new treatments

In accord with the advancement of therapeutic strategies and diagnostic tools in AD (46), it is required to define AD endotypes, which classify the disease based on pathogenesis through an analysis of both the clinical and molecular phenotypes (47). AD has been found to occur more frequently in Asian and Black individuals than in Whites. Therefore, a thorough understanding of the unique genetic, clinical and molecular features of AD across a broad range of ethnic AD subtypes is critical. Here, Kaufman et al. (48) review key factors in the epidemiology, pathophysiology, clinical presentation and treatment of AD in non-White ethnic groups and describe that variability in the frequency and type of mutations in genes involved in skin barrier function (ie FLG, FLG-2, SPINK5) and innate/ adaptive immunity (ie IL-4, IL-13, DEFB1) likely impacts the incidence and severity of AD in certain populations. The differential expression of inflammatory cytokines may explain the predominance of more lichenified, well-demarcated and scaly lesions in Asians compared to Whites (48).

AD comorbidities extend well beyond the march to allergic conditions (food allergy, asthma, allergic rhinitis, allergic conjunctivitis, and eosinophilic esophagitis), suggesting both cutaneous and systemic immune activation (49). AD and psoriasis are main inflammatory skin diseases, which are characterized by cytokine profiles, Th2 and Th17, respectively (19, 50). In this FTI, Guttman-Yassky et al. explore emerging evidence of shared and distinct systemic alterations in AD and psoriasis, including inflammation-related comorbidities and related opportunities for systemic treatment (51). Continued exploration of the systemic mechanisms of AD will suggest new approaches to AD treatment and may also modify existing strategies of psoriasis management (52).

Concluding remarks

Taken together with the approval of the first biologic treatment for AD, we have entered a new era in AD research and management. The high effectiveness of dupilumab against AD has confirmed the central role of Th2 cytokines, but the fact that the efficacy of dupilumab against AD is lower than that

of IL-17 blocking against psoriasis corroborates the heterogeneity and complexity of AD. The current FTI highlights several essential and emerging topics on novel developments in AD pathogenesis (especially in terms of microbiome involvement and pruritus), monitoring therapeutic interventions, possible biomarkers, AD endotypes, and comorbidities for new treatments. The wealth of information obtained recently from studies using omics have confirmed the heterogeneity of AD long ago observed by physicians. However, many pertinent research questions remain to be addressed to achieve a more precise understanding of pathogenesis and to reach improved treatment outcomes for AD patients. The current FTI of *Experimental Dermatology* instructively complements other AD studies recently published in this journal (e.g., 53-57) and highlights many of these exciting frontiers in translational AD research and management

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