001

Function of a psoriasis predisposition SNP: TNFAIP3 rs610604 SNP regulates IKKα binding to chromatin and sensitivity to TNFα

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So far 36 genomic loci for psoriasis predisposition have been identified, most located within or in proximity to innate and adaptive response regulating genes. Psoriatic keratinocytes are hypersensitive to inflammatory stimuli. On this scenario, treatment with TNFα enables the activation of IKKα, which induces the nuclear translocation of NFkB transcription factors, while its nuclear fraction is necessary for definite exit of keratinocytes from the cell cycle and termination of NFkB-dependent transcription of inflammatory genes. We found dowregulation of IKKα in psopriatic plaques, correlating with increased NFkB activation. ChIP-seq experiment performed on primary keratinocytes identified inflammatory genes involved in psoriasis pathogenesis as directly bound by IKKα. Moreover, siRNA mediated IKKα knock-out keratinocytes showed induction in IL6 gene expression, upon TNFα stimulation, while treatment of patients with TNFα blocking reagent etanercept induces re-expression of IKKα in keratinocyte nuclei, correlating with decreased NFkB activation. Preliminary results by ChIP-seq analysis of differentiating keratinocytes indicate that IKKα translocates to the nucleus to bind chromatin in intron 3 of the TNFAIP3 gene on the exact region where a psoriasis predisposition SNP (rs610604) is located. TNFAIP3 is rapidly activated by TNFα and encodes for a ubiquitin-editing enzyme that favours inhibition of NFkB activation and TNFα-induced apoptosis. We observed that TNFα failed to induce TNFAIP3 gene activation in IKKα-depleted keratinocytes and the TNFα stimulation caused preferential binding of IKKα on the G allele of rs610604 that has been described to favour a good response to all TNFα blockers and etanercept. These data suggest that IKKα molecule contributes to keratinocyte sensitivity to inflammatory stimuli in psoriasis.

002

Epidermal Protease-activated receptor-2 (PAR2) overexpression causes spontaneous atopic dermatitis-like skin disease: Neuro-Epidermal Communication

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Protease-activated receptor-2 (PAR2) activation has been implicated in the pathophysiology of atopic dermatitis, Netherton syndrome, pruritus, as well as impaired skin barrier regulation. With the aim to study the effects of epidermal PAR-2 function on skin inflammation and itch, we generated a mouse that overexpresses PAR2 in keratinocytes only (KC-PAR2OE). Although KC-PAR2OE newborns display no overt abnormalities, they spontaneously develop dry skin, severe pruritus, and subsequently eczematous skin lesions after several weeks. Analysis of barrier function and immune response in lesional KC-PAR2OE mice revealed the hallmarks of atopic dermatitis-like skin lesions including parakeratosis, downregulation of filaggrin and other epidermal structure proteins, a mast cell- and macrophage-driven inflammatory infiltrate. Of note, and in close correlation to patients with atopic dermatitis, repeated topical application of house dust mite allergens onto KC-PAR2OE mice induced earlier and more severe lesions and pruritus in these mice (as determined by increased skin lesion score, scratching bouts, TEWL, total IgE). Our electrophysiological, morphological and molecular studies show that KC-PAR2OE mice have an increased density of nerve fibers, increased NGF and endothelin expression levels in the skin, which may explain our findings of higher susceptibility of KC-PAR2OE mice to pruritogens and the development of spontaneously increased pruritus. In sum, our results suggest that certain proteases and KC-PAR2 are critically involved in the pathophysiology of pruritus and atopic dermatitis. KC-derived PAR2 seems to be an important link in neuro-epidermal communication with the keratinocyte-protease-PAR2 system as a forefront of sensory signaling and neuro-immune communication in inflammatory skin diseases.

003

Genome-wide comparative analysis of atopic dermatitis and psoriasis gives insight into shared and opposing genetic risk mechanisms

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HB & MH, SW, HJC & SJB contributed equally This study used genome-wide analysis data from the Wellcome Trust Case-Control Consortium and 17 additional co-authors who will be listed in the presentation. Atopic dermatitis (AD) and psoriasis are common chronic inflammatory skin diseases with strong heritability. Genome-wide studies have identified shared risk loci at chromosomes 1q21.3, and 5q31.1 but AD and psoriasis rarely co-occur within the same patient, indicating mutually exclusive pathogenic and immunological features. Shared risk loci for AD and psoriasis may represent overlapping pathophysiological mechanisms whilst opposing effects may indicate determinants of disease-specific susceptibility. We have used GWAS and ImmunoChip data from cohorts comprising >19,000 individuals to systematically compare and contrast AD and psoriasis on a genome-wide level, using methodologies adapted from meta-analysis. This approach has identified risk variants with opposing effects on AD and psoriasis in the epidermal differentiation complex on chromosome 1q21.3, the cytokine cluster on 5q31.1, the major histocompatibility complex on chromosome 6p21.32 and elsewhere in the genome. Our analyses demonstrate an opposing effect locus at RAD50, suggesting that DNA repair may play a role in controlling the differential Th2 response in AD and psoriasis. We have identified a previously unreported association within PRKRA showing opposing effects in each disease and a new risk variant for AD with an opposing effect on psoriasis in ANXA6. In contrast, there were no shared susceptibility loci with genome-wide significance. This statistical approach has shown strength to provide new insight into genetic variation leading to distinct but related complex traits.

004

Intra-individual genome expression analysis reveals a specific molecular signature of psoriasis and eczema

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Psoriasis and eczema are prevalent inflammatory skin diseases with high individual disease-burden and major socio-economic impact. Previous attempts to gain insight into their pathogenesis by using transcriptomic approaches were hampered by the high inter-individual variability of those complex diseases. In patients affected by both psoriasis and non-atopic or atopic eczema simultaneously (n=24), we compared the molecular signature of psoriasis and eczema by performing whole genome expression analysis of lesional as compared to autologous non-involved skin. Genes as well as signaling pathways regulated in common and exclusive for each disease across all patients were identified. Psoriasis-specific genes were crucial regulators of glucose and lipid metabolism, epidermal differentiation as well as immune mediators of Th17 responses, IL-10 family cytokines, and IL-36. In eczema, genes related to epidermal barrier, reduced innate immunity, increased IL-6 and a Th2 signature. Deeper analysis within eczema subtypes unrevealed a mutually exclusive regulation of epidermal differentiation. Besides, only contact eczema was driven by inflammasome activation, apoptosis and cellular adhesion. Based on this comprehensive picture of the pathogenesis of psoriasis and eczema, a disease classifier at the level of RT-PCR consisting of NOS2 and CCL27 was created. In an independent cohort of eczema (n=28) and psoriasis patients (n=25), respectively, this classifier diagnosed all patients correctly and also identified initially misdiagnosed or clinically undifferentiated patients. Since therapeutic strategies for psoriasis and eczema are distinct and sometimes opposing, this diagnostic tool could help to set the correct diagnosis in special cases.

005

A humanized NOG mouse model for atopic dermatitis

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The aim of this project is to establish a humanized mouse model for atopic dermatitis (AD). Previous studies have shown that human AD derived T cells injected s.c. into NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/JicTac (NOG) mice disappear from the skin within a few days. Concurrent injections of human IL-2 and IL-4 might prolong residence of the human cells in murine skin and promote development of a clinical phenotype. Human skin derived T cells from lesional skin of AD patients were cultured in media containing IL-2 and IL-4. NOG mice (n=60) were injected either i.v. or s.c. with human T cells from one of two donors or saline, followed by s.c. injections day 2, 4, 6, 8, and 10 with human IL-2 and IL-4 ± human chemokines (mix of CCL5/RANTES, CCL17/TARC, CCL27/CTACK) or saline. Mice were euthanized at day 1, 3, 6, and 10. At day 1, a clinical phenotype was visible as redness and oedema in 16/24 mice injected s.c. and in 0/24 mice injected i.v. with human T cells. At day 6, only faint redness was observed, and no symptoms were observed at day 10. Immunohistochemistry confirmed the presence of human T cells in the dermis of mice injected s.c., whereas human T cells were not detected in skin biopsies from i.v. injected mice at any time point, regardless of s.c. injections with IL-2 and IL-4 ± human chemokines. Thus, s.c. injection of human AD derived T cells can induce a clinical phenotype in NOG mice, which might be prolonged by more frequent s.c. injections of IL-2 and IL-4.

006

GATA3 is involved in the setup of a functional epidermal barrier and affects the expression of differentiation markers and inflammatory cytokines in human keratinocytes

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GATA3 is an important transcription factor and its role for the differentiation and maintenance of the Th2 cell subtype of lymphocytes has been already well described. However, GATA3 is not exclusively expressed by T cells but also amongst others by keratinocytes. In contrast to T cells the role of GATA3 in keratinocytes is still be poorly explored, especially in regard to inflammatory skin diseases like atopic dermatitis and psoriasis. Thus, it was the aim of this study to investigate the role of GATA3 in keratinocytes under different inflammatory conditions. For this purpose we silenced GATA3 in human primary keratinocytes by lentiviral transduction and performed microarray and qRT-PCR. Microarray data indicated that GATA3 is involved in the regulation of several genes important for the epidermal barrier like involucrin, loricrin and filaggrin. The silencing of GATA3 led to a down regulation of these molecules. Furthermore we found an impact on the expression of inflammatory cytokines like IL-6 and TNFα. Here GATA3 silenced keratinocytes showed an increased expression. Analysis by qRT-PCR confirmed these findings. Of importance, we could detect that the effects of GATA3 were dependent on the current micro milieu. The tendency to show a reduced filaggrin expression was only detectable in GATA3 silenced keratinocytes that were treated with IL-4/IL-13, whereas the effect on involucrin expression was only present under basal conditions. In contrast loricrin expression was found to be decreased under all tested conditions in GATA3 silenced keratinocytes as compared to controls. Our results demonstrate that GATA3 seems to be involved in the signal transduction during inflammatory conditions in the skin and that the modulation of its expression affects a broad range of different molecules which are of importance for the balance of an intact immunity in the skin.

007

IgA, IgG1 and IgG4 harbor clonally distinct desmoglein 3 autoreactive repertoires in pemphigus vulgaris

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Pemphigus vulgaris (PV) is a potentially fatal autoimmune disease of the skin and mucous membranes characterized by autoantibodies (autoAbs) to desmoglein (Dsg) 3. During active disease, the majority of PV autoAbs belong to the IgG4 and less so the IgG1 subclass. However, >50% of patients have serum anti-Dsg3 IgA, the role of which in PV has not been characterized. To better understand the clonal relationships among isotype-specific autoreactive B cells in PV, we cloned anti-Dsg3 IgA1, IgA2, IgG1, and IgG4 repertoires from a mucocutaneously affected PV patient by antibody phage display. We screened >$8x10^8$ combinatorial B cell clones per antibody subclass and characterized 55, 20, 40 and 88 clones from the IgA1, IgA2, IgG1, and IgG4 libraries after Dsg3 selection, which identified 17, 5, 15, and 29 unique anti-Dsg3 heavy chain sequences comprising 5, 4, 4, and 7 CDR3 clonal families, respectively. One clonal family was shared between IgA1 and IgA2. None were shared between IgA1/2, IgG1 and IgG4. Also, by CDR3-specific RT-PCR, we found no evidence of the IgA clones in the IgG4 repertoire. No anti-Dsg3 IgA were found in an unaffected individual. Interestingly, an expanded IgA1 family was identified that used the VH3-15 gene segment, the predominant VH gene used in the natural and vaccine induced response to *Haemophilus influenzae* type B capsular polysaccharide (HIB PRP). The VH3-15 anti-Dsg3 mAb specifically crossreacted to HIB PRP, but not other self-antigens such as RNA polymerase II or BP180 glycoprotein. This crossreactivity was encoded by the heavy chain and was lost after reversion of somatic mutations to the germline VDJ sequence. Additionally, a VH3-15 anti-HIB PRP IgA1 clone isolated by heterohybridoma from an adult after HIB infection showed crossreactivity with Dsg3 by ELISA and immunofluorescence. In summary,we find that anti-Dsg3 IgA, IgG1 and IgG4 repertoires in PV are clonally unrelated and that anti-Dsg3 IgA can harbor pathogen cross-reactivity. Our results suggest independent evolution of anti-Dsg3 isotype-specific B cell lineages in PV.

008

Tolerance break by immunoglobulin class switch recombination induces pemphigus vulgaris in mice

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Pemphigus vulgaris (PV) is an autoimmune blistering disease induced by IgG autoantibodies against desmoglein 3 (Dsg3). We previously isolated a pathogenic mouse monoclonal antibody against Dsg3, AK23 IgG_1, which is able to induce blisters by binding to the adhesive interface of Dsg3. In this study we generated a knock-in mouse expressing AK23 immunoglobulin which can undergo class switch recombination and analyzed the fate of the Dsg3-specific B cells and the mechanisms of the disease onset. Although Dsg3-reactive knock-in B cells producing AK23 IgM in the serum were detected in the peripheral lymphoid organs, the mice neither produced AK23 IgG_1 nor developed PV phenotype spontaneously under normal conditions up to 40 weeks. No *in vivo* binding of IgM on keratinocytes suggested that IgM was too large in size to bind Dsg3 in the core of desmosomes. IL-4 and LPS induced the class-switch from IgM to IgG_1 *in vitro*, and the class-switched B cells were able to induce PV phenotype when adoptively transferred to $Rag2^{-/-}$ mice. The immunization of AK23 knock-in mice with recombinant Dsg3 in complete Freund's adjuvant induced the IgG_1 class switch *in vivo* with the development of PV phenotype. Furthermore, the presence of contact dermatitis to 1-fluoro-2,4-dinitrobenzene also induced the class switch and PV phenotype. These observations indicate that Dsg3-reactive IgM^+ B cells can escape central tolerance mechanism and enter the periphery but do not exert pathogenicity until class-switching is induced. These findings suggested that the class-switching of self-reactive antibodies is one of the important checkpoints in the development of PV.

009

Screen and Identification of HLA-A*0201 Restricted CTL Epitope in Chinese Han Vitiligo Patients

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Vitiligo is a common depigmentation disorder resulted from the destruction of epidermal melanocytes by cytotoxic T lymphocytes (CTL). The identification of melanocyte-specific CTL epitopes is of great importance for understanding the pathogenesis and treatment of this disease. The aim of this study is to screen and identify the melanocyte-specific CTL epitopes of HLA-A*0201 Chinese Han vitiligo patients. PBMC samples were separated from 278 progressive vitiligo patients. HLA typing was performed to identify the HLA*0201 genetic candidates. We then synthesized melanocyte-specific T cell epitopes according to the predicting results of BIMAS and SYFPEITHI. Using ELISPOT, we identified the specific synthetic peptides from the selected synthetic peptides pools by HLA*0201 PBMCs. A transgenic human HLA-A*0201 mouse model was employed to confirm the immune effects of such identified melanocyte- specific epitopes. Six new positive melanocyte-specific peptides in HLA-A*0201 Chinese Han vitiligo patients were identified, including Gp100 P-112 QILKGGSGT, tyrosinase P-41 AMVGAVLTA, Gp100 P-118 QLIMPGQEA, tyrosinase P-24 SSADVEFCL, tyrosinase P-28 TLEGFASPL and Gp100 P-119 TLEGFASPL. After subcutaneous and intraperitoneal injection with selected positive peptides, the spleens and lymph nodes of HLA*0201 transgenic mice model were enlarged. Moreover, the number of $CD8^+$ T lymphocytes in mice spleens and lymph node increased though in a moderate degree. In conclusion, our experiments identified 6 new auto-antigenic peptides in Chinese Han HLA-A*0201 vitiligo patients. We found that synthesized melanocyte- specific peptides can cause T cell proliferation and activation. Further studies are needed to confirm the downstream effects by $CD8^+$ T lymphocytes using the synthesized melanocyte- specific peptides in HLA*0201 transgenic mice. Such observations may contribute to the understanding of the pathogenesis of vitiligo and provide potential targets for new treatments.

010

Genetic markers predictive for the outcome of anti-interleukin-12/23 therapy in psoriasis

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Recent studies agree on the need for pharmacogenetic data predictive for the outcome of the various therapeutic approaches in psoriasis. Following this direction, we sought to assess the potential of genetic variants of interleukin (IL) genes in predicting the outcome of anti-IL-12/23 therapy in psoriasis. In this study we included 64 psoriasis patients. All were put on an anti-IL-12/23 therapy due to failure of at least two conventional systemic therapies, or anti-tumor necrosis factor agents. We genotyped all patients for SNPs present in genes encoding for IL6 by restriction fragment length polymorphism PCR, for p40 and for IL23R using real-time PCR and for HLACw6 by allele specific PCR. All data was statistically assessed using SPSS. The response to therapy was evaluated using the PASI, which we recorded at baseline and weeks 4, 12, 28 and 40. The primary endpoint was considered reaching PASI 75. Results showed that a significantly higher percentage of HLACw6 carriers (p=0.004-0.005) reached the primary endpoint at the various intervals assessed, Statistical significance was present also for p40 SNP (p=0.002-0.004). Correlating IL6 and IL23R to the treatment outcome did not meet statistical significance. Such pharmacogenetic markers could be integrated in the daily therapeutic management of psoriasis.

011

Laser capture microdissection followed by next-generation sequencing identifies disease-related microRNAs in psoriatic skin that reflect systemic microRNA changes in psoriasis

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Psoriasis is a systemic disease with cutaneous manifestations. MicroRNAs (miRNAs) are small non-coding RNA molecules that are differentially expressed in psoriatic skin, however, only few miRNAs have been localized to specific cells or regions of psoriatic lesions. Using laser capture microdissection (LCM) and next-generation sequencing (NGS) we aimed to investigate the specific miRNA expression profiles in the epidermis (Epi) and dermal inflammatory aggregates (RD) of psoriatic skin (N=6). We identified 24 deregulated miRNAs in the Epi and 37 deregulated miRNAs in the RD of lesional psoriatic skin compared with non-lesional psoriatic skin (FCH>2, FDR<0.05). Among the identified miRNAs several have not previously been described in psoriatic skin. Interestingly, 9 of the 37 miRNAs in RD, including miR-193b and miR-223 were recently described as deregulated in circulating peripheral blood mononuclear cells (PBMCs) from patients with psoriasis. Using flow cytometry and qRT-PCR, we found that miR-193b and miR-223 were expressed in Th17 cells. In conclusion, we demonstrate that LCM combined with NGS provides a robust approach to explore the global miRNA expression in the epidermal and dermal compartments of psoriatic skin. Furthermore, our results indicate that the altered local miRNA changes seen in the RD are reflected in the circulating immune cells, suggesting that miRNAs may contribute to a systemic component in the pathogenesis of psoriasis.

012

PGD2-DP signaling facilitates the development of imiquimod-induced mouse psoriasis-like dermatitis by promoting IL-23p19 production by dendritic cells

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Psoriasis is a common chronic inflammatory skin disorder characterized by epidermal hyperplasia. Although it has been revealed that cytokines such as IL-23, TNF-alpha and IL-17 play pivotal roles in its development, the role of lipid mediators in its pathogenesis remains unclear. Prostaglandin D2 (PGD2) is a lipid mediator and exerts various physiological effects through two G protein-coupled receptor receptors, DP and CRTH2. Although the role of PGD2-DP signaling in allergic disease such as asthma or atopic dermatitis have been well studied, the role of DP signaling in the development of psoriasis remains unclear. In this study, we examined the role of PGD2-DP signaling in psoriasis using imiquimod-induced mouse psoriasis-like dermatitis. Firstly, we observed that the mRNA expression of H-PGDS, a PGD2 synthase, was up-regulated in the skin of psoriasis-like dermatitis. Mice lacking DP exhibited significantly reduced epidermal hyperplasia and dermal edema in skin than did WT mice. The cytokine mRNA expressions such as IL-23p19 and IL-17 in skin of DP deficient mice were significantly lower than those of WT mice. Immuno-histochemical analysis revealed DP was strongly expressed on dermal DCs. Stimulation of DP on bone-marrow derived DCs increased IL-23p19 mRNA expression in a cyclic AMP dependent manner. These results indicate that PGD2-DP signaling facilitates the development of mouse psoriasis-like dermatitis via inducing IL-23p19 production by dermal DCs, suggesting its involvement in the pathogenesis of psoriasis.