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Presented in LBN Session in Stockholm:

001

Critical Role for JNK in Mediating the Antagonistic Activity of TNF-α Against TGF-β/Smad Signaling and Collagen Gene Transcription

F. Verrecchia, C. Tacheau, E. F. Wagner, and A. Mauviel
Paris, France and Vienna, Austria

Given the physiopathological importance of TGF-β and TNF-α, we have focused our attention on the molecular events underlying their antagonistic activities regarding Smad signaling downstream of the TGF-β receptors, and expression of type I collagen (COL1A2). Using transient cell transfections of JNK1-JNK2 knockout ($JNK^{-/-}$) and I-κBγ/$NEMO^{-/-}$ fibroblasts, the latter being devoid of NF-κB activity, we have determined the specific roles played by the Jun N-terminal kinase (JNK)/AP-1 and NF-κB/Rel pathways in this phenomenon. We demonstrate that neither JNK nor NF-κB activities are required for TGF-β signaling through the Smad pathway. However, the absence of JNK activity prevents TNF-α-driven inhibition of both Smad signaling and COL1A2 promoter transactivation by TGF-β. On the other hand, the absence of NF-κB activity does not alter the TNF-α antagonistic effect against TGF-β. Among JNK substrates, c-Jun, and JunB to a lesser extent, but not ATF2, antagonize TGF-β/Smad signaling. Overexpression of JNK1 restores TNF-α/c-Jun inhibition of Smad signaling and COL1A2 promoter transactivation by TGF-β in $JNK^{-/-}$ fibroblasts. Overexpression of an antisense *c-jun* vector or that of a dominant-negative form of MKK4 blocks the inhibitory activity of TNF-α in $NEMO^{-/-}$ fibroblasts. These results suggest a critical role for JNK-mediated c-Jun phosphorylation in mediating the inhibitory effect of TNF-α on Smad signaling and up-regulation of type I collagen gene by TGF-β.

002

Congenital Lipodystrophy Berardinelli-Seip Reveals a New Gene and a New Protein

T. Gedde-Dahl Jr, M. Delépine,* E. Khallouf,† L. Van Maldergem,‡ E. Sobel,* J. Papp,* M. Meier, A. Mégarbane,¶ BSCL Working Group, M. Lathrop,* J. Capeau,§ and J. Magré§
*Departments of Dermatology, Internal Medicine and Pediatrics, Rikshospitalet and Institute of Forensic Medicine, University of Oslo, Oslo, Norway; *Centre National de Génotypage, Evry, Paris, France; †Service de Pédiatrie, Hôtel-Dieu de France, Beyrouth; ‡Centre de Génétique Humaine, Institut de Pathologie et de Génétique, Loverval, Belgium; §INSERM U.402, Faculté de Médecine Saint-Antoine, Paris, France; ¶Université Saint-Joseph, Faculté de Médecine, Unité de Génétique Médicale, Beyrouth*

The disease (BSCL) is characterized by near absence of adipose tissue from birth or early infancy, severe insulin resistance, *acanthosis nigricans*, hyperandrogenism, muscular hypertrophy, hepatomegaly, altered glucose tolerance or diabetes mellitus and hypertriglyceridemia. We have used clustered families from Norway and Lebanon, each presumed to represent a recessive mutation identical in descent, to reveal they both comply with genetic linkage to chromosome 11q13 DNA markers (Magré *et al*, 2001).

Using 25 additional dinucleotide repeat markers isolated from that segment, a 2.5 Mbase long overlapping segment was defined as having absolute disease association. None of 26 structural genes known there had detectable disease mutations. From a mouse gene (*Gng3lg*) of unknown function, residing in the mouse orthologue of HSA11q13, we sequenced a new gene (*BSCL2*) which appeared to have a null mutation in the Lebanon families and a missense mutation in the Norwegian families. Moreover, of world-wide collected BSCL patients, 47 had mutations in this gene, whereas families with patients with nonmutated *BSCL2* fitted linkage to 9q24 where a not yet cloned gene (*BSCL1*) is assumed to reside (Garg *et al*, 1999). *BSCL2* ORF is 1196 bases long and predicts a 398 aa long peptide named seipin. The *BSCL2* gene is most abundantly expressed in CNS and in testis, but its function is unknown up to now. It is a candidate for being one of the QTL genes for diabetes.

Magré J *et al*: Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. *Nat Genet* 28:365–370, 2001

Garg A *et al*: A gene for congenital generalized lipodystrophy maps to human chromosome 9q34. *J Clin Endocrinol Metab* 84:3390–3394, 1999

The following abstracts were presented as posters:

003

Effect of Vascular Endothelial Cell Growth Factor (VEGF) Deletion on Skin Biology and Mammary Gland Development and Function

H. Rossiter,* C. Barresi,* J. Ban, C. Mayer, M. Rendl,* J. Haigh,† E. F. Wagner,† and E. Tschachler*‡
**D.I.A.I.D., Department of Dermatology, University of Vienna Medical School, Vienna, Austria; †IMP, Vienna, Austria; ‡CE.R.I.E.S., Neuilly, France*

Vascular endothelial cell growth factor (VEGF) is a major inducer of angiogenesis. In the skin, an important source of VEGF is the epidermal keratinocyte (KC), so we wished to investigate the contribution of KC-derived VEGF to angiogenesis-dependent processes in this organ. We have inactivated VEGF specifically in mouse cells that express keratin 5, using a Cre/Lox-p system. Deletion of exon 3 of VEGF A in genomic DNA from K5 expressing tissues was demonstrated by PCR and Southern blotting, while cultured epidermal keratinocytes produced no detectable VEGF. Depilation-induced anagen resulted in sparser hair regrowth and fewer hair follicles in mutant mice, and wound healing was retarded compared to controls. Anti-CD31 staining of wounds demonstrated a reduced microvessel density immediately below the regenerating keratinocytes. In addition, mammary glands of lactating mutant female mice displayed significantly fewer alveolar ducts, and the weight gain of their pups was severely retarded. We thus show that KC-derived VEGF plays an important role in epidermal wound healing and hair regrowth, while deletion of VEGF in the mammary gland drastically compromises mammary gland development and function.

004

The All-*Trans* Retinoic Acid Receptor Binds to an Element in the Promotor of Hurpin (Proteinase Inhibitor 13, PI13) a Novel Serine Proteinase Inhibitor which is Overexpressed in Psoriatic Skin Lesions

Tanja Günther, Harry F. Abts, Carsten Carlberg,* and Thomas Ruzicka
*Department of Dermatology, Heinrich-Heine University, Düsseldorf, Germany; *Department of Biochemistry, University of Kuopio, Finland*

Retinoic acid and vitamin D_3 modulate keratinocyte proliferation and differentiation – processes that are disturbed in psoriatic skin – via binding to the respective nuclear receptors for retinoic acid (RAR-α,-γ), and vitamin D_3 (VDR) plus the common heterodimeric partners 9-*cis*-retinoic acid receptors (RXR-α,-β). In contrast to healthy skin, in psoriatic skin lesions the serine proteinase inhibitor hurpin (PI13) is over expressed. For those reasons it was interesting to address a potenial regulatory influence of all-*trans* retinoic acid (ATRA) or 1,25-dihydroxyvitamin D_3 (VD), respectively, on the hurpin promotor. RT-PCR experiments were carried out in HaCaT cells with these ligands to address their potential influence on the transcriptional activities of the hurpin gene. The incubation with ATRA led to a strong reduction of hurpin expression to 29.8%, wheras VD showed a repression to 63.2%. A computer based search for consensus binding sites revealed seven potential response elements (RE) in the hurpin promotor. In performed electrophoretic mobility shift assays, two of those potential hurpin response elements (a DR2- and a DR3-type element) showed their binding affinities to the receptor heterodimers RARγ/RXRα and VDR/RXRα, respectively. When ligated to a TK promotor and coupled to the pGL3 basic vector in transient transfections, only the DR2-type element confered a 6-fold increase of luciferase activity stimulated by ATRA, while the incubation with VD led to no effect. The VD mediated repression of Hurpin is thus not controlled by the HP-(DR3)1-RE. These studies suggest that the repression of hurpin mediated by ATRA on transcriptional level is correlated with the binding affinity of the HP-DR2-type retinoic acid RE.

005

Proteinases of Common Pathogenic Bacteria Degrade and Inactivate the Antibacterial Peptide LL-37

A. Schmidtchen, I.-M. Frick, and L. Björck
Lund, Sweden

Effectors of the innate immunity system, the antimicrobial peptides, have pivotal roles in preventing infection at epithelial surfaces. Here, we show that proteinases of *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Streptococcus pyogenes* degrade the antibacterial peptide LL-37. LC-MS analysis of fragments generated by *p. aeruginosa* elastase revealed that the initial cleavages occured at Asn-Leu and Phe-Leu, followed by two breaks at Arg-Ile, thus inactivating the peptide. Proteinases of the other pathogens induced similar fragmentations as determined by SDS-PAGE. The degradation was abolished by the metalloproteinase inhibitors GM6001, 1, 10-phenantroline (which both inhibited *P. aeruginosa* elastase and *E. faecalis* gelatinase), or the inhibitor E64 (which inhibited *S. pyogenes* cysteine proteinase). Furthermore, experiments showed that substances which bind LL-37, such as dermatan sulphate, and disaccharides of the structure [ΔUA(2S)-GalNAc(4,6S)] or sucroseoctasulfate blocked the degradation of LL-37. Thus, the degradation of the antibacterial peptide LL-37 by bacterial proteinases represent a virulence parameter which could be exploited in the development of novel antibacterial therapies.

006

Bone Morphogenetic Protein (BMP-1) Processes Procollagen VII to Mature Collagen VII

A. Rattenholl, M. Koch,* D. S. Greenspan,† and L. Bruckner-Tuderman
*Department of Dermatology, University of Münster, Germany; *Cutaneous Biology Research Center, MGH/Harvard Medical School, Charlestown, U.S.A.; †Departments of Pathology and Laboratory Medicine and Biomolecular Chemistry, University of Wisconsin, Madison, U.S.A.*

Collagen VII is the major structural component of the anchoring fibrils which ensure the cohesion between the epidermis and the dermis. This collagen is secreted by keratinocytes into the extracellular matrix as a precursor, procollagen VII. During polymerization and maturation of the anchoring fibrils, two procollagen VII monomers form an antiparallel homodimer with an overlapping C-terminus, and a C-terminal propeptide is removed. However, the mechanisms of anchoring fibril maturation, including the cleavage site and the enzyme processing procollagen VII have remained elusive. Genetic evidence has suggested the involvement of BMP-1 in this process, since a naturally occurring deletion in the COL7A1 gene, 8523del14, which eliminates a a putative cleavage site for BMP-1, prevents processing of procollagen VII in the skin. Here we show that recombinant BMP-1 cleaves full-length human procollagen VII *in vitro,* yielding a cleavage product of the same size as mature collagen VII in the dermis. In order to determine the cleavage site, a truncated recombinant procollagen VII containing an intact C-terminus was produced. This miniprocollagen VII was cleaved by both BMP-1 and by mammalian tolloid-like 1 (mTLL-1), another protease of the same enzyme family. N-terminal sequencing showed that miniprocollagen VII was processed at the predicted site. Analysis of collagen VII in the skin of BMP-1 deficient mouse embryos demonstrated that procollagen VII was processed to the same extent as in wild type embryos. This suggests that *in situ* the collagen VII precursor can be cleaved by at least two metalloproteases including BMP-1 and mTLL-1. The cleavage of the keratinocyte-derived procollagen VII by fibroblast-derived enzymes presents a novel mechanism of regulation of anchoring fibril formation and an intriguiging manifestation of epithelial–mesenchymal interactions.

007

Serum IL-4 and Acute Phase Proteins in Patients with Mycosis Fungoides

M. Pawlaczyk and M. Sobieska Poznañ
Poland

The aim of the study was to investigate the acute phase proteins concentrations and glycosylation profiles, as well as IL-4 during various stages of mycosis fungoides (MF). Sera samples were obtained from 45 patients with MF. Interleukin-4 concentration was determined by ELISA kit. Proteins concentrations were measured using rocket electrophoresis. The reactivity with Concanavalin A was estimated for alfa 1-acid glycoprotein and alfa 1-antichymotrypsin using crossed affinity immunoelectrophoresis. Increased concentration of IL-4 was observed in patients with tumors and erythtoderma. C-reactive proteine was found to be elevated in about half of subjects. The levels of alfa1-acid glycoprotein and alfa1-antichymotrypsin were increased in all patients except those in plaque stage and the difference was statistically significant. Glycosylation profiles were almost normal in patch stage, but in all other stages they changed towards glycoforms weaker reacting with Concanavalin A. These results prove the involvement of both specific immune reaction, concerning T-lymphocytes activation and nonspecific reactions, as acute phase proteins concentration reflect elevated IL-6 level. Though patients suffering from MF rarely show any abnormalities in rutine laboratory examinations from the begining of the disease, some features of inflammation are present. It may be described as a generalized inflammatory reaction, known as acute phase response, with the highest intensity for erythrodermic patients and lowest for patients in plaque stage

008

Second Line Vindesine Monotherapy in Advanced Stage IV Malignant Melanoma: Low Toxicity but no Amelioration of Disease Progression

S. Emmert, M. Zutt, H. Haenssle, C. Neumann, and L Kretschmer
Goettingen, Germany

Vindesine is a Vinca Alkaloid with mild toxicity. Anti-melanoma activity of vindesine as a single agent as well as in polychemotherapies has been reported. We investigated the usefulness of a second line vindesine monotherapy in stage IV melanoma. A total of 13 patients with progressive disease were treated with 3 mg per m^2 vindesine every 2 weeks (mean age 59.2 ± 11.9 years). Previous systemic treatment consisted predominantly of polychemotherapy or combined chemo-immune therapy (seven patients received 1 treatment, three patients 2, two patients 3, and one patient 4 treatments). All 13 patients suffered from visceral metastases besides regional skin and lymphnode metastases (three with lung, one with liver, one with pararenal, and eight with multiple visceral metastases). A mean of 2.7 ± 1.3 vindesine treatments were administered. Only one patient showed reversible hair loss and leucopenia. None of the patients developed neuropathy. In all of the 13 patients (100%) vindesine treatment was stopped due to disease progression. Median survival after first diagnosis was 42 months (8–151 months), survival after entering stage IV was 11 months (3–35 months), and survival after starting vindesine therapy was 4 months (1–22 months). We conclude that vindesine monotherapy is ineffective in stage IV melanoma patients previously treated with other chemotherapeutic agents.

009

Molecular Bases and Clinical-Functional Classification of Ectodermal Dysplasias

M. Priolo and C. Laganà
Reggio Calabria, Italy

The ectodermal dysplasias (EDs) are a large and complex nosologic group of diseases. To date, few causative genes have been identified for EDs. We recently proposed a new approach to EDs integrating both molecular-genetic data and corresponding clinical findings. This approach is the first attempt to integrate both systems of classification.

Two different groups are recognised, each likely to result from mutations in genes with similar function and possibly involved in the same mechanisms of regulation of development and/or pathogenesis. We now give an overview of molecular bases in EDs. A defect in epithelial–mesenchymal interaction can be recognised in group one. A structural protein defect can be recognised or postulated in group two. Finally, through an extensive revision of both molecular/functional mechanisms and clinical presentation of very well characterised EDs, we expand this approach to other forms speculating on possible candidate genes suggested by associated non ectodermal features.

Many signals triggered from proteins classified in the two group converge on the same intracellular factors, interact among them or regulate each other in complex pathways. These findings can explain common key signs sometimes observed in association with different types of EDs, such as cleft lip/palate or specific skeletal malformations (see ectrodactyly as an example). This approach is also a definitive improvement to better clarify the spatial and temporal activity of some EDs' causative genes during development, such as the recently proved altered paracrine signalling between EDA and EDAR when EDA can not be proteolytically cleaved by furin.

Our goal is to give both clinicians and researchers a lecture key in better understanding EDs and in dissecting complex phenotypes. We think that the accurate definition of key signs associated with the primary interest in pathogenetic processes are significant supports to researchers in a directed endeavour to find new candidate ED genes.

010

Preferential Usage of TCR-Vβ17 by Peripheral and Cutaneous T Cells in Nickel-Induced Contact Dermatitis

L. Büdinger, N. Neuser, U. Totzke, H. F. Merk, and M. Hertl
Aachen, Hamburg, and Erlangen, Germany

Nickel (Ni) is one of the most common contact sensitizers in man and Ni-induced contact dermatitis is considered as a model of hapten-induced delayed type hypersensitivity. Previous studies indicated that Ni-reactive T cells derived from Ni-allergic individuals preferentially express distinct TCR-Vβ chains. However, data on the TCR-Vβ repertoire of Ni-responsive T cells are not consistent. The aim of this study was therefore to identify the TCR-Vβ receptors of Ni-responsive peripheral and cutaneous T cells in a cohort of 17 donors with Ni-induced contact dermatitis in comparison to those of 6 healthy controls. Peripheral $NiSO_4$-responsive T lymphocytes showed a significant over-expression of TCR-Vβ17 and the frequency of TCR-Vβ17 + T cells correlated significantly with the *in vitro* reactivity of PBMC to $NiSO_4$. In addition, the cutaneous infiltrate of Ni-induced patch test reactions consisted primarily of TCR-Vβ17 + T cells. The majority of patch test-derived $NiSO_4$-responsive T cells of three allergic donors were TCR-Vβ17 + while patch test-derived $NiSO_4$ unresponsive T cells of four additional donors did not express TCR-Vβ17. Skin-derived Ni-responsive T cell lines from three donors uniformly secreted the Th2 cytokine, IL-5 but no IFN-γ or IL-10. These *in vitro* and *in vivo* findings strongly suggest that T cells with a restricted TCR-Vβ-repertoire, i.e. Vβ17, predominate in $NiSO_4$-induced contact dermatitis and may be crucial in the effector phase of Ni hypersensitivity. Ni-induced contact dermatitis thus holds great promise as a model system to establish therapeutic concepts to specifically modulate hypersensitivity reactions against haptens such as various ubiquitous and occupational allergens including metals, contact sensitizers, and drugs.

011

TP53 Polymorphism of Exon 4 at Codon 72 in Cutaneous Squamous Cell Carcinoma and Benign Epithelial Lesions of Renal Transplant Recipients and Immunocompetent Individuals: Lack of Correlation with HPV Status

O. Humbey, S. Cairey-Remonnay, C. Mougin, M. P. Algros, F. Mauny, J. Kanitakis, S. Euvrard, R. Laurent, and F. Aubin
Besançon and Lyon, France

Common polymorphism at codon 72 of exon 4 encoding either arginine (ARG) or proline (PRO) has been shown to confer a susceptibility to the development of skin tumor in renal transplant recipients (RTR). Moreover, this polymorphism may affect proteolytic degradation of p53 promoted by E6 protein from mucosal human papillomaviruses (HPV) and represent a risk factor for HPV-induced carcinogenesis. In this study, we analyzed the HPV presence and the TP53 allele distribution in cutaneous squamous cell carcinoma (SCC) of RTR and immunocompetent patients (ICP). 53 SCC from 40 RTR, 50 benign epithelial skin lesions from 50 RTR with no history of skin cancer, 51 SCC from ICP, and 29 blood samples from IC healthy individuals were investigated. HPV DNA was detected using pcr performed with 2 pairs of primers (MY09-MY11 and FAP59-FAP64). TP53 allele distribution was studied by denaturing gradient gel electrophoresis assay, followed by sequencing analysis. HPV DNA was detected in 64% of SCC and 79% of benign epithelial lesions from RTR (NS) and only in 37% of SCC from ICP ($p < 0.05$). Mucosal oncogenic hpv types were predominent in SCC from both RTR and ICP. Rate of arg homozygosity in SCC from RTR was significantly higher (85%) than in ICP with or without SCC (61% and 59%, respectively). Our results suggest that TP53 Arg/Arg genotype could represent a potential risk factor for the development of SCC in RTR as compared to ICP. However, no association between TP53 Arg/Arg genotype and HPV status could be determined.

012

***Ex Vivo* Isolation and Characterization of $CD4^+CD25^+$ T Cells with Regulatory Properties from Human Blood**

D. Dieckmann, S. Berchtold, H. Plöttner, T. Berger, and G. Schuler
Department of Dermatology, University of Erlangen-Nuremberg, Erlangen, Germany

It has been known for years that rodents harbor a unique population of $CD4^+CD25^+$ ''professional'' regulatory/suppressor T cells that is crucial for the prevention of spontaneous autoimmune diseases. Here we demonstrate that $CD4^+CD25^+$ $CD45RO^+$ T cells (mean 6% of $CD4^+$ T cells) are present in the blood of adult healthy volunteers. In contrast to previous reports these $CD4^+CD25^+$ T cells do not constitute conventional memory cells but rather regulatory cells exhibiting properties identical to their rodent counterparts. CTLA-4 (CD152), for example, which is essential for the *in vivo* suppressive activity of $CD4^+CD25^+$ T cells, was constitutively expressed, and remained strongly up-regulated after stimulation. The cells were nonproliferative to stimulation via their TCR, but the anergic state was partially reversed by IL-2 and IL-15. Upon stimulation with allogeneic (but not syngeneic) mature dendritic cells or plate-bound anti CD3 + anti-CD28 the $CD4^+CD25^+$ T cells released IL-10, and in coculture experiments suppressed the activation and proliferation of $CD4^+$ and $CD8^+$ T cells. Suppression proved IL-10 independent, yet contact dependent as in the mouse. The identification of regulatory $CD4^+CD25^+$ T cells has important implications for the study of tolerance in man, notably in the context of autoimmunity, transplantation, and cancer.

013

Isolation and Characterization of Dermal Lymphatic and Blood Endothelial Cells Reveal Stable and Functionally Specialized Cell Lineages

E. Kriehuber, S. Breiteneder-Geleff, M. Groeger, A. Soleiman, S. Schoppmann, G. Stingl, D. Kerjaschki, and D. Maurer
Vienna, Austria

Plexus of lymphatic vessels guides interstitial fluid, passenger leukocytes, and tumor cells towards regional lymph nodes. Microvascular endothelial cells of lymph channels (LECs) are difficult to distinguish from those of blood vessels (BECs) because both express a similar set of markers, such as CD31, CD34, podocalyxin, von willebrand factor (vWF), etc. Analysis of the specific properties of LECs was hampered so far by lack of tools to isolate LECs. Recently, the 38 kDa mucoprotein podoplanin was found to be expressed by microvascular LECs but not BECs *in vivo*. Here we isolated for the first time podoplanin$^+$ LECs and podoplanin$^-$ BECs from dermal cell suspensions by multicolor flow cytometry. Both EC types were propagated and stably expressed VE-cadherin, CD31, and vWF. Molecules selectively displayed by LECs *in vivo*, i.e. Podoplanin, the hyaluronate receptor LYVE-1, and the vascular endothelial cell growth factor (VEGF)-c receptor, Flt-4/VEGFR-3, were strongly expressed by expanded LECs, but not BECs. Conversely, BECs but not LECs expressed VEGF-c. LECs as well as BECs formed junctional contacts with similar molecular composition and ultrastructural features. Nevertheless, the two EC types assembled in vascular tubes in a strictly homotypic fashion. This EC specialization extends to the secretion of biologically relevant chemotactic factors: LECs, but not BECs, constitutively secrete the CCR7 ligand SLC/CCL21 at their basal side, while both subsets, upon activation, release mip-3α/CCL20 apically. These results demonstrate that LECs and BECs constitute stable and specialized EC lineages equipped with the potential to navigate leukocytes and, perhaps also, tumor cells into and out of the tissues.

014

Netherton Syndrome (NS) is not an Ichthyosis, but a Disease of Over-Desquamation: A Model of Skin Barrier Dysfunction

N. Komatsu, M. Takata, N. Ohtsuki, K. Takehara, and K. Saijoh
Kanazawa, Japan

NS is a congenital ichthyosis (i.e. dry rough skin resembling fish scale) associated with erhthroderma, hair-shaft defects and atopic features. Although the mutations of the secretory serine protease inhibitor Kazal-type 5 (SPINK5) gene have recently been identified in NS patients, how the genetic defects cause characteristic skin phenotype is unknown. Here we propose a model of inhibitory regulation of corneocyte desquamation by a set of peptides encoded by the SPINK5 gene. The SPINK5 gene encodes a proprotein which possesses multiple potential cleavage sites for a family of subtilisin-like proprotein convertases and carboxypeptidases and is subjected to limited proteolysis. The cleaved peptides inhibit serine proteases activated in the superficial stratum corneum that play a critical role in the corneocyte desquamation by degrading intercellular desmoglein-1. The defective inhibitory regulation of SPINK5-derived peptides in NS patients results in increased protease activity in the superficial stratum corneum leading to over-desquamation of corneocytes. This hypothesis is supported by a distinct phenotype-genotype correlation and the markedly increased stratum corneum hydrolytic activity in 3 NS patients from 2 unrelated Japanese families. Therefore, NS is not an ichthyosis, but a disease of over-desquamation causing severe skin permeability barrier dysfunction.

015

Vivo Phenotypic Reversion of Recessive Dystrophic Epidermolysis Bullosa Keratinocytes

C. Baldeschi, Y. Gache, A. Rattenholl,* L. Bruckner-Tuderman,* J. P. Ortonne, and G. Meneguzzi
*INSERM U385, Faculté de Médecine, Nice, France; *Department of Dermatology, University of Muenster, Germany*

Dystrophic epidermolysis bullosa (DEB) is an inherited skin disorder caused by mutations in collagen type VII, the major component of the anchoring fibrils of the dermal-epidermal junction. Gene therapy of DEB is justified because no other treatment is available. However, the size of the collagen VII cDNAs (> 9 kb) and the risk of immune response against the transgene product are serious hurdles. The identification of inbred dogs suffering from recessive DEB caused by expression of an abnormal collagen type VII gene offers the possibility of testing therapeutic assays based on gene replacement in immunocompetent hosts. Screening for genetic mutations in the affected animals detected a homozygous nucleotide change (5716G→A) in the *Col7A1* gene that cosegregates with the recessive DEB phenotype. The sequence variation predicts the substitution of a glycine residue (1906G→S) within the collagenous domain of the polypeptide. To perform a phenotypic reversion of recessive DEB keratinocytes, we have isolated and characterized the canine collagen type VII full-length cDNA from a wild type dog. The full-length cDNA was transfected *ex vivo* into immortalized and primary human collagen VII-null keratinocytes and fibroblasts using a panel of retroviral vectors. All the recombinant viruses efficiently expressed the canine collagen type VII molecules. The transduced keratinocytes synthesized pro-collagen VII molecules of the expected size that display the subcellular distribution and secretion pattern observed in normal human keratinocytes. The reverted keratinocytes also displayed decreased rates of proliferation and reduced motility. The reverted cells sustain expression of the collagen type VII transgene for at least four months in culture. These results demonstrate that retroviral vectors are promising tools for gene therapy of recessive DEB and for the functional analysis of the different domains of collagen type VII by site-directed mutagenesis of the corresponding cDNA

016

Targeted Ablation of the Murine LAMC2 Gene Recapitulates Human Herlitz Junctional Epidermolysis Bullosa Phenotype

J. Uitto, X. Meng, J. Klement, and L. Pulkkinen
Jefferson Medical College, Philadelphia, Pennsylvania, U.S.A.

The Herlitz junctional epidermolysis bullosa (H-JEB) is an autosomal recessive blistering disorder with poor prognosis. The underlying mutations reside in the genes encoding laminin 5 subunit polypeptides, most of them in LAMB3 (β3 chain) while some have been described in LAMA3 and LAMC2 (α3 and γ2 chains). To evaluate the pathomechanisms and tissue involvement of H-JEB in further detail, and to provide an animal model for development of gene therapy approaches, we have developed a LAMC2 null mouse (–/–) by targeted ablation of exon 8 of the γ2 chain gene of laminin 5, which results in out-of-frame deletion of 115 bp and creates a downstream premature termination codon for translation. Heterozygous mice were clinically normal, while homozygotes manifested with extensive blistering of the skin and mucous membranes and died within 1–2 day of birth. Histopathology revealed tissue separation at the cutaneous basement membrane zone. Immunohistochemistry of the skin and tongue at the dermal-epidermal junction (DEJ) was essentially negative for γ2 chain expression, and α3 chain expression was concomitantly reduced. The expression of other DEJ components (type VII and XVII collagens, α6 integrin, and plectin) appeared normal. RT-PCR clearly detected the shortened mRNA transcript in –/– mice, but Western analysis was unable to detect γ2 chains. PCR genotyping revealed that in 21 litters, 41 (24.4%), 96 (57.1%), and 31 (18.5%) mice were –/–, +/–, and +/+, respectively, consistent with autosomal recessive inheritance and no evidence for embryonic lethality. Collectively, these data, together with previously developed LAMA3 –/– mice and a naturally occurring LAMB3 null mouse, attest to the critical importance of each laminin 5 polypeptide subunit in epithelial-mesenchymal adhesion.