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Heidelberg, Cologne, Germany and Bucharest, Romania

INVITED LECTURES

I-01

Autoimmune bullous disorders of the dermo-epidermal junction

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During the last few years, considerable progress has been made in the understanding of the structure and function of various components of the dermo-epidermal junction. Bullous pemphigoid is a disease of the elderly characterized by the production of autoantibodies mainly directed against a 220–240 kDa polypeptide, the major BP antigen located in the hemidesmosome. Labib *et al* demonstrated that a 180 kDa protein was also recognized by a number of pemphigoid sera. The 180 kD BPA is a transmembrane molecule with a predicted type II orientation. The C-terminal domain is extracellular and consists of a series of collagen triple helical domains. Recent studies have located one of the major epitopes in a non collagenous stretch of the extracellular domain of the 180 kD BPA very close to the keratinocyte membrane. Recent evidence largely from DNA sequency suggest that multiple epitopes are present on the 180 kD BPA. Using rabbit antisera raised against different domains, we have demonstrated that the C-terminal domain was localized in the lower part of the lamina lucida. Double labelling performed with C-terminal antibodies and anti-kalinin antibodies have shown a common localization in the interface of the lamina lucida and lamina densa where it is believed to be associated with anchoring filaments. Our ultrastructural mapping confirm that the 180 kD BPA has a very long extracellular domain extending on the entire lamina lucida from the hemisdesmosome to the lamina densa. Recent report of non-lethal junctional epidermolysis bullosa have demonstrated that absence of expression of the 180 kD BPA may have a critical pathogenetic significance in adhesion between epidermis and the dermis. The 180 kD BPA which is mainly in its extracellular portion a collagenous molecule is probably a major component of the anchoring filaments network and certainly plays an important role in the adhesion process between basal keratinocytes and the dermis. The 97 and 120 kDa antigens recognized by IgA autoantibodies in linear IgA disease correspond to the soluble extracellular domain of BP 180. The development of Elisa systems using recombinant or synthetic proteins will be a very sensitive tool for the detection of antibodies and new antigenic sites allowing a better classification and understanding of the pathogenesis of subepidermal bullous disorders in the future.

I-02

Bullous disorders: the gray zone

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Numerous cases of bullous disorders with atypical clinical and histological features, and immunological findings characteristic of pemphigus or pemphigoid defy clinical classification. Their relationship with classic form of pemphigus or pemphigoid is unknown. 33-year-old female with clinical features of pemphigus herpetiformis, non diagnostic histological findings, and the presence of anti-cell surface antibodies of both IgG and IgA classes reactive exclusively with desmoglein 1 is presented. This atypical bullous disease with immunological pattern differing from pemphigus herpetiformis by presence of IgA antibodies, and from IgA pemphigus by coexistent IgG antibodies, could be classified as a novel atypical subset of pemphigus tentatively named IgA/IgG pemphigus. 54-year-old female with tense blisters forming targetoid-like lesions and the presence of *in vivo* bound IgG and C3 linear deposits along the basement membrane zone (BMZ) and circulating anti-BMZ antibody directed against 200 kD protein is presented. In contrast to previously described cases with bullous eruptions and psoriasis in which anti-BMZ antibodies were directed against 200 kD protein in the present case there is no history of psoriasis. *In vivo* bound antibodies are found to be localized at the lamina lucida-lamina densa border thus this case could be classified as atypical pemphigoid with antibodies against 200 kD protein. Two patients with scarring conjunctivitis and widespread mucous membrane involvement developed tense blisters and extensive well-defined pustules and vegetating erosions symmetrically distributed in the inquinal and axillary folds. Skin biopsy specimens showed acanthosis, papillomatosis, subepidermal blister and infiltrate consisting of leukocytes and eosinophils. Direct immunofluorescence of the perilesional skin showed linear distribution of IgG within BMZ, ultrastructurally localized at the lamina lucida-lamina densa border. Vegetating skin lesions have been described in autoimmune bullous diseases, most often in pemphigus vegetans, rarely in bullous pemphigoid, whereas present cases could be classified as vegetating cicatricial pemphigoid. Despite of advances in diagnosing of autoimmune bullous diseases the classification of some cases is still a matter of controversy.

I-03

Lysosomal Storage Diseases

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There are more than 60 enzymes in lysosomes in human cells. Each enzyme hydrolyses the substrate, i.e. unnecessary cellular substances such as intermediates of cellular metabolites, or exogenous foreign materials such as bacteria. If any one of them has a deficit in its enzymatic activity, the substrate, usually an intermediate metabolite, will not be hydrolysed. It leads to the accumulation of the metabolite in lysosomes, resulting in cell death and subsequently organ failure (lysosomal storage disease, LSD). There are more than 30 diseases which are known to be caused by known enzyme deficiency at the present time. The rest of them, about 30, are still unknown. There are 7 LSD's which will clinically present as angiokeratoma corporis diffusum. The most well-known angiokeratoma is Anderson-Fabry disease, which was reported in 1898 in England and Germany simultaneously and was revealed to be caused by a deficiency of alpha-galactosidase. Six additional diseases were reported after the mid 1960's. These are: beta-galactosidosis, fucosidosis, aspartyl-glucosaminuria (AGU), galactosialidosis, beta-mannosidosis and Kanzaki disease. Kanzaki disease (MIM#104170) is the most recently reported disease, which was revealed to be caused by a deficit of alpha-N-acetylgalactosaminidase (EC 3.2.1.49). Electron microscopically, only Anderson-Fabry disease presents electron-dense deposits in lysosomes and 6 other diseases will essentially show electron-lucent lysosomal dilatations. These are caused by lysosomal deposit of sugar-ceramide compounds in Anderson-Fabry disease and sugar-amino acid compounds in 6 other diseases. Therefore the former can be differentiated from the latter electron microscopically. To differentiate the 6 other diseases, biochemical (enzymatic or urinary) analyses are necessary.

I-04

Recent advances in our knowledge of the dermo-epidermal junction

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The dermo-epidermal junction or epidermal basement membrane (EBM) comprises a multimolecular complex that plays a pivotal role in the attachment of basal keratinocytes to the underlying dermis. A variety of recently developed immunoelectron microscopical techniques have contributed to determining the molecular organization of these EBM components. We now, not only understand the ultrastructural localization of each molecule, but also the more precise orientation of each epitope within the entire protein molecule. In this lecture, I shall present updated information on the precise orientation of crucial EBM molecules, such as type XVIII and VII collagen, and laminin 5, and their relationship to a variety of skin disease.

I-05

Hair and hair disorders: histological aspects

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Humans are naked only at first glance. Human bodies are covered almost entirely by hardly visible hairs. The soft, fine, lightly pigmented hairs that cover most of the body of children and adults are termed "vellus". Similar vellus-like hairs in a fetus are termed "lanugo". The long, pigmented hairs with large diameter (hairs on the scalp, eye-brows, eyelashes, etc.) are termed "terminal". Quantitative differences in hair between sexes or among races exist only in respect to the number of terminal hairs: the total number of hair follicles is about equal in the sexes and the races. Hairs can be divided into four morphologic categories: straight because hair follicles are vertically oriented, from their bulbs in the dermis or subcutaneous fat, to their ostia at the skin surface. Spiral: because follicles are curved so that the bases of their bulbs are aligned somewhat horizontally to the skin surface. Helical and wavy: follicular orientation is in between. In vertically oriented sections, a mature hair follicle in anagen, no matter if terminal or vellus, may be divided into three segments: an upper segment (infundibulum) that extends from the ostium of the follicle to the entrance of the sebaceous gland. An intermediate segment (isthmus), that goes to the attachment site of a muscle of hair erection and a lower segment (bulb) that goes from the site of attachment of a muscle of hair erection at the bulge to the base of the follicle.

I-06

Modern Dermatopathology

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More than 25 years after the advent of immunohistochemistry molecular methods have finally entered the routine laboratory. From the plethora of modern methods, PCR, PCR-ELISA, sequencing, FISH, and the detection of microsatellite instability will be shown as exceptionally helpful diagnostic techniques when working with formalin-fixed, paraffin-embedded tissue. PCR-ELISA is a particularly sensitive method to diagnose bacterial infections (syphilis, mycobacterioses). Sequencing may be paramount to many specific questions: vasculopathies with point (Leiden) mutations of factor V gene, septic vasculitides with precise characterisation of the infectious organisms. Fusion-genes in conjunction with FISH and PCR are gaining more and more diagnostic importance, starting with the hematopoietic and soft tissue lesions. ALCL, follicular BCL, myxoid chondrosarcoma, and clear cell sarcoma will be shown as specific examples for the importance of this diagnostic approach. Non-coding DNA-sequences, in particular microsatellites, are of practical diagnostic value. The detection of high microsatellite instability (MSI-H) in conjunction with mismatch-repair protein expression is paramount to the diagnosis of sebaceous tumors: cystic sebaceous tumors and other seemingly banal sebaceous lesions of HNPCC patients underline that standard H&E criteria may have reached their limits: dermatopathologists will be required "to look for the invisible", and apply molecular methods.

I-07

Dermatopathology: from light to ultrastructural microscopy

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Many skin diseases are histologically characterized by distinct intra- & extracellular changes that only can be explained by electron and immuno-electron microscopy (EM, IEM). Thereby, new re-embedding procedures are helpful to study paraffin material as available for routine histology at ultrastructural level. Using various ultrastructural techniques, we examined specific and unspecific findings in disorders of epidermal differentiation (Hailey–Hailey-like pattern of acantholysis, epidermolytic hyperkeratosis, confetti-like ichthyosis, pagetoid dyskeratosis, dyskeratosis congenita, granular parakeratosis, chemotherapeutic reactions), disorders of pigmentation (Hypo-melanosis guttata, Dowling–Degos disease, melanosis cutis, drug-induced hyperpigmentation), and cutaneous drug deposits (aluminum and hydroxyethylstarch). In addition, EM is able to specify alterations of connective tissue fibers (Pseudo-xanthoma elasticum) and basement membranes (Kindler-syndrome, collagenous sphaerulosis). Finally, differentiation of neoplasms, in particular adnexal and soft tissue tumors, can be precisely defined at the ultrastructural level. In conclusion, electron microscopy is still a valuable tool to understand histopathologic findings and to investigate skin diseases far beyond immunohistological and molecular techniques.

I-08

Pandora's box of cutaneous lymphomas

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Pandora's box of lymphomas contains a confusing plethora of concepts, classifications and nosologic entities. This applies especially for cutaneous lymphomas, which present some unique clinical and biological features. There are some diseases that present only in the skin, and are never primary in lymph nodes or other extranodal sites. Mycosis fungoides is one such example. Some cutaneous lymphomas morphologically resemble their counterparts in lymph node, but differ in terms of phenotype, genotype, and clinical behavior, suggesting that they represent an independent entity. Cutaneous follicle center lymphoma demonstrates such fundamental differences from nodal follicular lymphoma. Finally, some cutaneous lymphomas exhibit a different clinical behavior from their nodal counterparts, despite apparent phenotypic and genotypic similarities. These differences may be related to stage or tumour burden, or more fundamental biological differences. For example, some localized diffuse large B-cell lymphomas have an indolent clinical course when presenting as a localized cutaneous tumour. Nevertheless despite these differences, dermatologists, hematooncologists, and pathologists must use a common language. In this spirit a WHO/EORTC consensus classification has been elaborated, which takes into account the unique features of many cutaneous lymphomas to emphasize their distinctive clinical and biological characteristics.

I-09

Clinical-pathological aspects of acne

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Acne is an extremely common condition, affecting almost 80% of adolescents and young adults aged 11 to 30. In recent years, research has led to a greater understanding of the pathogenesis of this widespread disease. The pilosebaceous unit is the target organ in acne, explaining the distribution of acne primarily on the face, chest, and back-areas with the greatest concentration of pilosebaceous glands. The most notable pathophysiologic factors that influence the development of acne are: sebaceous gland hyperplasia with seborrhoea, altered follicular growth & differentiation, Propioni-bacterium acnes colonization of the follicle, inflammation & immune reaction. The primary lesion of acne is the microcomedo, that can evolve into either a noninflammatory comedo or becomes inflamed and present as a papule, pustule, or nodule. The improved understanding of the pathophysiologic features of acne has brought about changes in acne management. The pathophysiologic features of acne suggest that combination therapy should be used as early as possible preferably at the initiation of therapy to simultaneously attack two or three pathogenic factors.

I-10

Advances in cutaneous lymphoproliferative disorders

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The most recent advances in cutaneous lymphoma (CL) concern molecular diagnosis (including its relevance to staging), new clinico-pathologic entities, and treatment modalities. The main topics of interest in the molecular diagnosis of CL are early lesions and early involvement of lymph nodes and peripheral blood. The finding of a clonal rearrangement of TCR genes may be very important in the early diagnosis of cutaneous T-cell lymphomas (CTCL). In this regard, however, it has to be stressed that a careful and balanced combination of clinico-pathologic features with molecular findings is to date the only crucial key to final diagnosis of CTCL. The biological and prognostic significance of early molecular involvement of lymph nodes and/or peripheral blood are still debated, although there is an increasing evidence for the prognostic value of the finding of an identical clone in skin and in blood and/or in lymph nodes. Among newly described CL entities, epidermotropic cytotoxic CTCL is a distinct type of aggressive CL, characterized clinically by ulceronecrotic, rapidly spreading plaques and nodules, with extracutaneous spread to unusual sites (lung, oral mucosa, CNS) and rapidly fatal outcome. Histologically, the intraepidermal infiltration by medium-to-large pleomorphic T-cells expressing TIA-1 (specif. cytotoxic marker) is the clue to diagnosis. CD4+/CD56+(NK/T) CL is mostly characterized by disseminated plaques and nodules, dermal infiltration by pleomorphic or blast-like cells typically coexpressing CD4, CD56, & CD68 antigens, and bad prognosis. Regarding treatment, the most interesting advances concern the treatment of CTCL: new retinoids (oral bexarotene), nucleoside analogues (gemcitabine, pegylated doxorubicine), and targeted immunotoxins (DAB-IL2; anti-CD52 – alemtuzumab) can be usefully combined with other modalities. Among skin-directed procedures, monochromatic excimer light (308 nm) and imiquimod deserve particular attention in early CTCL. Rituximab (anti-CD20 immunotoxin) can be used systemically (pluri-relapsed or aggressive CBCL), or intralesionally (local recurrences in areas previously treated with radiotherapy).

I-11

Cutaneous lyme borreliosis: which morphological alterations can be detected?

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The aim of this study was to determine the general light and electron microscopic findings which lend support to the histopathologic diagnosis of the main cutaneous manifestations of Lyme borreliosis. The diagnostic criteria are delineated and illustrated. In culminating lesions of erythema migrans and acrodermatitis chronica atrophicans, a peculiar connective tissue reaction includes an increase in the number of fibroblasts, proliferation of collagen fibres and interstitial mucinous oedema. The cellular infiltrates are patchy and perivascular in erythema migrans and either patchy and/or band-like and interstitial in acrodermatitis chronica atrophicans. They consist of lymphohistiocytic cells with a variable admixture of plasma cells. The damage to elastic (and even collagen) fibres occurs in early acrodermatitis chronica atrophicans and is reflected by the phenomenon of elastophagocytosis with a fragmentation of elastic and oxytalan fibres. Reduction of the number or lack of pilosebaceous units is a constant finding. In advanced lesions of acrodermatitis chronica atrophicans a thinning of the dermal breadth is noticed, resulting from a decrease of collagen and elastic fibres. Fibrous nodules and morphea-like conditions are characterized by excessive formation of collagen. Borrelial lymphocytoma exhibits two different patterns of infiltration, accompanied by dermal fibrosis and increased numbers of fibroblasts. Recent tick bites show a predominantly neutrophilic infiltrate, with an admixture of eosinophilic granulocytes. By applying the results of this synoptic study, histopathologic diagnosis including the histological and ultrastructural findings of cutaneous borrelia infections should be possible without the absolute necessity of clinical correlation.

ORAL PRESENTATIONS

O 1

The ultrastructural localization of desmosomal components in Desmoglein3 knockout mouse

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Desmoglein3 (Dsg3) is known as a target antigen of pemphigus vulgaris (PV), a severe autoimmune blistering skin disease. Dsg3−/− mouse has been reported to present PV-like phenotype including oral erosion with suprabasal acantholysis indicating that loss of Dsg3 itself or its function is the main pathophysiological mechanism of the acantholysis in PV. However, keratin retraction from desmosomal attachment plaque after autoantibody binding to Dsg3 may be another explanation for the acantholysis. Although the molecular composition of desmosome is clarified, the intermolecular interaction of the desmosomal components is not fully elucidated. The purpose of this study is to clarify the molecular composition of Dsg3−/− mouse desmosomes to characterize the acanotholysis in this mouse. For this, we analysed the ultrastructural localization of well established members of desmosomal proteins in Dsg3−/− mouse epithelium using post-embedding immuno-EM and compared statistically with that in normal mouse. Samples were obtained from oral mucosa of Dsg3−/− mouse and normal control mouse, cryofixed with liquid propane at −190°, freeze substituted with methanol, and embedded in Lowicryl K11M. On-section immuno-staining was performed using six antibodies against Dsg1, desmocollin 1, desmocollin 3, plakoglobin, plakophilin 1, and desmoplakin (DP). The distances between the gold labelling of each molecule and the plasma membrane were measured and statistically analysed. As results, DP in Dsg3−/− mouse was localized 11 nm further from the plasma membrane than that in normal mouse. On the other hand, the localization of the other components were identical between Dsg3−/− and control mouse. Our results suggest a molecular interaction between Dsg3 and DP in desmosomes and may give insights to the mechanism of PV blister formation.

O 2

Ultrastructural assessment of the splice variant-specific function of Dsc1 in the epidermis

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Desmoglein 1 (Dsg1) and desmocollin 1 (Dsc1) are expressed in the suprabasal layers of epidermis, whereas Dsg3 and Dsc3, isoforms of the desmosomal cadherins are more strongly expressed basally. This differential expression pattern may have a function in epidermal morphogenesis and/or may regulate the proliferation and differentiation of the tissue. The Dsc1 gene encodes two proteins (Dsc1a and 1b) that differ with respect to their C-terminal cytoplasmic amino acid sequences. Previously, a study demonstrated the C-terminal domain of Dsc1a, not 1b, could recruit plakoglobin (PG) and desmoplakin (DP) to the plasma membrane of epithelial cells and serve as a nucleation site for the assembly of an electron-dense plaque with attached intermediate filaments. Therefore, one would expect that absence of the C-terminal domain of Dsc1a would affect desmosome (DM) assembly. We have recently generated a Dsc1 mutant mouse, which expressed novel 13 amino acid sequences instead of the Dsc1a- and 1b-specific C-terminal cytoplasmic domains. The purpose of this study is to assess the function of the C-terminal domain of Dsc1 in DM assembly and epidermal morphogenesis using this mutant mouse by electron microscopy (EM) and immuno-EM. Light microscopy revealed no remarkable phenotype in the mutant mice. EM revealed a mild epidermal spongiosis and hypergranulosis in the mucous membrane but no gross abnormalities in the structure of DMs. The ultrastructural localization of Dsc1 was specifically detected along the desmosomal plasma membrane and PG or DP was colocalized in the DMs at outer or inner plaques of DMs, respectively. These findings corresponded with the localization in control mouse. In conclusion, the C-terminal domain of Dsc1 is considered to be not essential for the DM assembly. In other words, the extracellular domain and transmembrane domain of the Dsc1 receptor is necessary to maintain the structural integrity of the skin.

O 3

Heterogeneous distribution of gli-1 expression in basal carcinoma cells

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GLI-1 overexpression has been observed in basal cell carcinoma (BCC) and has been considered a key event in its pathogenesis, leading to increased cell proliferation and expression of the anti-apoptotic molecule Bcl-2 as well as to a reduced expression of the adhesion molecule laminin. The aim of the study was to evaluate the expression of the above mentioned molecules in a series of BCCs and in a BCC cell line, TE 354.T. Immunohistochemical expression of GLI-1, Bcl-2, and laminin was evaluated in 15 sporadic BCCs of various subtypes and different clinical behavior (3 nodular, 3 superficial, 3 infiltrating, 3 metatypical, 2 recurrent and 1 metastatic), and, for comparison, in 20 benign hair follicle-related tumors and in 5 cases of basaloid hyperplasia overlying dermatofibromas. Suspended and adherent TE 354.T basal carcinoma cells were fixed with periodate-lysine-paraformaldehyde for immunohistochemistry and with Karnovsky's for electron microscopy. BCCs consistently showed GLI-1 immunoreactivity, the staining being quite heterogeneous in the neoplastic aggregates, with a stronger intensity at the periphery. BCCs also expressed Bcl-2, with a distribution similar to GLI-1. Laminin staining was observed at the interface between tumor nodules and adjacent stroma. Some GLI-1 immunoreactivity was observed in cases of basaloid hyperplasia and benign follicular tumors whereas the normal epidermis was negative. Cultured cells were flat and the cell surface adhering to the substrate was coated by a basal lamina. Different intensity of staining among cultured cells was found for GLI-1, Bcl-2, and laminin. The results support a direct correlation between the expression of GLI-1 and that of Bcl-2. However, GLI-1 expression, although rarely, also in hyperplastic basaloid proliferations and in benign follicular tumors suggests that in the pathogenesis of BCC, besides the deregulation of the patched-hedgehog signaling pathway, other mechanisms are implied.

O 4

Tacrolimus reduces the expression of GLI-1 by basal cell carcinoma

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The protein product of GLI-1 oncogene mediates the physiological effects of the intercellular signaling molecule Sonic hedgehog (SHH) and controls the cell proliferation and differentiation of subpopulations of epidermal cells during development and in mature organisms. Overexpression of GLI-1 is characteristic of basal cell carcinoma (BCC) and is considered pathogenetic for this disease. Tacrolimus (FK506) has extensive homology and uses the same receptor as rapamycin, which can antagonize the cellular transformation caused by GLI-1 overexpression. We have therefore addressed whether tacrolimus affects the expression of GLI-1 in a basal cell carcinoma cell line, TE 354.T. Total RNA was extracted with Trizol and the expression of GLI-1 was evaluated by quantitative RT-PCR with TaqMan polymerase. Cells lysates were analyzed by gel electrophoresis and Western blotting. Suspended and adherent cells were analyzed by immunohistochemistry and electron microscopy. The cell line TE 354.T had an approximate doubling time of 3.5 days; cells were flat and rich in organelles of the secretory pathway and intermediate filaments; the cell surface adhering to the substrate was coated by a basal lamina. Intermediate filaments contained vimentin. GLI-1, Bcl-2 and laminin were expressed with variable intensity among cells. The expression of GLI-1 was confirmed at the mRNA and protein levels. Three day treatment with Tacrolimus (0.5–50 ng/mL) led to a dose-related drop in the expression of GLI-1 mRNA (significant for 50 ng/mL) and, to a lesser extent, in the number of GLI-1 immunoreactive cells. The cell proliferation rate and fine structure were not affected by treatment. Tacrolimus affects the expression of GLI-1 in a basal carcinoma cell line; this result opens the pathway to investigate whether such effect correlates with altered expression of other genes related to neoplastic behavior.

O 5

Merkel cell carcinoma with atypical clinical presentation associated with chronic lymphocytic leukaemia

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A 71-year-old white woman presented with an 8 month history of a slowly growing painless nodular subcutaneous mass of the left arm. Her past medical history was unremarkable except for a recent onset thrombocytosis. At the beginning she tried to manually extrude the "content" of the growing "cyst". On examination teleangectatic skin overlying a subcutaneous roundish mass measuring 4 cm in diameter, poorly mobile and of elastic and lipomatous consistency was observed. The skin lesion was surgically removed and the histopathologic examination showed a diffuse infiltrating subcutaneous nodule characterized by a trabecular pattern composed of round to oval monomorphous cells with scant cytoplasm. The nuclei were large and vescicular, with multiple nucleoli. Numerous mitotic and atypical elements were present. Immunohistochemistry revealed diffuse positivity with chromogranin A, synaptophysin and anti-PGP 9.5. A sentinel lymph node biopsy was followed by a left axillary lymph node dissection and postoperative radiation therapy of the scar site in the left arm. After 11 months an osteomedullary biopsy showed a picture consistent with chronic lymphocytic leukaemia. The interest of the present case lies in the atypical clinical presenation of a Merkel cell carcinoma (MCC) and in the simultaneous association with a second neoplasia. Patients with chronic lymphocytic leukaemia (CCL) have a threefold risk of developing a second tumor, and this risk increases to eightfold when considering skin tumors only. In addition a high incidence of second neoplasms in MCC is also reported. Just a small number of cases of MCC arising in patients with CLL could be reviewed and the fact that this is more than coincidental remains to be established.

O 6

Dental alterations in junctional epidermolysis bullosa: report of a patient with a mutation in the LAMB3 gene

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In junctional epidermolysis bullosa teeth are often severely affected by abnormal dental development and/or dysplastic enamel (including pitting, furrowing and hypoplasia). Enamel hypoplasia is seen in 100% of individuals with junctional EB but is variably expressed. In addition this disease is associated with an increased risk of dental caries, caused by developmentally compromised enamel and external factors such as difficulties in maintaining oral hygiene or the patients' generally softer and more refined diet. We report on a patient suffering from junctional epidermolysis bullosa that was complicated by severe teeth decay, especially enamel defects. Immuno-fluorescence microscopy showed profound reduction of the linker protein laminin 5, which is located in the hemidesmosome. Transcriptomic analysis revealed homozygosity for the mutation 3009C → T in the exon 20 of the LAMB3 gene, which is coding for the beta3 chain of the laminin-5 protein. Electron microscopy showed blister formation between the keratinocyte plasma membrane and the underlying lamina densa, some degenerating basal keratinocytes, rudimentary hemidesmosomes and a discontinuity in the formation of electron dense plaques resulting in a complete absence of hemidesmosomes in some regions of the basal cells, which confirms the importance of laminin 5 for the formation and stability of hemidesmosomes. During early odontogenesis, the basement membrane is thought to be important in epitheliomesenchymal interactions and the subsequent interdigitation between inner enamel epithelium and the underlying dentine resulting in the highly complex amelodentinal junction. Mutations in the gene of one of the principal anchoring proteins, such as laminin 5, might therefore be expected either to seriously compromise ameloblast differentiation and/or interfere with normal basement membrane formation and the fixation of the ameloblasts to their underlying matrix.

O 7

Control Of Basement Membrane Formation In Skin-Organotypic 3d-Coculture

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Basement membrane (BM) formation was functionally dissected in 3d-cocultures of human keratinocytes (HK) and fibroblasts (human/mouse, HF/MFf) by either blocking interactions or implementing molecular deficiencies. This was supposed to complement knockout mouse studies, where loss or functional defects of collagen-IV, laminins, nidogen, or perlecan are causing embryonic or neonatal death. HK or HaCaT cells were grown on collagen gels harboring hf or mf from normal or ko-mice. To block nidogen-binding to laminin-10 the corresponding laminin-fragment (gamma1-iii3-5, L-gamma-f) was applied. BM-formation was surveyed by immunofluorescence (IF), regular (EM) and immuno-electron microscopy (IEM). In 3d-cocultures of HK and HF L-gamma-f blocked deposition of nidogen, laminin-10, and perlecan, while collagen-IV appeared normal. Although the hemidesmosome components laminin-5, BP180, and integrin alpha6beta4 were only mildly affected, EM and IEM revealed complete absence of BM, hemidesmosomes, and basal insertion of keratin filaments. To eliminate nidogen, made by fibroblasts, MF from nidogen1/nidogen2 ko-mice or crossbreds were employed. In 3d-cocultures with HaCaT cells nidogen1/2 (−−/++)-MF abolished nidogen1-staining, but (−−/+−)-mf reduced also largely nidogen2, collagen-IV, and drastically laminin-10. Total absence of nidogen (−−/−−) also deleted collagen-IV & laminin-5, integrins e.g. alpha6beta4 appearing still normal (IF). BM-formation could be entirely rescued by applying recombinant nidogens. In skin, perlecan can be apparently synthesized by both keratinocytes & fibroblasts. Accordingly, deficiency in either cell type did not affect BM-formation, demonstrated by combining either perlecan (−/−)-mf or HaCaT anti-sense-perlecan cells with respective normal partner cells. Thus, in this skin model BM-components are efficiently transported to their actual assembly site.

O 8

Two cases of CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) diagnosed by skin biopsy electron microscopy

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited cerebrovascular disease characterized by recurrent subcortical ischemic strokes starting in the third or forth decade as a result of mutations in the *Notch3* gene. Granular osmiophilic material (GOM) deposition around the vascular smooth muscle cells is a specific feature and electron microscopic observations of skin biopsies are useful for this diagnosis. A 39-year-old female with dizziness, abnormal visual field and hemiplegia, and a 42-year-old male with tinnitus and dizziness, were suspected of suffering from CADASIL based on MRI findings. Both cases were shown to have characteristic deposits of GOM, 200–800 nm in diameter, around the vascular smooth muscle cells of small arteries in the deep dermis, and thus the diagnoses of CADASIL were confirmed, although there was no family history of cerebrovascular disorders or dementia. Dermatologists should be aware of these ultrastructural findings because this disease may occur sporadically and might be more common than initially thought.

O 9

Exocytosis of haemostatic active von Willebrand factor multimers on human endothelial cells measured by using atomic force microscopy

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Von Willebrand factor (vWF) is the major player of the first response of primary haemostasis and inflammation. Immobilized to the subendothelial cell matrix vWF is exposed after endothelial cell (EC) damage to initiate platelet clotting. Our data indicate that vWF is also released at the luminal surface of EC mediating platelet adhesion. By using atomic force microscopy (AFM) we visualized the luminal surface topography of EC. Submembrane Weibel-Palade bodies (WPB; storage vesicles of vWF) can be imaged as humps characterized by decreased cell membrane stiffness. After cell stimulation (hyperosmolaric solutions or histamine) these docked WPB fuse with the plasma membrane forming large exocytotic pores (d $\sim$300 nm). Release of vWF from these pores was visualized by using immunofluorescence after staining the same cell surface with vWF-specific antibodies. Surprisingly, high molecular weight multimers of vWF were found to form elongated fibre-like structures ($>$200 μm in length) on the EC cell surface. After adding platelet-poor plasma during a 3 minute stimulation time cell surface attached platelets were found to be almost exclusively sticking to vWF fibres. Moreover, we confirmed that luminal release and surface attachment of high-molecular weight vWF fibres on EC at the intact human vessel wall. In contrast to the exocytotic pores we imaged endocytotic pores with sharp edges and a diameter of $\sim$200 nm.The study shows that AFM enables to measure and visualize cell surface topography with a nanometer resolution. The data indicate a new concept for a physiological function of vWF to effectively recruite platelets and leucocytes to the intact but stimulated vessel wall surface.

O 10

Dermatologic changes caused by arteriosclerosis in peripheral gangrene

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Peripheral gangrene was observed in 17 patients, most of them suffered in feet and one was in hand. The lesions and deriving processes were recorded carefully and specimen were examined by histological sectioning after amputation. The main gross lesions included discoloration, pale, scaly and exfoliation, congestion, cyanosis, hyper-pigmentation, putrefaction and gangrene. Microscopic lesions included coagulative necrosis with very severe hyperkeratosis in epidermal cells, purulent dermatitis with bacterial clumps was found in ulcerated area. Gangrene formation was found in very severe cases. All cases showed severe arteriosclerosis. The artery in dermis showed intima thickening by some amorphous substance infiltration, which was pinkish in H&E stain. The smooth muscles in media were hypertrophied. The vessel lumen was narrow, even obliterated. Old thrombus, organization or re-canalization was reveled in some arteries, some of them showed onion-like appearance in transverse section. The internal elastic membrane was broken or became birefringed. The entire picture of artery showed the thromboangiitis obliterans or arteriosclerosis obliterans.

O 11

Distribution of cannabinoid receptor 1 (CB1) and 2 (CB2) on sensory and autonomic nerve fibers and appendage structures in human skin

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Cannabinoid receptors mediate psychopharmacological, analgetic and immune functions of exogenous and endogenous cannabinoids. They are known to be localised in the central and peripheral nervous system as well as in immune tissues. Up to now, two cannabinoid receptors (CB) were cloned. Recent studies gave evidence for the presence of CB in the skin. To determine the precise localization of CB1 and CB2 in nerve fibers, epithelial cells of cutaneous appendage structures, epidermal keratinocytes and mast cells, we performed an immunohistochemical study in a series of normal human skin and mastocytosis. CB1 and CB2 immunoreactivity could be observed in cutaneous sensory and autonomic nerve fibers, mast cells, epidermal keratinocytes, epithelial cells of hair follicles, sebocytes, and eccrine sweat glands. Interestingly, in epidermal keratinocytes, hair follicle and sebaceous glands, CB1 and CB2 were distributed in a complementary fashion. Our study confirmed previous reports describing that cutaneous application of the selective CB1 and CB2 agonist HU 210 significantly reduced capsaicin-induced burning pain as well as histamine-evoked itch. Together, these findings suggest that CB act as inhibitory neuronal receptors diminishing excitation of skin nerves and release of neuropeptides. This has an interesting implication on the therapeutical use of cannabinoid agonists as antipruritic and analgetic agents. In conclusion, both cannabinoid receptors are widely distributed in the skin suggesting a central role for this receptor, e.g. in epithelial cell differentiation as well as in inhibition of nociceptive sensations.

O 12

Pseudomembranous angiomatoid papillomatosis of mucous membrane (PAPM) in bone marrow transplanted patients

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Cutaneous manifestation due to *Bartonella spp.* are regarded as severe, potentially life threatening disease especially in immunocompromised patients. AIDS patients, in particular, may present with the clinical pattern known as bacillary angiomatosis. We describe 3 patients, 2 children and an adult, who developed vegetating papillomatous lesions of oral mucosae, after bone marrow transplantation. They shared an identical history of haematologic disease, and all the three patients had received allogenic bone marrow transplantation. Later, they developed Graft versus Host Disease (GvHD), involving also oral mucosa. They were in relatively good general condition, and under Cyclosporine A treatment, associated with prednison (adult patient). All the patients, presented vegetating, pedunculated, exophytic lesions covered by yellowish membrane of fibrin on oral mucosa, associated with different severity of GvHD expression. Biopsies were taken in all three patients. Histology was characterized in all the cases by a granulation-like tissue with a mixed-cell infiltrate mostly composed by neutrophils with diffuse vascular proliferation. No vasculitis was present, although diffuse leukocytoclasis was noticeable. Despite their non-specific looking pattern, likely to be regarded as "granulation tissue secondary to focal ulceration of oral mucosa during oral GvHD", their histopathology similitude with the histopathology of Bacillary angiomatosis, prompted us to search for organisms. In all the cases, DNA of *Bartonella henselae* was detected by PCR and confirmed by the study of PCR product with sequencing reaction. Gram negative bacteria were also found in the deep portion of the dermis by electron microscopy. In all the cases, the lesions responded to systemic antibiotic therapy, recurred after weeks or months, and responded again to further systemic antibiotic treatment. In conclusion, we define these cases as Pseudomembranous Angiomatoid Papillomatosis of Mucous membranes (PAPM), a mucosal pattern caused by *Bartonella henselae* infection. It may affect haematologic and immuno-compromised patients with a previous history of allogenic bone marrow transplantation and mucosal GvHD, under immunosuppressive treatment.

O 13

Detection of high-risk human papillomavirus infection in penile lichen sclerosus

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Aim of the study was to investigate the prevalence of human papillomavirus (HPV) infections in patients with genital lichen sclerosus (LS), in order to highlight their possible role in enhancing the risk of penile cancer arising on LS. 46 adult patients (mean age 59.5 years; range 27–79 years) with histologically confirmed penile LS, randomly selected from our hospital pathology files, and an equal number of randomly selected control males, matched for age and with no history of LS, phimosis, chronic balanitis, anogenital warts or other HPV-related disorders, attending our referral center for non-genital complaints, were enrolled. HPV infection was assessed in paraffin embedded penile biopsies of genital LS patients and in brush cytology smears of penile healthy mucosa by a highly sensitive two-step nested polymerase reaction (PCR) technique based on general GP5+/GP6+ PCR primers and consensus primers MY11/MY09 followed by cycle sequencing. PCR disclosed the presence of HPV DNA in 17.4% of LS patients (HPV 16: 6 cases; HPV 18: 1 case; HPV 45: 1 case). There seemed to be no relationship between histopathologic features and presence of HPV infection. Among controls, incident HPV infection occurred in 8.7% of patients (HPV 16: 2 cases; HPV 53: 1 case; HPV 70: 1 case). Our results support the hypothesis of a causative link between genital HPV infection and a high risk of penile cancer development in men with genital LS. Further investigations to assess the significance of our findings are warranted.

O 14

Cellular reactions of malignant, semimalignant and premalignant conditions of the skin under immunostimulating therapy with imiquimod

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In this pilot-study we enrolled 12 patients including 4 squamous cell carcinomas, 6 basal cell carcinomas and 2 actinic keratoses. After histological confirmation of the diagnosis, treatment was performed 3 times weekly, applying imiquimod 5% cream (Aldara®) to the tumour site. 2 weeks after the last dosis of imiquimod 3 mm punch biopsies were obtained. Interest was payed as well to the histological regression of the tumour as to the quantitative and qualitative change in infiltrating cells under the therapy with imiquimod. In addition to a hematoxylin and eosin staining the paraffin sections were stained using a standard avidin-biotin-peroxidase technique for CD 3 (pan t-lymphocytes), CD 20 (b-lymphocytes), CD 56 (natural killer cells) and CD 68 (monocytes/macrophages). 10 out of the 12 enrolled cases experienced complete resolution of the tumour (83,3%). The number of infiltrating cells showed a great inter-individual variety for all marked cell-types before and after the end of therapy. In the collective of responders to therapy T-cells decreased over 68%, B-cells decreased over 37,5%, natural killer cells over 77,6% and monocytes/macrophages decreased over 41,3%. In contrast the two non-responders showed a mean increase in T-cells of 145%, in B-cells an increase of 190,4%, in natural killer cells an increase of even 974% and monocytes/macrophages an increase of 110,7%. Respecting the mechanism of action of imiquimod we should have expected an increase in inflammation cells in successfully treated tumours. The clinical control of the enrolled cases leads to the assumption that the responders to therapy had experienced an increase of inflammating cells to one point of treatment that had undergone our histological control. The individual different development of infiltrating cells might indicate an interindividual difference in the temporal course of induced cellular reactions. A high number of infiltrating lymphocytes before treatment might have a positive predictive value for a fast development of a sufficient immunreaction. Imiquimod proved to be an effective alternative to surgical treatment.

O 15

Lymphocytes subtypes expression and prognosis of squamous cell carcinoma of the lower lip

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Squamous cell carcinoma (SCC) of the lip is a relatively common malignancy of the head and neck region. Tumor thickness, grading and perineural invasion are significant prognostic indicators. However, there is still the need of new reliable biological markers able to predict the prognosis of the single cases with an unfavourable biological behaviour unpredictable by classic clinical pathological parameters. 32 cases of SCC of the lower lip were analysed for their clinicopathologic features, and immunohistochemical expression of Fas/Fas L in neoplastic cells and in inflammatory infiltrate. Moreover the density and phenotype of tumor-infiltrating lymphocytes(TIL) was analysed. The results were related with follow-up of the patients ranging from 2 to 5 years. The cases with overexpression of Fas/L in neoplastic cells and Fas+ in T cells preferentially showed a more aggressive clinical behaviour ($p < 0.01$). Moreover we found an alteration of the normal expression of CD4 and CD8 lymphocyte types in four cases. These data suggest that the Fas/FasL pathway is involved in the close relation between neoplastic cells and T cells and so in the biological behaviour of these tumors.

O 16

The role of immunohistochemistry in the grading of actinic keratosis

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It is known that actinic keratosis is a cutaneous lesion induced by UV sunlight exposure. It is considered a malignant lesion in the intraepidermal location, before its transformation into invasive carcinoma of the skin. Four intraepidermal stages are described and a tumoral progression phase too, according to the distribution of keratinocytic atypia. Also different histological varieties are described, like hypertrophic, acantolytic, bowenoid and atrophic. The immunohistochemistry plays a relevant role allowing the identification on paraffin blocks of different oncogenes and related products and of different extracellular matrix proteins, and also permits a count of some proliferation indexes. The substances more frequently studied are p53, PCNA, p16, bcl-2, ki-67 (MIB-1), MMP 2 and 3, and tenascin. PCNA, MIB-1 and p16 positivity, weak or strong, is always related to dysplasia. p53 is overexpressed in 80% of the cases; the percentage of positive cells is variable and seems to be related to the histological types. Regarding the extracellular matrix proteins, tenascin is present as a band under the dermo-epidermal junction and its thickness is related to the amount of dysplasia. MMP-2 is always positive in the downwards budding of the atypical cells, while MMP-3 has a variable response. These results are helpful in the evaluation of the grading of the lesion; in fact the positivity outlines the thickness of dysplasia in the epidermis, or the amount of the dysplasia as in the case of tenascin. Moreover the increased expression of some of these markers during the progression from actinic keratosis to invasive carcinoma confirm that these are the same lesions in different stages of evolution. Some results of MMPs appear interesting and, if confirmed by further studies, their use could represent an important tool for the identification of those forms of actinic keratosis potentially invasive.

O 17

Superficial acral fibromyxoma: 5 case reports

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Superficial acral fibromyxoma is a rare soft tissue tumor that has recently been described as a separate entity with a predilection for the hands and feet. We report 5 cases of superficial acral fibromyxoma at subungual location, 4 male and 1 female subjects ranging in age from 28 to 76. Three patients exhibited a nodular lesion of the great toe; two patients a nodule of a finger. X-ray examination of the involved phalanx showed a swelling of subungual tissue without infiltration of the bone. The gross pathology displayed a partially pseudocapsulated, round, pale and gelatinous mass. Histopathologic examination revealed a homogeneus proliferation of scattered spindle-shaped and stellate cells surrounded by a loose myxoid matrix. There was no evidence of nuclear atypia, mitotic activity and tumor necrosis. The tumors showed immunoreactivity for CD34, epihelial membrane antigen and CD99. No immunoreactivity was detected for actins, desmin, keratins, or HMB-45 and S100 protein. The histopathologic and immunohistochemical findings of these tumors are distinctive. All tumors have been fully removed with a frontal excision: the nail bed was excised entirely exposing the bone and regeneration properties of the nail bed were utilized. No recurrence in all 5 cases was observed. The neoplasm is seen in adulthood as solitary slow-growing mesenchymal mass involving the periungueal and subungueal areas of the fingers and toes. The tumor might recur if not completely removed. Malignant transformation was not observed.

O 18

Giant dermatofibroma: a case report and the review of the literature

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Dermatofibroma is a benign cutaneous tumor that generally ranges in size from 1 to 3 cm in diameter. However, these tumors sometimes become bigger and may be misdiagnosed as a malignant tumor. In this report, we demonstrate a case of giant dermatofibroma (GDF) and review similar cases in the literature. A 20-year-old man presented with a 3-year history of a slowly growing nodule on his lower right leg. Physical examination revealed a brownish, firm nodule, which was 5 cm in diameter and immobile due to subcutaneous tissue adhesion. MRI imaging suggested an invasion close to the fascia of the anterior tibial muscle. An initial incisional biopsy showed a dense irregularly arranged fibroblast proliferation throughout the dermis and superficial subcutaneous tissue, suggesting dermatofibrosarcoma protuberans. For both diagnosis and therapeutic purposes, we performed a simple surgical excision. Histopathological examination revealed a relatively sparse proliferation of fibroblasts without nuclear atypia that produced mature collagen bundles throughout the entire tumor. Immunohistological examination showed a lack of CD34 expression in the proliferative fibroblasts, leading to the diagnosis of GDF. GDF is an unusual dermatofibroma variant, usually exceeding 5 cm in diameter (Requena *et al*, JAAD 30: 714–719, 1994) that preferentially develops on the lower legs. Due to its clinical and histopathological characteristics, its distinction from malignant tumors is problematic. In our case, using the initial small biopsy specimen we failed to reach the correct diagnosis, and an excisional biopsy was required. According to the literature, GDF invariably has a benign clinical course and a simple excision is curative. However, an excisional biopsy of the whole tumor is recommended because of the diagnostic difficulties due to its clinical and histopathological appearance.

O 19

Macular purpura as a presenting sign of primary systemic amyloidosis

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The authors describe a case of a 79-year-old man admitted to our Department for a three months lasting asymptomatic ecchymotic eruption involving the face, neck and shoulders. Laboratory investigations revealed: IgG = 320 mg/dl (751–1550); hypoproteinemia with albumine level = 5,50 g/dl (6,00–8,00); urinalysis showed proteinuria and urine immunoelectrophoresis revealed a monoclonal light chain component (k/l = 1:3). Echocardiographic examination showed thickness of both left and right ventricular walls and pericardial effusion (consistent with thesaurismotic deposition). Chest X-ray revealed a right basilar nodular opacity. Abdominal CT scan showed hepatomegaly with multiple nodular lesions. An infiltrate of about 30% of atypical plasma cells was detected in the bone marrow aspirate. A cutaneous biopsy of an ecchymotic skin lesion revealed large depositions of amorphous material around the dermal dilated vessels associated with diffuse dermal red blood cell extravasation. Congo red staining showed a positive orange-brown reaction in the upper dermis, confirming the presence of perivascular amyloid deposition. A diagnosis of primary systemic amyloidosis associated with myeloma was done. The patient died two months later for heart failure. The term amyloidosis is referred to several diseases sharing an abnormal deposition of a fibrillar protein in several tissues. Clinically it can be classified into systemic and organ limited. The systemic amyloidosis can be further classified as primary idiopathic, primary multiple myeloma associated, secondary systemic and familial. Skin involvement is common in the primary systemic amyloidosis, but it rarely occurs in the secondary. Patients affected by primary amyloidosis associated with myeloma have a poor prognosis with a mean survival time of 13 months, which is lowered to 6 months when heart involvement occurs.

O 20

Fucosidosis with angiokeratoma. Light and electron microscopic study of a new case

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Fucosidosis (MIM# 230000) is a rare autosomal recessive lysosomal storage disease due to deficiency of the enzyme fucosidase, resulting in tissue accumulation of fucosylated glycoconjugates. It manifests with progressive mental and motor deterioration, coarse facies, growth retardation, recurrent infections, dysostosis multiplex, visceromegaly and seizures. Cutaneous findings include progressive, diffuse telangiectasias and angiokeratomas (52% of patients), and hypo- or hyperhidrosis. We present a new patient with fucosidosis, whose skin was studied by light and electron microscopy (EM). A 9-year-old Turkish girl born to consanguineous parents presented severe mental and growth retardation, recurrent respiratory infections, pectus excavatum and diffuse angiokeratomas predominating over the abdomen and the proximal part of the thighs. The diagnosis of fucosidosis was confirmed by biochemical testing of acid hydrolases within leucocytes (complete absence of alpha-L-fucosidase activity, normal activity of beta-galactosidase, hexosaminidase, arylsulfatase A and beta-mannosidase). Light microscopic study of an abdominal skin lesion showed an aspect of angiokeratoma. Immunohistochemically, endothelial cells showed strong expression of alpha-L-fucose residues but decreased expression of CD34. EM-examination showed the presence of cytoplasmic vacuoles within several cell types, mainly blood and lymphatic endothelial cells and eccrine secretory cells; however, vacuolisation was also seen in epidermal keratinocytes, melanocytes and Langerhans cells, pericytes, fibroblasts, Schwann cells and erythrocytes. The cytoplasmic vacuoles were round, limited by a single membrane and measured on average 0.5–2 micrometers; they were electron-lucent but contained frequently sparse granular, membranous, vesicular, flocculent or (rarely) myelinoid material. EM of clinically uninvolved skin of the forearm also showed vacuolisation of several dermal cell types as those seen in involved skin, but not in the epidermis. Despite the fact that our patient did not suffer from the most severe form of fucosidosis (type I), EM examination showed extensive and severe cell vacuolisation, affecting also epidermal keratinocytes, a finding reported only exceptionally.

O 21

Involvement of granulysin-producing T-cells in the development of superficial microbial folliculitis

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Granulysin is a recently identified antimicrobial protein expressed by cytotoxic T cells, NK cells and NKT cells. It is shown that granulysin contributes to defense mechanism against mycobacterial infection. Superficial microbial folliculitis is a common skin disease. In a previous report we could show, that as a first line of defense, alpha-defensin (human neutrophil peptides) and beta-defensin (human beta-defensin-2) were expressed in infiltrating neutrophils and lesional epidermal keratinocytes respectively in superficial folliculitis. As we also observed many infiltrating lymphocytes in lesional dermis, we hypothesized that infiltrating lymphocytes may possess antimicrobial substances such as granulysin and play a role in defense mechanism as a second line of defense. Seven specimens of superficial microbial folliculitis diagnosed clinically and histologically were examined by means of immunohistochemistry. To identify the phenotype of cells expressing granulysin, laser confocal microscopic examination was performed. Dense lymphoid cell infiltration was observed in pustules and perivascular regions. A large number of these lymphoid cells were positive for granulysin. The phenotype of cells consisted of CD3+T cells, CD8+T cells and UCHL-1+ T cells. CD20+ cells and CD56+cells were not observed. Laser confocal laser microscopic examination showed that the lymphocytes producing granulysin were CD3+, CD4+ T cells but not CD8+ T cells. We showed that many granulysin-bearing T cells infiltrated into affected follicles and perilesional dermis in superficial microbial folliculitis. However, few granulysin positve lymphoid cells were observed in sterile pustular lesions. Our observation indicated that adaptive immunity such as granulysin, lymphocyte-produced antimicrobial protein may play an important role in cutaneous defense mechanism.

O 22

Modelling a complex structure like the sweat gland requires the combination of light and electron microscopic investigations

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For the design of new effective cosmetic antiperspirants knowledge about the structure of human axillary sweat glands is an essential prerequisite. A complete morphological description of these skin appendages requires the visualization of their overall structure by light microscopy (LM) as well as the investigation of details by electron microscopy (EM). To get an overview of the complete biopsy and to preselect the specimens, the aldehyde-mediated autofluorescence of a Karnovsky-fixed biopsy is recorded by a confocal laser scanning microscopy (CLSM). Then, the sample is stained, cut into pieces and embedded in epoxy resin. Afterwards a confocal 3D image stack is recorded from the sample blockface and the confocal 3D data is used as a map for locating areas of interest within the ultrathin sections of the same sample for further TEM investigation. For the differentiation of eccrine, apocrine or apoeccrine sweat glands, immunofluorescence is an essential tool since apoeccrine glands cannot be classified by simple morphological features. Antigenicity of the specimen is normally not well preserved in Karnovsky-fixed samples, it is better preserved after cryoimmobilization. High-pressure freezing (HPF) followed by freeze-substitution (FS) is known for its optimal preservation of both, antigenicity and morphology, but this technique is restricted to very small sample sizes. As a comprimise between sample size, preservation of antigenicity and morphology and to have the same sample for investigations with LM, CLSM and EM as described above, we developed a preparation protocol with chemical fixation and cryoprotection prior to plunge-freezing, followed by FS and embedding in Lowicryl. In summary, a complete description of a giant complex tissue like the sweat gland requires a preparation protocol that allows to investigate the same sample with different microscopic approaches.

O 23

Immunolabelling is essential for the differentiation of human axillary apoeccrine glands

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Literature describing the structure and function of human axillary sweat glands only refers to eccrine and apocrine sweat glands. The existence of mixed glands or so-called apoeccrine glands has rarely been mentioned and an appropriate description of these glands is missing. Therefore, we performed immunohistochemical studies with antibodies described as eccrine or apocrine differentiation markers on cryosections in order to distinguish eccrine, apocrine and apoeccrine sweat glands. Considering the difference in structure and size most of the axillary glands can be classified as eccrine or apocrine. Apoeccrine glands may wrongly be classified as apocrine or eccrine because they show morphological characteristics from either gland type. The morphological differences between eccrine and apocrine glands, i.e. gland size, cell type and shape, and the presence of intercellular canaliculi become clearly visible with Phalloidin staining. Since antibodies against CD44 and S-100 label intensely the secretory portion of the eccrine gland, glands with a positive staining for CD44 or S-100 but with an apocrine morphology are apoeccrine. Antibodies against CD15 and HMFG1 have been established as markers for the apocrine gland. Therefore glands positive for these markers but with an eccrine morphology are apparently also apoeccrine or eccrinelike (according to Sato 1987). Sato *et al* [Am. J. Physiol. 252, 1987] classified axillary glands as apocrine, apoeccrine, eccrinelike, or eccrine and postulated that apoeccrine glands develop from the eccrine or eccrine-like precursor glands within the hairy area of the axilla. Further studies are necessary to clarify the development and the function of the apoeccrine gland. In our study, we examined the occurrence and the ultrastructure of these glands in comparison to eccrine and apocrine glands.

O 24

Sclerosing mucinous blue nevus

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Abundant mucin deposition is an unusual finding in melanocytic nevi. We report the first example of sclerosing blue nevus with an abundant mucinous stroma. This uncommon variant of blue nevus should be differentiated from desmoplastic-neurotropic melanoma, in which the presence of mucin stromal deposition is a more typical finding.

O 25

Ultrastructural observations in a pigmented nevus in Hermansky-Pudlak syndrome (HPS) type 1: abnormal melanosome formation in nevus cells is of significant diagnostic value

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HPS is an autosomal recessive disorder in which oculocutaneous albinism, a bleeding disorder, and ceroid lipofuscinosis in the lungs and gut occur. HPS is genetically heterogeneous and the most common variant of HPS is due to mutations in *HPS1*. The protein encoded by *HPS1* may facilitate the trafficking of melanocyte-specific gene products to the premelanosome. We report the ultrastructural (EM) findings in a pigmented nevus seen in an HPS patient harboring heterozygous *HPS1* mutations. The patient was a 17-year-old Japanese female with oculocutaneous albinism and bleeding diahesis. The patient had abnormally pale skin and gold hair since birth. She had a history of easy bruising. Her platelet aggregation test was abnormal. The diagnosis of HPS was made by detection of the novel mutation IVS6+1G → A and the mutation IVS5+5G → A in the patients' *HPS1*. Previous reports indicated that melanocytes of HPS patients exhibit aberrant ultrastructural melanosome formation. We performed a skin biopsy from a melanocytic nevus that presented as a pinkish papule on her neck in order to investigate the exact nature of this abnormal melanosome formation in the nevus cells. LM – observations showed that nevus cells in the dermis contained a small amount of melanin pigment. EM – observations revealed that nevus cells had abundant vesicles of a similar size to that of normal melanosomes, and that ceroid-like structures and electron-dense pigment were seen within these vesicles. Malformation of these vesicles due to protein trafficking defects was more clearly demonstrated in the nevus cells than in the epidermal melanocytes. Thus, EM analysis of HPS nevus cells might be a powerful diagnostic tool and could give us important clues to further our understanding of the pathogenesis of HPS.

O 26

Stem cell factor and melanoma cells: a never-ending story

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The Stem Cell Factor (SCF), through its ligand c-kit, controls growth, differentiation and homeostasis of melanocytes. The expression of c-kit decreases during melanoma progression and metastatization. The efficacy of SCF in reducing melanoma growth and metastatic potential of Metastatic Melanoma Cells (MMC) is likely to be decreased accordingly. MMC obtained from 5 c-Kit positive subcutaneous melanoma metastases were grown in presence of SCF. Their immunophenotype (HMB45, anti-melanoma protein, and S100) and the expression of molecules usually secreted by MMC during tumor progression (IL6, IL7, IL8, IL10, GM-CSF, TNF-alpha and TGF-beta) were controlled at each passage by immunohistochemistry (APAAP) and direct immunofluorescence. The quantitation of the same molecules on MMC surnatants was performed by ELISA. By EM, we documented the absence of apoptotic bodies and a well differentiated status reached by MMC; they were dendritic with a cytoplasm rich in organelles and melanosomes. Melanogenesis increased upon treatment with SCF. The increase in melanosomes did not correlate with a parallel increase in tyrosinase production as documented by PCR analysis. HLA-Dr molecules increased in the presence of SCF. The metabolic activity of MMC, assessed by means of NOS (NO synthase), was not diminished by SCF. Most cytokines and growth factors were downregulated in presence of SCF. Altogether, these data show that SCF is able to maintain MMC in a differentiated status, does not induce apoptosis and increases melanogenesis. SCF does not diminish MMC metabolic activity as shown by NO production, reduces or even suppresses the expression of cytokines and growth factors involved in melanoma progression. Therefore, a use of SCF in the melanoma treatment strategy might be hypothesized.

O 27

Fascin expression in thin melanomas : predictor of metastatic potential?

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Surgical excision of melanomas of less than 1 mm thickness is usually associated with a favorable outcome, compared to those that have extended deeper. However, some thin melanomas (<1 mm) are also paradoxically known to metastasize early and thus cofound thickness predictions. Numerous attempts have been made to identify phenotypic markers associated with this unusual behaviour pattern. So far no absolutely reliable marker has been identified. Fascin which is an actin-bundling protein has been shown to be associated with migratory abilities in *in vitro* studies of neural cells. In addition, in several tumour types including breast, ovarian and colonic carcinoma fascin expression has been shown by immunostaining to correlate with local invasive potential and subsequent metastasis. We investigated a series of pigmented skin lesions that included thin melanomas with the aggressive phenotype outlined above. Immunostaining of formalin-fixed paraffin-embedded clinical specimens for fascin, HMB-45, Mart1 and HLA-DR was achieved with antigen retrieval methods and ABC peroxidase methodology. Appropriate positive and negative tissue and reagent controls were used to ensure specificity of staining. Analysis of the staining included a quantitive assessment of stain intensity in cells at different depths in the lesions. A published algorythm applied to the Matlab analysis software program gave results as energy/pixel. Thin melanomas showed a significant expression of fascin compared to benign melanotic lesions and to other melanomas of <1 mm with clinical histories indicating the more usual phenotype and subsequent progression. In particular cells at the leading edge of the positive thin melanomas showed elevated expression of fascin. Fascin may prove to be a useful indicator of early metastatic potential.

O 28

Histological features of cutaneous melanoma. An italian association of dermatopathology study

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The diagnostic efficacy of the histological parameters on which the diagnosis of melanoma is based is still to be defined. Nine dermatopathologists, affiliated to the Melanocytic Lesion Group of the Italian Association of Dermatopathology (A.I.DE.PAT.), from eight Italian Institutions, studied 64 melanomas and 86 melanocytic nevi to perform a quantitative analysis of 13 histological parameters used in the diagnosis of melanoma (dimension >6 mm, asymmetry, poor circumscription, irregular and confluent nests, single melanocytes predominating, absence of maturation, suprabasal melanocytes, asymmetrical melanin, melanin in deep cells, cytological atypia, mitoses, dermal lymphocytic infiltrate, necrosis). The concordance among the 9 observers resulted excellent ($k=>0.75$) for 10 of the 13 examined features. The k values obtained by comparison with the *majority diagnosis* were generally good. Results showed that in melanomas, the investigated histological features were not constant (except for cytological atypia) and tended to have a higher incidence as melanoma progressed. More than 93% of melanomas showed 7 or more investigated features. The efficacy of single histological features was generally poor, because of low sensitivity or specificity; suprabasal melanocytes was the only single reliable feature, contemporary showing a high sensitivity and a high specificity (0.94). Cytological atypia, dermal lymphocytic infiltrate, and asymmetry were rather sensitive ($\geqslant 0.92$), but poorly specific features of melanoma ($\leqslant 0.86$); absence of maturation, asymmetrical melanin, melanin in deep cells, mitoses and necrosis were rather specific ($\geqslant 0.92$), but not sensitive ($\leqslant 0.55$); single melanocytes predominating, irregular and confluent nests and poor circumscription were poorly sensitive ($\leqslant 0.89$) and poorly specific ($\leqslant 0.72$). The univariate logistic analysis showed that parameters useful for discriminating melanomas from controls included suprabasal melanocytes, asymmetrical melanin, asymmetry, dermal lymphocytic infiltrate, melanin in deep cells ($p=<0.05$). The multivariate analysis showed that two parameters (suprabasal melanocytes and asymmetry) yielded independent diagnostic information ($p=<0.05$).

O 29

Generalized Argyria

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Argyria is a rare cutaneous discoloration due to silver deposition. It can be localized or generalized depending on the different mechanisms of silver absorption. We describe two cases of diffuse argyria secondary to uncontrolled use of silver protein in vasomotor rhinitis over a period of 30 years in two sisters. A blue/grey discoloration over sunexposed areas, more prominent on the face, less intense on the "V" of the neck, forearms, hands, was the more remarkable clinical feature.

O 30

Modulation of the phenotype of dermal fibroblast

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Dermal fibroblasts are subjected to changes according to biomechanical and molecular stimuli during physiological and pathological conditions. The demonstration that fibroblastic cells acquire contractile features during the wound healing and, thus modulating into myofibroblasts, has opened a new perspective in the understanding of mechanisms leading to fibrocontractive diseases. Myofibroblast is the predominant cell type present in granulation tissue of contracting wounds and fibrocontractive diseases, and is also present in some developing or normal adult tissues. Myofibroblasts synthesize extracellular matrix components and during normal wound healing disappear by apoptosis when epithelialization occurs. The main function of myofibroblasts is generating force and altering tissue tension during wound healing. In some dermal pathological conditions, however, abnormal expression of contractile phenotype is also observed. Myofibroblasts were initially characterized by the presence of microfilament bundles (stress fibers) that are not present in dermal fibroblasts. Morphologically, the contractile apparatus of myofibroblasts is organized as bundles of microfilaments similar to the stress fibers present in cultured fibroblasts. These actin bundles terminate at the myofibroblast surface in the fibronexus, a specialized adhesion complex that uses transmembrane integrins to link intracellular actin with extracellular fibronectin domains. It provides a mechanotransduction system capable of transmitting the force that is generated by stress fibers to extracellular matrix. Generally, fibroblasts *in vivo* lack the contractile microfilamentous apparatus that is observed in myofibroblasts. The transition from fibroblasts to myofibroblasts is influenced by mechanical stress, TGF-beta1 and extracellular matrix molecules. Two types of myofibroblasts can be characterized: protomyofibroblasts, which contain stress fibers but lack alpha-smooth muscle actin, and differentiated myofibroblasts, which contain both stress fibers and alpha-smooth muscle actin. A better knowledge of the mechanisms influencing modulation of dermal fibroblast phenotype is crucial for preventing the development of fibrocontractive changes as well as the stromal reaction to epithelial tumours.

O 31

Dermoscopy (epiluminescence/surface microscopy) and histopathology: a two-way information flow?

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The introduction of dermoscopy (epiluminescence/surface microscopy) in clinical practice has disclosed a new morphologic dimension in the study of melanocytic skin lesions (MSLs). Dermoscopy always refers to histopathology for working up its diagnostic criteria; however, we also think that it is now going to raise new problems in clinicopathologic correlation, as summarized below.

1. The lack of a dermoscopic counterpart for dysplastic nevi. MSLs belonging to the histopathologic spectrum of the so-called 'dysplastic nevus' fail to show any peculiar dermoscopic feature. Presumably benign MSLs showing some architectural irregularity are best regarded to as 'atypical nevi' on both dermoscopy and histopathology. An interesting subset of these atypical nevi, often discovered on the trunk of young or middle-aged males, shows dermoscopic and histopathologic features of regression.
2. The inconsistency of some traditional pathologic entities. There is no clear-cut clinical and dermoscopic correlate for Reed nevus: this can be best considered as belonging to the clinicopathologic spectrum of (pigmented spindle cell) Spitz nevus.
3. The reappraisal of forgotten pathologic entities. Some variants of blue nevus – namely, 'compound' blue nevus and 'hypopigmented' blue nevus – deserve special histopathologic mention, because they often appear as melanoma look-alikes on dermoscopy.
4. The dermoscopic features of MSLs used as a guide for gross sampling protocols. Dermoscopy can draw attention of histopathologists to suspicious areas of MSLs. Moreover, histopathologic symmetry/asymmetry can be best studied by sampling a given MSL according to the symmetry planes seen at dermoscopy.
5. In conclusion, while learning from histopathology, dermoscopy is also expected to give new ideas and working protocols to histopathology.

POSTER PRESENTATIONS

P 1

Alterations of cutaneous skin microvasculature in lipoid proteinosis

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Lipoid proteinosis is a rare autosomal recessive disorder characterized by deposition of hyaline-like material in several organs, including skin. Pathogenic mutations have been found in the extracellular matrix protein 1 gene (ECM1). Some abnormalities in dermal blood vessels in this condition have been reported but these changes have not been fully assessed. In this study, we labelled skin from a 51-year-old man with lipoid proteinosis, and three control subjects, using an antibody to type IV collagen and laminin-1. Three-dimensional reconstruction of the skin microvasculature using laser confocal microscopy and computer imaging in lipoid proteinosis revealed reduplication of basement membranes surrounding blood vessel walls and a very abnormal architecture to the dermal vasculature. Notably, there were enlarged vessels in the mid and deep dermis that were orientated parallel to the dermal-epidermal junction. In addition, the normal capillary loop network in the dermal papillae, as well as the subcutaneous plexus and transverse connecting vessels were lacking in lipoid proteinosis. The study demonstrates that the skin microvasculature is grossly altered when ECM1 is targeted by inherited mutations and that this glycoprotein appears to have an important role in regulating blood vessel physiology and anatomy in the skin.

P 2

Primary and metastatic epidermotropic pigmented breast carcinoma: histological, immunohistochemical and ultrastructural analysis of four cases

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The appearance of breast carcinoma as a pigmented lesion, primitive or metastatic, is an exceedingly rare event. The histomorphological, immunohistochemical and ultrastructural aspects of this pattern, seem to be the result of a mechanism developing each time the neoplastic cells with epidermotropic properties surpass the dermo-epidermal junction. We report 4 cases of pigmented skin lesions, apparently primitive in one case, secondary in 3 cases, of breast carcinoma, clinically mimicking malignant melanoma. Histomorphology and immunohistochemical analysis with the Alkaline-Phosphatase anti-Alkaline-Phosphatase (APAAP) method, using the following mono- and polyclonal antibodies: S100 protein, HMB45, epithelial membrane antigen (EMA), Cytokeratin A, estrogen and progestagen receptors, ki67, PCNA, were performed. Retrospective ultrastructural investigation was performed by reprocessing the paraffin embedded material. Neoplasm was found positive for both epithelial and melanocyte markers and a histomorphological pattern indicative for malignant melanoma was observed. Ultrastructurally, neoplastic cells were seen to contain melanin pigment but at the same time presented characteristics of epithelial glandular cells. Our data suggest that breast carcinoma sometime shows a polyvalent phenotype and the neoplastic elements are moreover capable of acquiring antigenic characteristics typical of the melanocyte. This peculiar behavior must be kept in mind for the correct diagnosis, especially when the pigmented skin lesions are the first manifestation of the tumor.

P 3

Benign glandular schwannoma

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The benign glandular type is a rare variant of schwannoma, described for the first time by Woodruff in 1976, in which glandular inclusions with intestinal, respiratory, and ependimal differentiation were observed. The vast majority of tumors containing these glandular structures are malignant. The case of a 32 year old woman is reported, with an asymptomatic nodule on the distal phalange of the right hand third finger. The lesion had a diameter of 1 cm, a hard elastic consistency and was removed in toto, revealing the presence of a pseudocapsule. On histological analysis it had a compact aspect and was composed of spindle cells that were lined up in irregular layers having different orientations. The neoformation had no atypical cytological characteristics nor did it present unusual mitotic activity, vascular invasion or necrosis. In several areas, cystic structures of variable dimensions were present and were lined by glandular epithelium composed of elongated, cubic or cylindrical cells having oval nuclei. These cells had a pale cytoplasm and were located directly on the basal membrane. Immunohistochemical analysis showed positivity for S100 protein in the schwannoma proliferation and for cytokeratin on the glandular structures. Focal positivity was observed also for Leu-/, CEA and chromogranin on the glandular epithelium. The histogenesis of the glandular elements is still debated, but they probably derive from pluripotential neural crests, as well as the spindle cell component. Our data further support this neuroendocrine origin. In contrast with malignant glandular schwannoma, the benign variant is not associated with von Recklinghausen neurofibromatosis. Simple excision is curative, but gentle and careful enucleation is necessary to preserve normal function of the attached nerve, especially on the extremities.

P 4

Nanometric changes of hair shafts measured by atomic force microscopy in a patient with Comel-Netherton syndrome

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Comel-Netherton syndrome is an autosomal recessive disorder characterised by typical skin and hair changes such as ichthyosis linearis circumflexa or congenital ichtiosiform erythroderma and trichorrhexis invaginata. The disease is due to mutations in the gene SPINK5. These mutations result in a deficiency of the serine protease inhibitor LEKTI which is strongly expressed in hair follicles. The diagnosis can be confirmed by light-microscopy of altered hair-shafts which amount to approximately 20% of total hair. However, anomalies of hair-shafts in the submicrometer range cannot be detected by conventional light microscopy. In the present study we measured and imaged hair-shafts of a patient with Comel-Netherton syndrome and hair of healthy people by using atomic force microscopy (AFM). AFM, a new tool in medicine and cell biology, enables to measure surface topography with a nanometer (10^{-9}) resolution without any further chemical sample preparation (i.e. dehydration, fixation). Next to the 3-dimensional high-resolution images of surface topography AFM was used to measure local stiffness of hair shafts. Hair of healthy people show a regular pattern of overlaying cuticula cells with a spatial frequency of about 10 μm. Step height between two cuticula cells was 600 nm. In contrast, hair shafts of Comel-Netherton syndrome show a loss of the regular periodic cuticula pattern as well as amorphous keratinic globules with diameters between 500 nm and 1500 nm and a height between 300 nm and 500 nm. These globular-like structures can aggregate to plaques of more than 100 μm^2. In addition some cuticula cells were splitted in single pieces. The data show that by using AFM structural changes of hair shafts could be imaged and measured with a nanometer resolution. This procedure enables us to identify new pathological features that can be disease-specific characteristics and already visible before developing trichorrhexis invaginata.

P 5

Early response in human breast skin cultures after a single dose of gamma-rays: an ultrastructural study

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Radiation therapy with ionising radiations (gamma-rays) is the main non-surgical treatment for cancer, but skin reaction is the most common side effect of such a treatment. At present, the scientific rationale of the management of skin response is still lacking. Though the radiation-induced dermal fibrosis was histologically characterized, little is known about the epithelial compartment overlaying fibronecrotic lesions, i.e. epidermis, and the early keratinocyte response are not yet fully understood. In this study we have investigated early effects of gamma-irradiation in organotypic breast skin cultures obtained after aesthetic surgery of healthy women (30–40 years old) ($n = 4$). Skin biopsies in triplicate were placed epidermis upwards on Transwell plates and underwent a single dose of gamma-irradiation (200 cGray). Samples were harvested 24 hours after irradiation and routinely processed for either Araldite or Lowicryl embedding for immunohistochemistry of cytokeratin 10 (CK10), i.e., a major marker of suprabasal keratinocytes. In araldite ultrathin sections, no difference exists in epidermal architecture between non irradiated and irradiated fragments. In particular, basal cell degeneration was not apparent. However, in irradiated epidermis a marked condensation of keratin intermediate filaments was evident in the basal layer and in the lower spinous layer, where scattered pyknotic nuclei were also present. Desmosomes were abundant and present throughout all epidermal layers. CK10 immunogold confirmed the occurrence of a cytoskeletal rearrangement induced by a single dose of gamma-rays. Together with our previous observation by immunofluorescence analysis indicating that, in these experimental conditions, a strong inhibition of cell proliferation occurs, these results demonstrate that gamma-rays induce a very early alteration of epidermal homeostasis. A topical therapy before the irradiation can be thus a suitable approach to limit and/or avoid the onset of the early epidermal response, with particular regard to keratinocytes.

P 6

Histopathological Evaluation Of Injection Site Reactions In Patients Treated With Enfuvirtide

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Enfuvirtide is the first of a new class of antiretroviral agents. Injection site reactions are the most common adverse events. The aim of the present study was the histopatologic evaluation of injection site reactions in patients treated for 80 weeks. Five patients were submitted to cutaneous biopsies using a 4 mm punch. Sections were stained with haematoxylin-eosin, periodic acid-Schiff stain and Verhoeff's stain. Moreover, immunohistochemical studies were carried out using CD20, CD45Ro and CD34 antibodies. Histological examination showed three patterns: 1) an acute urticarial/vasculitis-like pattern associated with inflammation of the fat tissue; 2) a subacute pattern with an initial derma sclerosis; 3) a chronic scleroderma-like pattern with connective tissue disposed around the adnexa, whose structure was intact. The immunohistochemical study evidenced a prevalence of T lymphocytes and a moderate neoangiogenesis. Conclusions: In our experience, after a rather long period of treatment with enfuvirtide, cutaneous reactions comprised a variety of features largely independent of the virological and immunological outcome. The adnexa were unaltered in all patients, indicating a tendency to a possible regression of the sclerotic lesions. Therefore, patients should be encouraged to rotate the sites of injection thus permitting the tissues to regenerate.

P 7

Disseminated epidermolytic acanthoma of the scrotum

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The term epidermolytic hyperkeratosis (EHK) describes a characteristic histopathological pattern with granular degeneration caused by alterations of tonofilament organisation and breakdown of intracytoplasmic skeleton with unaffected desmosomes. EHK has been applied to a spectrum of mostly dominantly inherited dermatoses, but it is also a feature of some acquired cutaneous disorders, e.g. epidermolytic acanthoma, and an incidental finding, showing the pathological feature in single rete ridges, and in perilesional regions e.g. of epidermal neoplasms, scars and inflammatory diseases. We report the case of a 76-year old white male with itching papules of the scrotum existing for one year. By electron microscopy, the lesions revealed the typical morphological features of EHK with generalized clumping, aggregations and more or less loss of fibrillar character of tonofilaments in suprabasal epidermal cells. These features are the counterparts to the partly degenerative partly dyskeratotic pattern by light microscopy. On the basis of the clinical history, the morphological changes as well as the absence of human papilloma virus DNA, the final diagnosis was disseminated epidermolytic acanthoma of the scrotum. Mucocutaneous and genital EHK eruptions are rare and may easily be misinterpreted. In hereditary conditions, mutations in genes coding differentiation-specific keratins underlie EHK. These are suprabasal keratins 1 and 10 in bullous congenital ichthyosiform erythroderma and nevus verrucosus affecting non ridged skin, and keratin 9 in palmoplantar keratoderma Voerner affecting ridged skin. The pathogenesis of EHK in acquired conditions is still unknown. Interpretations and speculations include mechanisms of immunosuppression and decreased immune surveillance, trauma, and metabolic disturbances. Forms of mosaicisms due to somatic mutations, i.e. postzygotic neoplastic DNA changes, cannot be ruled out either. Recent *in vitro* results indicate that several mechanisms seem to be able to interfere with the highly dynamic keratin filament cytoskeleton.

P 8

Malignant melanoma arising in the mucosa of the oral cavity: a morphological, ultrastructural and cytogenetic study

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Malignant melanoma of the oral cavity is rare. We describe the first cytogenetic study of a case. The patient was a 65 year old Italian woman, who presented with a brown lesion on the gingival margin of the hard palate. At histology the lesion was diagnosed as malignant melanoma, with a Breslow depth of 2.196 mm and the presence of many mitosis and moderate pigmentation. At immunohistochemistry the neoplastic cells were strongly positive with anti MART-1 and anti HMB-45 antibodies. Surgical resection was complete and regional lymph nodes were free of metastases. Cell culture of fresh material was performed according to the protocol currently in use. The results revealed the presence of 4 clones: the most representative was 49,XX, +1, +2, +3, −5, −6, +8, +9 (with trisomy of 1,2,3, 8 & 9, and monosomy of 5 & 6). For electron microscopy, paraffin embedded material was rehydrated, post-fixed in $Os0_4$ and embedded in Epon. Ultrastructure confirmed the histological observations, showing large clear cells with prominent nuclear and the presence of melanosomes. The cytogenetic results are particularly interesting, showing anomaly in more than 85% of metaphases. Furthermore, different chromosomes are involved (such as chromosome 9) from those usually observed in familial cutaneous malignant melanoma.

P 9

Anaplastic large T cell CD30+ lymphoma (ALCL)

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Anaplastic large T cell CD30+ lymphoma (ALCL) represents a distinct subtype of non-Hodgkin's lymphoma that typically involves multiple nodal and extranodal sites at the time of presentation, including the skin. A 59-year-old man came under our observation for asymptomatic red nodules on the neck, the chest and on the upper back that had appeared 2 months earlier for the first time. Family history was negative. Physical examination revealed multiple purplish nodular lesions of a mean size of 1 cm in diameter on the neck, arms, chest and upper back that had progressively increased in number and size during the last months. Histological examination revealed a diffuse infiltrate of large cell with highly pleomorphic nuclei containing one or more prominent nucleoli. Some Reed-Sternberg like cells were identified. Immunocytochemical analysis revealed the tumour cell to be positive for CD30 and negative for anaplastic lymphoma kinase (ALK-1). Staging investigations, including CT scan of neck, chest, abdomen and pelvis, were negative. On the basis of the clinical aspect and histopathologic features a diagnosis of cutaneous anaplastic large T cell lymphoma was made. After three months lymphadenopathy appeared on the right axilla. Lymph node biopsy showed identical features to those noted in the previous skin biopsy. The patient was referred to a Haematology department where he is actually under treatment with polychemotherapeutic drugs.

P 10

Cutaneous basement membrane formation in organotypic culture

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The cutaneous basement membrane (BM) consists mainly of polymeric collagen-IV and laminin-10 and associated mono-/oligomeric laminin-5, nidogen, and perlecan. Since BM-defects in transgenic or knockout mice are mostly lethal at early developmental stages, we have studied the role of nidogen specifically in 3D-cocultures of human keratinocytes (HK) and fibroblasts (human/mouse, HF/MF) by either blocking interactions or implementing molecular deficiencies. HK or HaCaT cells were grown on collagen gels harboring HF or MF from normal or ko-mice. Nidogen-laminin interaction was blocked by the laminin-fragment (gamma-1-III3-5, L-gamma-f) binding nidogen. BM-formation was surveyed by immunofluorescence (IF), regular (EM), immuno-electron microscopy (IEM), and Western blots of protein extracts of separated epithelial and 'dermal' tissue. In 3D-cocultures of HK and HF L-gamma-f blocked mainly deposition of nidogen, laminin-10, and perlecan. Whereas the hemidesmosomal/BM components laminin-5, BP180, and integrin alpha6beta4 were still detectable (IF), by EM and IEM any BM-structures or hemidesmosomes (insertion of keratins) were absent. The fibroblast-made nidogen was eliminated by employing MF from nidogen-1/-2 ko-mice. In 3D-cocultures with HaCaT cells nidogen1/2 (−−/++)-MF abolished nidogen-1 staining, but (−−/+−)-MF reduced additionally nidogen-2, collagen-IV, and laminin-10. Absence of nidogens (−−/−−) further abolished collagen-IV and laminin-5; integrins such as alpha6beta4 appear normal (IIF). BM-formation could be reinstalled with recombinant nidogen-1 or -2. BM-perlecan, for comparison, is apparently synthesized also by keratinocytes. Thus, deficiency in either cell type did not affect BM-formation, demonstrated by growing perlecan (−/−)-MF or HaCaT antisense-perlecan cells with normal keratinocytes or fibroblasts, respectively. Accordingly, BM-components are efficiently recruited for ultrastructural assembly in this skin model.

P 11

The thermo phobic foams: pharmacokinetic properties and clinical efficacy data

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Traditional topical formulations are creams, ointments, gels and lotions. These formulation have some limits and drawbacks. Recently a new topical formulation has been available: the thermo labile foam. The main characteristics of this foam is that when it is dispensed is stable but when applied to skin the body heat causes the foam structure to break down and the active ingredient is deposited on the skin with little residue. Most of the vehicle components (alcohol and water) quickly volatises and active ingredients are rapidly absorbed into the skin. Pharmacokinetic data: Several *in vitro* studies (*Franz 1999, Franz 2001, Bonina 2003*) have shown that the thermo labile foam has a 2–3 fold increase in absorption rate in comparison with traditional formulations such as lotion and cream. The formulation of the foam is modulable in term of alcohol contents (high, low concentration, alcohol-free): in this manner a wide range of active ingredients could be incorporate in the foam. So far several foams containing different active compounds are available such as corticosteroid (betamethasone, clobetasol) vitamin E, pediculocide substances and antifungal products. Clinical Efficacy: In patients with scalp dermatoses, such as psoriasis, seborrheic dermatitis and alopecia areata, corticosteroid-based foams have demonstrated a greater clinical efficacy in comparison with both placebo and active lotions (*Franz 1999, Andreassi 2000, Mancuso 2003*). A thermo labile foam containing natural pyrethrin 0.1% has shown to be at least as effective as permetrhin cream 5% in the treatment of scabies (*Capizzi 2003*). Foam is also the preferred formulation, over the cream, gel and ointment vehicles, to be used for patients with dermatoses (*Salam 2002*). The foam is easily to applied, odourless and residue free. Therefore the foam formulation, due to its efficacy and its main characteristics could provide a good patient's compliance to the treatment.

Announcement on behalf of the Society for Cutaneous Ultrastructure Research (SCUR)

32nd Annual Meeting of the SCUR, 2–5th June 2005, HAMBURG – Germany
Joint Meeting with the International Society of Skin Pharmacology and Physiology (ISP)

For a preliminary information, please contact

Dr. Roger WEPF, Local Host Organizing Committee via e-mail: **Roger.Wepf@Beiersdorf.com**
or
Dr. Sonja STÄNDER, SCUR-Board Member via e-mail: **Sonja.Staender@uni-muenster.de**

Deadline for the Abstract submission is April, 2nd 2005.

Find the First Announcement for 2005 within the website of SCUR at
http://www.dermis.net/org/scur/index.htm

SCUR is an International Society, the aims of which are:

- Research in electron and light microscopy, other combined visualization techniques and molecular biology tools applied to cutaneous biology and pathology.
- Understanding of ultrastructure and function of human skin in health and disease.

The SCUR Annual Meetings are open to all interested scientists.

The plenary and poster sessions comprise presentations and discussions on ultrastructural and other new morphological findings: Light Microscopy and Electron Microscopy – Immunohistochemistry – *In Situ* Hybridisation/ PCR – Confocal Laser Scanning Microscopy – Scanning Probe Technique – Fluorescence microscopy – Atomic force microscopy – Dermatopathology.

AWARDS

The Society awards the best oral and the best poster presentation. In addition, a travel grant is offered to young members (up to 35 years) of the SCUR. This grant can be requested by applying to the Secretary. An Application Form (see Website) must be sent to the SCUR Secretary at least 1 month before the beginning of the Annual Meeting.

The **ABSTRACTS** of the meetings **are published in "The Journal of Investigative Dermatology".**

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