O1

Image Analysis as a Tool for Comparing Complex cDNA Array Hybridization Patterns

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The development of malignant melanoma is most probably a process caused by an accumulation of poorly characterized alterations of gene expression. In order to analyse these complex changes of the molecular determinants involved in this progression we combined the SMART technology (Clontech) with cDNA array hybridisation. Previously, we have shown, that the SMART protocol allows the generation of reproducible and representative cDNA probes for array hybridisation from 5 ng of total RNA. From RNA extracted from melanocytic cells, obtained contamination-free by a laser-capture microscope, we generate probes for hybridisation of high density cDNA arrays (RZPD, Berlin). Each array carries 27648 human cDNA clones from the I.M.A.G.E. consortium spotted as *E. coli* colonies. The probes generated according to our protocol from melanocytic nevi and malignant melanomas were hybridized against replica cDNA arrays. To allow reproducible and reliable quantification the hybridization signals were normalized, segmented and measured by a specially designed image ana1ysis software. We could detect reproducible patterns of hybridization signals of about 800 cDNAs. Roughly 1% of these cDNAs showed a different hybridization signal comparing different stages of melanoma initiation and progression. Our data clearly show that the combination of the new technical resources such as the laser-capture microdissection, microarray hybridization based on SMART -PCR and image analysis will help to develop molecular diagnostics and prognostics of melanocytic tumors.

O2

Eph-Receptors Regulate *In Vivo* Cell Sorting and Patterning: Clues for Understanding Morphogenesis

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Eph receptors (Eph-RTKs) and their ligands represent the largest (14 receptors and eight ligands) and most complex system of membrane-located receptor protein tyrosine kinases in vertebrates. They have been implicated in regulating fundamental processes such as developmental patterning events, including assembly of the vasculature, axonal pathfinding in brain development, and segmentation of embryonic tissues into boundary zones defined by reciprocal spatial gradients of ligands. The functional meaning of Eph-RTKs in the adult organism are still unknown. Possibly, they may be essential for regeneration of tissues after injury and may also be involved in the fomation of tumour architecture and establishing a tumour vasculature. Recent data argue for a central effector system that transduces a complex biochemical input (zytokines, hormones, matrix composition, cellular neighbours) into an output that determines, where a cell migrates to, where a cell dedifferentiates and proliferates, and where not. These functions are engineered by internetting the pathways of Eph-RTKs with integrins and other cell surface receptors directly with cytoskelletal GTPase cascades as well as the JNK/SAPK-pathways. Our group has set up a unique novel tool to further analyse the complex pattems of Eph/Ephrin-expression profiles: We produced the first comprehensive Eph/Ephrin cDNA miniarray that will help to characterize Eph-expression profiles in cultivated tissues as well as in microdissected *ex vivo* material.

O3

DNA Image Cytometry in Cutaneous Adnexal Tumors with Follicular Differentiation

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We could demonstrate in previous studies that DNA image cytometry (ICM-DNA) can be helpful in detecting (prospective) malignancy in recurrent clear cell hidradenoma, sebaceous tumors of Muir–Torre syndrome, and sweat gland tumors. Studies concerning ICM-DNA in adnexal tumors with follicular differentiation have not been conducted to date. In the present study, a series of 50 benign and eight malignant follicular tumors was analysed by ICM-DNA. All cases were clear-cut benign or malignant, respectively, by histopathological criteria. ICM-DNA was performed according to the current recommendations of the European Society for Analytical Cellular Pathology (ESACP). DNA-aneuploidy was detected by the stemline interpretation according to Böcking and/or at least three 5c-exceeding events (single cell interpretation). DNA-aneuploidy was detected in 100% (eight of eight) of the carcinomas. The single cell interpretation (5c-exceeding events) proved to be more sensitive than the stemline interpretation: Using the single cell interpretation, DNA-aneuploidy was detected in eight of eight carcinomas, whereas only two of eight carcinomas were detected as DNA-aneuploid by use of the stemline interpretation. DNA-aneuploidy was not detected in any of the benign tumor cases (specificity = 100%). In conclusion, using a single cell interpretation, the DNA image cytometric detection of DNA-aneuploidy in follicular adnexal tumors is a sensitive and specific indicator of (prospective) malignancy.

O4

Nitric Oxide Fully Protects from UVA- or Singlet Oxygen-Induced and Ros-Mediated Apoptosis or Necrosis

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UVA-irradiation of human skin leads to expression of the inducible nitric oxide synthase (iNOS) in keratinocytes and vascular endothelium. Despite its known cytotoxic activity NO may also act as a protective compound. We recently found that iNOS activation or exogenously applied NO when administered 24 h prior to the light stimulus protected endothelial cell from UVA-induced apoptosis and protection was due to NO-induced increase expression of the antiapoptotic protein Bcl-2. We now looked for the effect and molecular mechanism of NO on UVA-or Rose Bengal-induced and reactive oxygen species (ROS)-mediated apoptosis or necrosis of endothelial cells (EC) when present during or after the toxic insult. Using the NO-donor S-nitrosocysteine (SNOC), we found that at concentrations = 100 μM SNOC protection from apoptosis as well as necrosis was significant (10% apoptosis with 1 mM SNOC vs. 80% without SNOC and 5% necrosis with 1 mM SNOC vs. 85% without SNOC). The NO-mediated protection is not due to scavenging of ROS but was due to complete inhibition of ROS-induced lipid peroxidation (6-fold increase in MDA formation without SNOC vs. 1.2-fold with 1 mM SNOC) which, in the case of apoptosis as induced cell death, strongly correlates with caspase-and Bcl-2-independent inhibition of mitochondrial cytochrome c release into cytoplasm, a key signal in apoptosis initiation. The experiments presented here demonstrate a new protective mechanism of NO upstream of caspase and Bcl-2 activation and underline the protecting capacity of NO against ROS as formed during UVA-irradiation or inflammatory processes.

O5

Detection of Differential Expression of a Novel Tumor Suppressor Candidate Gene by mRNA *In Situ* Hybridisation in Cell lINES of Malignant Melanomas

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Tumorigenesis of malignant melanomas as well as other human tumors is paralleled by a disrupted cell cycle control. One of the important players in cell cycle control is the retinoblastomaprotein (pRB) that blocks cell cycle in G1 phase in its active hypophosphorylated form. A machinery of kinases phosphorylates pRB and permits transition from G1 to S phase. Known tumor suppressors like p16 or p53 stabilize the active (hypophosphorylated) pRB via indirect upstream modulation of those cyclin dependent kinases. In contrast, we have found a representative of a new family of proteins that might bind directly to pRB and mediate cell cycle control. This protein is most closely related to retinoblastoma binding protein 2 (rbbp2) and was named rbbp2-homologue 1 (rbbp2-h1). Rbbp2 binds to pRB and modulates the cell cycle, but until now no tumorigenic or tumorprotective effect of rbbp2 was observed. For rbbp2-h1 we observed differential expression in malignant melanomas vs. nevi suggesting an involvement in tumorigenesis. These findings were confirmed by *in situ* hybridisation of melanocytes and melanoma cells. An additional homologue found by an other group was called plu-1 gene. This gene is identical with rbbp2-h1 in almost all domains except one ~ 150 bp region that seems to be spliced in the plu-1 gene. The plu-1 gene has a potential role in tumorigenesis of breast cancer. In summary, a new family of cell growth regulators is emerging that may directly effect on pRB-function.

O6

Reconstructed Pigmented Epidermis as a Model to Study Melanogenesis

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Reconstructed pigmented epidermis has been established by coseeding melanocytes with keratinocytes on a dermal substrate and culturing up to 6 wk at the air–liquid interface. Melanocytes can be recognised as dendritic cells, with a lighter coloured cytoplasm containing many melanosomes. In 2-wk-old cultures, the melanocytes can be seen distributed evenly throughout the basal cell layer forming close contacts with the surrounding keratinocytes. No melanocytes are found in suprabasal layers. After 4 wk of culture melanosomes are distributed evenly within the keratinocytes of the lower epidermal layers. After 6 wk of culture, the melanosomes are also seen capping the keratinocyte nuclei in the upper cell layers. In 4 and 6 wk air-exposured cultures basal layer keratinocytes have a columnar shape very similar to that found *in vivo*, in contrast to 2-wk-old cultures where the basal layer cells are more rounded. Modulation of melanogenic activity can be induced by administration of growth factors, hormones and IBMX thus providing a useful *in vitro* tool to study melanogenesis.

O7

Human Stem Cell Factor (HSCF) Does Not Affect the Expression of Nitric Oxide Synthases (NOS) by Metastatic Melanoma Cells (MMC) *In Vitro*

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HSCF is a pleiotropic factor playing an important role in the survival, proliferation and migration of melanoblasts during their differentiation, and contributes in the homeostasis of normal melanocytes. The effect of HSCF on subcutaneous MMC expressing c-kit, the natural HSCF ligand, was evaluated. The MMC were cultured through a modified explant technique. The expression of typical melanocytic markers (S-100 and HMB45) and specific cytokines differentially expressed during the tumor progression of melanoma (interleukin-6, IL-6; IL-8; IL-I0; granulocyte macrophage-colony stimulatig factor, GM-CSF; and integrin beta3 subunit) were evaluated baseline and after HSCF addition. The metabolic activity of MMC was evaluated as well, according to the expression of two NOS isoforms: constitutive NOS (cNOS) and inducible NOS (iNOS), both leading to the production of Nitric Oxide (NO), an important biological messenger involved in carcinogenesis. The addition of HSCF to the culture medium did not affect S-100, IL6, IL8, IL-IO expression, completely abolished HMB45 expression and integrin beta-3 subunit; conversely HSCF increased GM-CSF expression. Concerning cNOS and iNOS, both expressed baseline, their activity was not affected by HSCF addition to the culture medium. The results of this study suggest that HSCF is not able, in this experimental model, to affect significantly the metabolic activity of MMC evaluated through the NO production capacity.

O8

Defects of the Basement Membrane Zone in Nude Mouse Skin Tumors and Surface Grafts of Malignant HaCaT-Ras Cells

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The malignant human HaCaT-ras cell line II4, derived from epidermal HaCaT cells by ras-transfection, grows invasively as surface transplant on nude mice and forms squamous cell carcinomas (SCCs) upon s.c. injection. Herein we have analysed early ultrastructural changes during the assembly of basement membrane (BM) and hemi-desmosomes (HD) by regular transmission (EM) and immunoelectron microscopy (ImEM) in correlation to indirect immunofluorescence (IIF) data. Thus, in contrast to grafts of normal or HaCaT cells, epithelial lining by BM- or HD-specific antibodies was largely marginal by IIF including early II4-grafts before onset of invasion. Accordingly, by EM only short stretches of BM-material were detectable, while the fine structure of HDs was defective lacking especially the *inner dense plaques* responsible for anchorage of keratin filaments. Our mild fixation procedure allowed a satisfactory ultrastructural location of collagen IV, laminin-5 and −10, integrin β4 (α6β4), bullous pemphigoid antigens 1/2, and HD1 (plectin) in the BM-zone by ImEM. Different from the regular patterns in skin, both II4-tumors and surface grafts revealed a focal restriction of all BM-components and a dramatic reduction of HD-proteins especially the plaque components, only β4 being regularly located below cell membranes. Keratin filaments were totally disconnected and displaced towards the apical pole of those cells facing the extracellular matrix. Presumably this increases cell motility, at least by compromising attachment, while HD-association with the cytoskeletal migratory apparatus remains to be proven.

O9

Ultrastructural Alterations in Keratinocytes after Irradiation with UVA 1, UVB or PUVA-a Morphometric Analysis

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The physiological protection of the human skin against exposure to UV radiation involves the epidermis, melanocytes as well as keratinocytes. In this study the effect of chronic exposure to therapeutic dosages of UVA 1, UVB and PUVA was investigated. In 16 patients the cell size of keratinocytes, the number of nucleoli per nucleus and the amount of heterochromatin was evaluated using computer-assisted image analysis program in 16 patients before and after UV-irradiation. Following UVA 1 irradiation the number of nucleoli increased while the amount of heterochromatin in the nucleus decreased suggesting a proliferation of keratinocytes with high proteinbiosynthesis. UVB – irradiation caused an increase of cell numbers per area indicating acanthosis; parakeratosis was not observed. Following PUVA irradiation the number of nucleoli per nucleus increased, but the amount of heterochromatin did not change after irradiation. Keratinocytes take part in the physiological protection against UV–irradiation. The reactions of keratinocytes differ depending on the applied wavelengths.

O10

Familial Ultrastructural Connective Tissue Alterations in Patients with Spontaneous Cervical Arterial Dissections

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In a previous study ultrastructural dermal connective tissue aberrations were demonstrated in a majority of young to middle-aged patients with spontaneous cervical artery dissections (CAD). Subsequently it was now intended to clarify whether these findings are reproducible hereditary features. Skin biopsies of eight first relatives of four index patients with CAD were investigated by electron microscopy for similar changes of the extracellular matrix. None of the patients or relatives had skin, joint ore skeletal symptoms of a known heritable connective tissue disorder. In three children of one index patient collagen and elastic fibre aberrations were present similar to those of the index patient. Elastic fibre alterations similar to those of the index patient were found in the dermal connective tissue of the father of a second index patient. Within the families of two more index patients, no relative has been identified with ultrastructural dermal connective tisse alterations so far. In some families at least ultrastructural dermal connective tissue aberrations seem to be familial and consistent with an autosomal-dominant trait. On the basis of a morphologically defined phenotype, possibly predisposing cervical artery dissection, the analysis and identification of both underlying linkage and mutation should be faciliated.

O11

The Lateral Lipid Organization in Diseased Skin in Relation to Barrier Function

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Skin diseases are known in which the lipid composition is different from that of healthy human skin. For instance, patients that suffer from Lamellar Ichthyosis (LI) have a decreased amount of long-chain free fatty acids. The skin of patients that suffer from Atopic Dermatitis (AD) has a reduced amount of ceramides, especially ceramide I. Possibly these differences lead to an altered lateral and/or lamellar organization which in turn may be responsible for impaired barrier function. In the present study, electron diffraction (ED) was used to compare the lateral organization of the lipids in the stratum corneum (SC) in a control group with that of LI or AD patients. The results of this study show that lipids in the SC of the control group are mainly organized in an orthorhombic (*ort*) lattice. AD patients have an increased presence of the hexagonal (*hex*) lattice, while the *ort* packing could regularly be detected. The SC of LI patients showed predominantly *hex* ED patterns, whereas the *ort* packing was only occasionally observed. This is in good agreement with earlier model studies in which we could demonstrate that the occurrence of the *ort* packing depends on the presence of long chain free fatty acids. Furthermore, we studied the lamellar organization in the same control and patient groups using freeze fracture electron microscopy. It was found in AD patients that in contrast to healthy SC, the lipid surface had rough areas and irregular corneodesmosomes. In LI patients, next to the presence of irregular corneodesmosomes, the fracture plane was altered. There was an increased amount of steps, indicating that the connection between lamellae has changed. The changes in lateral and lamellar lipid organization, and in corneodesmosomes may explain the abberations in SC barrier function in the LI and AD patients.

O13

Congenital Reticular Ichthyosiform Erythroderma a Histologic, Immunohistochemical and Ultra-Structural Study of a Rare Keratinization Disorder

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Congenital reticular ichthyosiform erythroderma (syn. Ichthyosis en confetti, Ichthyosis variegata) is a rare genodermatosis with characteristic clinical and histologic features. We report on a 8-year-old girl that was born with a scaly erythodermic skin. In the following years, characteristic patches of normal skin appeared in a reticular arrangement apart from hypertrichosis and hyperkeratosis on palms and soles. Multiple biopsies were investigated by histology, histochemistry, electronmicroscopy, and immunohistochemistry. Histopathology showed a hyperplastic and parakeratotic epidermis and pale and multinucleated suprabasal keratinocytes with perinuclear vacuolisation. Expression of epidermal keratins was regular but the arrangement of the keratinskeleton appeared to be highly disturbed. Immuno-electronmicroscopy confirmed complete dissolution of keratin filaments in large parts of the cytoplasm. As recognized by both ultrastructural features and nick end labeling (TUNEL) for DNA fragmentation, the number of transitional cells was increased. In contrast to lamellar ichthyosis, an in-situ assay for transglutaminase activity was normal. Furthermore, keratinocytes variably showed an irregular uptake and processing of melanosomes thus contributing to the reticulated clinical aspect of the disease. In conclusion, our investigations suggest a basic deficiency in the assembly of keratin proteins in congenital reticular ichthyosiform erythroderma with an unknown genetic defect.

O15

Tissue Counter Analysis – Principles and Applications

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Tissue counter analysis is a specific approach to the automated evaluation of complex images. Instead of discriminating different structures for feature extraction, a lattice of circular or square elementary measuring masks (elements) of equal size and shape is randomly placed over the section and the contents of each element is evaluated by a set of colour and texture features.

Forty cases each of melanoma and nevus were consecutively sampled. After creation of a learning set based on 20 cases each, discriminant analysis of background vs. tissue elements, tumor vs. other tissue elements, and benign vs. malignant tumor elements was performed. The discriminant functions were used to assess the amount of benign and malignant tumor elements in each case. Discriminant analysis facilitated recognition of 99.6% of tissue vs. background 90.7% of tumor vs. other tissue components, and 85.6% of malignant vs. benign tumor elements. In the whole set, the percentage of malignant tumor elements was $8.7 \pm 7.7\%$ for benign nevi and $77.4 \pm 16.2\%$ for malignant melanoma. Based on these measurements, the correct diagnosis could be established in all cases ($chi^2 > 0.0001$).

In subsequent experiments, tissue counter analysis was also successfully applied to surface microscopy images and clinical slides, illustrating the general applicability to diagnostic problems.

O16

Multi-Dimensional Histo-Morphology in Basic and Applied Research

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Recently several techniques have revolutionized the three-dimensional imaging of tissue samples. The progress in the design of microscopes usage of appropriate light sources as well as computer equipment and peripherals inspired the development. The multidimensional methods have shown its full potential in basic and applied research in the past and lead to an unexpected renaissance of microscopical imaging techniques. Here we present a rapid segmentation and visualization procedure for histological sections which allows the full multidimensional data analysis on an acceptable time scale. Some representative samples taken from routine dermatological pathology will be discussed. Whereas usage of this technique is mainly limited to low magnification non destructive sectioning techniques can be used for high resolution studies. In particular a confocal setup using single or multi photon excitation yields high resolution information deep in the specimen minimizing alteration of the sample. A brief overview on state of the art laser scanning microscope experiments will be given showing representative examples.

O17

Laser Scanning Confocal Microscopy in Differentiation Cicatricial Pemphigoid from Bullous Pemphigoid and Epidermolysis Bullosa Acquisita

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We studied three patients fulfilling clinical and immunelectron microscopic criteria for cicatricial pemphigoid (CP), with the use of laser scanning confocal microscopy (LSCM) technique. The localization of in vivo bound IgG antibasement membrane zone antibodies (IgG-BMZAbs) in the patients' skin was compared to the localization of BMZ markers. *In vivo* bound IgG-BMZAbs were visualized by labeling with FITC-conjugated goat antihuman IgG antibody, whereas BMZ markers were labeled with appropriate TRITC-conjugated antibodies. Immunofluorescence images were overlaid by an image processing system integrated in the LSCM and photographed. Skin biopsies from patients with bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA), found positive for IgG-BMZAbs, served as controls. In all CP cases IgG-BMZAbs were *in vivo* bound to the dermal-epidermal junction between localization of laminin-1 (marker of the lowest part of lamina lucida) and collagen IV (lamina densa marker), and this corresponded to their ultrastructural localization in the upper part of lamina densa. In contrast to CP, *in vivo* bound IgG-BMZAbs in BP were localized at the epidermal side of laminin-1, whereas in EBA IgG-BMZAbs were found at the dermal side of collagen IV. Our study showed that application of LSCM allowed for precise localization of *in vivo* bound IgG-BMZAbs and thus for differentiation of CP from BP and EBA. This technique is of special value for diagnosing cases in which circulating autoantibodies are not detectable.

O18

Environmental Influences on Skin and Changes by Ageing can be Evaluated by Histometric Mesurements by Confocal Laser Scanning Microscopy *In Vivo*

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The Vivascope, a confocal laser scanning microscope, allows to study especially the epidermis *in vivo* and real-time. The system is used with a 830-nm Laser and a water-immersion objective. (a) Age related changes in skin structure were examined ($n = 10 < 25$, $10 > 60$ years). E_{min} (minimal thickness of the epidermis), the number of dermal papillae per area as indicators for the flattening of the dermal-epidermal junction and the thickness of the basal layer were measured. (b) Skin was irradiated repetitively with UVA in nonerythemal doses over a period of 4 weeks.

Parameters Index of Melanisation (MI), a quotient (brightness of the basal layer/brightness of the spinous cell layer), which represents the amount of melanin, E_{min} and DSC (the thickness of the horny layer). (c) Changes after occlusion and dryness caused by silica gel for 4 h ($n = 10$) were investigated mea-suring E_{min} and DSC and their difference (E_{min} – DSC). (d) 0.5% SDS was applied occlusively for 2 h and 4 h (dorsal side of the forearm, $n = 8$) and E_{min} was compared to water treated sites.

Results (a) With age E_{min} increased, the number of dermal papillae and the thickness of the basal layer decreased. (b) Under the influence of UVA, MI, E_{min} and DSC increased. (c) After occlusion E_{min} and DSC increased (E_{min}-DSC) did not change. The skin treated with dryness showed a decrease in E_{min} and (E_{min}-DSC) and no sign. change in DSC. (d) E_{min} increased (0.5%,2 h, 4 h after 48 h), which we would interprete as an edema. Parakeratosis could be identified (0.5%,4 h). The influences of environmental factors can be substantiated by histometric parameters *in vivo*. In our opinion, the Vivascope is a suitable instrument for basic research and routine measurement in the cosmetic science.

O19

Modern Stereological Tools in Dermatopathology: Quantification of Epidermal Langerhans Cells Using the Confocal Laser Scanning Microscope and the Disector Method

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Since Langerhans cells (LC) play an important role for the immune surveillance a certain distribution of LC as well as a specific numerical relationship to other epidermal cells (EC) can be expected. Extensive studies have been performed using two-dimensional quantification methods on vertical sections or epidermal sheet preparations. These methods were biased by the dendritic shape and the nonrandom distribution of LC resulting in contradictory and mostly non reproducible results.

To get unbiased data the 3-dimensional disector method in combination with confocal laser scanning microscopy (CLSM) has been applied to quantify the number of LC (stained by anti-CD1a) and EC nuclei (stained by propidium iodide) per volume unit in cryosections of 24 punch biopsies of breast skin of eight women. Furthermore, the ratio of LC to other EC, their total number per biopsy and per skin surface area unit were calculated. To minimize the bias by shrinkage the reference volume was estimated using Cavalieri's principle.

A constant ratio of one LC to 53 other EC was identified in breast skin (interindividual correlation coefficient: 0.952, $P < 0.0001$). LC represent 1.86% of all epidermal cells. However, a wide interindividual range was found for the number of LC per mm^2 (912–1806; mean 1394; SD ± 321) and for the corresponding number of other EC per mm^2 of 47,315–104 588 (mean 73 952; SD ± 19 426), explaining the conflicting results achieved by conventional morphometric assessments where cell numbers have been related to skin surface area, ignoring the varying thickness of the epidermis. The surprisingly constant relationship of LC to other EC stresses the hypothesis of an epidermal LC unit, where one LC seems to be responsible for the immune surveillance of 53 epidermal cells.

O20

Human Resting Langerhans Cells Constitutively Bear the Fas-L Molecule, but do not Express it on the Plasma Membrane

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Two different groups recently showed that dendritic cells (DC) having tolerogenic properties exert such an immunosuppressive potential through the expression on their plasma membrane of the CD95-ligand (Fas-L) molecule, which is able to trigger apoptosis of CD95 (Fas)-positive T cells. We therefore asked whether epidermal Langerhans cells (LC), which are DC resident within the skin, could constitutively express the Fas-L molecule. For such a purpose, we carried out an 'in situ' immunoelectronmicroscopy procedure on ultrathin frozen sections, obtained by ultra-cryomicrotomy of human normal skin specimens. A double-step method was performed, by using an antihuman-Fas-L monoclonal antibody, followed by a specific gold-conjugated antibody. All the observed LC showed gold particles within the cytoplasm, whilst this was not the case of negative control sections; nevertheless, no gold granules were visible along the LC plasma membrane. This study demonstrates that in normal human skin LC bear indeed Fas-L, which might possibly be expressed even on the surface in pathophysiological conditions, but do not constitutively express such a molecule on the membrane. The lack of constitutive expression of Fas-L on the plasma membrane of resting LC can be presumably aimed to avoiding autoreactive phenomena, e.g. apoptosis of Fas-expressing keratinocytes, in normal unstimulated epidermis, in order to maintain cutaneous homeostasis.

O21

Monocyte-Derived Dendritic Cells (MODCs) and Dermal Dendritic Cells (DDCs) Express CD1d

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The CD1 family represents an antigen-presenting molecule system for T cell recognition of lipids and glycolipids. Different from classical MHC molecules, CD1 proteins (a, b, c, d) are nonpolymorphic. CD1d-restricted T cells are suspected to play an important role in immune response to bacteria, autoimmune disorders and tumors. We investigated whether MODCs and skin antigen-presenting cells expressed CD1 molecules focusing on CD1d. Immunohistochemical studies on normal skin samples, using a panel of 9 specific monoclonal antibodies, demonstrated the expression of each of the four previously characterized human CD1 proteins. Expression of CD1a was observed in Langerhans cells, as expected, whereas DDCs were found to express CD1a, -b and -c. In addition, CD1d was expressed on numerous DDCs in the papillary dermis. CD1d expression on DDCs was further confirmed by FACS analysis using dermal cell suspensions obtained from skin biopsies. Western blot analysis on microdissected skin sections revealed the presence of a 50–55 kDa CD1d molecule in the dermis. Both immature and mature MODCs, grown in presence of autologus plasma, expressed CD1 molecules including CD1d. When autologous plasma was replaced with fetal calf serum, a downregulation of CD1d was observed on mature MODCs. In conclusion, MODCs and DDCs express different subsets of CD1 molecules and potentially present lipids and glycolipids to T cells. Preliminary studies performed in psoriasis and atopic dermatitis showed a clear-cut increase in CD1d + DDCs, suggesting a potential role of this molecule in inflammatory skin diseases.

O22

Effects of Serum on Human Langerhans Cells within Epidermis *In Vitro*

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While the factors promoting precursor development to Langerhans cells and maturation of the latter to efficient antigen presenting cells begin to be unravelled, much less is known on the requirement of specific stimuli to maintain the differentiated state of Langerhans cells within epidermis, an issue addressed by this study *in vitro*.

Epidermal laminae were isolated with EDTA from skin biopsies obtained at plastic surgery and were incubated in Dulbecco's modified minimum Eagle's medium with or without 10% fetal calf serum. The laminae were fixed with acetone or with phosphate buffered lysine-periodate-formaldehyde mixture (PLP) and immunolabeled for CD1a and major histocompatibility complex class II molecules (MHC-II). The labeled cells upon acetone fixation were counted and their surface area and perimeter length measured by computerized image analysis followed by analysis of variance, with significant $P < 0.05$.

With either immunolabeling, the number of cells per unit epidermal surface area appeared to decrease with time, significantly more without than with serum. The mean cell surface area decreased in all cultures within 48 h, then recovered significantly within 72 h only upon culture with serum. Similar results were obtained for cell perimeter length upon labeling for CD1a, whereas upon labeling for MHC-II a recovery was observed in all cultures. At confocal laser scanning microscopy, the labeling appeared at the cell surface and in small intracytoplasmic vesicles. Incubation for 48 h led to perinuclear addensation of CD1a labeled vesicles, more markedly in cultures without serum, whereas MHC-II labeled vesicles were more uniformly distributed in the cytoplasm in all cultures.

The results suggest that CD1a and MHC-II molecules behave independently of each other in Langerhans cells and that serum factors are required to maintain the differentiated state and in part the number of these cells within human epidermis.

O23

Clinical and Immunopathological Heterogeneity of Linear IgA Dermatosis in Adults

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Linear IgA dermatosis (IgALD) is defined by predominance of IgA deposits on the basement membrane zone (BMZ) by direct immunofluorescence (DIF). Recent progress in the knowledge of epitopes involved in bullous disorders suggest a disruption of this entity. We report herein a series of 15 patients presenting with exclusive IgA deposits on the BMZ (Medium age = 67). All patients have been carefully clinical evaluated using a standardized form. Circulating autoantibodies have been characterized by indirect IF and western blot on epidermal and dermal extracts. Directimmunoelectronmicroscopy (IEM) was performed in 10 patients. Eight patients had a clinical phenotype of bullous pemphigoid (BP) with cutaneous widespread bullous eruption. Seven of eight patients had circulating autoantibodies anti 180 kDa (two), anti 97 kDa (two), anti 230 kDa (one) and anti 200 kDa (one). By direct IEM deposits were located in the lamina lucida (LL) (two), under the lamina densa (LD) (one), on both sides of the LD (1).4 patients had a clinical phenotype of cicatricial pemphigoid (CP) and anti 180 or 97 kDa autoantibodies on epidermal extract. By direct IEM deposits were located on the lamina densa (2).3 patients were younger (medium age 38) and presented with widespread herpetiformis vesicular eruption. Circulating antibodies were anti 180 kDa (two) or anti 97 kDa (one). Direct IEM showed a reactivity with the LL (one) sub LD (one) or deposits on both sides of the LD.

These findings confirm the clinical and immunopathological heterogeneity of IgALD. The first group of patients correspond to genuine bullous pemphigoids with an IgA response whereas the second group correspond to cicatricial pemphigoids. The third group of patients is clearly differentiated with clinical and immunological and evolutive patterns patterns close those observed in IgALD of the infancy.

O24

Automatic Analysis of the Epidermal Silhouette of Junctional Clark's Nevus and Melanoma *In Situ*

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Although reliable histopathological criteria for the diagnosis of junctional Clark's nevus from melanoma *in situ* have been established, the significance of architectural features for the diagnosis of these lesions is still controversial.

One hundred consecutive cases of junctional melanocytic lesions were sampled (84 Clark's nevi, 16 melanoma *in situ*). Immunohistological slides stained against cytokeratin were fed into an image analysis system. After several steps of image enhancement the epidermal silhouette was discriminated automatically. Finally the thickness of the epidermis and the number of rete ridges were calculated.

Comparing the thickness of the epidermis in Clark's nevi (mean thickness, $124 \pm 27\,\mu m$) with the thickness of the epidermis in melanoma *in situ* ($137 \pm 56\,\mu m$) there was no significant difference (*t*-test: $P = 0.18$). However the number of rete ridges was significantly higher ($P = 0.001$) in Clark's nevi (mean value 8.4 ± 1.3 per mm) than in melanoma *in situ* (6.5 ± 1.6). Multivariate analysis using logistic regression taking into account age, sex, location, thickness of epidermis, and number of rete ridges, revealed thickness of epidermis, number of rete ridges and age of the patient as significant criteria predicting the diagnosis of melanoma *in situ*. The epidermal silhouette of benign and malignant junctional melanocytic lesion differs due to the altered pattern of rete ridges. These different patterns can be easily quantified by image analysis and probably reflect different biological properties.

O25

Non-Random Spatial Association of Melanoma and Naevi – A Morphometric Analysis

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Previous studies have shown that congenital as well as acquired melanocytic naevi indicate an increased risk for developing melanoma in individual patients. Most studies stress the importance of melanocytic naevi as melanoma markers, while in some studies they are considered to be direct melanoma precursors. The latter opinion is favored by the common co-occurrence of melanoma and naevus within one biopsy. The present study examines the question of whether the co-occurrence of melanoma and naevus is a random event or whether melanomas significantly colocalize with pre-existent naevi, which would suggest a precursor role for these naevi. Seven hundred biopsies of primary melanoma were examined for the presence of congenital or acquired naevi according to standard histological criteria. A naevus was found in 143 of the 700 biopsies (20.4%), of which 90 were acquired (12.9%) and 53 were congenital (7.6%). Within each biopsy the exact location of the melanoma and the naevus was determined using an ocular micrometer at a final magnification of 20 x. From these data the frequency of finding a naevus with increasing distance from the melanoma margin was calculated. The frequency of finding a naevus decreased from 2.6% immediately at the melanoma border to 0.3% at a distance of 4.5 mm and 0% at a distance of 5.0 mm. This decrease was statistically highly significant ($P < 0.001$). Similar results were obtained when congenital and acquired naevi were evaluated separately. These data strongly indicate that melanoma and naevi are nonrandomly distributed and that both congenital and acquired naevi may be precursors of melanoma.

O26

Tissue Counter Analysis of Surface Microscopy Images of Melanocytic Skin Tumors

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In tissue counter analysis a lattice of elementary measuring masks (elements) of equal size and shape is randomly placed over the image and the contents of each element is evaluated by a set of colour and texture features. In this study we tested the efficiency of tissue counter analysis in the diagnostic discrimination of benign and malignant melanocytic skin lesions in surface microscopy images. Twenty cases each of nevus and malignant melanoma were sampled. Multivariate discriminant functions were calculated derived from a learning set of 10.000 elements. Based on these functions, the proportion of benign and malignant tumor elements was assessed in each case, with 2500 elements classified per case.

The percentage of malignant tumor elements was 18.5 + 5.3% in benign nevi and 79.9 + 5.6% in malignant melanoma ($P < 0.001$). Discriminant analysis facilitated a correct diagnosis in 90% of nevi and 87.5% of malignant melanomas (sensitivity 85%, specifity 90%).

Tissue counter analysis may be a useful method for user-independent, automated diagnostic procedures in surface microscopy.

O27

Automatic Measurement of Immunhistochemically Stained Leukocytes in Adhesion Slides

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Classification and counting of immunhistochemically stained leukocytes in adhesion slides is time consuming and subjective. Thus we developed an automatic procedure to facilitate this procedure.

Measurement procedure. Automated counting of cells in the adhesion slides was performed using a KS 400 3.0 image analysis system (Zeiss Vision, Hallbergmoos, Germany). An Axioskop bright field microscope was mounted with a scanning table (Zeiss Vision) and a 3-chip digital colour video camera (Sony, Tokyo, Japan). A 20 × objective was used, yielding a final magnification of 0.66 μm per pixel. Illumination was kept constant at a mean grey value of 200 + – 4 in a white field. Each image was automatically focused, enhanced by additive shading correction and automated contrast enhancement by grey level rescaling and Delinate filtering were performed. A binary mask image was created by fixed thresholding of the red image component and subsequent binary image operations (opening, scraping of small and large objects, dilation). Based on these mask, grey level and colour parameters were assessed.

At first, a learning set was created by interactive labelling each cell as either being immunohistologically positive or negative, with a total of 254 positive and 230 negative cells in the data base.

For automated counting of positive and negative cells, each well of the adhesion slides was scanned with an appropriate meander consisting of 41 fields of 512 × 512 pixel size and a measurement frame of 450 × 540 pixels, and each cell was classified according to the learning set by multivariate linear discriminant analysis as implemented in the KS 400 3.0 program package. Number of all cells and of immunohistologically positive cells was recorded in each well, and the percentage of positive cells was calculated. In all slides the positive cells were correctly discriminated and the results were very well reproducible.

O28

Congenital Exfoliative Dermatitis, Thrichorrhexis Invaginata, and Recurrent Septicemia: A Case of Netherton-Syndrome?

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A female baby from consanguineous parents of Turkish descent exhibited from birth on a generalised exfoliative erythroderma (GEE). Fine, translucent scales were accentuated at the elbows, knees, cheeks, and scalp. Mucosal membranes and nail plates appeared normal. Also the baby developed recurrent infections, e.g. otitis media. A high serum IgE level and an aminoaciduria were detectable. Other Ig levels and T-cell functions were normal. Light microscopy of a skin biopsy revealed a moderate psoriasiforme acanthosis with lack of granular layer and a thin parakeratotic horny layer. Spongiosis in the lower epidermis was accompanied by a mild perivascular infiltrate in the dermis. Electronmicroscopy confirmed these findings demonstrating highly undifferentiated keratinocytes (e.g. low amounts of tonofilaments and keratosomes, no keratohyalin granules) which were suggestive of a severe epidermal barrier defect. At the age of 2 months hair shafts had signs of torsion nodes resembling thrichorrhexis invaginata. The skin worsened severely when adding urea and lactic acid to the topical applied emollients. There were episodes of hypernatremia that resolved after partially compensating the epidermal barrier defect with topical emollients 3–4 times a day. The baby continued to suffer from recurrent septicemia and developed a progressive hepatomegaly. Despite of maximal intensive care support the baby died at the age of three month due to multiorgan failure secondary to a candida sepsis. Our case had typical clinical, histological, and ultrastructural findings as described for Netherton-Syndrome (NS). Recurrent severe infections and fatal clinical courses are reported for NS especially when GEE is present at birth. GEE may be understood as the severest expression in a broad clinical spectrum in NS. In contrast, despite similiar skin findings the divergent clinical course in NS with GEE or mild ichthyosis linearis circumflexa Comèl may also argue for different molecular defects in NS.

O29

Necrobiotic Xanthogranuloma: Histological and Ultra-Structural Aberrations Related to a Novel Concept on Non-Langerhans-Cell-Histiocytoses

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Necrobiotic xanthogranuloma (NXG) is defined as a non-Langerhans cell histiocytosis (*n*-LCH) clinically characterized by indurated yellow plaques, atrophy, teleangiectasia and facultative ulceration. Recently, *n*-LCH were classified according to the predominant mononuclear (vacuolated, spindle-shaped, xanthomatized, scalloped, and oncocytic) and/or multinucleate (Touton, ground-glass appearance, Langhans and foreign body) histiocytic cell types. Variable mixtures produce a polymorphous or monomorphous reaction pattern.

We report on a 75-year-old female patient with NXG and multiple myeloma of the IgM-type. Marked hyperpigmentation was an additional prominent feature of our patient. Histology showed the epidermis with flattened rete ridges, a dense infiltration of xanthomatized histiocytes and giant cells of the Touton-type from the papillary to the deep reticular dermis. Focally a pauci-cellular connective tissue, lymphocytes and plasma cells could be observed. Immunohistochemistry showed histiocytes positive for CD 68 and Mac 387. Ultrastructurally the foamy histiocytes contained numerous lipid droplets and myelin bodies. Thus, adult NXG shows a polymorphous reaction pattern with a mixture of various mononuclear histiocytes. The clinicopathological and ultrastructural findings are discussed in the view of a novel concept on *n*-LCH.

O30

Structural Instability in Skin, Muscle and Nerves in a Case of Epidermolysis Bullosa Simplex due to a Mutation of Plectin: Transmission Electron Microscopic Observations

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The role of Plectin – a cytoskeleton linker protein – as an essential intermediate filament binding protein recently has been supported by reports on the development of blistering disease (Epidermolysis bullosa/EB simplex) with late onset muscular dystrophy in humans. We report on ultrastructural TEM-findings in skin, skeletal muscle, and intramuscular nerves of a now 6-year-old patient, offspring of unaffected parents with a compound heterozygous one amino-acid insertion/nonsense mutation in the plectin gene. Increased protein degradation possibly caused by increased self-assembly of plectin molecules in skin seems to result in reduced and hypoplastic formation of hemidesmosomes in the DE-BM-zone, and irregular, if not missing insertion of tonofilament bundles into the hemidesmosomes. On the other hand, skeletal muscle shows moderate fibrillar Z- and I-band alterations, at places a disoriented and "dystrophic" filament system, focally missing or defect sarcoplasmic membrane besides degenerative mitochondria, suggestive of moderate myopathic changes. Additionally, there was increased endo-and perimysial collagen proliferation and several intramuscular nerve cross sections exhibited dystrophic and/or degenerative alterations. These findings provide evidence for the central role of plectin in tissue integrity. Additionally, it suggests a role of altered innervation in the variable onset of muscular weakness in such patients.

O31

Acquired Epidermolysis Bullosa with Involvement of the Upper Intestinal Tract

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There are few literature reports about the association of an acquired epidermo-lysis bullosa (AEB) and inflammatory lesions in the gastrointestinal tract.

We report on a case of a 38-year-old white male suffering from AEB who had ubiquitious skin lesions for more than 10 years and complained about gastroenterologic symptoms for 5 years. Immunofluorescence studies of the jejunal mucosa showed linear immune deposits at the mucosal basement membrane. These deposits did not coincide with collagen VII localization neither in the affected nor in the normal intestinal mucosa. No collagen VII antibodies could be found in the patient's serum in direct immunofluorescence investigations under therapy. Electron microscopy of the skin showed typical subepidermal bullae and immune deposits. In the intestinal mucosa detachment with separation of the subbasement membrane zone in mucosal lesions could be seen without identifiable deposits.

The involvement of the intestines in the course of AEB must be kept in mind in AEB patients with abdominal complaints. Apart from collagen VII obviously other – in our case yet unidentified – autoantigens at the basement membrane are supposed to be involved in the autoimmunologic pathogenesis of this disorder. Due to the general similarity of the histopathologic bowel lesions in AEB to Crohn's disease, AEB with intestinal involvement is an additional differential diagnosis of Crohn's disease.

O32

Contact Dermatitis by Metals *Versus* Non-Metals

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Contact dermatitis (CD) by metals or nonmetals differs in antigen presentation and in the induction of proteins *in vitro*. In an *in vivo* study, we aimed to study heat shock proteins (HSP) induced by, e.g. metals, TNF-α, the adhesion molecules ICAM-1, VCAM-1 with regard to inflammation and neurohormonal markers, alpha-melanocyte-stimulating hormone (α-MSH) with respect to downregulation of the immune response, qualitatively, and the number of Merkel cells (MC) quantitatively. Positive patch test reactions at day 3 after application ($n = 8$ metals; $n = 12$ nonmetals) were characterized by avidin-biotin-complex method and the indirect immunoperoxidase technique with mAb for HSP 27, HSP 72, ICAM-1, VCAM-1, TNF-α, cytokeratin (CK) 19 and a polyclonal Ab for α-MSH applied to 10 sections of every specimen and to controls. All these proteins showed an increased staining pattern in CD, except HSP 72 and α-MSH, but a reduction of MC from a mean of 36,5 in normal back skin to 16,1 per mm^2 interfollicular epidermis in CD. Metals and nonmetals revealed no differences in labeling and only minor ones in quantification of MC (metals mean 13,5; nonmetals mean 16,9 MC per mm^2 interfollicular epidermis, not significant).

In vivo we could not confirm differences in the expression of the proteins studied or in the number of MCs between metal or nonmetals CD by means of immunohistochemistry.

O33

Preservation of Skin Structures of Bog Bodies from North Germany. An Ultrastructural Study

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Due to the conservation mummies are a good material for investigations of ancient tissues. For the first time skin samples of six bog bodies from North Germany were examined by transmission electron microscopy. Calibrated radiocarbon dating of the bodies and artifacts specified their lifetimes to between 340 BC and 400 AC. As the mummified materials had dried out and shrunk, they were rehydrated. The skin samples were fixed with glutaraldehyde (3,5%) and H_2O_2 for 90 min. The samples were then fragmented into small pieces and again fixed in glutaraldehyde (3,5%) for 24 h. A washing with cacodylate buffer for three times in 15 min and a washing with osmium for 90 min (2%) followed. Removal of materials for embalming was not necessary as bog bodies are naturally mummified. Embedding in Epoxy medium followed.

It was possible to observe the excellent preservation of the dermal structures. The epidermis was not preserved. In the dermis, the collagen fibrils formed bundles and showed the characteristic axial periodicity.The collagen fibres had a diameter of 45–110 nm. Elastic fibres were not recognized. Throughout the dermis, a number of round structures were found. They had the typical appearance of spores of bacteria. In the centre, they had an electron dense core surrounded by a spore coat.

Only the collagen fibrils resisted to the bog for a period of about 2000 years. The presence of spores of bacteria was previously reported on mummies from Egypt. Investigations performed on skin obtained from Eskimo mummies even showed the preservation of melanocytes, vessels and nerves, due to the extremely cold and dry polar weather.

O34

Eosinophilia in Biopsies of Atopic Dermatitis. A Correlation with Histopathological Parameters of Inflammation Using Quantitative Image Analysis of Immunostaining

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Extracellular tissue deposition of toxic eosinophilic granule proteins (EGPs) is crucial for the functional effect of eosinophilic granulocytes *in situ* and a marker of their complete activation. We recently described a method for the quantitative morphometric assessment of cell independent EGP-deposition by image analysis of immunostaining (IAI) using colour translation, linear combination and automatic thresholding.

In the present study, 31 lesional skin biopsies of atopic dermatitis (AD) were evaluated using this technique. With the antibodies EG1 and EG2 against EGPs, the mean as well as the maximum focal immunostained area fraction (Mean-IAF and Max-IAF) were determined. Histopathological parameters of inflammatory tissue reaction like spongiosis, epidermal hyperplasia and dermal infiltration by lymphoid cells were scored and correlated with Mean-and Max-IAF. EGP-deposition was detected by IAI in nearly all biopsies (30/31). A positive correlation between EGP-deposition and spongiosis could be shown for Max-IAF of EG1, but no correlation with the score of dermal lymphoid infiltration was found. However, epidermal hyperplasia was positively correlated with Mean-and Max-IAF of both, EG1 and EG2. These results provide further evidence by a quantitative *in situ* approach, that eosinophils are important effector cells in AD. As epidermal hyperplasia can be considered to be a histopathological parameter of chronic dermatitis, these results are another hint, that activated eosinophils may be involved in the development of chronic AD.

O35

Miner's Scars and Decorative Tattoos: Immunohistochemical and Electronmicroscopical Investigations

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A possible association between exposure to cristalline quartz and progressive systemic sclerosis is controversly discussed. Aim of our study was to describe pathological reactions of the skin of miners after exposure to quartz in comparison to pathological seen reactions of the skin after decorative tattooing. Tattoo dyes are not officially controlled, various components as for example heavy metals may be responsible for unwanted and probably chronical skin reactions.

Material and methods Skin excisates of 20 miners with macroscopical obvious incorporated dark particles, excisates of five decorative tattoos and different native tattoo dyes (red, green, blue, black) were investigated. Light and immunohistochemicaly (antibodies: CD 68. Factor VIII) the reaction pattern of the skin against different incorporated particles were described. Using scanning electronmicroscopy in combination with X-ray microanalysis the chemical nature of the incorporated particles were determined.

Results All foreign particles incorporated in the miners' skins contained coal particles in combination with varying amounts of aluminium and silicates. Activations of macrophages up to formation of granuloma-like structures were found. Silicate-rich particles were predominantly located perivascular and perifolicular. Coal particles were seen in all layers of the dermis. Reactions after decorative tattooing were weaker than after occupational incorporation of quartz containing particles.

Conclusion All alterations seen have to be interpreted as unspecific reactions of the skin.

Guest Lecture I.

Ultrastructural Telepathology – EM-Diagnostic Via Internet

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EM evidence of specific cell organelles or products (e.g. neurosecretory granules) can be essential in differentiating certain neoplasms and can assist in determining the primary site for some metastatic tumors. Other strong indications for ultrastruct. pathology are numerous renal, skin, muscle, nervous system, ciliar and storage diseases, as well as rapid viral diagnosis. In difficult cases, similar to light-histopathology, the consultation of experts is essential. It is of great importance for the expert to examine the original specimen and not to interpret at some later time a number of photographic prints of the tissue in question.

The ability for the expert to examine original samples live can now be realized by modern automated and digitally controlled EM using telepresence microscopy techniques. A live demonstration of a remotely via Internet operated digitally controlled EM at the ORNL/USA and/or the EM-Lab/Regensburg will present the possibilities of the ultrastructural telepathology in modern health care.

Guest Lecture II.

3-Dimensional Dermatopathology: A Tool and a Paradigm for the Study of Structure and Function

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3-Dimensional computer reconstruction of tissues from serial sections whether at the light or electron microscopic level provides information about structure and function that cannot be obtained from 2-D light and electron microscopy alone. *In vivo* studies such as capillary microscopy of appropriate animal and human tissues can demonstrate physiologic functions such as blood flow but it cannot show the morphologic infrastructure responsible for the real-time observed phenomena. Investigations into mechanisms underlying any physiologic function ultimately requires specific morphologic information for a complete understanding of the mechanisms. 3-D reconstruction techniques provide the essential bridge between 2-D information and physiologic function in whole tissue. As an example, this presentation will focus on the dermal microcirculation and the information on structure and function that could only have been obtained by the use of 3-D computer assisted reconstruction of the cutaneous microvasculature. The specific areas to be covered are the 3-D organization of the vessels in the dermis; the formation of endothelial cell gaps in response to inflammation; and the role of the periadventitial veil cell in health, aging, and diabetes. Each of these examples will illustrate both the efficacy of 3-D computer reconstruction techniques and how it serves as a paradigm for the study of structure and function of any given tissue.

Guest Lecture III.

Loricrin and Human Skin Diseases: Molecular Basis of Loricrin Keratodermas

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The cornified cell envelope (CE) is a tough structure formed beneath the plasma membrane of terminally differentiated keratinocytes. Recent progress in understanding the molecular organization of the CE has disclosed the complex, yet orderly structure that functions as a protective barrier against the environment.

We have recently demonstrated that two inherited skin diseases, Vohwinkel's syndrome (VS) and progressive symmetric erythrokeratoderma (PSEK) may result from mutations in the gene encoding loricrin, a major constituent of the CE. In adult human epidermis, loricrin is diffusely distributed within the superficial granular cells. In the cornified cells, loricrin is associated with CEs. In some patients with VS and PSEK skin, however, granular cells contain many intranuclear granules which are labeled with an amino-terminal loricrin antibody. CEs are thinner than normal and sparsely labeled with the loricrin antibody. Parakeratotic cornified cells contain loricrin-positive granules. Sequencing of the loricrin gene has disclosed heterozygous mutations; insertion of one nucleotide (730insG, 709insC) that shifts the reading frame in these patients. Consequently the carboxyl-terminus are replaced by highly charged missense sequences that override the endogeneous termination codon extending the protein with an additional 22 amino acids. Elucidation of the molecular biology of ''loricrin keratodermas'' adds to our understanding of the complexity and biological significance of the CE.

P1

Increased Expression of Aminopeptidase N (APN, CD13) and Neutral Endopeptidase (NEP, CD10) in Primary Malignant Melanoma

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Cell-surface peptidases are widely distributed in human tissues and function to hydrolyze peptides in the extracellular space, including peptide hormones and growth factors. They also have been implicated in the degradation and invasion of the extracellular matrix (ECM) by metastatic tumor cells. We used peptidase-specific monoclonal antibodies (mAbs) and immunohistochemical techniques to determine the expression pattern of aminopeptidase N (APN, CD13; mAb F23) and neutral endopeptidase (NEP, CD10; mAb J5) in cryostat sections from seven benign melanocytic nevi as well as 15 primary and one etastatic maligriant melanoma (MM). Benign melanocytic nevi demonstrated APN and NEP expressjon in four of seven and two of seven specimen, respectively. In contrast, APN was expressed in > 50% of tumor cells in 15 of 16 MM specjmens. NEP was expressed by > 50% of tumor cells in 15 of 16 of primary MM. These data show that expression of APN and NEP is increased in MM. Increased expression of these cell-surface peptidases presumably would either activate precursor forms of biologically active peptides to interact with MM cells or degrade inhibitory peptides. Alternatively, they could be directly jnvolved in ECM degradation and thus potentially affect MM growth and metastasis.

P2

Localization of PAF-R (RECEPTOR) in Human Epidermis, Dermis and Cell Cultures

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PAF (platelet activating factor) is a proinflammatoric, vasoactive, platelet activating and angiogenetic phospholipid in eosinophils and neutrophils of the peripheral blood. We studied the expression of PAF-R in human epidermis and dermis, outer root sheath (ORS)-keratinocytes, primary human keratinocytes and fibroblasts in culture and in embryonal cell-lines immunohistochemically.

We used a monoclonal antibody mouse IgG2a against the human PAF-R in a concentration of 1:300 and an anti-idiotypic primary antibody in controls.

PAF-R was shown in 0,2% ± 0,1 per 1000 cells in cultures of human epidermal keratinocytes. Human keratinocytes of plantar epidermis revealed 3% ± 0,5 per 1000 cells positively labeled for PAF-R. Human ORS-keratinocytes were in 0,5% ± 0,1 per 1000 cells PAF-R positive. Embryonal keratinocytes of human plantar epidermis (20. PW = pregnancy week) were negative for PAF-R, but showed in 4,2% ± 0,5 per 1000 cells PAF-R positive cells in the 21. PW. Human epidermal fibroblasts of the 23. PW labeled PAF-R positive cells in 0,6% ± 0,1 per 1000 cells. The cytoplasmic labeling of the embryonal keratinocytes and from cell lines of adults showed brown perinuclear spots. The embryonal fibroblasts and cell lines of adults presented a very fine-grained and fibrillary cytoplasmic staining pattern. In the tissues of human epidermis single cells of the basal cell layer and in the dermis endothel cells of the sweat glands and keratinocytes of the inner root sheath of an anagen hair and blood cells expressed PAF-R. In human skin PAF-R is expressed in normal, noninflammatory skin conditions.

P3

Expression of Inducible Nitric Oxide Synthase (iNOS) is Linked to Hyperproliferative Epidermal Diseases

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Previous studies from our laboratory demonstrated that nitric oxide (NO) is a pleiotropic mediator of keratinocyte growth and differentiation *in vitro*. Low or intermediate levels of NO promote keratinocyte proliferation, whereas high levels of NO arrest cell proliferation and initiate the switch to terminal differentiation.

In the present study, expression of the inducible nitric oxide synthase (iNOS) was explored *ex vivo* in several hyperproliferative epidermal diseases. Lesional skin specimens from patients with psoriasis ($n = 10$), seborrheic keratosis ($n = 5$), actinic keratosis ($n = 5$) and basal cell carcinoma ($n = 5$) were stained with monoclonal iNOS antibodies to characterize the tissue distribution of iNOS protein expression. As control, we used skin specimens from patients with atopic dermatitis ($n = 5$) and healthy volunteers ($n = 5$) as well as isotype-matched control antibodies. Immunostaining revealed high levels of iNOS protein in the proliferating compartment of psoriasis, seborrheic keratosis, actinic keratosis and basal cell carcinoma, but only weak immunoreactivity for ncNOS protein in some suprabasal and occasional basal keratinocytes. No or weak staining for iNOS could be detected in basal epidermal keratinocytes of normal human skin.

Collectively, these data suggest that expression of iNOS may represent a general feature of hyperproliferative epidermal diseases. Future studies should show whether iNOS is inappropriately activated in hyperproliferative diseases or, on the other hand, counterregulated by as yet undefined growth-stimulating signals.

P4

Extraordinary Case: Subcutaneous Manifestation of Invasive Thymoma

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Thymoma is a rare tumor entity and generally seen as a mediastinal mass. Invasive thymoma with extrathoracic manifestation is seldom and was observed in sites such as vertebrae, liver, thyroid and nervous system. A deep cutaneous and subcutaneous manifestation of invasive thymoma has not been reported yet.

A 71-year-old man was referred to our clinic with history of an amelanotic malignant melanoma at the right thigh (Breslow 1,55 mm, Clark Level IV, $pT_3\ N_0\ M_0$). Due to severe coronary artery disease with two myocardial infarctions the patient underwent multiple thoracic surgery with coronary artery bypass grafting in 1974, 1988 and 1990. In 1999 a soft, presternal subcutaneous node (diameter 5 cm) was noted. Histopathological examination of the subsequently excised tumor revealed unforeseen results: a lobulated tumor partially surrounded by a fibrous capsule; tumor parenchyma was composed of two different cell types. Epithelial neoplastic cells with a high admixture of lymphocytes. The large epithelial cell (cytokeratin +) tended to round or oval as well as polygonal configuration. The lymphocytes were dominantly small and mature (CD20 and CD3 +), some of them however, appeared polymorph with enlarged nucleus (MIB1 +). Immunohistochemical staining with HMB45 and S100 was negative. Histological diagnosis was thymoma of the mixed type. Computed tomography of the thorax demonstrated a great tumor encompassing the sternum with intrathoracic extension into the anterior mediastinum. The invasion of multiple mediastinal structures such as the lateral pericard, myocard of the right ventricle and aorta ascendens, furthermore adherence to the coronary arteries, bypass grafting and infiltration of the sternum became evident intraoperatively. Ensuing the patient received chemotherapy with 6 cycles of the ADOC scheme (doxorubicin, cisplatin, vincristine and cyclophosphamide) at 3-week interval. The therapeutical success has been indicated by the partial remission of the postoperatively remaining tumor. It remains debatable whether multiple thoracic surgery may have scattered parts of thymic tissue into the above described structures, or if a spread

P5

Incidence of Malassezia sp. In Immunodeficient Rhesus Macaques (Macaca Mulatta)

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Infection of rhesus macaques with SIV (simian immunodeficiency virus) is an established animal model for research on human HIV-induced acquired immunodeficiency syndrome (AIDS) and both diseases share a wide range of similarities. In humans, there is a prevalence of seborrhoeic dermatitis together with Malassezia sp. in HIV-patients compared to otherwise healthy persons. We describe the occurrence of skin lesions together with mycological evidence of *Malassezia* sp. in SIV-infected rhesus macaques with AIDS–like syndrome. Prevalence of yeast organisms on the skin was tested by mycological methods and histological investigations. Cultures were grown on oil-covered Sabouraud-Gentamycin-Chloramphenicol-Agar and classified on the basis of macroscopic colony appearance and microscopical yeast morphology. 37 animals were monitored throughout the infection, with eight animals showing scaly skin lesions during the course of AIDS–like syndrome. In three of these animals round budding cells typical for Malassezia could be observed and mild seborrhoeic dermatitis was detected using histological methods (H. & E., Giemsa, PAS, Grocott). In two of these cases cultures were positive. An invasion of yeast organisms into the dermis or hyphae could not be found. For the first time the occurrence of *Malassezia* sp. is described in the skin of rhesus macaques. Whether these yeasts represent a saprophyte belonging to the normal physiological flora of the skin, and what role it possibly plays during the development of immunodeficiency remains to be clarified.

P8

Subtypes of Collagen in Hypermobile Syndrome. An Ultra-Structural Analysis

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Hypermobile syndrome (HS) is a tentative term in the authors' clinic. The syndrome designs for the patients demonstrating incomplete cardinal symptoms of Ehlers Danlos Syndrome (EDS). The patients of HS showed Beighton's Score index 0–4 accompanied with a few symptoms of skin and vessels. Ultrastructurally the patients demonstrated constantly twisted collagen fibrils in dermis with variable thicknesses and disarray in the bundles. This study showed collagen subtypes I, III and V in dermis of EDS and HS. Skin specimens were prepared for immuno electron microscopy. Immuno reactants were visualised by biotinylated IgG and streptavidine gold. The gold particles for collagen types I and III were counted and calculated I/III. The ratio for EDS was less than 1.0 and 1.0–1.5 for HS, if compared to normal range of 2.0–2.5. The low ratio corresponded to thinner fibrils than 50 nm across. Twisted collagen fibrils and ratio I/III was still unclear in relation. Type V collagen was found on the dermal surfaces of vascular basal lamina and in the filamentous ground substance. It seems likely that HS and EDS can be divided by the score index 4.

P9

Collodion Baby and Ichthyosiform Erythroderma: A Report of 5 Patients

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Collodion baby describes a transient skin condition to ichthyosiform erythroderma in new-born baby. The condition develops either non bullous ichthyosiform erythro-derma or lamellar ichthyosis. Sporadically, self-healing cases have been also reported. Ultrastructural changes of keratinization may predict further development of the condition. Based upon the previous descriptions on the ultrastractural changes of ichthyosis, present paper reports ultrastructures of three female, 10, 11 and 14-day-old collodion babies, and a 1 4/3-year-old female and 25-year-old male with non bullous ichthyosiform erythroderma (NBIE). The 25-year-old male was born as collodion baby in anamnesis. Skin biopsis were prepared for routine electron microscopy. Pathological ultrastructures were found mainly in horny cells and granular cell layers. Two of three collodion babies were mature, probably with NBIE following up those patients. One was immature, born 2 weeks ago, not concluded diagnosis. The other two patients were clinically NBIE but one of them showed ultrastructure of lamellar ichthyosis.

P10

NO-Induced Modulation of Proliferation and Differentiation in Human Keratinocytes

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The hyperproliferative skin disease psoriasis has been reported to be associated with local iNOS expression. Using immunhistochemistry we have recently demonstrated that NO mediates a biphasic response on proliferation and differentiation in primary human keratinocytes in contrast to fibroblasts as demonstrated by modulation of Ki67 and cytokeratin 6 expression. With the present data, we now confirm these results on the gene expression level using reverse transcriptase-polymerase chain reaction (RT-PCR) and primers specific for proliferation (Ki67) and epidermal differentiation (involucrin). Keratinocytes and fibroblasts were incubated for up to 3 days in the presence of the NO-donor DETA/NO at concentrations ranging from 0.1 to 1 mM. The RT-PCR analysis with the Ki67 primer revealed a cell-type specific modulation of cell growth: In keratinocytes a biphasic response is found with increased proliferation at low concentrations and a decrease at concentrations of > 0.5 mM, whereas in fibroblasts Ki67 mRNA downregulation is significant at the lowest concentration of NO-donor. Involucrin-specific RT-PCR analysis revealed an increase of mRNA levels at higher NO-concentrations, confirming the NO-mediated induction of keratinocyte differentiation.

In conclusion, studying the gene expression pattern in primary human keratinocytes maintained at relatively high levels of NO-generating donors, we confirm that NO acts as an active signal for epidermal differentiation even in the presence of growth promoting substances present in the keratinocyte growth media.

P11

Collagenous Vasculopathy with Luse Bodies, an Unrecognized Form of Generalized Primary Cutaneous Telangiectasia

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Telangiectasia of skin denotes permanent dilatation of the microvasculature of the subpapillary plexus which may be a secondary or idiopathic disorder.

A 54-years-old male presented with a 5-years history of asymptomatic cutaneous telangiectases that started on the lower limbs and gradually spread to the upper extremities and trunk. Biopsies showed dilatation of the superficial dermal vessels with marked thickening due to deposition of collagen in and around the vessel walls. Immunohistochemically, the vessels lacked actin staining and showed increased matrix glycoproteins. Ultrastructurally, the vessels affected were post capillary venules which showed marked deposition of collagen around the basal lamina. In addition to regular collagen fibres and reticulin fibrils, there was a prominent component of abnormally banded long spacing collagen or Luse bodies with typical 100–150-nm periodicity. Veil cells or fibroblasts were embedded within the collagen, suggesting a source for collagen synthesis. Pericytes were sparse and lacked intracytoplasmic "contractile" filaments. This rare condition may be due to a defect in collagen formation and/or degradation, associated with deposition of abnormal collagen in the cutaneous microcirculation.

P12

Hyalinosis Cutis ET Mucosae. A Case Report

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A patient, 20-year-old male, with hyalinosis cutis et mucosae was reported. The patient showed typical signs of skin and mucous membrane for hyalinosis cutis et mucosae, however, no clinical signs were found in the eyes, central nerve system and intestine. The cultivated fibroblasts from the skin lesion showed increased secretion of collagen types III and IV, while collagen type I was reduced. Ultrastructurally, hyalin material was seen as an irregular network of 6–7 nm thick filaments. Increased amounts of basal laminae and masses of hyalin material were demonstrated in and around the vascular walls and on the surfaces of the infiltrated fibroblasts. Besides, basal laminae of perineural cells, Schwannian cells, smooth muscle cells and adipose cells were also hyalinized, while the multiplication and hyalinisation of the basal laminae in the epidermis and adnexes were not prominent. Seemingly, the pericytes and fibroblasts secreted an excess amount of collagen type IV. The formed basal lamina was altered to hyalin material in hyalinosis cutis et mucosae. Presumably, the pericytes and (myo-) fibroblasts have inherited abnormalities.

P13

Eels-Analysis Revealed Negative Metal and Mineral Evidence in GOM-Deposits in a Cadasil-Patient

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Since 1993 CADASIL (Cerebral Autosomal Dominant Arteriopathy with Sub-cortical Infarcts and Leukoencephalopathy) is defined as an autonomous CNS disease with transient or permanent ischemic strokes, dementia, and migraine. Specific osmiophilic granular material (GOM) were visualized by EM in the basal lamina of vascular smooth muscle cells of the CNS, peripheral nerve, and skin.

We report a 62-year-old woman which showed in the MRI-scan subcortical infarcts and a diffuse signal hyperintensity of the white matter. The EM revealed multiple GOM-deposites in the arterioles of the corium with a size of 0.1–1.8 μm. The deposites displayed a density gradient to the periphery of the vessels. The EELS-analysis (Electron-Energy-Loss-Spectroscopy) revealed only protein complexes without any metal or mineral evidence in the deposites examined.

P14

X-Ray Microanalysis as a Tool to Study Melanogenesis in Cultured Melanocytes and Clinical Atypical Naevi

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Two types of melanin can be recognized in human skin: the light colored pheomelanin, predominantly present in skin type I and the dark colored eumelanin mainly present in skin type VI. There are indications that in atypical naevi the melanogenesis in melanosomes is disturbed and that the pheomelanin content of these melanosomes is enhanced. Using transmission Electron Microscopy and X-ray microanalysis, pheomelanin can be detected by its sulphur signal.

Human melanocytes can be cultured *in vitro*. The type of melanin and the melanin content of the melanosmes can be modulated by changing L-tyrosine concentrations in the medium. We compared the pheomelanin content of melanosomes from skin type I, II and VI, cultured in low and high L-tyrosine concentrations with that of melanosomes in clinical atypical naevi. The results show that melanocytes cultured under low L-tyrosine conditions have a low sulphur concentration in their melanosomes, whereas the same melanocytes cultured under high L-tyrosine conditions show increased sulphur.

A relatively high sulphur signal was also obtained from clinical atypical naevi, indicating that clinical atypical naevi have an increased pheomelanin content suggesting a disturbed pheomelanogenesis.

We hypothesize that this is mainly the result of oxidative damage caused by an overproduction of H_2O_2. Furthermore the present study indicates that cultured melanocytes are a suitable model to study melanogenesis in relation to oxidative stress, as induced for example by UV radiation.

P15

Expressiongenetics of Melanoma Cell Lines: Bridging the Gap Between Tumour Characterization and Functional Analysis

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The progression of malignant melanomas (MMs) is accompanied by fundamental changes of gene expression profiles. Yet, the sca1e of changes that can be expected in MMs displaying different biological phenotypes has not been determined. Therefore, we investigated profiles of gene expression in MM-cell lines using both hybridisation to a high density E. coli colony filter carrying 27 648 human cDNA clones from the I.M.A.G.E. consortium and RNA arbitrarily primed PCR (RAP-PCR). Cell lines used were (a) isogenic MM-cells that were selected for increased tumourigenicity, (b) selected for increased invasiveness, and (c) selected for an increased metastatic potential in nude mice. With both techniques we found that regulated transcripts amount to about 20% of all expressed mRNAs. 55 genes were identified that showed differential regulation in a progression-related fashion. Whereas hybridisation to cDNA arrays offered the most rapid access to expression-profiling of heterogeneous biological phenotypes in MMs, RAP-PCR was also an efficient tool to find novel and rare partial mRNA transcript-sequences. Based on our data it can be estimated that shifts in the biological phenotype of MM towards higher malignancy affects up to 400–800 genes of the whole transcriptome. We conclude that these expressiongenetical tools help to find more potentially usefu1 molecular markers for diagnosis and prognosis and provide the basis for further functional analyses of genes that may be relevant for tumor progression.

P16

Confocal Laser Scanning Microscopy a Suitable Tool for *In Vivo* Measurements of Epidermal Structures

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The so called VivaScope™1000 provides the user the ability to observe structures of the Epidermis in confocal mode, without any harming of the investigated tissue. In our research, we did investigations, e.g. about the size of capallary diameter and thickness of different epidermal layer types. The different look of the keratinocytes caused by keratinization, will be also shown by focussing from stratum corneum to the papillary dermis, as the flow of erythrocytes and blood plasma through the capillaries.

Objective a.) The effect of UVA-and UVB-radiation in skin was examined in a study with 14 volunteers. b.) The density of melanin were measured after SDS treatment at three volunteers, with help of the so called index of melanisation. This index is the quotient between the intensity of basal and the neighboring spinous layer. c.) Flattening of Papillas caused a decrease in papilla density. Two different age collectives were investigated to detain a age influence. d.) Measurement of minimal epidermis thickness should change after treatment with different SDS-Solutions, caused by spongiosm.

Results a.) The UV-exposition induced a significant thickening of str. corneum. The UVA radiation causes a faster thickening as the UVB does, but this will be equalized after a longer period of treatment. b.) The index of melanisation shows an increase between treated compared to untreated areas. c.) The results showed a decrease in density with increasing age. d.) As shown in former histological examinations, we could see in most times a signficant increase in epidermal thickness, depending on duration and dosage.

Conclusion Together with the easy handling, the device is quite usefull for dermatological research to make examinations about skin conditions depending on cosmetic treatments, volunteer ages or special questions.