Abstracts for the Society for Cutaneous Ultrastructure Research (SCUR) 
22nd Annual Meeting 
Heidelberg, June 23–24, 1995

Sessions

<table>
<thead>
<tr>
<th>Session 1:</th>
<th>Structure and Function</th>
</tr>
</thead>
</table>
| Session 2: | Guest Lecture: K. Beyreuther 
Alzheimer’s disease: molecular and cellular aspects |
| Session 3: | Dermatopathology Club |
| Session 4: | Workshop: Diagnostic Electron Microscopy |
| Session 5: | Keratins and Keratinization |
| Session 6: | Guest Lecture: B. Dobberstein 
Protein translocation across the membrane of the endoplasmic reticulum |
| Session 7: | Special Lecture: G. von Hagens 
Whole body sheet plastination for topographical studies in macroscopy and histology |
| Session 8: | Special Techniques |
| Session 9: | Junctions and Adhesion Molecules |

Abstract Numbers

1–5
6–12, P1–P6
13–30
21–26, P8
27–29, P9–P10
30–34, P11–P12

Congress Honorary President
Detlef Petzoldt, Heidelberg

Scientific Program Committee
Ingrid Anton-Lamprecht, Heidelberg
Ingrid Hauser, Heidelberg
Jean Kanitakis, Lyon
Hans-Joachim Schulze, Cologne

Congress Secretariat
Wiebke Tubbesing, Heidelberg

Organizing Committee
Ingrid Anton-Lamprecht, Heidelberg
Marie-Luise Arnold, Heidelberg
Ingrid Hauser, Heidelberg
Hans-Joachim Schulze, Cologne

Three prizes for the best oral and poster presentations at the Annual Meeting of the SCUR 1995 in Heidelberg were awarded to: Giorgio Pasolini (oral presentation no. 7), Andreas Jäckel (oral presentation no. 23), and Ulrike Ebschner (poster no. 12) and their coworkers.

We greatly acknowledge the sponsorship of the industry that helped in realizing this meeting:

Financial Support: Dako Diagnostica, Hamburg 
Fumedica, Wiesbaden 
Hoechst, Frankfurt/Main 
Lederle, Wolfratshausen 
Marion Merrell-Dow, Berlin 
Phillips, Eindhoven 
Widmer, Rheinfelden

Essex Pharma, München 
Glaxo, Hamburg 
Hoffmann-La Roche, Grenzach-Wyhlen 
Leica, Stuttgart 
Olympus Optical Europe, Hamburg 
Sandoz, Nürnberg 
Zeiss, Oberkochen

Congress Maps: Boehringer, Mannheim

0022-202X/96/$10.50 • Copyright © 1996 by The Society for Investigative Dermatology, Inc.
PROTEIN TRANSLOCATION ACROSS THE MEMBRANE OF THE ENDOPLASMIC RETICULUM. Bernhard Dobberstein, Zentrum für Molekulare Biologie (ZMBH), Universität Heidelberg, Germany.

Most secretory proteins in mammalian cells are translocated cotranslationally across the membrane of the endoplasmic reticulum (ER). This process can be divided into 1) the targeting of the nascent polypeptide to the ER membrane and 2) the actual translocation across the membrane. Targeting involves the signal sequence on the nascent polypeptide, cytosolic (signal recognition particle (SRP) and membrane integrated components (SRP-receptor). Three GTPases regulate the different stages of targeting and docking of the ribosome / nascent chain complex to the ER membrane. Translocation of the nascent polypeptide across the ER membrane occurs through a protein conducting channel which is open laterally towards the lipid bilayer. Such a channel explains also how membrane proteins are inserted into the ER membrane.

Targeting and translocation has been studied in an in vitro system. Special focus will be on the role of SRP in protein targeting and the molecular architecture of the protein conducting channel as revealed by site specific photo-crosslinking.

1 NEUROFILAMENT IMMUNOREACTIVITY IN NAHMALIAN MERKEL CELLS - A LIGHT MICROSCOPIC IMMUNOHISTOLOGIE AND ELECTRON MICROSCOPIC IMMUNOHISTOCHEMISTRY STUDY. Wolfgang Hartschuh1, Ulman Schu2, Ursula Kersz1, Andreas Jaegel1, Eberhard Weih1, Universitätsklinikum, Universität Heidelberg, Pathologisches Institut, Klinikum Heilbronn der Universität, Heidelberg1, Anatomisches Institut, Universität Marburg1.

Neurofilament (NF) neurofilaments (NF) previously have been described by others in human Merkel cells (MC) of plantar skin by light microscopic (LM) immunocytochemistry (IC) using epithelial sheet preparations. The aim of this study was to contribute more precisely to the cellular and subcellular localization of NF in MC from various species applying LM- and electron microscopic (EM)-IC. A monoclonal antibody directed against 70, 160 and 210 KD NF proteins and a polyclonal NF-antiserum were used for LM-IC on deparaffinized sections of aldehyde-fixed human, pig and ex-vivo rat skin. Part of the sections were treated by microwave prior to incubation. Visualization of the IC was performed by a nickel-enhanced diaminobenzidine reaction. For preembedding EM-IC the immunogold technique was applied. LM-IC revealed that human and porcine MC were weakly NF-IR. The signals were enhanced by microwave pretreatment. MC from rat sinus hair follicles were heavily stained for NF. EM-IC exhibited an intense labeling of wavy filaments of MC, mainly in the periphery of the cells. In numerous studies NF-IR has been found in Merkel cell carcinomas (MCC) but not in MC. We have confirmed a recent report that MC are NF-IR and were able to demonstrate for the first time at the ultrastructural level that NF-IR is bound to MC filaments. These findings might be an important clue that MCC are derived from MC although differences in the molecular patterns of NF proteins are likely to exist.

3 IMPAIRED SYNTHESIS OF TYPE I AND TYPE III COLLAGEN IN JUVENILE HYALINE FIBROMATOSIS. Friedrich Breier, Susanne Fang-Kirchner, Wolfgang Jurecka. Department of Dermatology and Institute of Medical Chemistry, University of Vienna, Medical School.

A 14 months old infant presented with the clinical diagnosis of juvenile hyaline fibromatosis. This condition has been considered as a result of disturbed metabolism of ground substance and/or type VI collagen. As skin harbours mainly type I and type III collagen, morphological and biochemical investigations of these collagens have been performed. Dermatopathology revealed numerous epithelial cysts in the reticular dermis embedded in a homogenous connective tissue. By electron microscopy the connective tissue cells showed a gyrated nucleus and a highly developed Golgi apparatus. Extracellularly and within the cells a membrane-bound finely fibrillar material could be seen. Examination of supernatants of control-matched fibroblast cultures by determining collagen type I and III metabolism detected an increased metabolism of type I and a 50 % decreased type III metabolism indicating a crucial role of these collagens in the pathogenesis of juvenile hyaline fibromatosis.

WHOLE BODY SHEET PLASTINATION FOR TOPOGRAPHICAL STUDIES IN MACROSCOPY AND HISTOLOGY. Günther von Hagens, Anatomisches Institut I, Universität Heidelberg. INF 207, 69120 heidelberg.

"Plastination" is a method of preserving putableb species. It is a vacuum process in which biological specimens are impregnated with a reactive polymer like silicone rubber, epoxy or polyester resin. Sheet Plastination is a special application of Plastination. Organs are cut with a meat slicer, body species are cut with a band saw in 2-4 mm. Whole body slices require extensive machinery and auxiliaries such as a high speed band saw with a cooled guide and a vacuum system. Plastination is a time-consuming process during dehydration and impregnation. After impregnation the slices are cured between foils or glass plates or are casted with additional resin in a flat chamber composed of glass plates. Transparent body slices are superior to opaque slices like plastic sheets for detailed inspection at the magnification of up to 100 x is possible. The region of interest can be inspected thoroughly by simply adjusting the microscope through a sequence of focal planes. In this way structures can be followed in its course in one or in several serial slices. The area of interest can be cut out, glued upon a wooden block using hot melt glue and cut into thin sections of 4-10 microns for histological purposes as done with other plastic embedded tissue. Fixed macroscopic slices as well as plastic sections processed thereof can be stored.

Whole body sheet plastination offers the possibility to study in one large and in successive large slices all tissues of functional and pathological units, such as skin with infiltrative tumorous growth.

Electron microscopy from plastinated slices of animals requeues perfusion fixation and freeze substitution by shock freezing in order to avoid visible autolysis and ice crystal formation.


Next International Meeting: 8th International Conference on Plastination, July 1996, Department of Anatomy, University of Queensland, Brisbane, Australia.

2 ULTRASTRUCTURAL ABERRATIONS OF DERMAL CONNECTIVE TISSUE COMPONENTS IN PATIENTS WITH SPONDOANEUROARTERIAL DISECTIONS. Ilja Haustmann, T. Brandt, M. Müller-Küppers, E. Oboh, H. Anton-Lamprecht1 Institut für Ultraschallforschung der Haut1, Universitäts-Hautklinik, und Neurologische Universitätsklinik1, Heidelberg, FRG.

Heritable disorders of connective tissue may be accompanied by neurovascular manifestations, i.e. carotid-cavernous fistulae, intracranial aneurysms, cerebral ischaemia and arterial dissections. In a small minority of patients with neurovascular diseases, heritable connective tissue disorders (HCTD) are recognized, classically Ehlers-Danlos-Syndrom (EDS), Marfan-Syndrom, pseudoxanthoma elasticum and osteogenesis imperfecta. Cervico-cerebral dissections are an increasingly recognized etiology of younger stroke patients, and therefore vice versa the question arises, if correlation exists between cervico-cerebral dissections and HCTD abnormalities of the extracellular matrix (ECM). In a first approach, skin biopsies of 8 young and middle-aged patients with spontaneous dissections of vertebral arteries were investigated for the morphology of dermal connective tissue components. None of them showed clinical skin, joint or skeletal abnormalities of the HCTD mentioned above. 5 of them, however, revealed more or less pronounced but regular ultrastructural abnormalities in middle and deep dermal regions with collagen bundles containing composite fibrils and signs of elastic fibre degeneration. In two cases, the abnormalities were even comparable to EDS type II. The role of pathologically altered ECM-components in the etiology of arterial dissections is discussed.

4 COMPARATIVE STUDY OF DUPYRTREN TISSUE AND HEALTHY PALMAR APONEUROSIS CONDUCTED WITH SCANNING ELECTRON MICROSCOPE AND MICROANALYSIS. Stefano Calvieri, Alfredo Rossi, Marcello Pozzi, Paolo Bonacorsi, Nicola Scuderi. Department of Dermatology, University of Rome "La Sapienza".

Dupuytren's contraction, together with plantar fibromatosis and Peyronie disease, constitutes a small group of superficial, collagenous fibromatoses whose aetiology and pathogenesis are unknown. The condition involves irregular or nodular subcutaneous thickening of the palm, the fascia either unilaterally or bilaterally. Over time, attachment to the underlying skin causes puckering and dimpling, resulting in a slowly progressive flexion contracture, mainly of the fourth and fifth fingers of the hand. The aim of the present study was to differentiate the Dupuytren tissue from healthy palmar aponeurosis on the basis of their morphology and trace element composition. To this end, tissue from 5 Dupuytren patients and 3 healthy controls was subjected to microscopic and microanalytical examination. Results showed that the affected tissue has a higher calcium content than healthy palmar aponeurosis. There were also differences between cords and nodules, which, as known, represent different stages in the evolution of the pathology. In fact, we found a high sulphur content in cords, and a high silicon content in nodules. The authors are reporting these results because of the help they may provide in understanding the pathogenesis of the disease. Moreover, in the future and with the support of further investigations, this technique could be used to assess the degree of therapy.
5 SPERM TAIL DISTURBANCES AS A MAJOR CAUSE OF MALE SUBFERTILITY. G. Haid, K.-J. Friedrich, M. Uerich, H.-W. Kreysel. Department of Dermatology, Univ. of Bonn, Germany.

Sperm motility is one of the most important prognostic factors for male fertility. One frequent cause of impaired motility is a particular type of sperm tail disturbances. On the light microscopic level, such flagella are characterized by poor outline, varying diameter and/or missing endpiece. Transmission electron microscopic investigations revealed poorly developed outer dense fibers as a major cause of these alterations which was particularly confirmed by means of negative staining technique. Fibrous sheath (FS) and outer dense fibers (ODF) are substructures of sperm tails that are involved in the regulation of sperm motility. For clarification of the cause of defective ODF their biochemical properties were studied. On SDF-PAGE, polypeptides with apparent molecular masses of about 90, 55, 50, 34, and 30 kDa were found. As ODF are locked upon as resilient, elastic structures, immunogold electron microscopy for demonstration of cytokeratin was performed. Preliminary results indicate that cytokeratin 8 probably represents a major component of FS and ODF. Exact characterization of these substructures is of high importance for further clarification of sperm motility disorders due to defective ODF.

7 THE EXPRESSIOIN OF CD36 BY LANGERHANS CELLS AND LANGERHANS CELL-LIKE DENDRITIC CELLS EXHIBITS PHENOTYPIC PLASTICITY. AN "IN SITU" IMMUNOELECTRON MICROSCOPY STUDY OF NORMAL HUMAN SKIN. Giorgio Pasolini, Michele A. Menin3a, Antonietta Leon1, Antonio Lazzara1, Giuseppe De Pastis2. Dept. of Dermatology, and 1Istituto Zoopatologico, Brescia, Italy; 2Dep. of Dermatology, Leiden, The Netherlands; 3Dalhousie University, Halifax, NS, Canada.

Langerhans cells and Langerhans cell-like dendritic cells (LC/DC) are known to express the CD36 molecule in some cutaneous pathological conditions, such as atopic dermatitis and mycosis fungoides, and during the LC/DC repopulation phase in the period of immunologic reconstitution after allergenic bone marrow transplantation. On the other hand, the possible constitutive expression of CD36 by LC/DC in normal human skin has to date not been definitely assessed, especially due to the lack of precise identification, at the single cell level, of LC/DC in routine immunocytochemical sections or in FACS analyses. The aim of the present study was therefore to detect the constitutive presence of CD36 molecules in LC/DC within normal human skin, using the "in situ" ultrastructural immunoelectron microscopy technique, permitting both to reveal even minimal antigenic amounts and to reflect the "in vivo" situation. In normal human skin LC/DC did not show any gold granule along the plasma membrane, although showing gold labelling in some intracytoplasmic organelles. Such results suggest that LC/DC, although not constitutively expressing CD36 on the cell surface in resting skin, may be equipped to express the CD36 molecule on the plasma membrane if appropriately stimulated, as shown in culture conditions and in pathological states. In conclusion, we favor the hypothesis that LC/DC exhibit for the CD36 molecule some phenotypic plasticity, which can be deeply influenced by the cutaneous microenvironment.

9 DERMAL MYOFIBROBLASTS REACT WITH ANTIBODIES TO FACTOR XIIIa, MYOFIBROBLAST AND VIMEMENTIN. Takaki Kobayashi, Tommy Karlmark. Dept. of Dermatology, University of Copenhagen. Rigshospitalet.

Histioctic cells in the dermis have been found to react with an antibody to factor XIIIa. These cells have been recognized in dermal histiocytoma and juvenile xanthogranuloma, and tentatively called dermal dendrocytes (1). However, the nature of dendrocytes is obscure. Biopsy specimens of scleroderma, granulation tissue of leg ulcer and cultured human skin pieces were fixed in 2% glutaraldehyde-0.3% glutaredyldehyde in PBS and embedded in Technovit 7100. The authors have produced an antibody to myofibroblasts from human dermal fibroblasts in vitro (2). This antibody as well as antibodies to factor XIIIa (Calbiopham) and vimentin (DAKO) were used for immune staining in living and electron microscopy. For detecting, histiolyzed IgG and streptavidin-peroxidase or -gold techniques were used. Dermal dendrocytes reacted with those three antibodies. It seems that the factor XIIIa positive dermal dendrocytes are myofibroblasts.

6 TRANSIENT BULLOUS DERMOLYSIS OF THE NEWBORN. J. Kanitakis1, C. Leguille1, M. PJeux1, H. Roger1, P. Dechelotte1, P. Souteyrand1. (1) Dept. of Dermatology, Ed. Herriot Hospital, Lyon (2) Dept. of Dermatology and (3) Pathology, Hôp. Hôtel-Dieu, Clermont-Ferrand, France.

Transient bullous dermolysis of the newborn (TBDN) is a rare disease first described in 1989 of which up to now less than 20 cases have been reported. Our patient was a female baby born to non-consanguineous parents. At birth she presented a bullous eruption initially localized on the limbs that extended over the next days to areas subjected to friction; neither mucous membrane nor nail lesions were present and growth of the baby was uneventful. Light microscopic examination of a lesion showed a subepidermal blister containing, along its roof, the main dermal-epidermal junction antigens. Electron microscopy showed a cleavage occurring below the lamina densa, to which remnants of anchoring fibrils were attached. Basal keratinocytes exhibited cytoplasmic vacuoles containing a fibrillogranular material of medium electron density, also seen (although to a smaller extent) within adjacent suprabasal keratinocytes. Within one month all lesions had disappeared spontaneously; at three months age some residual milia were observed over previously blistering areas. No relapse has been noted for a follow-up period of two years. TBDN is a rare (although probably under-reported) congenital bullous disease very similar to dystrophic epidermolysis bullosa (DEB), it occurs within families with an unnoticeable history or in association with dominant or recessive DEB. It is known that the DEB fraction of its rapid spontaneous regression and the presence, within basal keratinocytes, of distinct vacuoles containing type VII collagen, seen ultrastructurally as fibrillogranular material.

8 DIFFERENTIATION OF THE SPECIFIC CELLS IN LANGERHANS CELLS HISTIOCYTOSIS. H.-J. Schulze, K. Barlag*, G. Mahrie. Dept. of Dermatology, Univ. of Köln and *Düsseldorf, Germany.

Langerhans cell histiocytosis represents a clinical spectrum of diseases and includes intermediate and poorly elucidated forms. We present a 19-year-old Turkish woman who manifested a diabetes insipidus at the age of 14. Neurosurgery had shown an intrasellar tumor diagnosed as hamartoma, 4 years later she developed gingival hypertrophy with ulceration and bleeding on account of which all teeth were extracted. Because of embarrassment she did not indicate the granulomatous ulceration of the vulva.

Biopsy of the persistent hyperplastic gingiva showed clusters and sheets of large acid cells with abundant eosinophilic cytoplasm, pseudotrabeculae, stromal myxomatous stroma, and an intimated, eccentric nucleus, a variable admixture of lymphocytes and a few eosinophils. The tumor cells were lysosyme-positive and expressed CD4 and S100 protein, however, there appeared to be some immunophenotypic heterogeneity. Contrasting with the immunophenotype, only approximately 20% of the tumor cells contained Birbeck granules. Some of these cells also possessed concentrically laminated dense core bodies, a finding that has been proposed as specific for congenital self-healing histiocytosis.

Reevaluation of the ptirous lesion revealed similar findings and confirmed the diagnosis of Langerhans cell histiocytosis.

10 ULTRASTRUCTURAL STUDY ON THE PATHOGENESIS OF PURPURA FULMINANS WITH EVIDENCE ON A BACTERIAL "FACTOR" AFFECTING ENDOTHELIAL CELLS. Wolfgang H. Mueh, Ines Keller. Institute of Pathological Anatomy & Department of Dermatology, St. Johannis-Spital, LKA Salzburg, Austria.

In a case of purpura fulminans due to meningococci, sequential evaluation not only of routine parameters but also of skin biopsies taken for TEM examination (day 0 of admission before treatment and day 3) was performed. In addition, specimens of the patient's blood culture, positive for meningococci, were processed for TEM. Two different types of fixatives were used and compared.

LM (semithin sections) and correlative TEM revealed subepidermal and vascular destruction reported to be typical of acute bacterial sepsis. Moreover, a close relationship of an electron dense, "shed" material (lipoproteinaceous-polysaccharide in nature) produced by circulating (as well as cultured) meningococci to "blobs" of endothelial cells could be demonstrated. The occurrence of such a material seems to be fixation dependent.

We suggest this material to be - at least part of the "meningococcal lipopolysaccharide fraction" interacting with and inducing necrosis of endothelial cells which in fact is one major property of vessel structure in purpura fulminans.
11 INVOLVEMENT OF ECCRINE AND APocrine SWEAT GLANDS IN NEUROPHILIC HidRADENITIS INDUCED BY CHEMOTHERAPY. Sonia Reilmann, Randolf Brehler, Gisela Bomsann, Thomas Luhr, Dieter Metze, Dept. Dermatol., Univ. Münster, Germany.

Neutrophilic eccrine hidradenitis is a reversible inflammatory dermatosis induced by chemotherapy treatment of malignant diseases. We report on a case of a 43 year old patient treated with cytarabine, daunorubicin and mitoxantrone for acute myelogenous leukemia who developed painful, red nodules in both axillae on the third day of chemotherapy. The lesions healed spontaneously and recurred once when chemotherapy was continued. Biopsies were examined histologically, ultrastructurally and immunohistochemically using antibodies specific for CEA, EMA, S100, keratin, α-smooth muscle actin and phenotypic markers. A nick-end labeling technique was applied to evaluate apoptotic cell death. Single lobules of sweat glands showed necrosis of secretory epithelia without evidence of apoptosis. Immunohistochemical staining suggested involvement of both eccrine and apocrine glands. Neutrophils, histiocytes and lymphocytes of T and B cell type surrounded and infiltrated the sweat glands. Ultrastructural examination revealed signs of severe cellular degeneration of the secretory epithelia and coiled ducts of eccrine sweat glands. Furthermore, a prominent necrosis of apocrine sweat glands was evident. However, myoepithelial cells remained intact. In conclusion, neutrophilic hidradenitis has been demonstrated to be associated with necrosis of both eccrine and apocrine sweat glands presumably caused by processing of cytotoxic drugs. Uninvolved of distal ducts and myoepithelial cells may account for rapid regeneration of the glandular structures after discontinuation of chemotherapy.

13 ULTRASTRUCTURAL RECOGNITION OF CHILDHOOD NEURODEGENERATIVE DISEASES IN SKIN BIOPSY SPECIMENS. Hans Hilmar Goebel, Irene Wall, Division of Neuropathology, Johannes-Gutenberg University, Mainz, Germany.

There are certain neurometabolitic and neurodegenerative diseases in children which require electron microscopic documentation or confirmation for precise diagnostic recognition. The cytological wealth of the skin and the frequent morphological manifestation of these primarily brain-based conditions render the skin an easily accessible and most useful biopsy target, in addition to the possibility to culture dermal fibroblasts for eventual genetic and biochemical studies. Basically, these disorders are classified as lysosomal diseases and certain non-lysosomal diseases, the latter comprising infantile neuroaxonal dystrophy, giant axonal neurophy, Laffo disease, and, foremost, the neuronal ceroid-lipofuscinoses. Occasionally, even skin lesions lends itself to such a diagnostic approach. Many, but not all lysosomal disorders can be confirmed ultrastructurally in biopsied skin, especially those of a vacuolar lysosomal type, but others as mucolipidosis type-IV, Niemann-Pick disease, and Faber disease as well. Thus, skin biopsy is now an indispensable procedure in the diagnostic regimen to recognize certain neurodegenerative diseases in children.

15 DIAGNOSTIC ELEcTRON MICROsCOpy: ULTRAstructural MARKERS OF SELECTED HERITABLE CONNECTIVE TISSUE DISORDERS. J. Hauser, Institut für Ultraschalluforschung der Haut, Universitäts-Hautklinik, Heidelberg, FRG.

While increasing numbers of different molecules are revealed to contribute to the extracellular matrix of connective tissue and biomolecular defects underlying the heterogeneous group of heritable connective tissue disorders (HCTD) are continuously elucidated, there are still classical HCTDs eluding their molecular deciphering. For HCTDs affecting the skin, electron microscopic investigation of the dermis may, as a preparatory work, give at least hints for diagnosis and classification: Ehlers-Danlos syndrome types I-III are defined by differential collagen alterations; various cutis laxa and some premature aging syndromes are characterized by distinct aberrant deposition of elastic fiber components; pseudoachondroplasia elastica can be diagnosed by typical elastin and collagen changes even in individuals with minimal skin involvement and in preclinical stages; hyaline basophilic etheroskeletal (M.Ulrich-Werthe, lipoid proteinosis) in newborns revealing blistering is delineated from bullous diseases by the deposition of hyaline material around basal laminae of vessels and appendages and at the dermo-epidermal junction. The identification of ultrastructural dermal connective tissue aberrations could give clues to the identification of the molecular defects.

16 TWISTED SHAPES OF COLLAGEN FIBRILS. SIGNIFICANCE FOR DIAGNOSIS OF EHLERS-DANLOS SYNDROME (EDS). Takasi Kobayasi, Susanne Ullman, Dept. of Dermatology. Univ. of Copenhagen, Rigshospital.

Twisted shapes of collagen fibrils are stigmata for Ehlers-Danlos syndrome. These characteristic shapes were common among clinical variants of EDS (Clin Gen 25 477 1984). The study has been proceeded to evaluate the stigma for diagnostic purposes. Eighty-six EDS patients, solitary and familiar cases, and normal family members were studied by routine electron microscopy. All the patients have manifised symptoms of skin and joints, some having complications of eyes, teeth, heart, aorta, lung and colon, and in pregnancy. The twisted collagen fibrils were divided in 6 groups by the intensities, forms, and distribution. All the patients had shapes of collagen fibrils. Forty-nine of 86 cases showed mild twisted shapes. Various twisted shapes were insignificant for differentiating clinical variants. Some of the normal family members also had the twisted shapes. EDS could be separated from other heritable connective tissue disorders, if elastic fiber changes were taken into consideration. However, Marfan syndrome and hypermobility syndromes in some families were sometimes be confused with EDS by their dubious twisted shapes of collagen fibrils. Seemingly, the twisted collagen fibrils are useful criteria for diagnosis of EDS.
17

DIAGNOSTIC ELECTRON MICROSCOPY: ULTRASTRUCTURAL MARKERS OF INHERITED ICHthyoses
M.L. Arnold, Institute for Ultrasound Research of the Skin, Department of Dermatology, Heidelberg, FRG

Inherited keratinization disorders are a heterogeneous group of diseases with a broad spectrum of clinical manifestation, severity, and recessive or dominant transmission. Systematic ultrasound investigations of large series of patients have led to the delineation of distinct markers that permit identification of the various genetic types of ichthyoses independent from age and possible therapy. Based on these ultrastuctural markers, diagnostic electron microscopy is performed in Heidelberg since about 1978. In the recessive ichthyosis congenita group these markers concern deposition of different lipid entities. Inhibitable during terminal differentiation (lipid droplets in horn cells - type I; cholesterol clefts in horn cells - type II; abnormal keratinosomes, vesicular complexes, and elongated membranes in granular and horn cells - type III; lenticular membrane aggregations in granular and horn cells - type IV). In ichthyosis congenita gravis (Harlequin ichthyosis) the basic defect concerns a specific abnormality of keratinosomes and their accumulation in the horny layer. In dominant type mutations genes directly affect structural proteins, i.e. keratinolysin in autosomal dominant ichthyosis vulgaris, the keratin cytoskeleton in epidermolytic hyperkeratosis, ichthyosis hyaluronic and congenital reticular ichthyosiform erythroderma, ichthyosis in Los Angeles, and in epidermolytic ichthyosiform discolabs. Electron microscopy is inevitably required for a reliable diagnosis of inherited ichthyoses, for genetic counselling, and as a basis for identification of the underlying gene mutations.

19

ICHthyOSIS CONGENITA TYPE III: REPORT OF A CASE
A. Brusasco, S. Camozzi, L. Restani, G. Tadini, Institute of Dermatological Sciences, University of Milan, Italy

A 31 year old man was referred to our department with a diagnosis of generalized Epidermolytic Hyperkeratosis (EH) based on a skin biopsy performed when he was 18. The specimen showed a marked hyperkeratosis with chilblain-like aspects, but no true focal of epidermolysis were present. The clinical features were also not typical of EH and suggested the diagnosis of non-erythodermic ichthyosis congenita, with an impressive reticulated skin pattern. Thus, we performed a new punch biopsy for the ultrastructural examination. The horny layer was composed by 30-40 layers of corneocytes containing several membrane structures and keratinoosomes debris, and in the stratum granulosum membrane structures and membrane-bound vacuoles and vesicular keratinoosomes, often aggregated, were observed. These typical ultrastructural criteria allowed us to confirm our suspected diagnosis of ichthyosis congenita type III. This diagnosis dramatically changed the life of the patient, who, according to previous genetic counselling had decided not to have children. This case is a further example of the reliability of the ultrastuctural markers in the diagnosis of keratinization disorders.

20

DIAGNOSTIC ELECTRON MICROSCOPY: EPIDERMOLYSIS BULLOSA
Jasmin Anton-Lamprecht, Institute for Ultrasound Research of the Skin, Department of Dermatology, University of Heidelberg, Germany

Based on systematic ultrastuctural studies of large series of EB patients, diagnostic EM is routinely performed in Heidelberg since nearly 20 years. For diagnostic purposes it is important to distinguish between unaggregated and secondary changes, first encountered in biopsy samples, and the disease-specific, genotype-dependent basic abnormalities and pathomorphogenetic patterns that identify the individual EB subtype.

Recent molecular genetic analyses have confirmed our previously proposed classification of the three major EB groups with intraepidermal, junctional, and dermal dystrophic formation as a natural, biologically based classification. The specific planes of blister formation result from structural and molecular defects of three major target structures common to the subtypes of either group: keratins (EB simplex and allied disorders), hemidesmosomes and anchoring filaments (EB atrophicans group), and anchoring fibrils (EB dystrophica group). For diagnostic purposes it is not sufficient to determine the plane of blistering, that is merely group-specific, but to check for the specific basal defects to identify the respective genetic subtype sufficiently reliable to ensure correct diagnosis and genetic counselling, as well as to indicate the most promising candidate genes for future molecular genetic attempts in the patients and their families.

The markers of the specific abnormalities of the most important EB types shall be outlined. Moreover, pitfalls in the interpretation of secondary changes often present in older lesions due to remodelling processes, artefactual damage, and selection of optimal biopsy sites for diagnostic EM are to be discussed.

21

TRANSGENIC AND FOCAL PALMOPRANT KERATODERMA: EVIDENCE FOR KERATIN FILAMENT ABNORMALITIES USING IMMUNOGOLD ELECTRON MICROSCOPY
J. Spence, C. Strooman, J.R. McMillan, R.C. Ramamurthy, J.P. Stearns, L.J. Leigh, IMA Eady, 1St John's Institute of Dermatology, St Thomas' Hospital, London; 2-Experimental Dermatology Laboratory, London Hospital Medical College, London, U.K. 3-German Cancer Research Centre, Heidelberg, Germany

Previously, keratin 9 gene mutations were described in patients with the Voerker form of palmoplantar keratoderm (PPK). In other forms of heritable PPK the underlying defect remains to be defined. To see whether keratins represent candidate proteins in some of these disorders we performed an ultrastructural study including postembedding immunogold electron microscopy. The autosomal dominantly inherited transgenetic (n=3), diffuse (n=4), and focal (n=3) forms of PPK were investigated. By light microscopy signs of epidermolytic hyperkeratosis were present in all three forms of PPK. Ultrastructurally abnormal keratin filament aggregates were observed in suprabasal cells of lesional ridged epidermis. Differences in the ultrastructural appearance of keratin aggregates were noted in the transgenetic and diffuse form and less pronounced between the diffuse and focal form of PPK. Keratin aggregates labelled with several anti-keratin antibodies including A11 (K9), KA12 (K6), and LL025 (K16). This study shows that ultrastructural changes of epidermolytic hyperkeratosis are not only present in diffuse PPK but may also be seen in the transgenetic and the focal form of PPK. Immunolabelling data indicate the involvement of keratins 9, 6, and 16 in the formation of keratin aggregates, which should be considered candidate proteins and genes in these disorders.

22

SPECIFIC EXPRESSION OF CYTOKERATINS IN NON-EPIDERMOLYTIC PALMOPRANT KERATODERMA (JUPA)
L. Lehn-Meisner, Board for Research on Human Genome, Christine Grand, Werner W. Enders, German Cancer Research Center, Division of Cell Biology, Heidelberg, and "Department of Dermatology, Mannheim Medical School, Mannheim, Germany

Palmoplantar Keratoderm (PPK) is a common and distinctive disorder characterized by a prominent hyperkeratinosis without an cytolytic keratoacanthoma and loss of cytoplasmic keratin (CK) filaments, typically found in PPK of the Voerker type. It is ultrastructurally characterized by a marked increase of tightly arranged CK filaments in the suprabasal layers of the thickened hyperkeratized epidermis. As known from other keratinization disorders, where point mutations of specific CKs are involved, some CKs are known to be caused by mutations of the CK9 gene, which is topologically expressed within the palmoplantar (PP) epidermis. Using a panel of monoclonal antibodies (CKs 1, 2, 6, 7, 8, 9, 10, 14, 16, 17, 18, 20), it was determined that the PPK type of Upa possess a remarkable difference in cytoskeletal expression as compared to normal PP epidermis. The CKs 5 and 14 are more strongly restricted to the basal epidermal compartment. The expression of CK 6 and 16 is strongly enhanced and is found in the keratin layer also, whereas CK 17 expression is limited to a few cells. The CK 2,4 expressing cells are increased both in number and in location, and they are found to be located already in lower suprabasal cell layer (Stratum spinosum). As studied by pre-embedding immunogold electron microscopy, increasing keratin expression and detection in in situ hybridization the expression of CK 9 is significantly increased in the diseased epidermis. In contrast to normal PP epidermis, where it is found heterogeneously expressed in the upper suprabasal layers (Stratum spinosum/intermedium), in the PPK this CK is found to be strongly enhanced and already seen in the first suprabasal layer and even in some few basal cells. Here the expression of CK9 is in general no longer distinguishable from that in the stratum granulosum. It is also highly expressed in CK16 very tightly arranged keratin filament aggregates. Our results strongly indicate the special morphological participation of CK 9 in disorders of CKs in the course of the Upa type of PPK.
IMMUNOELECTRON MICROSCOPIC EXPRESSION OF KERATINS IN
Palmoplantar Keratoderma with Tubular Keratins Anjaek Jokel, Lotia Lassam&, Ingrid Anem-Janssen, Institute for Ultrastructure Research of the Skin, Department of Dermatology, University of Heidelberg, and Division of Cell Biology, German Cancer Research Center, Heidelberg, Germany.

Palmoplantar keratoderma (PKP) with tubular keratins (TK) is a dominant disorder that clinically resembles PKP of the Voerst type and of the Unna type with diffuse palmoplantar hyperkeratosis and a sharply defined, thickened, red-eruption margin. Ultrastructural examination of TK keratinocytes isolated from skin biopsies revealed a keratin expression pattern identical to that of keratinocytes from the skin of normal donors. The observation clearly demonstrates that keratin synthesis is not limited to the superficial layers of the epidermis but also occurs in the deep layers. In a number of keratin skin diseases, point mutations in keratin (K) genes have been identified recently as the underlying cause. Of these diseases, the keratin disorder seen in the patients with TK keratoderma is the only one due to abnormalities in keratin synthesis by some unexplained mechanism. We performed postembedding immunoelectron microscopy in vivo and in vitro using a panel of antibodies directed against K1 (347 B4), K3 (cACK53), K5 (15B16), K9 (RKY 1013), K10 (DE10), K1,10,11 (K4,60, K14 (L, 002, cACK14) and anti-proliferating/cyclindependent (LOT 502) to examine K expression in clumped and free TKs. The results are compared with our findings in normal tubular skin. Normal labelling of basal keratin networks is visible with K3, K5, and K14. Strong immunolabelling for K14 (cACK14), K1, K9 and K10,11 is present in both, aggregated and nonaggregated supranuclear TKs. The intensity of labelling clearly increased with the degree of cell maturation except of cACK14 that gradually decreased. A patchy focal labelling was observed for K10. Supranuclear labelling for K5, K14, and K10 was often observed, although there were no obvious differences in the other regions. Neither aggregated nor nonaggregated TKs in the living cells were labelled for prolif/epithelial. Our results indicate that the clumps contain different keratins. It seems that the structural abnormality includes a profound defect of at least one keratin that is preferentially expressed in ridged skin, involving various other keratins secondarily.

SIZE AND COMPOSITION OF KERATOCYTES IN COMPARISON TO PROLIFERATION RATE AND EPIDERMAL THICKNESS IN ACANTHOTIC AND SCLOEROTIC DERMATOMAS. Martin Bacharach-Buhles, Barbara Panz, Stefan el Gamal, Peter Altaner, Dermatologische Klinik der Ruhr-Universitét Bochum, Germany.

Only few authors studied the size of single keratinocytes in different dermatoses.

The aim of this study was to measure the size, the proliferation rate and ultrastructural composition of single keratocytes in acanthotic and sclerotic epidermis.

In 158 psoriatics with active psoriasis, 21 patients suffering from cutaneous sclerosis, 38 healthy controls the size of the ridged epidermis was measured as well as the size of keratocytes by the use of computer supported image analysis.

The proliferation rate was determined by the use of MIB1. Ultrastructurally the composition of keratocytes concerning the different cell organelles was analyzed.

In psoriatic skin acanthosis takes place mainly in the region of the ridged epidermis. In comparison to healthy skin the area of a single keratocyte takes up 206.5 μm² in contrast to 82.6 μm² in healthy skin. In sclerotic skin the thickness of the epidermis equals the thickness of the ridged epidermis with the exception of the underlying corneum. The area of a single keratocyte diminishes to 16.38 μm². Proliferation rate is highest in acanthotic skin with 4.9 %, while it runs about 5.5 % in healthy and 2.9 % in epidermis of scleroderma. Parallel to the reduction of cell size, the proportion of organelles and cytoplasm increases, so does the electron density in the keratocytes.

In psoriatic skin acanthosis is the result of increased number and size of keratocytes while in scleroderma proliferation and size of keratocytes is reduced with increasing epidermal thickness. Cell size and proliferation activity correspond to the vascular supply of the epidermis.


For the visualization of intercellular lipid bilayers in the stratum corneum (SC), ruthenium tetroxide is now commercially used as a post-fixative. Investigation of the multilamellar lipid sheets in large areas of human epidermis is hampered by the poor penetration characteristics of ruthenium tetroxide. We prepared 50μm thick vibratome sections of paraform/glutaraldehyde-fixed normal human skin tissue. These sections were post-fixed in 1% osmium tetroxide followed by 0.5% ruthenium tetroxide in aqueous solution or 0.2% ruthenium tetroxide 0.1 M cacodylate buffer supplemented with potassium ferrocyanide. The 50μm vibratome sections assure an optimal penetration of the post-fixatives throughout all levels of the SC-lipid bilayers and the alternating electron-dense and electron-transparent lamellae were visible between all cell layers, from the stratum granulosum-stratum corneum interface up to the outermost cell layers of the stratum disjunctum. Optimal preservation of lipid structures of lamellar bodies and intercellular lamellae was achieved by a further adaption of the preparation procedure by reducing the dehydration to 70% ethanol only, before embedding in EPO.

In summary, we here describe a simple and rapid method which allows complete penetration of ruthenium tetroxide in normal human SC and consequently improves the visualization of the ultrastructure of lipid bilayers considerably.

THE INTERMEDIATE FILAMENT OF CULTURED HUMAN KERATOCYTES ARE AFFECTED BY CYCLOSPORIN-A. F. Prignano, I. Domenici, G. Gerlini, N. Pinnelli, P. Ronaglia. II Dermatology Clinic and Department of Human Anatomy and Histology, University of Florence, Italy.

Many factors can influence the antigenic and molecular features of the cytoskeleton of normal keratinocytes in culture. The effects of cyclosporin-A (CSA) on keratinocytes in culture have been widely investigated especially on pathological human keratinocytes and found to inhibit proliferation. The aim of our study was to find out if CSA has any effect on the differentiation of normal human keratinocytes, including the molecular and morphological organization of the cytoskeleton. We have investigated the influence of CSA (1.6 μg/ml) on the expression of cytokeratin and vimentin in primary cultures of keratinocytes harvested from healthy skin obtained at plastic surgery. Control keratinocytes grown without CSA were flattened; the cells which had not reached confluence stained intensely for vimentin and weakly for cytokeratins; confluent cells stained with intermediate intensity for both types of proteins and the cells adhering on the top of others - interpreted as the best differentiated ones - stained for cytokeratins but not for vimentin. CSA inhibited the growth of keratinocytes, which never reached confluence and appeared small and roundish; only some cells stained for cytokeratins and none for vimentin. By electron microscopy, the meshwork of intermediate filaments could not be recognized in CSA-treated cells, at variance with controls. These results indicate that the molecular composition of intermediate filaments of cultivated human keratinocytes is affected by confluence and stratification, i.e. by differentiation in vitro, and that the addition of CSA inhibits both cell growth and the correct organization of the cytoskeleton of keratinocytes at a molecular and electron microscopic level. We would suggest that the interference of CSA with the cytoskeleton may be one pathway by which this agent affects cell proliferation.
A MODIFIED CULTURE SYSTEM FOR HUMAN EPIDERMAL CELLS IN TRANSPLANTATION AND FUNCTIONAL TESTING. Annette G.M. van Dorp, Mary C.H. Verhoeven, A. Meike Mommaerts, Henk K. Koerten, Maja Poesen. Department of Dermatology and Electron Microscopy, Biomechanics Research Group, University Hospital Leiden, The Netherlands

Autographs of cultured epithelial cell sheets are widely used for the clinical treatment of full-thickness skin defects. The most commonly used technique involves the culturing of suspended keratinocytes on a feeder layer of lethally irradiated 3T3 mouse fibroblasts. The keratinocytes will divide and individual colonies are formed. At confluency the epithelium can be enzymatically detached from the dish as a coherent sheet of cells, consisting of approximately 4-6 cell layers. Nevertheless, morphological differentiation is not complete in the cultures; the cell layers are flattened and distinct granular and cornified layers do not develop.

In the present study normal human keratinocytes were grown on different inert filter substrates (Costar) at various culture conditions. Vertical sections stained for histology and indirect immunohistochemical studies (LM and EM level) showed a correct stratification and expression of major differentiation markers. Transplantation of these cultured sheets to the patient is much easier as compared to the conventional method. The epithelial sheets cultured on filter substrates are less fragile because of the presence of a stratum corneum. Furthermore, they can be removed from the filter, by gentle mechanical handling, without Disperse treatment resulting in the presence of an intact basement membrane. Next to transplantation purposes, the culturing system allows the complete differentiation pattern of human epidermis and can therefore be used for investigations of molecular mechanisms underlying terminal differentiation, as well as for testing transdermal drug delivery, epidermal toxicity, non-irritancy, and specific activities of cosmetic and pharmaceutical creams or ingredients.

PEMPHIGUS VULGARIS AND PEMPHIGUS FOLIACEUS SERA SHOW AN INVERSELY GRATED BINDING PATTERN TO EXTRACELLULAR REGIONS OF DESMOSOMES IN DIFFERENT LAYERS OF HUMAN EPIDERMIS. Hiroshi Shimizu, Takuii Masunaga, Akira Ikido, Arata Kikuchi, Takashi Hashimoto, Takashi Nishikawa. Department of Dermatology, Keio University School of Medicine, Tokyo 160, Japan

We analyzed the location of binding sites for pemphigus vulgaris (PV) antigen and pemphigus foliaceus (PF) antigen in the human epidermis using serum samples obtained from 3 patients with PV and 3 patients with PF. Confocal laser scanning microscopy, immunoelectron microscopy, examination of ultrathin cryosections, and immunoperoxidase EM demonstrated discontinuous dots along the epidermal cell surfaces. Immunogold EM of ultrathin cryosections showed specific binding of PV and PF autoantibodies only to desmosomes. Post-embedding immunogold EM using cryofixation and cryotributination permitted the whole depth of the epithelium to be examined and the binding of PV and PF autoantibodies was demonstrated by counting gold particles. Both PV and PF autoantibodies bound to all desmosomes in the epidermis, but not to the non-desmosomal surface of the keratinocyte. The majority of the autoantibody binding occurred in the extracellular domain (PV: 62%; PF: 69%). The statistical analysis of two-way analysis of variance regarding the number of gold particles labeling a single desmosome confirmed a significant interaction between subtypes of pemphigus (PV and PF) and the different epidermal cell layers (p=0.044). The results indicate that the number of gold particles bound to individual desmosomes with PF sera was significantly higher in the lower epidermis than in the upper epidermis, and that of PF sera showed a reciprocal pattern. This inversely graded binding pattern suggests a heterogeneity of the composition of the desmosomes, which may explain the differences in the level of acantholysis between PV and PF.


Hailey-Hailey disease is a blistering genodermatosis that has been successfully treated by dermabrasion (Hamm et al, Arch Dermatol., 130:1143-1149). The aim of our study was to investigate the structural alterations of the epidermis in Hailey-Hailey patients before and after surgery. Our study confirmed histologically, ultrastructurally, and immuno-electron microscopically. Cryosections were stained for desmosomes, adherens junctions, actin filaments and actin associated proteins and investigated with a confocal laser scanning microscope (CLSM). Histologically acantholytic dyskeratosis was not confined to lesions, but was also detectable in clinically unaffected skin. Although continuous internalization of desmosomes and perinuclear collapse of the keratin cytokeleton they were partially linked together by well preserved adherent junctions. Staining for actinfilaments with phalloidin proved a remarkable formation of actin stress fibers in response to the tension generated across the adherent junctions. The effective therapy with dermabrasion depended on a sufficient eradication of the surface epidermis since the lower portions of the hair follicles and sweat ducts never were affected by the intrinsic defect of cell adhesion. Accordingly, resphenialization from adnexal structures resulted in an intact epidermis not susceptible to blistering. In conclusion complete acantholysis can be demonstrated in both lesional and non lesional skin of Hailey-Hailey patients and is apparently based on the compensatory function of the actin-adherens junction system.


Cicatricial pemphigoid (CP) is a rare autoimmune bullous disease characterized by a chronic scarring course with skin and mucosal involvement. CP antigen has been shown to be extracellular and in the anchoring filament zone of the lamina lucida. Previous molecular analysis has demonstrated that the bullous pemphigoid (BP) 180 antigen is a transmembrane protein with various immunogenic sites of which SAI is recognized by most of BP sera and is adjacent to hemidesmosomes. The non-collagenous C-terminal domain is another candidate for antibody reactivity. To further elucidate this question we have compared the reactivity of 4 CP sera with affinity purified rabbit antibodies against recombinant proteins representing either the C-terminal domain, using a post-embedding immunogold (5nm) immunoelectron microscopy procedure. On immunoblotting using epidermal extracts, all CP sera recognized a 180 kDa band and 3 sera reacted with the C-terminal domain of the molecule. Six BP180-positive BP sera were used as controls. With all CP sera examined, the gold particles were found in the lower lamina lucida facing the hemidesmosomes and very close to the lamina densa. Some gold labelling was associated with anchoring filaments. The same labelling was found with the rabbit antioxidant raised against the BP180 C-terminal domain. Controls performed with BP180-positive BP sera or anti SAI showed a very different labelling with gold probes mainly situated in the upper lamina lucida and on the hemidesmosome plaque. These findings suggest that the C-terminal domain of the BP 180 antigen is an important immunogenic domain for CP and is a part of the anchoring filament network.

IMMUNOCYTOCHEMICAL LOCALIZATION OF PROTEINS OF ADHERENS JUNCTIONS IN HUMAN EPIDERMIS. Mark Haftek, Martin Hansen*, Hans-Wilhelm Kaiser*, and Daniel Schmitt, U. 346 INSERM / CNRS, Hôpital E. Herriot, Lyon, France and *Dept. of Dermatology, University Hospital, Bonn, Germany.

Adherens junctions, a multi-filament 'associated structures involved in cell-cell and cell-matrix adhesion and signalling. Those which are present at the dermo-epidermal interface, called focal adhesions (FA), comprise integrin β1 heterodimers, whereas cell-cell adhesion is mediated by E-cadherin -expressing zonula adherentes (ZA).

We have studied several proteins known to be expressed by each type of adherens junctions using post-embedding immunogold labeling of mouse jejunal (the reference tissue) and normal human epidermis. Antibodies to actin and vinculin, considered as markers for both FA and ZA, permitted localization of the two types of actin-associated junctions. Anti-pp125FAK and anti-talin or anti-β catenin and anti-α catenin antibodies were used for detection of FA or ZA-specific proteins, respectively, also localized to the corresponding structures. Antibody against plakoglobin showed the same subcellular localization as anti-desmoglein and did not react with jejunal ZA. Our current results show the presence of FA and ZA in human epidermis. Epidermal adherens junctions are distinct from hemidesmosomes and desmosomes but their localization in the immediate vicinity of their keratin-associated counterparts suggests an important role of the former in the modulation of mechanical junction expression.

CELL-CELL CONTACT IN THE EPIDERMIS MEDIATED BY VLA-2 IS NOT RELATED WITH ADHERENS JUNCTIONS. Czady Kowalski, Eberhard Klein, Stefania Jablonska, Stanislaw Majewski. Department of Dermatology, Warsaw School of Medicine, Poland. *Department of Dermatology, University of Ulm, Germany.

Desmosomes (DES) and adherens junctions (AJ) are cell-cell contact (CCC) mediating structures. VLA-2 is an adhesion molecule which was found in basal layer keratinocytes in areas of CCC. It is, however, not known whether this protein is linked with DES or AJ. Our aim was to investigate immunohistochemically and a monoclonal antibody against VLA-2 was performed on biopsies from normal human skin and from patients with acantholytic skin diseases (ASD): pemphigus vulgaris and Hailey-Hailey disease. In the normal epidermis VLA-2 was found in basal layer keratinocytes, whereas in ASD a loss of expression of the protein to the basal layer but was also present in acantholytic cells. Post-embedding immunogold electron microscopic studies on LR White-embedded normal skin revealed the presence of VLA-2 in basal keratinocytes in the areas of CCC. The labeling was confined to both cytoplasmic and extracellular parts of the cell membrane. Our results showed that VLA-2 is not present in areas of desmosomes. The study has shown that VLA-2 is involved in the cohesion of plasma membranes of neighbouring basal keratinocytes. Thus it presents another type of CCC in the epidermis in addition to the proteins of DES and AJ. The expression of VLA-2 in acantholytic keratinocytes lacking CCC mediated by cadherin-associated structures represents a regulatory role of VLA-2 in cell adhesion.
P1


Dyskeratosis congenita (DC) is a multisystem disorder characterized by cutaneous, mucosal, ocular, gastrointestinal and hematological abnormalities, and an increased risk of cancer.

A 28-year-old man presented the classical diagnostic triad: 1) reticulocyte poikilocytosis with hypersegmentation involving most of the neutrophilic series; 2) dysplastic nail, and 3) a large area of leukoplakia on the dorsa of the tongue. Moreover, the patient showed a palmo-plantar hyperkeratosis, absence of fingerprints, sparse eyelashes, and echymosis on the lower limbs. Finally, thrombocytopenia and anemia were noted.

Morphological observations of poikilocytemic skin were performed at the light microscopic level. The most relevant feature was the focal thickness of the basal lamina. This was confirmed by the observation, under the TEM, of a multilayered basal lamina. Moreover, hemidesmosomal hypoplasia associated with keratin filament disruption, and cytoplasmic vacuolization were detected.

It seems conceivable to hypothesize the structural defects here described being the consequence of genes encoding structural proteins in the DC dermal-epidermal junction. Immunohistochimically as well as with immunoelectron microscopic investigations dealing with hemidesmosome-related components could bring further insight into the pathogenesis of this disorder.

P2


For efficient presentation of foreign exogenous antigens, antigen presenting cells need to retain and present the antigen and degrade them into immunogenic peptide fragments that can bind to MHC class II. A compartment for peptide-loading, called MIC, has recently been identified. Using immunogold labeling on ultrathin cryosections, we have previously demonstrated that human Langerhans cells (LC) in situ possess a regular MIC in which class II molecules and endosomal/lysosomal markers such as LAMP-1, CD63 and H-lysosomal co-localize. We now report that further analysis, however, revealed striking differences in the distribution of these markers in LC and EBV-transformed B cells. Whereas in B cells class II molecules were found to co-localize with H-lysosomal, LAMP-1 and CD63 in LC the class II molecules were preferentially found in vesicles that were strongly positive for H-lysosomal but only weakly positive for LAMP-1 and CD63. Since LAMP-1 is a marker for early endosomes these results suggest that such organelles are less abundant in LC, which may relate to lower endocytic activity of LC compared to EBV-transformed B cells. These results indicate that professional antigen presenting cells can differ with respect to the nature of their MIC which may reflect differences in the state of activation of the cells examined.

P3

ULTRASTRUCTURAL STUDY OF AN EARLY JUVENILE XANTHOGRANULOMA: A HELPFUL TOOL FOR THE DIAGNOSIS Ghislaine Daste, Geneviève Samalens, Muriel Camouflé, Bernadette Gorguet, Jacques Bazex. Department of Pathology and **Department of Dermatology, Purpan Hospital, Toulouse, France.

We report here the case of an 18-month-old girl with a vulgar early juvenile xanthogranuloma mimicking an autoinvolutive histiocytosis on the basis of clinical and histological presentations. The skin biopsy specimen showed a focal dense intradermal infiltrate of histiocytes with numerous hyperchromatic nuclei. No Touton cells were seen. By immunohistochemistry on paraffin sections, most cells were reactive with anti-CD68 KPI or PGM1 antibodies but unreactive with anti-P510 antibody. On frozen sections, only rare scattered cells were CD1 positive.

Ultrastructurally a dense histiocytic infiltrate was found. The nuclei were oval or kidney-shaped with no nucleoli evident. The cytoplasm contained smooth and rough endoplasmic reticulum. Rare lipid droplets and lysosomal structures were seen. Langerhans cells granules were observed only in about 1% of cells. The ultrastructural findings confirmed the diagnosis of juvenile xanthogranuloma. Differential diagnoses such as histiocytosis X and auto-involutive histiocytosis in childhood are to be discussed.

P4

EM OBSERVATIONS OF PIGMENTED DERMATOFIBROSARCOMA PROTUBERANS (BEDNAR TUMOR). Mieaki Tan, Shiniho Rba, Hiroshi Sendo, Department of Dermatology, Nihon University Surugadai Hospital, Tokyo, Japan.

A case of pigmented dermatofibrosarcoma protuberans (Bednar tumor) is reported. The histopathological findings of a storiform pattern composed of fusiform tumor cells were typical of dermatofibrosarcoma protuberans, but the presence of melanin-containing cells scattered in a few areas of the lesion was unusual. Ultrastructurally, the proliferating tumor cells possessed extensive cytoplasmic processes, convoluted nuclei, and intercellular junctions, but lacked a basal lamina. Tumor cells were surrounded by dense amorphous materials, and were arranged in masses containing melanocytic cells which were also surrounded by amorphous substance and in which were seen numerous melanosomes. Further examination of the dense amorphous material has suggested that it is collagen, and immunoelectron microscopy is now being used to identify its type.

P5

ULTRASTRUCTURAL EVIDENCE FOR EOSINOPHIL ACTIVATION IN INFLAMMATORY SKIN DISEASES. Gabriële Zeeck-Kopp, Christa Kübler-Pestoni, Alexander Kopp. Dept. of Dermatology, University of Freiburg, and *Hannover Medical School, Germany.

Eosinophil (EO) activation is a characteristic feature of certain inflammatory skin diseases. Based on their capacity to release toxic granule proteins EO are thought to be potent effector cells in the inflammatory tissue response. In the present study EO activation was evaluated by transmission and immunoelectron microscopy (EM) using the monoclonal EO-antibody to detect the activated form of the cationic protein (EP) in patients with bullous pemphigoid (BP), N-IgA dermatitis herpetiformis (DH, N-IgA), arthropod reactions (N=6). The diagnosis was confirmed by routine HE staining and direct IF. Furthermore, ED1- and ED2-staining were performed in parallel by immunohistochemistry based on APAAP-technique. In BP numerous ED1+ and ED2+ eosinoids were found in the inflamed tissue, particularly in the dermis, showing characteristic signs of activation: formation of vesicles in the cytoplasm and in the matrix of secondary granules as well as the appearance of dense small granules. Activated ED1+ and ED2+ eosinoids were observed in the dermis and the bulla. ED1 was detected in the matrix of secondary granules and small dense granules in tissue ED2 pointing to an intracellular translocation of activated ECP, but not a release of granules in the tissue. Similar results were obtained in biopsies of BP patients. In all biopsies specimens obtained from arthropod reactions a marked eosinophil infiltration was observed. As compared to BP or DH, tissue eosinoids were significantly increased and showed, based on light microscopy, an intracellular ED1- and ED2-activation in the matrix of secondary granules detected. In contrast to BP or DH, no intracellular translocation of ECP could be observed. However, "activ" secondary granules were seen in the dermis and in between collagen fibers. In histiocytic tissue eosinoids, eosinoids with reduced numbers of secondary granules were seen in the tissue. Our data clearly show that eosinoids are differentially activated in bullous skin diseases and arthropod reactions suggesting different pathophysiological mechanisms of the cellular activation process.

P6

ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDY OF CHONDROID SYRINGOMA WITH EXUBERANT OSSIFICATION. Satoko Shimizu, Hiroshi Han-yuku, Sachiko Fukushima, Hiroshi Shimizu. Divisions of Dermatology and Pathology, Dokkyo Hospital and Department of Dermatology, Keio University School of Medicine, Tokyo, Japan.

Cutaneous ossification is sometimes associated with certain skin tumors though it rarely occurs in chondroid syringoma. We report an unusual case of chondroid syringoma associated with exuberant ossification arising on the upper lip of 68-year-old Japanese woman. The tumor, having a 5-year history, presented as an elastic but hard mass measuring 3x2x2 cm and sited over the orbicular muscle of her mouth. Histologically, the tumor was well circumscribed and contained exuberant bone which shared a quarter of the specimen with narrow-like structure and internalizes a remaining cartilage. The main tumor structure shared three-quarters of the mass and exhibited the typical histological features of tubulo-crest type chondroid syringoma. Immunohistochemically, the majority of tumor cells were keratin-positive, with some being positive for CEA or smooth muscle actin. Electron microscopy demonstrated intercellular canaliculi and multiple villus formation within the tumor. Also seen were the so-called hyaline cells containing numerous filaments. Although these results were clearly compatible with chondroid syringomas, to the best of our knowledge, no similar report on this condition associated with such marked bone formation has appeared in the English literature.
P10

cULTURED HUMAN MELANOCYTES AS A MODEL TO STUDY DIFFERENCES IN PHLOGISTIC AND EUMELANOCYTES. J. van der Meulen, N.P.M. Spith, A.M. Moomans, H.K. Kroon and S. Peer. Laboratory for Electron Microscopy and Department of Dermatology, University Hospital Leiden, Leiden, The Netherlands.

Phagocytosis in melanocytes is a morphologic process that seems to have as major purpose a rapid production and release of light absorbing material to protect the body against harmful effects of ultraviolet light (UV). Melanomas produce two types of melanin: eumelanin and phaeomelanin, which may not only be chemically but also in their physical properties. When UV irradiated in vitro, eumelanin has been described to produce free radicals, whereas in contrast, eumelanin was shown to act as a free radical scavenger. In vitro experiments also showed that a number of metal ions, such as Fe³⁺, Ca²⁺ and Zn²⁺, have a catalytic effect on the production of melanosomes. To investigate in more detail the possible differential role of phaeo- and eumelanin in protection against UV, and the influence of metal ions on the development of these melanocytes, we studied cultured human melanocytes of different skin types using transmission EM. Two melanocyte cell lines, established from foreskin of neonates with skin type I and VI, were studied. These cells were cultured in Ham’s F-10 medium either under standard conditions or by adding 10 mM zinc sulphate. In both cultures with and without addition of zinc the melanocytes were very active in the formation of melanosomes. As judged by ultrastructural morphological criteria only, no preferential presence of phaeo- or eumelanin in melanocytes of skin type I and VI was observed. Furthermore, the melanosomes population could be modulated by changing the zinc concentration of the culture medium. Future experiments with X-ray microanalysis will be performed to establish the specific nature of the melanosomes in the melanocytes of different skin types.

P11


In close contacts between keratinocytes adherens junctions play an important role. Previously we have shown the disturbances in E-cadherin distribution in transformed cells. The aim of the present study was to examine the expression and localization of adherens junctions in human keratinocytes (NHK) and in tumorigenic (TU) and nontumorigenic (NONTU) cell lines harboring HPV16 DNA (SKV). We found vinculin expression in all cells but the pattern of immunofluorescence was different. In NHK the interrupted linear staining was present at sites of cell-cell contact, whereas in SKV (especially in TU) cells this staining was more diffuse. Postembedding immunogold labeling of TU-SKV cells with vinculin antibodies showed the presence of gold particles in the area of adherens junctions, however, there was no connection with the cell membrane. In NONTU-SKV cells vinculin was detected at the cytoplasmic side of adherens junction. In TU-SKV cells there were also less amounts of actin "stress fibers" but very pronounced protrusions of the plasma membrane. The differences between NONTU-SKV and TU-SKV cells in organization of adherens junctions in cell-cell contacts could facilitate cell-cell detachment of tumorigenic cells.

P12

EXPRESSION OF ANCHORING FIBRIL ULTRASTRUCTURE IN COMPOUND HETEROZYGOTOUS TWINS WITH SEVERE DYSTROPHIC EB DUE TO A RECESSIVE AND A DOMINANT COL7A1 GENE MUTATION COMPARED TO THE DOMINANT MATERNAL SKIN STATE. U. Eihsenbre, I. Anton-Lamprecht, A. Christiani, S. Amano, R.E. Burgeson, and J. Utt. Institute for Ultraceutural Research of the Skin, University of Heidelberg, Germany, *Department of Dermatology, Thomas Jefferson University, Philadelphia, PA, and Cutaneous Biology Research Center, Harvard Medical School, Charlestown, MA, USA.

In two heterozygotic twins suffering from severe scarring EB with mutilation tendency, mutational analysis had identified, as expected, a recessive frameshift-downstream PTC due to a 2 bp deletion/insertion in exon 56 of the paternal COL7A1 allele, and, unexpectedly, a dominant glycate-to-arginine substitution in exon 90 of the maternal COL7A1 allele present as well in the mother and her father. Involvement of both with mild dystrophic EB was only then revealed by careful questioning and an additional skin biopsy of the mother. Electron microscopy (EM) and immunogold EM (IEM) using anti-collagen VII antibodies were applied to analyse the expression of anchoring fibris (AF) in the dermo-epidermal junction of both twins and their mother. Raster electron microscopy and ultrathin sections demonstrated the presence of severely hypoplastic AF and low degree of IEM labelling in the twins corresponded to the extremely low amount of collagen 7 protein and lack of truncated protein as found by immunoprecipitation of radiolabelled polypeptides from cultured fibroblasts. A comparison of normal and affected parts of skin in agreement with the possible combinations of mutant and normal collagen 7 protein molecules in the triple helices of AF.
This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.