Abstracts for the Society for Cutaneous Ultrastructure Research (SCUR) 24th Annual Meeting
Warsaw, Poland, April 24–26, 1997

Sessions

<table>
<thead>
<tr>
<th>Sessions</th>
<th>Abstract Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session 1: Gene mutations and their manifestation on ultrastructure</td>
<td>1–5</td>
</tr>
<tr>
<td>Session 2: Invited lecture: Jean-Paul Ortonne (Nice, France) The molecular dissection of hemidesmosomes, consequences for the understanding of hereditary junctional epidermolysis bullosa</td>
<td>L1</td>
</tr>
<tr>
<td>Session 3: Stratum corneum and epidermal barrier</td>
<td>6–9</td>
</tr>
<tr>
<td>Session 4: Melanosomes and melanocytes</td>
<td>10–13</td>
</tr>
<tr>
<td>Session 5: Invited lecture: Gerard Orth (Paris, France) Epidermodyplasia verruciformis: the virological and immunogenetic aspects</td>
<td>L2—no abstract</td>
</tr>
<tr>
<td>Session 6: The effects of UV radiation on various cell types and cancerogenesis</td>
<td>14–17</td>
</tr>
<tr>
<td>Session 7: Experimental immunofluorescence</td>
<td>18–21</td>
</tr>
<tr>
<td>Session 8: Invited lecture: Robert A. Briggaman (North Carolina, USA) Linkin, a new family of extracellular matrix adhesion molecules</td>
<td>L3</td>
</tr>
<tr>
<td>Session 9: Comparative immunohistochemistry and electron microscopy</td>
<td>22–25</td>
</tr>
<tr>
<td>Session 10: Dermatopathology club</td>
<td>26–30</td>
</tr>
<tr>
<td>Session 11: Invited lecture: Jan Steffen (Warsaw, Poland) Genetic predisposition to cancers: current status and perspective</td>
<td>L4—no abstract</td>
</tr>
<tr>
<td>Session 12: Antigen presenting and effector cells</td>
<td>31–34</td>
</tr>
<tr>
<td>Poster session</td>
<td>P1–P19</td>
</tr>
</tbody>
</table>

Congress Honorary President
Stefania Jablonska, Warsaw

Scientific Programme Committee
Marek Haftek, Lyon
Jean Kanitakis, Lyon
Henk Koertjen, Leiden
Cezary Kowalewski, Warsaw
Mieke Mommaas, Leiden

Organizing Committee
Cezary Kowalewski
Maria Blaszczyk
Anna Gorkiewicz-Pekow
Wiesław Gliński
Marek Haftek

Three prizes for the best oral and poster presentations at the Annual Meeting of the SCUR 1997 in Warsaw were awarded to: Gonke Pilgram (oral presentation no. 7), Jacek Bartosik (oral presentation no. 15), and Cezary Kowalewski (poster no. 15) and their coworkers.

We gratefully acknowledge the sponsorship of the organizations and industry that helped in realizing this meeting:
State Committee for Scientific Research (KBN)
Ministry of Health
Yamanouchi Europe (Warsaw, Poland)
Galderma (Nice, France)
1 EPIDERMIS AND FOLLICLES IN CATHEPSIN K-KNOCKOUT MICE

Schener, W. Roth, C. Peters, E. Anton-Lamprecht.* Institute for Ultrastructure Research of the Skin, University of Heidelberg, # Dept. of Internal Medicine, University of Freiburg, Germany.

The aim of the study was to elucidate the cutaneous ultrastructure of transgenic mice, which do not express the gene for Cathepsin K, a cysteine protease, by experimental knock-out. 12 Various important in vivo functions have been ascribed to Cathepsin K, such as tumor progression and metastasis, bone remodelling, hair growth, and immune response. The transgenic mice were obtained from female agouti-colored transgenic and control mice. The knock-out mice pass through a special kind of hair cycle with eight different important stages from 5 to 50 days. It is a repeated change between hair growth and hair loss which is total at about 23 days and alopecia areata-like after regrowth in later ages. Epidermis and hair follicles are the most important structural components. Changes in the epidermal and follicular ultrastructure during the age-related fur changes were discussed. Acanthosis and hyperkeratosis of epidermis and follicles are major findings in knock-out mice. By comparing the transgenic mice with control animals it may be concluded that hyperkeratosis is a consequence of loss of regulatory function of the Cathepsin K gene.

1 Roth W.: Thesis Nov.95, Göttingen.
2 Roth W., Peters C.: Presentation on 11th Int. Conf. on Proteolysis and Protein Turnover, Turkú, Finland, Sept.96.96

3 CORRELATION OF ULTRASTRUCTURAL COLLAGEN FIBRIL ABERRATIONS IN THE COL5A1 GENETIC SYNDROME TYPE I. Hauser, A. de Pascual, L. Merino, P. Noguera, J. Anton-Lamprecht.* Institute for Ultrastructure Research of the Skin, Department of Dermatology, University Heidelberg, FRG. # Dept. of Medical Genetics, University Hospital Hamburg-Eppendorf, Germany.

 Pronounced morphological alterations characterize the dermal connective tissue in Ehlers-Danlos syndrome (EDS) type I, most remarkable are chaotically arranged collagen bundles consisting of abnormally assembled fibrils. Collagen V plays an important role in collagen I fibrillogenesis and forms heterofibrillotopic fibrils with collagen I in many tissues. Recent evidence based on linkage and transgenic mice studies suggests that collagen V is causally involved in human EDS. Some reports proved or excluded, respectively, linkage of the COL5A1 gene with EDS types I and II; however, the classification of the respective patients had mostly been done by an assessment of the clinical severity and not by an ultrastructural investigation of connective tissue alterations. In four independent studies of the skin of EDS patients and/or of knock-out mice described an association of a COL5A1 type I in two members of two generations by the ultrastructural features and identified a mutation in the COL5A1 gene encoding for the proc(IV) collagen chain and segregation with the affected members. The association causes a cysteine to serine substitution within a highly conserved domain and a reduction of collagen V by preventing incorporation of the mutant chains in the collagen V trimers. The mechanisms causing functional haplo-insufficiency of COL5A1 and functional absence of collagen V, respectively, are likely to account for aberrant composite collagen fibrils and the deposition of highly disorganized collagen masses characteristic of EDS I.

4 IDENTIFICATION OF A COL7A1 SPICE MUTATION AFFECTING PROCOLLAGEN VII PROCESSING IN A FAMILY WITH PREVIOUSLY REPORTED DYSMORPHIC EPIPHYSIS AND HYDRALACRIMAL SYNDROME TYPE II. Deba Hill, J. Coste, Patricio Pastorino, Manut Schubert, Lenam Bruckner-Tuderman, Daniele Castiglia, Dermoatology Clinic of Dept. of Spec. and Exp. Medicine, "Dept. of Exp. Pathology, University of Bologna, Italy, # Dept. of Dermatology, Univ. Münster, Münster, FRG, 1Instututo Dermoestetico dell'Infermiera, IRCCS, Rome, Italy.

Hydralacrimosalivary biliou (DEB) is an inherited blistering disease of the skin characterized by loss of dermal-epidermal adhesion and by abnormal anchoring fibrils. Pathogenetic mutations in the type VII collagen gene COL7A1 have been identified in patients affected by both dominant and recessive forms of DEB. Here we describe a prebiatal DEB patient with a heterozygous 14bp deletion in the genomic intron 15 of the COL7A1 gene and demonstrate its segregation in two non-affected members of the family. The mutation results in the skipping of exon 115 with the elimination of 29 amino acids from the proc(VII) procollagen chain which consequently fails to be processed to the mature form and accumulates protein of the upper dermal lamina. Ultrastructurally, deposition at the dermal-epidermal junction was shown by immunofluorescence analysis with antibodies recognizing the NC-2 domain of the molecule. The identification of this unusual splice mutation in both affected and non affected members of the family is in keeping with similar reports in two other families and indicates that a second mutation is required to cause the pathogenetic phenotype.
5 EB-SIMPLEX (PSUEDOFUNCTIONAL EBI IN A MALE PATIENT WITH PLECN
DEFICIENCY: TRANSMISSION ELECTRON MICROSCOPIC OBSER-
VATION. W.H. Hökfelt, H.J. Wahlström, G. Bäckström, D. Westin, O. Pohls-Dahlgren, H. Hamnnerå 2 and H. Hinkner 2 2 Dept. of Pathology, EM-Lab; 2 Dept. of Dermatology, Gen. Hospital LKA, Salzburg, Austria; 2 Inst. of Bio-
chemistry & Molecular Biology, Umeå University, Umeå, Sweden.
We report on a turkish boy 3 years of age, offspring of unaffected parents. Generalized blistering occurs since birth as a result of minor trauma. Blistered and desquamated skin is characterized by a diffuse infiltration of small blood blebs on fingers and toes. These symptoms suggest the diagnosis of Epidermolysis Bullosa hereditaria (EBH). Clinically there is no sign of tenseae weakness. Immunofluorescence-optical investigation of patient's skin revealed normal staining with an antibody against an R-terminal portion of the rod domain of plecin, a cytoskeletal interme-
diate filament-associated protein, as well as staining with antibodies di-
tected against other components of the dermo-epidermal basement mem-
brane zone. However, two antibodies, which bind at a more C-ter-
minally located intermediate filament sites detected the plane of cleavage just above the basement membrane zone indicating the diagnosis EB simplex. HD's are hypoplastic and reduced in number. Cyto keratin (fonio)-filamentes are disturbed & do not show proper insertion into the HD's.

6 STUDY ON THE ORGANIZATION OF LIPIDS PRESENT IN HUMAN STRATUM CORNEUM. G.S.K. Pilgram, A.M. van Pel*, H.K. Koerten* and J.A. Bouwstra* * Laboratory for Electron Microscopy, # Leiden/Amsterdam Center for Drug Research, Leiden University, The Netherlands.
The stratum corneum (SC) plays a key-role in the barrier function of the skin and it is generally accepted that the intercellular lipidic matrix is responsible for that property. The major classes of lipids in the SC are ceramides, cholesterol and free fatty acids, an unusual lipid composition that results in an exceptional lipid organization. Wide angle (WAXD) and small angle X-ray diffraction studies have examined the presence of (i) crystalline and hexagonal lateral packings and (ii) lamellar phases of 13.4 nm and 6.4 nm. Since the SC is such a complicated heterogeneous system, lipid model systems have been developed to investigate the importance of the lipid composition in relation to the behavior of lipid phases. It has been found that cholesterol is an essential component in the SC although its concentration may vary. In the present study, lipid mixtures were prepared from ceramides, cholesterol and/or fatty acids, resembling the lipid composition of human SC. The lateral packings of the lipid mixtures were studied by electron diffraction (ED) at -17°C. We find that doublet reduced stacking in contrast, in cultured keratinolysis only the most C-terminally located antibody shows clearly reduced stacking. This stacking is seen in the different patterns; the diffraction- cium and immunohistochemistry. Electron microscopy (leptosomal-per-
itesional-tensional sites) detected the plane of cleavage just above the basement membrane zone indicating the diagnosis EB simplex. HD's are hypoplastic and reduced in number. Cyto keratin (fonio)-filamentes are disturbed & do not show proper insertion into the HD's.

7 ELECTRON DIFFRATION AS A TOOL TO STUDY LATERAL PACKINGS OF HUMAN STRATUM CORNEUM LIPIDS. G.S.K. Pilgram, A.M. van Pel*, H.K. Koerten and J.A. Bouwstra*.* Laboratory for Electron Microscopy, # Leiden/Amsterdam Center for Drug Research, Leiden University, The Netherlands.
The stratum corneum (SC) plays a key-role in the barrier function of the skin and it is generally accepted that the intercellular lipidic matrix is responsible for that property. The major classes of lipids in the SC are ceramides, cholesterol and free fatty acids, an unusual lipid composition that results in an exceptional lipid organization. Wide angle (WAXD) and small angle X-ray diffraction studies have examined the presence of (i) crystalline and hexagonal lateral packings and (ii) lamellar phases of 13.4 nm and 6.4 nm. Since the SC is such a complicated heterogeneous system, lipid model systems have been developed to investigate the importance of the lipid composition in relation to the behavior of lipid phases. It has been found that cholesterol is an essential component in the SC although its concentration may vary. In the present study, lipid mixtures were prepared from ceramides, cholesterol and/or fatty acids, resembling the lipid composition of human SC. The lateral packings of the lipid mixtures were prepared from ceramides, cholesterol and/or fatty acids, resembling the lipid composition of human SC. The lateral packings of the lipid mixtures were studied by electron diffraction (ED) at -17°C. We find that doublet reduced stacking in contrast, in cultured keratinolysis only the most C-terminally located antibody shows clearly reduced stacking. This stacking is seen in the different patterns; the diffraction-composition and immunohistochemistry. Electron microscopy (leptosomal-per-
itesional-tensional sites) detected the plane of cleavage just above the basement membrane zone indicating the diagnosis EB simplex. HD's are hypoplastic and reduced in number. Cyto keratin (fonio)-filamentes are disturbed & do not show proper insertion into the HD's.

8 ULTRASTRUCTURAL LOCALIZATION OF CALCIUM IN RECONSTRUCTED HUMAN EPIDERMIS. Jana Vitanova1, A. Mieke Mommaas2, Aat A. Mulder2, Johannes K.empers2, Lucia Line1, Pirjo Laine1, Onno Pone1 and Hend K. Koeppen1 1 Curves, Charles University, Prague, The Czech Republic 2 Department of Dermatology and Laboratory for Electron Microscopy, Leiden University Hospital, The Netherlands.
Calcium ions have been demonstrated to be essential for the correct functioning of cell structure, they can induce cellular proliferation and differentiation. The cellular distribution pattern of calcium ions in normal human epidermis and in reconstructed human skin in culture were compared in normal granulomas. This distribution is abnormal in skin disorders with known proliferation and differentiation defects (psoriatic lesions). Since reconstructed epidermis shares some common features with hypoproliferative epidermis, we employed ion capture cymochrome (the potassium oxalate-pyroantimonitate method) for analysis of ion calcium distribution over human skin sections in an attempt to understand the role of cellular differentiation in reconstructed epidermis cultured under air-exposed conditions. On ultra-thin sections of the skin, calcium containing deposits appeared as electron dense granules, the specificity of which was checked by X-ray microanalysis. The cellular distribution pattern of calcium ions in reconstructed epidermis cultured under optimal conditions in gynaecological skin showed that normal human epidermis, human epidermis, both keratinocytes and keratinocytes, primary and with a peculiar similarity to those observed using X-ray diffraction. Promising distribution patterns the intercellular lipid domains have been recorded in the SC as well. Therefore, electron diffraction is a tool to study local packings of lipids in the SC, which we will use to study the influence of penetration enhancers on the permeability of the SC.

9 X-VILES: A SEGMENTARY EXPRESSION OF X-LINKED INTHYROID VARIANT NOT RELATED WITH THE STEROID SALTASE DEFICIENCY. Mark Huthke, Michel Dugue, Patrick Conforte, Gay Serre and Daniel Schmitt U2436 / CNRS, E.Henriot Hospital, Dept. Dermatology, HIA Desgenteres, Lyon, and **Dept. of Biology and Pathology of the Cell, CHU Purpan, Toulouse, France.
X-linked ichthyosis is a recessive hereditary disorder transmitted by women and expressed in men. Retention of the pigmented horny layer had been related to a mutation or deletion of the steroid saltase gene, situated at the locus p22.3 on chromosome X, responsible for abnormal processing of the cholesteryl ester in the stratum corneum. We have observed a segmentary expression of clinically typical skin lesions of an X-linked ichthyosis in a male patient presenting with characteristic cornoid maculee but revealing neither systemic nor localized steroid saltase deficiency. The case appeared to be isolated, with no family history, suggesting a mosaic expression associated with the instability of the distal portion of the X chromosome's short arm. 
Ultrastructurally, the epidermis was hyperpigmented, with a thick granular layer and hyperkeratotic, compatible with X-linked ichthyosis. Cordornosudinon expression was investigated by its persistence Cult Cell Biology, Biocenter, University of Heidelberg on the neuronal epidermal surface, as it is also the case in fully expressed X-linked ichthyosis. Ultrathin section labelling with anti-filaggrin and anti-profilaggrins antibodies indicated a deficient content of the protein in the layer, suggesting a type II keratinocyte ichthyosis.
Our observation is in favour of a possible existence of X-linked ichthyosis not correlated with steroid saltase deficiency but showing a typical phenotype, possibly, another enzymatic abnormality.

The aim of the study was to compare two methods quantifying eu- and pheomelain, pigments synthesized by melanocytes. One is based on high pressure liquid chromatography (HPLC) quantification of specific degradation products of each meam. The other requires image analysis, transmission electron microscopy (TEM) and stereoology. This study was carried out in cultured human melanoma cells of different tumorigenic and nontumorigenic potential. The results were measured by HPLC and cells were fixed and embedded as pellets for TEM. Ultrathin sections were treated or not by the alkali dissolving method allowing the elimination of pheomelain from sections. Cells were also cut in frozen sections and were analyzed with our image analysis program permitting the estimation of 3 primary parameters useful for stereoology. Stereology and quantification of the melanization, intracellular melanin content and the number of melanised melanosomes (MB) per cell and this, for total melanin, eu- or pheomelanin. The results obtained revealed a good correlation of methods for total melanin and particularly when using the cytoplasmic volume density of melanin (r=-0.93). Moreover, we report the existence of a granulomatous type of melanization, responsible for the differences observed between the different cell lines in total melanin (quantified by HPLC). These results demonstrate the relevance of the stereological method, but the ultrastructural analysis is essential for the quantification. Both methods are correlated in the case of eu- and pheomelanin alone. Several hypothesis are put forward and the recent improvements in the HPLC determination of eu- and pheomelanin could help us to explain these differences.
11

OCCURRENCE OF MELANOCYTES AND MELANOMAS IN EPIDERMIS OF LONG STANDING STATIONARY VITILIGO. J. Bartošk, J. Kubánský and H.C. Wu. Laboratory of Photobiology, Department of Dermatology, Bispebjerg University Hospital, Copenhagen, Denmark.

To examine the occurrence of melanin and melanocytes (MC) in stable vitiligo, skin biopsies from five patients with vitiligo lesions lasting for 1 to 17 years were explored. Areas of upper dermis and epidermis apart from hair follicles were extensively investigated under the electron microscope. All non-keratinocytes present in the epidermis were scrutinized and identified in series of 200-300 consecutive sections. Neither active or inactive MC, nor MC dendrites were found. Melanomas type III and IV, mostly in the form of melanosome complexes (MCc) occurred in few, grouped keratinocytes (KC) located in the stratum basale in vitiligo lasting for 1-3 years. They carried up to 21 MCc each, whereas the remaining KC in the epidermis were completely devoid of melanin. Small amounts of melanin were also observed in a few dendrites in all vitiligo cases.

The results show that melanin can be found in non-negligible amounts in the epidermis as late as 3 years after the vitiligo outbreak. The origin of that melanin is unknown but it seems unlikely that melanomas could be transported to KC from other skin regions. It appears that either there is a small population of basal KC able to prevent their loss of melanin by postponing their mitosis or there are other mechanisms enabling melanomas to persist for a few years in some basal KC.

13


Lisch nodules are melanocytic hamartomas of iris stroma that represent the most common clinical feature of patients with peripheral neurofibromatosis (neurofibromatosis type 1, or Von Recklinghausen disease). Lisch nodules are prevalently bilateral and it is widely accepted that they increase in number with age. In our study, 35 patients (20 females and 15 males) affected by NF1 with a relative ranging from 7 mounts to 58 years were evaluated. Iris nodules were observed in 22 out of 35 cases (63%) of patients with NF1. We analyzed the histopathological and ultrastructural aspect of Lisch nodule of a 50 year old woman biopsied at the time of intraocular cataract extraction. Electron microscopical observations showed three main cell types in a dense collagenous matrix: a) melanocytes with well-developed organelles such as mitochondrions, Golgi complexes, rough endoplasmic reticulum and a variable number of melanosomes and granules; b) discontinuous basement membrane-like structures were also present along their plasma membrane, and c) fibroblast-like cells, characterized by a large nucleus and long cytoplasmic processes and d) some mast-cells which showed their typical ultrastructure morphology with a centrally placed nucleus and numerous granules with a poorly defined limiting membrane. This ultrastructural aspect is similar to that described by other investigators. In our case we underline the presence of mast-cells, sometimes in contact with fibroblast-like cells, because this aspect, also observed in NF1 neurofibromatosis, could be related with the pathogenesis of these tumors.

15

MORPHOLOGICAL FORMS OF APOPTOSIS IN CULTURED HUMAN KERATINOCYTES.

Robert Gajdecki*, Jack Bartosik*, Barbara Gajkowskat, Michael Hansenat

*Department of Dermatology, University of Copenhagen, Bispebjerg Hospital; tDepartment of Microbiology, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark; tLaboratory of Electron Microscopy, Center of Experimental and Clinical Medicine, Polish Academy of Sciences, Warsaw, Poland.

Condensation of nucleus (pyknosis), nuclear fragmentation (karyorrhexis), and cell surface blebbing (exocytosis) have been considered to be characteristic for apoptosis in any cell type. We studied the morphological aspects of apoptosis in cultured keratinocytes using DAPI and TUNEL staining procedures for DNA, combined with confocal laser-scanning microscopy, and scanning or transmission electron microscopy. Three qualitatively different stimuli were used: an ultraviolet B radiation (UVB) which provides a mutagenic factor, a membrane-penetrating ceramic analogue inducing apoptosis (I-keratocytes) and cell suspension being a model of differentiations-induced apoptosis (differtiation). Nuclear condensation and karyorrhexis were extremely rarely encountered among the cells treated with UVB or ceramic. Blebbing was a frequently observed phenomenon, in some blebs fragmented DNA was seen. In contrast, suspended cells demonstrated primarily pyknosis without karyorrhexis or blebbing. These observations indicate that the morphological features of apoptosis depend on the initial stimulus. It is likely that the signaling machinery activated in differtiation differs from that induced by ceramic or UVB.

12

CLEAR CELL SARCOCOMA. D. Innocenti, V. Silipo, A. Rizziotta, C. Bonnan and S. Calvieri.* Istituto di Clinica Dermatologica Universita di Roma "La Sapienza" - Roma, Italy.

Clear cell sarcoma (CCS), an uncommon tumor of soft tissues, was first described by Enzinger in 1965 as CCS of tendons and aponeuroses. Although there is some controversy about its histogenesis, the best hypothesis today is that it is derived from melanocytes. Accordingly, Chung and Enzinger, in 1983, changed its name to malignant melanoma of soft tissue. We report a case of CCS in a 9 year old boy who presented a nodular lesion on his left foot. Surgical resection of the tumor was performed and in situ immunohistochemical study disclosed involvement by melanocytes. Under the light microscope, the pattern of the tumor consisted of compact nests or fascicles of pale cells surrounded by bundles that involved the deeper part of the dermis and subcutis. The cells were not connect to the overlying epidermis. The cells were non pigmented and presented round or ovoid vesicular nuclei with prominent nucleoli. Their clear appearance was the result of the presence of abundant glycogen They were fusiform in shape or had sometimes a more epithelioid aspect. No multinucleate cells were seen, and rare myotic figures were found. Cytoplasmatic melanin granules were not observed in the neoplastic cells. Immunohistochemically cells were positive for vimentin, S100 and HMB-45. The ultrastructural study showed cytoplasmatic melanomas at various developmental stages and some of them were partially melanized.


UVB (Ultraviolet B) light has been shown to suppress the (skin) immune system. To study the mechanism of immunosuppression in vivo in man, we investigated the effect of UVB on the mixed epidermal cell lymphocyte reaction (MECLR). Part of the right flexor forearms of healthy volunteers were exposed to an erythrogenic UVB dose of 160 mcald/m2 for 4 consecutive days. Four days after the last irradiation, epidermal sheets were obtained by the suction blister method and epidermal cells were used as stimulator cells in the MECLR. Responses were expressed as percentages of the non-irradiated controls. Using this high dose, short term UVB protocol, we found a significant increase of the MECLR responses of 205%. In a previous study, however, in which we used a low dose, long term UVB protocol, we found a strong reduction of the MECLR responses to 20% of control values. These conflicting results could be explained by the appearance in the epidermis of CD36® cells II CD1a macrophage-like cells after erythrogenic UVB doses. FACS analysis of the epidermal cells indeed showed a strong expansion of CD36® cells in sunburned epidermis. For a more detailed study to the nature of these cells, biopsies were taken from both arms and examined by electron microscopy (EM). The results showed the presence of a cell-type in irradiated epidermis, that was not, or only seldom present in the non-irradiated epidermis. These cells are morphologically very similar to Lc, but lack Birbeck granules and are located near the basal membrane in contrast to Lcs which are located more suprastratally. Immun EM showed that these cells are strongly HLA class II, especially on the cell surface, while Lc express HLA class II primarily intracellularly. These strong HLA class II expressing macrophages can probably efficiently stimulate responder T cells in the MECLR, explaining the increase of the MECLR responses that we found after an erythrogenic UVB dose. In contrast to Lc, UVB-induced macrophages activate pathways, including T suppressor cells, that can induce antigen-specific tolerance. The mechanism of the differences in T cell activating capacity of these two cell types is subject of future studies.

16

SKIN PROTECTION AGAINST UV RADIATION: ULTRASTRUCTURAL CHANGES IN KERATINOCYTES AND MELANOCYTES. Bacharach-Rubles M, Lubowska M J, Gammal S, Ahmney P. Dermatological Department of the Ruhr-University Bochum, Germany.

UV-radiation induces a physiological protection against UV light. Interestingly skin protection is specific for different wave lengths. In this study we investigated the alteration of keratinocytes and melanocytes after irradiation with UV B- (group 1), UV A1 (group 2) and PUVA (group 3) in therapeutic dosage. Each group contained 5 patients, each patient was biopsied twice: before the beginning of irradiation and 4 weeks after the start of UV therapy. Apart from morphological alterations the content of keratinocytes and melanocytes was determined by the help of computer-supported image analysis.

After UVAl and PUVA treatment, intercellular spaces between keratinocytes increase. The number of cell layers in the epidermis rises. An increase of melanin granules can be observed especially after irradiation with UVAl and PUVA. After PUVA irradiation a microinfiltration can be observed focally in melanocytes. The cell-size of keratinocytes is increased after irradiation with UVA1. There are no measurable cell changes after a low dose therapy of UVB. Focally some fibrillary bodies can be seen in the upper corneum.

We conclude that the reaction of the epidermis to different UV wavelengths differs. The most clear result is the increase in cell size of keratinocytes and melanocytes after UV-A 1 irradiation parallel to the increase in the number of melanosomes in both cell types. Histologically skin-adaptation to UV exposure occurs with an increase of melanosomes and the number and cell size of keratinocytes.
17 EXPRESSION OF THE P53, BCL-2 AND BAX PROTEINS IN MALIGNANT AND BENIGN LESIONS OF EPIDERMOSPYDIMALIS VERRUCIFORMIS. Kamila Padlewskia**, Michel Fayolle, Nicolas Ramond, Michel Barrieu, Marie-Luce Le Rhésis, Guy Rootz, Alain Remy. *Department of Dermatology, School of Medicine, University of Liège, Belgium. **Department of Dermatology, School of Medicine, Warsaw, Poland. —Étude de la P53, BCL-2 et BAX dans les lésions de l’épidermodysplasie verruciforme (EDV). Étude d’un groupe de patients ayant développé des tumeurs malignes sur les lésions cliniques de l’EDV. Cette étude a pour objectif d’évaluer l’expression de la P53, BCL-2 et BAX dans les lésions malignes et bénignes de l’EDV. Les résultats montrent que l’expression de la P53, BCL-2 et BAX varie en fonction de l’état histologique des lésions. Les lésions malignes présentent une expression plus importante de ces protéines que les lésions bénignes. —Les résultats de cette étude peuvent être utiles pour la prise en charge des patients avec EDV.

18 FLUORESCENCE OVERLAY ANTIGEN MAPPING (FOAM): A NEW TOOL FOR DIFFERENTIATING BULLOUS PEMPHIGOID AND EPIDERMOLYSIS BULLOSA ACQUISITA. Marcelas C.J.M. De Jong and Siert Bruins. Dept. Dermatology, University Hospital, Groningen, The Netherlands. —The FOAM technique combines multiple wavelength band immunofluorescence and digital overlay imaging procedures, should enable reliable identification of relative antigen distributions close to the resolving power of the light microscope. —Aim and Design: To study whether FOAM can distinguish between the ultrastructurally different sites of skin-bound IgG deposits at the basement membrane zone in bullous pemphigoid (BP) and epidermolysis bulla aquisita (EB). FOAM was applied to skin specimens from seven patients with BP and six with EB, defined by clinical, immunohistochemical, NaCl-split skin and ultrastructural criteria. —Results: The FOAM images showed characteristic 'non-overlap' patterns of green-stained IgG and red-stained type VII collagen in BP, and 'overlap' patterns of IgG and type VII collagen in EB. —Conclusion: FOAM offers an attractive diagnostic tool in problematic cases of BP and EB showing absence of circulating anti-BMZ autoantibodies and/or inconclusive immunoelectron microscopic images.

19 CICATRICIAL PEMPHIGOID Versus EPIDERMOLYSIS BULLOSA ACQUISITA-FLUORESCENCE OVERLAY ANTIGEN MAPPING STUDIES. C. Konakowla, K. Wozniak, A. Gorkiewicz-Petrok, A. Kowalczyk, S. Jahnwala. Department of Dermatology, University of Warsaw, Warsaw, Poland. —In light of the similar clinical features of cicatricial pemphigoid (CP) and epidermolysis bulla aquisita (EB), fluorescence overlay antigen mapping technique (FOAM) was employed to examine the distribution of the two collagen types in the basement membranes. —The aim of the study was to assess the potential of FOAM in distinguishing between the two diseases. —The results showed that the distribution of the two collagen types in CP was different from that in EB. —In summary, FOAM may be a useful tool in differentiating CP from EB.

20 SMOOTH MUSCLE ACTIN-BASED STRESS FIBERS IN CULTURED FIBROBLASTS FROM HYPERTROPHIC SCARS AND KELOIDS. F. Pignone**, G. Gerini*, L. Borgognoni*, N. Pimpinelli**, U.M. Reali** and P. Romagnoli**. *Dept. of Human Anatomy and Histology, **Dermatology Clinic and *Unit of Plastic Surgery, University of Rome "La Sapienza," Rome, Italy. Wound healing involves relevant modifications of the extracellular matrix. Fibroblasts provide for the synthesis and contraction of this matrix and their function depends on their molecular and supramolecular architecture. In this study, we analyzed the expression and distribution of α-smooth muscle actin (α-SMA), a contractile protein, in fibroblasts grown in vitro from normal dermis, eutrophic and hypertrophic scars and keloids. All the fibroblasts from keloids expressed α-SMA actin, with fibroblasts from younger lesions showing more intense expression than those from older ones; this protein was found to be involved in stress fibers, which in turn were more prominent in keloids than in normal dermis or eutrophic scars. The results of this study show that fibroblasts from keloids and, to a minor degree, those from hypertrophic scars are especially well equipped for both synthesis and contraction of extracellular matrix, at variance with those of eutrophic scars. Therefore, these cells might be primarily responsible for the pathogenesis of hypertrophic scars and keloids.

21 ACTIN ASSOCIATED JUNCTIONS ARE PRESENT IN HUMAN EPIDERMIS AND DERMIS. T. Kowalczyk, L. Takeda, M. Vejey**, M. Halak, J. Konakowla,*** E.O. Keefe**, H.W. Kreykel**, D. Schlafer** and M. Halak*. Departments of Dermatology University *) Bonn, Germany; # Warsaw, Poland; **) Chapel Hill, USA; and **) INSERM U346/CNRS, Edouard Herriot Hospital, Lyon, France. —Despite their presence in ankylosing spondylitis and various connective tissue diseases, the full biological role of membrane and cytoskeletal proteins associated with cell-cell and cell-matrix contacts as well as their role in cell functions is not known. —The authors investigated the distribution of membrane and cytoskeletal proteins associated with cell-cell and cell-matrix contacts in normal human epidermis and dermis, and in tissues from patients with various connective tissue diseases. —The results suggest that membrane and cytoskeletal proteins associated with cell-cell and cell-matrix contacts are involved in the regulation of cell-cell and cell-matrix interactions.
23 PALMAR AND JAW CYSTS IN BASAL CELL NEVUS SYNDROME: A COMPARATIVE HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL INVESTIGATION
M. Reine, D. Metze.
Department of Dermatology, University of Minnesota, U.S.A.
Basal cell nevus syndrome is an autosomal dominant disease featuring multiple basal cell carcinomas, rib and vertebral anomalies, calcification of the falx and a high frequency of various neoplasms. In addition to odontoegenic jaw cysts, 50% of the patients present with asymptomatic cutaneous cysts, predominantly found on the face, neck, and preauricular region. A series of jaw and palmar cysts was studied histologically, immunohistochemically and ultrastructurally. The cysts were lined by a stratified epithelial cell wall. The differentiating epithelium was flattened and did not show any formation of stratum granulosum. The luminal surface was characteristically corrugated. The cysts contained compact orthokeratin. Some of the cutaneous cysts were surrounded by a dense fibrous tissue. In both jaw and palmar cysts, immunostaining for filaggrin was negative. Expression of involucrin and epidermal keratin was regular. Ultrastructural examination confirmed the absence of keratohyalin granules and the orthokeratotic configuration. In conclusion, both palmar and jaw cysts found in basal cell nevus syndrome possess a distinct cyst wall differing from other cutaneous cysts or seatoxidomas underlying their unique genetic origin.

25 LINEAR FOCAL EALOSTIS: LIGHT AND ELECTRON MICROSCOPIC STUDY OF A NEW CASE
I. Kantaki*, B. Chouery*, M. Dupin†, A. Flechette‡, P. Combemale‡.
* Dept. of Dermatology (P.Y. Claudy), Hosp. Ed. Herriot; † Dept. of Pathology (E. Henrion), Hosp. Ed. Herriot, Lyon, France.
Linear Focal Ealostis (LFE) is not yet well-known entity since 12 cases have only been reported to date after the first description in 1989. Our patient was a 25-year-old Frenchman referred to us for evaluation of herogenous yellowish, infiltrated, striate-like asymptomatic, longstanding lesions of the right lower abdominal wall. Histologic examination showed densification of dermal collagen. Ostein staining showed a well-developed elastic network within the superficial and mid-dermis consisting of fine fibres; within the deep dermis these were more sparse, but thicker, with irregular contours. Electron-microscopy showed within the superficial dermis a granular material amorphous in nature and densely packed fragments, with geographic contours; some of them presented a flag-like staining, whereas others were found within the cytoplasm of mononuclear cells (elastophagocytosis). New biopsies performed five years later showed, in comparison with the first ones, dermal sclerosis and a diminished elastic network; von Kossa staining and direct immunofluorescence were negative. An immunohistochemical study with antibodies to collagen types I, III, IV and VII did not reveal obvious differences in comparison with controlateral skin. LFE is a rare but probably underreported entity, likely misdiagnosed as stria distensae; the description of more cases will hopefully lead to the elucidation of its nature (connective tissue naevus of late onset).

27 EFFECT OF TOPICAL 1, 24-(R)-DIOHYDROXYVITAMIN D3 ON HARLEQUIN ICHTHYOSIS
Y. Taniguchi*, H. Hanochof, and M. Shimizu*.
* Dept. of Dermatology, Mie University School of Medicine, Tsu, Japan; # Dept. of Pediatrics, Suzuka Hospital, Suzuka, Japan.
Harlequin ichthyosis is a severe and usually fatal hereditary skin disorder. To rescue these patients, retinoids may be effective. However, side effects are unavoidable. Severe scaling and fissure formation are the biggest problem when patients survive. We investigated the clinical efficacy of topically applied 1, 24-(R)-dihydroxyvitamin D3 in a patient with harlequin ichthyosis. Slight unilateral improvement of scaling in favour of the 1, 24-(R)-dihydroxyvitamin D3-treated site was observed in a month. Slight thinning of the horny layer was seen in H & E sections. Multiple layer formation in vaculated vesicles were prominent in the treated sites by Electron microscopy. Since there are no side effects, 1, 24-(R)-dihydroxyvitamin D3 ointment may be useful in harlequin ichthyosis in some extents.

24 MERKEL CELLS IN NEVUS SEBACEUS: AN ELECTRON MICROSCOPIC STUDY
W. Hartwich, T. Schalme. Dept. of Dermatology, University of Heidelberg, Germany
Hyperlaxation of Merkel cells (MC), sometimes arranged in clusters, has been recently reported to occur in primitive follicular germs and trichothelomas of nevus sebaceous. The aim of this investigation was to affirm these light microscopic immunohistochemical (IHI) findings by electron microscopy (EM).
Routine EM of formalin-fixed superficial tumour tissue which had developed on a nevus sebaceous localized at the left parietal region in a 27 year old woman. By LMI, using antibodies against cytokeratin 20 and chromogranin A, no positive staining could be seen in the epithelial or the connective tissue elements of the tumour.
EM revealed many typical non-intervected MC in the neoplastic epithelium containing abundant dense-core granules of variable electron density concentrated mainly within long dendritic cell processes, a characteristic feature of MC from hair follicles and facial skin. Unlike the distribution pattern in normal skin, the neoplastic tissue MC were frequently found juxtaposed side by side or located directly on the basal membrane. No cells showing characteristics of both keratinocytes and MC (so-called transitional cells) were found.
Our results show that typical non-intervected MC, partly in an unusual arrangement, represent integral constituents in trichothelomatomatos areas of nevus sebaceus. MC hyperplasia is likely to reflect another facet of hamartomatous hyperplasia in nevus sebaceus and may play a regulatory role in the growth and development of this adenoid tumor.

26 ICHTYSOSIS BULLOSA OF SIEMENS. Ultrastructural study of a new case
C. Bédéz*, N. Delpeugt-Bertin*, B. Gillet†, M. Leboeuf‡, J. Mathieu‡, J. Boumeimiet.*
Dept. of Dermatology *Pessac, Toulouse, FRANCE; † Dept. of Pathology, Hospital, Toulouse, FRANCE; ‡ Dept. of Pathology, Hospital, Toulouse, FRANCE.
A 7-year-old boy was referred to us for evaluation of large areas of cutaneous xerosis and hyperkeratosis on skin-exposed parts of the body. The skin at birth was normal. Physical examination revealed diffuse xerosis markedly increased on the face and forearm with a linear pattern and a milium-like papular pattern. Pseudovesicul areas were also involved. Circinate areas of denuded skin and skin peeling could be seen on the dorsum of the hands corresponding to previous superficial blisters (moulting phenomena). Questioning of the parents did not reveal any skin disorder in the family and their physical examination was normal. Light microscopic examination of a biopsy specimen revealed hyperkeratosis and a prominent granular layer with cytoplasmic oedema. Electron microscopy examination showed large irregular ruffles in the stratum spinosum representing abnormal tonofilament aggregates. In the granular layer, abnormal tonofilaments were seen associated with keratohyalin granules and had lost their connections with desmosomes. Cytoplastic oedema was also seen within the granular layer. Numerous intracellular splits were found within corneocytes. Ichtysosis bullosa of Siemens is a rare subtype of autosomal dominant inherited epidermolytic hyperkeratosis. The precise diagnosis is based on the lack of erythroderma associated with hyperkeratosis of the flexural areas and localized peeling of the skin corresponding to intracorneal blistering. Ultrastructurally the level of blistering is localized in the upper layers of the epidermis sparing in most cases within the granular layer. These findings explain the delay in the diagnosis which is usually made during late infancy. Recent linkage analysis studies have suggested that the mutation is localized on the same genomic region as keratin 25 in ichthyosis bullosa of Siemens. Our patient seems to be the only member involved in his family which could account for a spot mutation in an autosomal dominant inherited epidermolytic hyperkeratosis.

28 A CASE OF UNCONTROLLED HYPERPLASIA OF THE EPIDERMIS AND DUCTS OF ECCrine SWEAT GLANDS
Panz J, Barabath-Bulles M, El Gammal S, Stucker A, Almeyer P.
Dermatologica Department of the Ruhr-University Bochum, Germany
A 56-year-old male patient developed multiple epidermal tumors, a basal cell carcinoma and several epidermoid hyperplasias in the face and neck. The skin of the face appeared indurated, induration of the eyelids had caused a physical occlusion of the eyes. Histologically apart from epidermal hyperplasia the corium contained multiple ducts of eccrine sweat glands. In this study we examined 1. the three-dimensional organization of the ducts of the eccrine sweat glands with the help of a computer-supported D-3 reconstruction technique: 2. the ultrastructure of the single newly formed ducts of the sweat glands and here especially 3. the blind ends of these ducts below the basal part of the epidermis. In the D-3 reconstructions the ducts of the sweat gland take a winding course. We found the number of sweat gland ducts increased. Most of them end below the epidermis in large lobules, missing any drainage through the epidermis. Ultrastucturally cells of these sweat gland ducts resemble as large clear cells with enlarged intercellular clefts. The covering stratum corneum is not observed. There are multiple decussating intercellular lumina within a single duct. The surrounding basement membrane is interrupted focally or missing completely. A fine fibroelastic material is seen within the eccrine ducts but also in small plagues in the upper corium. In the whole corium dense packed collagen fibres are observed. We suppose that in this clinically unusual case epidermal growth factors induce an eccrine and sweat gland hyperplasia. The parallel increase of collagen, fibroblasts and fine fibroelastic material may point to an activation of fibroblasts, as well.

Stewart-Treves angiosarcoma (STA) is a rare vascular malignant tumor developing on chronic lymphedemas, usually complicating surgical treatment of breast carcinoma. Our patient was a 82-year-old woman who had undergone in 1980 mastectomy, axillary lymph node dissection and radiation therapy for breast carcinoma. In early 1996 she developed, on her lymphedematous forearm, a rapidly growing, violaceous, hard, multinodular growth. Histology showed a diffuse, dense-cellular proliferation invading the whole dermis. Tumour cells were large, polyhedral, occasionally spindle-shaped, with hyperchromatic nuclei; they formed solid masses occasionally containing slit-like vascular spaces. The vascular origin of the tumour was univocal, as they were CD34+, CD31+ and factor VIII-related antigen positive, a feature not present in lymphedema fibrosis. Ultrasound examination showed the tumour cells to form solid cords, occasionally surrounding (pseudovascular) clefts; the cells had large nuclei, usually voluminous, with abundant cytoplasm, swollen mitochondria, multivesicular bodies and rare myelinoid bodies. Weibel-Palade bodies, desmosomes or basal membranes were not seen. Tumour cells were positive for factor VIII-related antigen, urokinase-type plasminogen activator receptor and CD31, giving them a sarcomatous aspect.


Treatment of TNF-alpha and GM-CSF induces the differentiation of precursors into dendritic cells, including expression of CD1a. In order to investigate whether these cytokines also play a role in a continuum supporting the differentiation state of LC in the epidermis, keratinocytes of normal human skin were stimulated separately with GM-CSF (20 ng/ml) and cytokines in the presence of TNF-alpha (20 ng/ml). GM-CSF (0.1 ng/ml), or both cytokines and without cytokines (controls). In control sheets, LC had fewer, shorter and less branched dendrites than in the freshly harvested sheets. The cells migrated into the medium treatment with TNF-alpha, monocyte or in combination with GM-CSF, but not in the other experimental conditions, appeared with smooth cytoplasm, adherent to each other into clusters and expressing the adhesion molecules intercellular cell adhesion molecule-1 (ICAM-1) and the TNF-alpha together which are effective in maintaining well-developed dendrites to LC within the epidermis, while TNF-alpha is able to change the shape and adhesive properties of these cells once they are deprived of the contact with keratinocytes. Since the expression of ICAM-1 is relevant to T-cells dimmision on TNF-alpha apparently induces further LC differentiation outside the epidermis.

32 IMMUNOELECTRON-MICROSCOPY IDENTIFICATION OF SUBPOPULATIONS OF CD8+ T-LYMPHOCYTES. G. Pagliaro*, A. Locati, M. Marselli, M. Cazzaniga, G.C. Maroni (1), C. Ferrari (1) and G. De Paolis (1). (1) Dept. of Dermatology, Brescia, Italy; (2) Zoopneurophysiology Inst, Brescia, Italy.

The cell population of T lymphocytes, was recently subdivided into "Th1-like" CD8+ subset versus "Th2-like" CD8+ subset, the former being postulated as "cytotoxic", the latter as suppressor. On the other hand, the CD8+CD28+ subpopulation had been previously demonstrated as showing "cytotoxic" capability while the CD8+CD28- displayed an antigen specific T cell receptor, but had been asrophoricated as "suppressor" functional capacity. We therefore tried to identify the "Th1-like" CD8+ subset versus the "Th2-like" CD8+ subset by recognizing their different immunophenotypic characteristics. To pursue this purpose the following antibodies: anti-CD4, anti-CD8, anti-CD28 and anti-CD11b were used on a number of subpopulations recognized by immunofluorescence from E-rosetted ( T lymphocytes, isolated from peripheral blood of healthy donors), immunoenzyme-microscopy was further performed using peroxidase or silver-enhanced-collodial gold as immunomethods. It was thus possible to split the CD8+ population in two further subdivisions: a) almost 60% of the cells were CD8+CD28+CD11b+, presumably corresponding to "cytotoxic" CD8+ lymphocytes; b) nearly 20% of the cells were CD8+CD28-CD11b-, presumably corresponding to "suppressor" CD8+ lymphocytes; c) over 20% of the cells were CD8+CD28+CD11b-. The interpretation of this last subset being uncertain. These qualitative and quantitative results may somehow clarify the problems currently linked to the classification of the CD8+ subset of T lymphocytes. Nevertheless, functional studies investigating the role of the different cytokines released by the "Th1-like" CD8+ subset versus the "Th2-like" CD8+ subset are necessary to clarify this problem.


Dendritic cells (DCs) efficiently take up antigens by macrophagy and mannosce receptor mediated endocytosis. In both cases, antigens are delivered to endocytic comparttments where they can be processed to antigenic peptides which are transported to the cell surface as peptide-class II complexes. To answer the question whether the mannosce receptor mediated endocytosis of antigens involves the DCs endosomal function, DCs, derived from normal skin, were labeled following in vitro ICAM-1 and the TNF-alpha together which are effective in maintaining well-developed dendrites to LC within the epidermis, while TNF-alpha is able to change the shape and adhesive properties of these cells once they are deprived of the contact with keratinocytes. Since the expression of ICAM-1 is relevant to T-cells dimmision on TNF-alpha apparently induces further LC differentiation outside the epidermis.

34 FUNCTIONAL ROLE OF LANGERHANS CELLS IN THE REGENERATIVE EPIDERMIS OF A HUMAN SKIN WOUND L.E.Kostruchenko, A.J. Kostruchenko, P.M. Volodyak, Dept. Pathology, Med Institute, Chernivtsi, Kh. "Dept. Dermatology, Medical Academy for Postgraduate Education, Kiev, Ukraine.

Langerhans cells (LC) are the main acting members of the immune system in epidermis and they are expected to play a critical role in the development of the regenerative epidermis (RE) of the immunological and functional properties of a normal epidermis. We performed an immunohistochemical study of a skin wound of a 10 day duration of patients (n=4) undergoing reparation. The bioderivedreinervated immunoregenerate stinging was used to evaluate cryostat sections of RE. Monoclonal antibodies to human antigens included: CD1a, HLA-DR, CD3, CD4 and CD8 (DAKO). The total number of keratinocytes (KC) and LC (CD1a and HLA-DR positivty) were counted in each wound segment at different times of maturation. The results revealed (1) at the tip of RE the first appearance of LC was constantly noted in the RE segment of 3 day maturity; (2) at the rate of RE growth of 0.5mm per day the number of LC progressively rose from 0.5% in the segment of the 1-3 day maturity up to 7.9% in the segment of 4 day maturity; (3) the migration of LC into RE could not be correlated with HLA-DR expression by KC or with the presence in RE of CD3+, CD4+, CD8+ lymphocytes because KC of RE did not show HLA-DR positivity, and the lymphocytes were not noted in RE, (4) it seems that LC were much attracted by the newly formed KC and that the increase in LC numbers within the matured segments of RE coincided with the reparation in the proliferative activity in RE and with KC functional differentiation and apoptosis.

30 A CASE OF TRAUMA-INDUCED BULLOUS PEMPHIGOID: EVALUATION OF AN ATIVID- REACTIVE EPITHELIOID CELL TECHNIQUE WITH AN ANTI-TYPE IV COLLAGEN AUTOREACTIVE ANTIBODY IN DIFFERENTIAL DIAGNOSIS OF BULLOUS PEMPHIGOID. Marian Demidova*, Inga Bubelis, Rigmunds Ferencis, Ina Rudzute, Gvido Kriemers, Dept. of Dermatology, University School of Medicine, Riga, Latvia; # Dept. of Pathology, University of Latvia.

An 82-year-old male presented with hemorrhagic blisters localized around the mouth, fresh erosions of the oral mucosa, and areas of tender erythematous fructation of the right forearm. Blisters soon spread to the skin of the back. Initial biopsies had been healing with milia. Single courses of pulse therapy with intravenous methylprednisolone (1 g daily for two consecutive days followed by 5 g on third day) and topical corticosteroids were not effective. His mucoso-mucous and skin lesions. The diagnosis of bullous pemphigoid (BP) was confirmed by indirect immunofluorescence on sal-}

477
P1


Darier's disease (DD) or dyskeratosis follicularis is a rare autosomal dominant disorder characterized by abnormal keratinocyte adhesion. A nine year old girl, with no apparent family history of DD, presented with small yellow-brownish papules located on the neck, scalp hair line, around the nose, and eyebrows. It was considered initially as juvenile seborrhoeic eczema. Histologically acantholytic dyskeratosis was observed. At the ultrastructural level the basal layer desmosomes were found to be intact. Acantholysis was observed in the suprabasal layers with condensation of tonofilaments and formation of dense perinuclear rings. In the granular layer dyskeratotic keratohyalin containing cells were found, together with characteristic corps ronds and granules.

P3

ELASTIC FIBERS IN DERMIS OF JUVENILE ELASTOMA. T.Karlmark, S. Bartolok and T. Kobayasi. Department of Dermatology, University of Copenhagen, Bispebjerg Hospital, Copenhagen, Denmark. Juvenile elastoma is a nevoid changes of elastic fibers and usually appears combined with other nevoid changes of connective tissue. By light microscopy, elastic fibers in elastoma were seen normal and distributed unevenly in the dermis. Previous ultrastructural studies of connective tissue nevi have shown either increased amounts of normal elastic fibers or homogenized structureless elastic fibers. The authors have studied three patients with juvenile elastoma and found that structureless homogenized matrix of elastic fibers were the real nevoid change of elastic fibers.

P4

ELECTRON MICROSCOPY STILL MAY SERVE AS A USEFUL DIAGNOSTIC TOOL IN INFECTIOUS SKIN DISEASES. M. Tronnier, Department of Dermatology, Medical University of Lübeck, Germany. For recognition of infectious agents in infectious skin diseases several approaches are possible. In most cases the clinical picture combined with histology and microbiology allows the specific diagnosis. In some cases the proof of the suspected diagnosis by ultrastructural recognition of the infectious agent may be obtained by electron microscopy. The confirmation by ultrastructural investigation may be of special value in cases with a very small number of microorganisms and in cases with an unusual clinical presentation. In a case of cutaneous leishmaniasis the diagnosis was confirmed by ultrastructural detection of bacteria in lesions of bacillary angiomatosis where the diagnosis was proved. Despite of other possible specific diagnostic procedures, electron microscopy is an easy and reliable method for the confirmation of the diagnosis in the reported cases.

P5

CONFLUENT AND RETICULATED PAPILLOMATOSIS GOUGEROT-CARTEAUD. M.J. Arnold, J. Antillon-Lampré. Institute for Ultrastructure Research of the Skin, Rupeheuts-Karls University, Heidelberg, Germany. Confluent and reticulated papilomatosis, first described by Gougerot and Cartaud (1927, 1932), is a relatively rare dermatosis clinically characterized by brownish papules which coalesce to confluent reticulated plaques. Although a series of patients has been reported its etiology still remains unknown. Various hypotheses have been put forward including endocrine disturbances with obesity and diabetes mellitus, an abnormal reaction to Pityriasis rubra infections, a genetically determined defect of keratinization, and a form of localized skin amyloidosis. Definite proof of a genetic disposition is not yet available. Two reports of familial occurrence exist, but the majority of cases are sporadic.

Until now, characteristic markers pathognomonic of this disease are missing in the literature. Three patients (2 females, 19y/ 24y; and 1 male 25y) were investigated by EM. No amyloid deposits were present in our patients. The epidermis showed slight acanthosis with papillomatosis, a reduced granular layer, enhanced numbers of keratohyalines and a loose hyperkeratotic horny layer. A peculiar finding in the keratinized layer of the upper granular layer of all three patients was the presence of perinuclear hollow spaces probably enlarged during the embedding procedure. Depending on the cutting plane, fine membranes were recognizable in these spaces. Their remains were demonstrable in the keratinized cells of the horny layer in the form of stacks of elongated membranes and membrane cells. To the best of our knowledge, this is the first time that these characteristic markers allow to confirm a disturbance of epidermal differentiation and keratinization for this disease and to delineate Gougerot-Cartaud disease as a distinct entity.

P6

ANGIOLYMPHOID HYPERPLASIA WITH EOSINOPHILIA LIGHT MICROSCOPIC AND ULTRASTRUCTURAL STUDY. E. Zombai and M. Magyarlaki. Dept. of Dermatology, Medical University Pécs, Hungary. The histology and ultrastructure of angiolyphoid hyperplasia with eosinophilia in two cases are presented. The diagnosis was based on histology, which showed abnormal vascular proliferation, infiltration with eosinophils and proliferation of lymphoid cells. Ultrastructural studies supported the vascular nature of this disease, the lesions contained mature-appearing vessels, clusters of atypical endothelial cells with pseudo-lumen formation and surrounded by basement membrane and the presence of large bundles of the cytoplasmic filaments in isolated cells. The results of immunoperoxidase studies using endothelial cell markers revealed that the proliferating cells were of endothelial origin. The histologic and ultrastructural findings suggested that angiolyphoid hyperplasia with eosinophilia is a vasoproliferative lesion with a specific inflammatory cell response.
P7 DERMAL MAST CELLS AND ACTIVATION OF THE EPIDERMAL MELANIN UNIT IN MAST OCYTOSIS (IMMUNOHIStOChemICAL - UTRALSTRUCTURAL STUDY).
M. Pec, I. Plank, K, Belej, J. Jakubovska, V. Vilo, J.Pec. Jenevus Faculty of Medicine, Comenius University, Martin, School of Medicine, Comenius University, Bratislava, Slovakia.

Ten children with mastocytosis were divided into: systemic mastocytosis (SM) - infiltrates of skin, bone marrow, lymph nodes, liver (3 cases), and urticular-pigmented (UP) - mast cell infiltrates of the skin (7 cases). Skin lesions were investigated dermoscopically, showing discrete pigmentation and intense vasodilated capillaries of the corium (SM), and hyperpigmentation (hyperpigmented network of the skin) and mild vasodilation (UP). L-DOPA reaction demonstrated numerous melanocytes also in the upper and intermediary corium (more in UP). The findings correlated with the melanin detection (Warthin - Starry, Fontana). PCNA labelling indicated of keratinocytes proliferation. Langerhans cells (CD1a+) were numerous. Mast cells expressed CD45, vimentin, NSE, alpha-DAT, alpha-D-AC, lysozyme, alpha-naphtol-CHAE, but were negative for CD20, CD45RO, substance P, serotonin, S-100 protein. In TEM mast cells (SM) showed spongy thimbeloid shape, large oval or biconvex nuclei, a markedly reduced number of small granules situated in the peripheral part of the cells, but also some giant atypical granules, particularly with lamellated structures. Villi were very long, creating a network, and contained immature granules and fine or coarse granular matrix. The results of the TEM were evaluated also by morphometric analysis.

P9 BULLOUS PEMPHIGOID WITH EXTENSIVE MILIA FORMATION IN A PATIENT WITH THIN SUPERSFICIAL SPREADING MELANOMA.
I. Botte, S. Vasileva. Department of Dermatology, Alexander's University Hospital, Sofia 1431, Bulgaria.

Bullous pemphigoid (BP) is a subepidermal blistering disease, that infrequently shows milia formation. Some cases have been associated with visceral malignant neoplasms. A 74-year-old male presented with disseminated urticarial-like and figure erythematous rash with multiple vesicles and bullae up to 1 cm in diameter arising both on erythematous and normal skin, some bullae were haemorrhagic. Histology showed subepidermal blister with an intact epidermis forming the roof and dense inflammatory infiltrate containing many eosinophils. Direct immunofluorescence revealed linear deposits of IgG and C3 along the basement membrane zone. On 1.6 ml NaCl-split skin they were located at the epidermal site of the blister. By immunoelectron microscopy using peroxidase-labelled antibodies deposits were located in the upper part of lamina lucida and in some areas occupied the whole lamina lucida. The condition was well controlled with systemic steroids. At the sites of blisters multiple milia formed being agranular or arranged in an annular pattern. Five years previously, a growing mole was excised from patient's left forearm. Histology showed a superficial spreading melanoma (SMM). Clark level II, Breslow thickness 0.65 mm. No signs of recurrence were detected. In conclusion, we report a case with SMM followed by severe blistering disease with extensive milia formation, otherwise typical for BP. It is not clear whether the association of BP and SMM is responsible for the unusual extensive milia formation in our case.

P10 EVIDENCE OF PERICAPILLARY COLLAGEN FIBRILS IN THE BRAINS OF RATS AFTER EXPERIMENTAL CARDIAC ARREST AND IN SENILITY.
M. Walski, Medical Research Centre, PAS, Warsaw, Poland.

The problem of reparative processes in the central nervous system has been investigated extensively so far. The possibility of the transformation of cells other than fibroblasts into the multipotential cell of matrix proteins production has not been elucidated sufficiently as yet. The current opinion is that extracellular matrix serves as a background for all the reparative processes. Cerebral cortex of rats surviving from 6 to 12 months after experimental cardiac arrest and of senile 4-year-old rats was studied electronmicroscopically. As fixatives we used histochemical markers: alcin-blue, tannic acid or potassium ferrocyanide. In the capillary and precapillary spaces adjacent to the basement membranes single collagen fibrils lie parallel to the long axis of endothelial and pericyte bodies. Sometimes pericytes or astrocytes containing collagen fibrils were observed at the side of an adjacent neurons. The transverse diameter of the fibrils was approximately 40 nm. Further investigation included the precapillary vessels that were connected with the brain parenchyma by collagen fibrils. Such a location suggests the anchoring of the fibril to the pericytes, astrocytes and smooth muscle cells from one side and to the basement membrane mesh from the other side. In the cerebral cortex of senile 4-year-old rats, in enlarged spaces between capillary vessels and brain parenchyma we observed the giant forms of collagen fibrils of a diameter about 100 nm exhibiting irregular profiles on transverse sections. We interpret these findings as evidence of a reparatory process in the border zones between capillaries and precapillaries and the adhering parenchyma brain cells. According to our view non fibroblastic cells in the rat brain are capable to synthesize collagen protein and collagen fibrils anchored to adjacent cellular compartments.

P11 KAPOSI’S SARCOMA - ELECTRON MICROSCOPIC STUDY.
C. Tukaj*, T. Wzrzkowka*, and J. Roszkiewiczow, Lab. of Electron-Microscopy, # Dept. of Dermatology, Medical University of Gdańsk, Poland.

The purpose of this study was to examine the ultrastructure of Kaposi’s sarcoma (KS). Punch biopsies of skin were obtained from four HIV-seronegative patients with clinically and histologically confirmed KS. The phenotypic expression of neoplastic spindle cells and vascular components in nodular stage of KS were examined in electron microscopic sections. The vessel-forming cells presented different stages of maturity. Stromal cells were morphologically identified as macrophages, smooth muscle cells or fibroblasts in various stages of differentiation. Stromal cells showed phagocytic activity manifested by presence of lysosomes containing intact or partially digested erythrocytes (erythroplagosomes). Masses of haemosiderin were seen in vascular endothelial cells and in spindle cells of KS. The present results support the opinion that KS is derived from primitive mesenchymal cells which differentiate either into vessel forming cells, or express other mesenchymal phenotypes.

P12 THE ULTRASTRUCTURAL FEATURES OF MAST CELLS IN ALLERGIC CONTACT DERMATITIS.
J. Roszkiewicz, M. Crubel, M. Lange. Department of Dermatology, Medical University of Gdańsk, Poland.

Changes in the ultrastructural features of mast cells in the skin of patients with allergic contact dermatitis were characterized by degranulation of these cells with releasing of their contents into the interstitial space. This process manifested itself by the disruption of the cell membrane continuity, by the presence of deeply reaching intracytoplasmic cisternae which communicated with the extracellular spaces, and by the presence of secretory granules out of the cells. A diminution of electron density of the whole granules or their part was also observed. The mast cells participating in allergic reactions were in a direct contact with adjacent lymphoid cells, surrounding them with long, thin processes which were arranged parallel to the surface of the lymphoid cells, often in several layers.
P13

THE ULTRASTRUCTURAL FEATURES OF LANGERHANS CELLS IN PATIENTS WITH CHRONIC ALLERGIC CONTACT ECZEMA. J. Roszkiewicz, A. Roszkiewicz, A. Babicka-Siedlecka, Department of Dermatology, Medical University of Gdansk, Poland.

The number of Langerhans cells (LCS) in patients with eczema was considerably higher than that in the epidermis of healthy people. The alterations in the ultrastructural aspect of LCS were characterized by a distinct membrane of the Birbeck granules and a significant increase in the number of granules accompanied by the increase in volume of smooth- and rough-surfaced endoplasmic reticulum. These changes may be regarded as a morphological expression of the increase of the synthetic and secretory activity of LCS. In subjects with eczema LCS in the epidermis rarely occurred singly forming small groups of cells associated with immediately adjacent precursors cells (the so-called indeterminate cells), lymphoid cells and keratinocytes; forming morphologically visible and functionally units. Similar morphologic units consisting of LCS, indeterminate cells, lymphoid cells and microphages were observed in the dermis.

P15

EXPRESSION OF GAP-JUNCTION PROTEIN CX43 IN DARIER DISEASE AND HAILEY-HALLIEY DISEASE. E. Kwalewska 1, A. Wdzieclak 2, B. Schmidt 2, M. Rojanowski 1, A. Jablonska 1,2. 1 Department of Dermatology, Medical University of Warsaw, Warsaw, Poland; 2 Department of Pathology, Hospital Kanselaria, Bydgoszcz, Poland.

Recently we and others have found normal expression of desmosomal and adherens junction-associated proteins in uninvolved epidermis of both Darier disease (DD) and Hailey-Halliey disease (HHD), and the dissolution of desmosomal proteins in the cytoplasm of acantholytic cells. These suggest that the acantholysis in both diseases is not due to a primary defect in the synthesis of proteins forming cell-cell contact (CCC) but to the dissolution of desmosomal proteins by unknown cytoplasmic process. Gap junctions, in addition to desmosomes and adherens junction, are forming CCC in epidermis. In contrast to desmosomes and adherens junction gap which are responsible for mechanical adhesion, gap junction are important for communication and ionic exchange between the cytoplasm of neighboring cells. In this study we investigate the expression and ultrastructural localization of connexin 43 - gap junction protein - in apparently normal and tessellated epidermis of DD and HHD with the use of immunohistochemistry (IHC) and post-embedding immunogold electron microscopy (IEM). The IF studies showed that granular deposits of connexin 43 in the area of CCC in the upper part of uninvolved epidermis of both DD and HHD. The perilesional epidermis of HHD the distribution of CX43 was observed in CCC and at the periphery of the cytoplasm. In acantholytic keratinocytes of both disease CX43 connexin showed a dotted pattern and clumps in the peripheral cytoplasm. IF staining was stronger in HHD in contrast to DD. The IEM confirmed the presence of connexin 43 in gap junction in uninvolved epidermis of HHD and DD. In the acantholytic keratinocytes of both HH and DD accumulation of gold particles was observed in the perinuclear cytoplasm. However, in the keratinocytes of both disorders gap junction structures were randomly detected, which suggests a disorganization of gap-junction structures. These results support the supposition that changes in CCC structures of acantholytic cells in HHD and DD are rather secondary, and not due to a primary defect in the synthesis of CCC proteins.

P16

TRANSIENT BULLOUS DERMOLYSIS. NORMALIZATION OF ULTRASTRUCTURAL CHANGES AFTER A FOUR-YEAR FOLLOW-UP. J. Kanitakis 1, M. D'Inca 2, R. Roget 3, P. Souteyrand 3, P. Vanlierdeghem 3, J.-P. Buquet 3. 1 Dept. of Dermatology, Ed. Herriot Hospital, Lyon; 2 Dept. of Dermatology, University of Lyon; 3 Pathology, Hop. l'Hotel Dieu, Clermont-Ferrand, France.

Transient bullous dermatolysis (TBD) is a rare disease of which fewer than 20 cases have been reported to date. Our patient was first presented at the 1995 SCUR meeting (JID 1996, 107, 265). It was a female baby born in 1992 to non-consanguineous parents; she presented the typical features of TBD (a congenital mecanobullous eruption repressing within one month with residual milia). Changet in dermis and epidermis manifested after this UVA dose. Elastic fibers disappeared almost completely. Occasionally, single disrupted fibres were seen. Regular collagen fibre bundles with small long spacing collagen foci were observed. The endothelial cells of terminal vessels were of low activity and around the vessels there were active histiocytes with an abundant rough reticulum. Melanophages proved to be the most characteristic cells in all specimens. There was an increase in the number of melanosomes in the basal layer.

P17

STUDIES ON THE FUNCTION-RELATED STRUCTURE OF LANGERHANS CELLS IN MOUSE EPIDERMIS. Z. Karas, I. B. Wierchol. Dept. of Radiology and Cell Biology, Karol Marcinkowski School of Medicine Poznani, Poland.

In this work we studied the biogenesis of Langerhans cells and the role of keratinocytes in their function. The data suggest that the function of keratinocytes is mediated by the signal transduction pathway involving the release of cytokines and other factors that can stimulate the maturation and migration of Langerhans cells. The results also showed that the number of Langerhans cells increased in the epidermis of mice with a high number of keratinocytes. However, the number of keratinocytes in the epidermis of mice with a high number of Langerhans cells was significantly lower than in the epidermis of mice with a low number of Langerhans cells. The results obtained in this study suggest that the function of keratinocytes is mediated by the signal transduction pathway involving the release of cytokines and other factors that can stimulate the maturation and migration of Langerhans cells.
P19


*) Cussons International Ltd, Stockport, England; #) Unoda S.A., Warsaw, Poland.

Recently using in vitro corneocyte release assays it has been established that moisturisers facilitate desquamation by enhancing the enzyme mediated lysis of cornodesmosomes in the stratum corneum. In moisturisation efficacy studies using simple prototypes it has also been demonstrated that neither a barrier lipid complex alone or glycerol alone effectively relieve skin xerosis but when both are combined a dramatic and synergistic relief of scaling occurs. In the present study comparing the effects of the barrier lipid and glycerol in a fully formulated emulsion to its corresponding placebo in newly established in vivo corneocyte release assays, a dramatic four fold increase in the cell exfoliation process was observed with the lipid and glycerol emulsion (P<0.05). In moisturisation efficacy studies the lipid and glycerol containing emulsion relieved skin xerosis faster and to a greater extent than the corresponding placebo (2 grading scale unit separation; P<0.05) and subjects were also able to perceive the effects of the formulations on their skin condition. We ascribe the benefit of the lipid and glycerol containing emulsion to its effects on cornodesmosysis.
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.