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001

Immunofluorescence and Immunoelectron Microscopic Evidence that Pemphigus Vulgaris IgG Causes no Steric Hindrance in Desmosome Formation in Cultured Keratiocytes

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Low-Ca^{++} (0.05 mM) grown keratinocytes (KC) produce no desmosomes (DS), but they form "half-DS", which can couple to form DS within 2 h when Ca^{++} is switched to high (1.2 mM). We studied the effects of pemphigus vulgaris IgG (PV-IgG) on this Ca^{++} induced-coupling of "half-DS" to form DS. Low Ca^{++} normal human (NH) keratinocytes and human carcinoma (DJM-1) cells were pretreated with PV-IgG (confirmed to be specific to Dsg3) or NH-IgG for 30 min in low Ca^{++}, and Ca^{++} concentration in the medium was switched to high. After 2-h incubation in high Ca^{++}, cells were studied by immunofluorescence (IF) and immunoelectron (IEM) microscopy, using anti-Dsg3 affinity purified polyclonal, Dsg1/2, desmocollin 3 (Dsc3) and desmoplakin 1/2 (Dpk) monoclonal antibodies. In low-Ca^{++} grown cells, IF showed a punctate distribution of Dsg3, Dpk and Dsc3 (but not Dsg1/2) in the cytoplasm and/or on the cell membrane, but not at cell-cell contacts, and IEM revealed Dsg3 on the cell surface "half-DS". The existence of Dsg3, Dpk and Dsc3 in low Ca^{++} cells was confirmed by Western blotting. Double-staining IF revealed that high-Ca^{++} switched cells after pretreated with PV-IgG for 30 min in low Ca^{++} showed a punctate-linear pattern of PV-IgG, which were colocalized with Dsg3, Dsc3 and Dpk, at cell-cell contacts. When low Ca^{++} cells were treated with PV-IgG for 5 min and labelled with antihuman IgG-5 nm gold for 5 min in a low Ca^{++} medium, and followed by incubation in high Ca^{++} without antibodies for 2 h, gold-labels were detected in the newly formed DS as well as on "half-DS", by IEM. These IF and IEM results demonstrate that PV-IgG causes no steric hindrance in DS formation.

002

Photodynamic Therapy and Apoptosis in Cutaneous Basal Cell Carcinoma

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The effects of photodynamic therapy (PDT) on epidermis and dermis were evaluated by electron microscopy and affinity cytochemistry on punch biopsies of cutaneous basal cell carcinomas (BCC) between 15 min and 72 h treatment. All patients healed within 30 days. The epidermis showed intercellular edema at each time point. At 15 min, some basal keratinocytes had nuclei?) with dense, marginated chromatin and condensed cytoplasm with clusters of translucent vesicles, suggestive of apoptosis. At later time points, these modifications were observed also at progressively higher epidermal levels. At 4 h, granulocytes were found scattered in the basal layer and collected into aggregates within prickle and granular cell layers; together with damaged keratinocytes. The latter cells had nuclei with dense, marginated chromatin, clear cytoplasm with organelle remnants and interrupted cell membranes.

These features are suggestive of keratinocytes apoptosis deranging into cell lysis. Few granulocytes were scattered throughout epidermis at 24 h; at this time point, many mitotic figures were seen in the basal cell layer. TUNEL-positive cells were found in the basal cell layer at 4 h and, in higher amounts, in basal and prickle cell layers at 24 h; at later time points, TUNEL-positive cells were distributed in all epidermal cell layers, but in lower numbers. Some Bcl.2 positive cells and annexin-V positive cells were scattered among all epidermal layers, most numerous at 24 h. The dermis contained many mast cells underlying the epidermis at 15min and at later time points was infiltrated by leukocytes (mostly granulocytes at 4 h, mostly lymphocytes and large, pale monocytoid cells later on). At 72 h, cells of the last type prevailed, and many were rich in lysosomes, i.e. they were activated macrophages. TUNEL positive cells and annexin-V positive cells were found in the dermis at 24 h. The results suggest that apoptosis and leukocyte infiltration occur in the skin during the early stages of BCC healing upon PDT, and lead us to hypothesize that these processes are related to the mechanisms of healing.

003

Semi-Quantitative Analysis of the Constitutive Fas-L Expression by Keratinocytes

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In a previous study we demonstrated that basal keratinocytes (KC) in resting normal human skin express the ligand of the CD95 receptor (Fas-ligand, Fas-L), a molecule able to trigger apoptosis of CD95 (Fas)-expressing target cells. Since a consistent Fas-L expression by epithelial cells is able to preserve the homeostasis of certain epithelial tissues, we intended to investigate to what extent the homeostasis of the normal human epidermis can be preserved by KC Fas-L expression.

For such a purpose, we performed a semiquantitative analysis of the Fas-L expression by KC in normal human skin. "In situ" gold-immunoelectronmicroscopy on skin ultracryosections was carried out, and thereafter the number of gold particles expressed at the KC surface per cell section (midplane) was evaluated. Relatively few, namely, 51.55 ± 28.61 ($n = 100$), 10-nanometers sized, colloidal gold particles were observed at cell suface of KC resident in the basal layer of epidermis. Extending preliminary results, gold granules were detected even at the plasma membrane of KC resident in the spinous layer, namely, 25.41 ± 15.80 ($n = 100$) particles per KC section in the lower spinous layer, and 13.99 ± 14.03 ($n = 100$) particles per KC section in the upper spinous layer. By contrast, the KC labeling of ultracryosections prepared as controls for method specificity was not significantly different in various epidermal layers, namely, 0.75 ± 0.88 ($n = 100$) per KC section in the basal layer, 0.81 ± 0.88 in the lower- and 0.76 ± 0.87 in the upper-spinous layer. Thus, KC in resting epidermis express Fas-L in relatively moderate amounts, and such a low expression is not presumably capable, alone, to fully preserve the homeostasis of human epidermis.

It is temping to speculate, however, that Fas-L expressing KC may possibly exert at least some cytolytic activity against both Fas-expressing inflammatory cells infiltrating the epidermis and Fas-expressing epidermal cells bearing viral/neoplastic antigens. In conclusion, the herein demonstrated expression of Fas-L by KC, although moderate, may, at least within the limits of low antigenic stimuli, favour the maintenance of epidermal homeostasis.

004

Reductions in Desmosome Size and Number in Skin Fragility Syndrome (SFS) with Homozygous and Heterozygous Defects in the Desmosomal Protein Plakophilin 1 (PkP 1)

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Mutations in the gene encoding the desmosomal plaque protein plakophilin 1 (PkP1) are now known to underlie the skin fragility syndrome (SFS) in which epidermal desmosomes are small and reduced in number with a consequent reduction in cell-cell cohesion. We undertook a detailed quantitative electron microscopic examination of epidermal desmosomes in the lower suprabasal layers, where PkP1 is most highly expressed. The study was performed in an SFS patient with homozygous splice site mutations in the PkP1 gene and in an unaffected heterozygous sibling with a defect on a single allele. Three skin biopsies from healthy, normal subjects served as controls. The size and number of desmosomes per high power field (HPF) ($n > 50$) was assessed at a magnification of $\times 30$ K. The suprabasal desmosome size was 350.2 ± 9.3 nm (mean $\pm$ SEM) in control skin. Desmosomes in the affected SFS patient's skin (167.9 ± 7.5 nm) and the unaffected sibling (231.6 ± 9.6 nm), both showed significant ($p < 0.01$) reductions in desmosome size, to 47% and 66% of normal epidermis, respectively. The mean number of desmosomes per HPF was 2.3 ± 0.1 in the SFS patient's skin (39% of the control value), 4.0 ± 0.3 in the unaffected sibling (66%) and 6.0 ± 0.3 in control skin. Both the SFS patient and the unaffected sibling showed significant desmosomal changes compared to control skin ($p < 0.01$), despite only the homozygous patient exhibiting any clinical symptoms.

Desmosome adhesion in the unaffected sibling therefore may be compensated for by sufficient cell-cell binding and desmosomal stability, due to the expression of at least some wildtype PkP1. These findings attest to the important role of PkP1 in stabilizing desmosome structures, influencing plaque size and the frequency of such junctions.

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Expression of CD1 Family Molecules in Primary and Metastatic Melanoma

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CD1-restricted-T cells have been suggested to play an important role in immune responses against tumors. The CD1 proteins are molecules able to present lipids/glycolipids, including gangliosides, to T cells. Melanoma cells are rich in gangliosides. We wished to know whether CD1 molecules might play a role in melanoma pathology.

To address this question, we investigated by immunohistochemistry (ICH) the expression of CD1 molecules on cryosections of primary melanoma (n = 10), metastatic melanoma (n = 10) and normal skin (n = 10). In normal skin, few scattered dendritic cells (DC) were positive for the CD1 molecules. In the mononuclear infiltrate of primary melanoma, an important intra/peritumoral expression of CD1 molecules by DC was observed.

Even though a similar number of HLA-DR$^+$ DC were present, there was a significant reduction in the expression of CD1 molecules in metastatic melanoma. ICH using anti-IL10 antibody revealed a strong expression in metastatic but not in primary melanoma. Culturing CD1$^+$ DC in a medium containing IL10 resulted in the down-regulation of CD1 expression in a dose dependent manner. Supernatant from melanoma cells, but not from fibroblasts, down-regulated CD1 expression on DC. Neutralizing anti-IL10 antibody blocked this effect. We speculate that expression of CD1 molecules by dermal DC might play a role in the immune response to primary melanoma. However, this mechanism down-regulating the CD1 proteins via an IL10-dependent pathway could disappear in advanced melanoma stages.

006

Ultrastructural Features of Hereditary ''White Nails''

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Hereditary subtotal leukonychia is a rare nail disease. We have analyzed microscopically and ultrastructurally the white nails of a patient from a family in which the trait is inherited in an autosomal dominant manner as an isolated symptom. No skin lesions or hair abnormalities could be detected.

A longitudinal surgical biopsy of the nail from a big toe was split in two parts. One part was fixed in formalin and processed for histopathology. Another part was further subdivided and embedded either in Epon, following fixation in 2% glutaraldehyde, or in Lowicryl K4M, after fixation in 3% paraformaldehyde. Dewaxed nail sections and Lowicryl ultrathin sections were stained with several antikeratin antibodies, in search for abnormalities of keratinisation. The nail matrix presented an abnormal hypergranulosis. The upper part of the nail plate, originating from the proximal nail matrix, had a nonhomogeneous lamellar appearance, with numerous intracellular ''lipidic'' vacuoles and ''empty'' spaces separating keratin filament bundles. These cells were progressively shed at the nail surface. The cell loss was compensated by hyperproliferation of the distal matrix and of the nail bed keratinocytes, with persistent marked parakeratosis and loose arrangement of keratin bundles. The lower plate made up 80% of the nail thickness and presented the characteristics of a tissue composed of soft keratins. Our morphological findings suggest a mutation in one of the hard keratin genes as a possible cause of the hereditary leukonychia. Genetic linkage studies of the family are in progress.

007

Structure of Skin Appendages During the Prenatal Development in Skin

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The evolution of hair, apocrine and eccrine sweat and sebaceous glands during foetal development has been the subject of research for more than 100 years.

The aims of our research have been both the estimation of the time of the hair germs' development and the observation of the initial stage of sweat and sebaceous gland development from epidermis and hair germs, including topographic differences and a detailed analysis of the periods of gland formation.

110 skin biopsies were taken from foetuses aged 8–36 gestation weeks, as well as 4 from newborns. The samples were obtained from hands, heads, perianal and pubic regions. The samples were stained using H-E, Mallory, Masson, PAS and Bielschowsky-Lawrentive methods.

The earliest visible germs of hair were observed in lips and forearms in the 8th-9th week of foetal life. The first sebaceous glands appear in the 3rd-4th week of the third month. There are 8 stages in the development of hair. The formation of eccrine gland germs was observed in the 4th month and the differentiation in the 6th-8th months. There are 3 stages of that process: The first concerns palms and soles, the sceond the axillar region and the third the whole surface of the skin. The apocrine glands are formed in the 3rd-4th months of foetal life. The sweat glands of axillar, perianal and pubic region have typical characteristics of both eccrine and apocrine glands. The nervous apparatus of hair develops in the fourth month of life from hair germs, which have mature bulbs and papillae.

008

Control of Basement Membrane Assembly by Nidogen–Laminin Interaction in Organotypic Coculture (Skin Equivalent)

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Regular basement membrane (BM) formation can be observed in organotypic cocultures (OTC) of epidermal keratinocytes and fibroblasts by indirect immunofluorescence (IIF) and *trans*-mission electron microscopy (EM) within about two weeks. Cross-linking of type IV collagen (C IV) and laminin (LN) by nidogen (ND) is apparently crucial for the regular assembly of epidermal and other BM's. For a direct proof in OTC, we have competitively inhibited ND–LN interaction with a recombinant laminin γ1 fragment (Lγ1f) covering the ND binding site. By applying of Lγ1f either on top of the air-exposed epithelium or with medium deposition of ND at the epithelial-matrix, the interface was completely abolished. There was also a dramatic reduction of LN-10 (seen by LN- or LNγ1-antibodies) and perlecan at the matrix interface, while C IV appeared quite normal initially (at 10 days), becoming diffusely distributed later on. Other components of the BM zone were only mildly or not at all affected, such as LN-5, C XVII (BP180), and integrin α6β4. Furthermore, differentiation was largely normal and there was no apparent cytotoxic effect. Accordingly the inhibition was reversible when treatment was discontinued, while already formed BM-complexes could be still dissociated by Lγ1f added later. According to EM, the blocking of the LN–ND interaction by Lγ1f suppressed BM and also hemidesmosome formation and caused the displacement of keratin filament bundles from the basal cell aspects. Subtle changes in the molecular distribution became manifest by immuno-EM using antibodies against C IV, integrin β4 chain, BP230, and pan-keratin which underlines the crucial role of proper BM-assembly for the functional integrity of the epidermis.

009

Laser Scanning Confocal Microscopic Study in Lichen Sclerosus and Morphea

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The study included three cases of morphea and three cases of lichen sclerosus (LS) diagnosed according toclinical and histopathological criteria. Biopsies from patients' skin and a control biopsy from normal human skin were labelled with antibodies against β-4 intergin (lamina lucida marker), collagen IV and N-terminal end of collagen VII (lamina densa-LD markers) and C-terminal end of collagen VII (sublamina densa-SLD marker), and studied with the use of laser scanning confocal microscopy.

The three-dimensional reconstruction of various regions of basement membrane zone (BMZ) showed a decrease in the number and size of the dermal papillae in both LS and morphea as compared to normal skin. In the morphea, there were numerous invaginations of flattened BMZ at the level of lamina lucida and lamina densa, whereas numerous holes were present in LS BMZ. The vascular skin network systém, visualized by labelling with anticollagen IV antibody, revealed increased angiogenesis in morphea, as compared to LS and normal skin. The three-dimensional reconstruction of BMZ and skin vascular networks revealed the different alterations of BMZ and vessels in morphea and LS. Our studies provide further evidence that these two diseases are separate entities – in spite of the not infrequent overlapping of their clinical and histological patterns.

010

Alteration of the Lower Part of the Basement Membrane Zone in Epidermolysis Bullosa Acquisita: Three-Dimensional Reconstruction of BMZ in Laser Scanning Confocal Microscopy

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Our previous laser scanning confocal microscopy (LSCM) study showed a discrepancy between the localization of the target antigen for antibasement membrane zone antibody (aBMZAb) and *in vivo* bound IgG in epidermolysis bullosa acquisita (EBA). The circulating aBMZAb is a recognized antigen present between the localization of N-terminal and C-terminal ends of collagen VII, whereas *in vivo* bound deposits of IgG were localized below the N-terminal end of collagen VII, colocalized and extended below the C-terminal end of collagen VII. The presence of *in vivo* bound IgG below C-terminal end of collagen VII could be due to damage to the BMZ and the production of antigen de novo. To prove this hypothesis in this study, the skin of EBA patients was labelled with antibodies against beta-4 integrin (lamina lucida marker), collagen IV and N-terminal end of collagen VII (lamina densa-LD markers), C-terminal end of collagen VII (sublamina densa-SLD marker), followed by three-dimensional reconstructions of different regions of BMZ using LSCM. Our study showed numerous invaginations of BMZ at the level of lamina lucida and lamina densa. The antibody labelling for C-terminal end of collagen VII revealed discontinuous staining along BMZ and the presence of large deposits of this protein orientated vertically to the BMZ and extending from areas of invaginations to the deep dermis. Our study suggest that the discrepancy between the localization of target antigens for aBMZAb and *in vivo* bound IgG in EBA is due to the alteration of the lower part of BMZ.

011

Giant Cell Tumor of Tendon Sheath – Localized Type (Pigmented Villonodular Tenosynovitis). Case report

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The authors describe the case of a 57-year-old woman presenting with an enlargement of her right 4th toe without any bone involvement on X-ray examination. Punch biopsy findings included nodules separated by collagenous septae formed by middle-sized polygonal mononuclear and foam cells, positive for anti-PGM positive (macrophage marker) with an admixture of B and T lymphocytes. The diagnosis of a giant cell tumor of a tendon sheath was suspected. Because of the tumor extension the amputation of the whole digit had to be performed. Histological examination revealed additional features consisting of scattered multinucleated giant cells, xanthoma cells, cleftlike spaces and hemosiderin pigment which confirmed the diagnosis. Bone invasion was not present. Two years after the surgical intervention there were no signs of tumor recurrency.

012

Neutrophilic Eccrine Hidradenits Secondary to Serratia Marcescens Infection Demonstrated by Electronmicroscopy

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Neutrophilic eccrine hidradenitis (NEH) is a rare dermatosis of unknown origin, developing usually after the administration of chemotherapeutic treatments for haemopoietic malignancies. Very rarely, NEH may have an infectious origin. We report the case of a 31-year-old man operated for ependymoma who presented a typical eruption of NEH, although he had not received chemotherapy. The eruption was comprised of successive crops of small, erythematous papules that began on the legs and progressively spread to the thighs and the abdomen. Histological examination of a skin biopsy performed under strictly aseptic conditions showed focal necrosis of eccrine secretory coils extending to the excretory ducts and to adjacent blood vessels; these were surrounded by a dense infiltrate of pycnotic neutrophils.

Electronmicroscopic examination of a skin biopsy showed a dense, mainly neutrophilic inflammatory infiltrate surrounding eccrine sweat glands. Several neutrophils contained cytoplasmic inclusions with a granular content of variable electron-density (phagosomes), and some of them contained membrane-bound round or oval-shaped bacteria. Culture of a skin biopsy revealed Serratia Marcescens (SM) in the absence of other bacteria. The dermatosis improved after antibiotherapy (ceftriaxone plus tetracyclin) but recurred twice and cultures isolated again showed SM. Up to now, only three cases of NEH of infectious origin have been reported. (One of them was due to SM). Our case demonstrates the usefulness of EM, as it showed for the first time the presence within the lesions of the responsible microorganism and suggested antibiotherapy as an appropriate treatment.

013

Olmsted Syndrome. Light and Electronmicroscopic Study of a New Case

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Olmsted syndrome (OS) is a rare keratinization disorder (18 patients published so far), associating a mutilating congenital palmoplantar keratoderma (PPK) with periorificial and intertriginous erythematokeratotic lesions. We report herein a new case of OS, that was studied by light and electronmicroscopy (EM). The patient was a 7-year-old boy born to consanguineous parents of Algerian origin, presenting with the major features of OS, i.e. a thick, mutilating PPK with ainhum-like constrictions and flexion contractures of the fingers, nail dystrophy, perianal, scrotal, axillary, inguinal, and perioral erythematokeratotic lesions. He also suffered from severe growth retardation, muscular atrophy and joint laxity of the lower limbs.

A skin biopsy from the palm showed hyperkeratosis, hypergranulosis and psoriasiform hyperplasia of the stratum malpighii. The mitotic index and nucleolar organiser regions (AgNORs) of the epidermis were increased as compared with a control skin specimen.

Immunohistochemically, there was normal expression of high MW keratins, subnormal expression of filaggrin, and highly increased expression of involucrin and calprotectin. S100 + epidermal Langerhans cells were sparse, and one of them was seen by EM to be in mitosis. By EM, corneocytes contained a dense matrix, lipid droplets and occasional remnants of cytoplasmic organelles. Keratohyalin granules were coarse, polygonal or star-shaped and occasionally absent. Keratinosomes seemed slightly diminished in number. Epidermal keratinocytes had large nuclei with visible nucleoli. Several keratinocytes contained centrioles, even at the level of the stratum malpighii. Mitochondria were abundant and occasionally contained myelinoid inclusions. The mode of inheritance (autosomal or X-linked recessive?) and the mechanisms responsible for skin lesions are not known. Our results suggest that the cutaneous lesions develop as a result of epidermal hyperproliferation.

014

Congenital Gengival Granular Cell Tumor (CGGCT)

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The term ''congenital epulis'' should be correctly referred to as ''CGGCT'', a disease which is frequently confused with the granular cell tumor or Abrikossoff tumor; this neoplasm is actually considered of Schwannian origin and can easily be identified by immunohistochemistry, due to the positive staining of its cytoplasm with S100 protein, CEA, CD57 and collagen IV. The CGGCT, evenly built up from eosinophilic granular cells, represents a different nosographic entity, with its immunohistochemical pattern negative for S100 protein and positive for vimentine, in relation to its histiocytic origin. At the ultrastructural level, in both tumors the granular aspect of the cytoplasm is due to a large number of autophagosomes, each containing myelin-like electron dense lamellae. The cells in CGGCT are evidently of histiocytic type and thus deprived of basal lamina; on the other hand, this lamina is consistently well evident, and sometimes multilayered, in cells of Schwannian origin. This case report refers to a 3-month-old baby showing a congenital gengival tumor. The diagnosis was achieved by histologic, immunohistochemical and ultrastructural examination.

015

Multiple ''Spitz'' Naevi in a Boy with Oculocutaneous Albinism

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A 7-year-old boy affected by oculocutaneous albinism (OCA) recently and progressively developed multiple (up to 40elements) cutaneous pink salmon papular lesions, widely disseminated over the trunk and the limbs, each of about 5 mm in diameter. One of these lesions was biopsied and evaluated with light microscopy, immunohistochemistry and electron microscopy. The lesion proved to be a nonpigmented Spitz naevus, whose melanocytes showed as strongly positive for S 100 protein and weak for HMB 45 cytoplasmatic reaction. In the cytoplasm of melanocytes, ultrastructural investigation showed aberrant melanosomes in the form of spherical organelles containing granular electron-dense material or concentric electron-dense lamellae. To our knowledge, this report represents the first case of multiple Spitz naevi associated with oculocutaneous albinism.

016

Ultrastructural Demonstration of Skin, Muscle and Other Organ Involvement Caused by Borrelia Burgdorferi Persistence

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Because the localization of the causative spirochete (B. burgdorferi) in infected tissues is unknown, we used electron microscopy to find spirochetes in the skin (erythema migrans and acrodermatitis chronica atrophicans lesions), muscles, hearts, synovial membranes, placentas (and in the brain authopsy of humans with active or advanced Lyme disease (LD).

Spirochetes were cultured only from skin and placenta samples in a special BSK medium. Ultrastructural findings were analyzed antigenetically with monoclonal antibodies and by molecular biology techniques. Ultramorphologically, the spirochetes were often situated between collagen fibers and along fibroblasts, some of which were deeply invaginated by these Borrelial organisms. In the active phase of LD, they were found in or around blood vessels in dermis, heart and other muscle tissue. Spirochetes were generally not observed in or near areas of inflammatory infiltrates. They are able to adhere and invade human peripheral monocytes, leucocytes and B-lymphocytes. Features which may be related to the persistence of Borrelia were the occurrence of unknown phagocytosis ,coiling'and ,tube'included the segmental uptake of spirochetes with leaky lysosomes and invagination of a large cell's membrane area. The studies extend our understanding of the behavior of the spirochete *in vivo* by identifying common locations of Borrelia and by finding the disparity between infection and inflammation.

017

Persisting Erythematous Symmetric Orbital Swelling: Ultrastructural Features Disclosing Breast Cancer Metastasis

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A 61-year-old female patient presented with progredient erythematous swelling of upper and lower lids persisting for half a year. The histopathological diagnosis had been xanthelasma. Because of progredience and additional skin tumours arising in perianal, inguinal, temporal and mandibular regions as coarse nodules, new deep biopsies were taken from lesions of the lower lid and from the neck. Histological and immunohisto-chemical investigations of the lid biopsy revealed an unusual, rather monomorphous infiltrate consisting of histiocyte-like cells, similar to those found in xanthelasma but also of signet-ring cells, resulting in the suspicion of metastasis of a signet-ring cell carcinoma. By electron microscopy, numerous individual or grouped tumour cells were found within the reticular and deep dermal region of the neck nodule biopsy; they revealed characteristic intracytoplasmatic lumina as well as a secretory vesicle pattern typical for invasive lobular mamma carcinoma. Indeed, the cells showed a strong expression of Gross Cystic Disease Fluid Protein-15 (GCDFP), an apocrine differentiation marker. The following examination established an invasive lobular mamma carcinoma with signet-ring cell carcinoma portions, disseminated bone metastases, and a signet-ring cell carcinoma of the stomach expressing Epithelial Membrane Antigen (EMA) which is especially present in breast tissue (human milk fat globule proteins).

In this rare variant, skin metastases are the primary symptoms of an invasive lobular breast carcinoma. This case presents an example of tumour diagnostics in which electron microscopy presents a valuable tool, in combination with other techniques, to improve diagnostic accuracy.

018

Photosensitivity to Amiodarone – Clinical and Ultrastructural Changes

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Amiodarone is an antiarrhytmic drug with many side-effects including the induction of photosensitivity and the development of blue-greyish pigmentation in the sun-exposed skin areas. We examined 64 patients treated by Amiodarone (mean dose 200 mg/day) and 32 controls without any photosensitivity. We established the skin phototype, the minimal erythema dose – MED (using polychromatic light of xenon lamp), early onset of erythema and its lasting, pigmentary response after phototests. Obtained data were evaluated by two-sample t test and by the X2-test of independence in contingency table. Skin biopsy specimens were obtained from the hyperpigmented facial skin area of a 59-year-old women treated with amiodarone (400 mg/day for 3 years). The specimens were processed for light and electron microscopy. For paraffin sections, routine staining methods as well as techniques detecting various pigments were used. Ultrathin sections for transmision electron microscopy were stained with uranyl acetate and lead citrate. Moreover, semithin epoxy resin sections were prepared for light microscopical examination.

The incidence of clinical photosensitivity (erythema, burning, papules) as well as hyperpigmentation related to amiodarone on sunlight-exposed skin areas were present in 9.4% (6 patients). The average MED, early onset and delayed duration of erythema and pigmentary response after polychromatic light irradiation were not statistically different (p below 0.05) between amiodarone treated patients and controls. Light microscopy revealed mononuclear cellular infiltrates situated in the upper and middle dermis, predominantly in a perivascular location. In these infiltrates, numerous cells showed macrophage morphology together with prominent cytoplasmic accumulations of granules. The granules had staining properties similar to lipofuscin.On electron microscopy, the granules were membrane-limited and dense, showing a nonhomogeneous content.

The appropriate patient's education and sun protection is necessary, especially if the amiodarone dosage is more than 200 mg/day.

019

Subcutaneous Benign Triton Tumor

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Giant cell fibroma, pleomorphic hamartoma, neuromuscolar hamartoma or choristoma are all synonims of a rare entity also called the benign triton tumor. Most of the case reports in literature concern the neural localization; rarely, the subcutaneous fat can be involved. We report the case of a 35 y old man showing two cutaneous nodules in his left forearm. Both were indolent and freely movable on the subcutaneous planes. The histologic examination of the two excised specimens revealed in both the nodules were well circumbscribed, containing giant spindle cells dispersed in an abundantly collagenized stroma. The spindle cells with plump, serpiginous, often multiple large nuclei were interponed among the collagen bundles. There was no mitotic activity or necrosis. Immunohistochemistry showed a hybrid immunophenotype. The spindle cells were concurrently reactive for vimentin, S 100 protein, CD 57 (Leu 7), myelin basic protein, desmin, GFAP, CGR and negative for NF, SYF and smooth muscle specific actins.

The ultrastructural examination of samples retrieved from paraffin inclusions revealed some spindle cells, which contained in their cytoplasm parallel bundles of contractile filaments merging in electron dense bands resembling Z lines. More numerous giant cells, with multiple or labyrinth nuclei and devoid of basal lamina, showed a broad cytoplasm containing clusters of ergastoplasmic reticulum, intermediate filaments, neurosecretory vescicles and occasional clusters of centriols, as if they were neuroectodermic in nature with occasional ependimal differentiation. In some cases, these cells showed under the citoplasmatic membrane bundles of thin actin-like filaments, as if they had a double neurologic as well as muscular ultrastructural phenotype.

020

Acquired Progressive Kinking of the Hair: Diagnosis by Scanning Electron Microscopy

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Acquired progressive kinking of the hair (APKH) is a rare hair disorder of unresolved pathogenesis characterized by kinking, curling, darkening and thinning of the hair in a circumscribed scalp area. Male patients are predominantly affected with a predilection site at the frontal and parietal region. APKH usually occurs in adolescence and early adulthood. In male patients it often eventuates into androgenetic alopecia, whereas in females it tends to spontaneous resolution. Hair changes similar to APKH may develop after retinoid treatment and hair transplantation. We describe a 35-year-old female patient with APKH of 8 years' duration in the occipital, retroauricular and parietal region. (She also had schizophrenia.) By light microscopy the hair appeared twisted. Scanning electron microscopy disclosed canalicular grooves along the shaft of abnormal hairs (pili canaliculi). Focally, a desquamation of cuticular scales was observed. Some spontaneous improvement was observed at a follow-up examination one year later.

021

Furosemide-Induced Lichen Planus Pemphigoides. Ultrastructural Study

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Furosemide is a thiazide diuretic and one of the most commonly prescribed diuretic drugs. Cutaneous side-effects occur in 0.05% to 0.5% of patients treated with furosemide. We report herein a case of lichen planus pemphigoides (LPP) induced by furosemide.

A 92-year-old white woman complained of severe pruritus of 7 months' duration, and also a generalized lichenoid papular eruption which occurred within a month of her furosemide treatment commencing. Histological examination showed a dense lymphocytic infiltration of the upper dermis with some aspects of vacuolization of the basal layer associated with eosinophils. Direct immunofluorescence (IF) revealed the deposition of IgG and C3 on the basal membrane zone. Circulating autoantibodies were located on the upper surface of the blister by indirect IF on Salt split skin. Immunoblotting analysis showed that antibodies recognized a 230-kDa band on epidermal extract. Electron microscopy showed the infiltration of eosinophils through the basal layer with focal disappearance of the lamina densa. By immunoelectron microscopy, immune deposits were located in the lamina lucida facing the hemidesmosomes as reported with typical bullous pemphigoid. Spontaneous healing occurred after the withdrawal of furosemide without any corticosteroid treatment.

We report a new cutaneous adverse effect of furosemide. To date only one case of drug-induced LPP has been reported with Ramipril and treated with corticosteroids. In our case, the spontaneous healing after withdrawal of furosemide without any therapy was a strong argument to consider that the drug intake triggered the lichenoid and autoimmune process.

Moreover, we can speculate that furosemide had first induced a lichenoid dermatitis which had secondarily furthered the development of an autoimmune blistering disease after exposition of the bullous pemphigoid antigen.

022

Feline Orthopoxvirus-Infection Transmitted from Cat to Man: a Case Report

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We report on a 56-year-old female patient who presented at the Department of Dermatology with an inflamed, exulcerated lesion on the left side of her neck measuring about 1.5 × 4 cm. There was deep subcutaneous infiltration without signs of abscess formation. She had already been treated by a general practitioner with systemic antibiotics for about three days without success. Further exploration revealed an initial contact (scratch) with a cat living in the patient's house. Differential diagnoses included tularemia, cat scratch disease, scrofuloderma and orf. Satellite lesions developed despite local treatment and parenteral clindamycine. Immunofluorescence testing for herpes simplex virus type 1 and 2 proved negative, although histologic examination was found to be consistent with herpes-induced ulceration. Initially, negative staining of scrap tissue and material from a fresh pustule was negative for bacterial and viral structures.

Subsequent transmission electron microscopical (TEM) examination of the same material – fixed, pelleted, and embedded in resin – clearly showed multiple typical poxvirus particles, predominantly in remnants of scaled-off layers of degenerated keratinocytes, as well as virus-particles in intermingled phagocytes, leading to the diagnosis ''orthopox (cowpox) virus infection''. These results of the TEM examination, which were verified by PCR and sequencing (performed at the Institute of Virology, University of Veterinary Sciences, Vienna) again demonstrate the value of using electron microscopical techniques in the diagnosis of infectious skin diseases.

023

Apoptosis Resistance in Human Melanoma Cells

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The CD95/Fas system is known as an important pathway for the induction of apoptosis in cells and tissues. Human melanoma cells have recently been shown to be heterogenously sensitive to CD95-induced apoptosis. Defective cytochrome *c* release and the resulting loss of caspase-3 activation was recognized to be essential for the susceptibility of human melanoma cells to CD95/Fas-induced apoptosis. Cytochrome *c* release from mitochondria is regulated by the relative amounts of apoptosis-promoting and -inhibiting Bcl-2 proteins in the outer membrane of these organelles. The assignment of Bax/Bcl-2 ratios by quantitative Western blotting in 11 melanoma cell populations revealed a relation to the susceptibility to CD95-mediated apoptosis. We could show that a relative low Bax/Bcl-2 was characteristic for resistant cells and a relative high Bax/Bcl-2 ratio for sensitive cells. Furthermore, Bcl-2 overexpression abolished apoptosis triggered by CD95L, confirming the critical role of the Bax/Bcl-2 ratio in melanoma cells.

We assume that the Bax/Bcl-2 ratio creates a rheostat in melanoma cells determining the sensitivity to apoptotic signals acting upstream of mitochondria. We therefore tested betulinic acid, a cytotoxic agent selective for neuroectodermal cells like melanoma, directly influencing mitochondrial functions. In fact, betulinic acid induced mitochondrial cytochrome *c* release and DNA-fragmentation in both, CD95-resistant and sensitive melanoma cell populations, independent from the Bax/Bcl-2 ratio. We suggest that apoptosis resistance in human melanoma cells can be circumvented either by gene therapy altering the Bax/Bcl-2 ratio or by the drugs directly targeting mitochondria as, e.g. betulinic acid.

024

Effects of a Ceramide-Containing Emolient in an Experimentally Induced Skin Barrier Dysfunction

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Recently a new generation of emollients, containing lipids chemically related to the physiological content of the intercellular domain of the horny layer, has been introduced. In the present study we investigated the efficacy of a ceramide-containing emollient (CCE) which is claimed to be aformulation containing an optimal composition of natural skin lipids. We compared the effect of CCE with a positive (Vaselinum album/cremor lanette ana) and a negative control (untreated damage skin) using clinical, bioengineering and immunohistochemical methods in an experimentally induced skin barrier dysfunction.

17 healthy volunteers participated in two different models (A,B) of barrier dysfunction. In model A, skin barrier dysfunction was inflicted at three investigation sites by tape stripping. In model B, the volunteers were patch tested at three investigation sites with sodium dodecyl sulfate (0.2%) for 4 h a day for 5 consecutive days. The investigation sites were treated once a day with the above-mentioned agents. Daily, irritant reaction was assessed by erythema scoring and measurements of transepidermal water loss (TEWL). After 5 days, punch biopsies were taken from all sites. Immunohistochemical assessment was carried out with respect to epidermal proliferation, keratinization and epidermal differentiation.

Tape stripping resulted in an erythematous reaction and an increase of TEWL associated with up-regulation of proliferative cells and expression of cytokeratin 16. Both the CCE and the positive control significantly decreased the erythematous response ($p < 0.01$ and $p < 0.03$ resp.). TEWL was also influenced significantly by treatment with CCE ($p < 0.01$), but not significantly by treatment with the positive control ($p > 0.05$). Concerning immunohistochemical markers, CCE significantly suppressed the amount of proliferating cells (Ki-67) compared to the control site.

Repetitive exposure of SDS induced a variable degree of erythema. The erythematous response enhanced in intensity up to day 4 in four participants. The gradual increase of TEWL values associated with up-regulation of proliferative cells was observed in all participants. Treatment with both CCE and the positive control did not significantly prevent erythema. Barrier dysfunction, however, was prevented significantly ($p < 0.01$) by the positive control, but not by CCE ($p < 0.5$). The immunohistochemical analysis in this model showed a significant suppression of proliferating cells by the positive control.

Both investigated emollients enhance the recovery of the skin barrier from mechanical and chemical damage. However, the unique properties of CCE are best expressed following mechanical damage, suggested that its shielding from chemical damage is not optimal. Further research in various skin conditions is required to find out to what extent the use of CCE has a clinical advantage.

025

UV-Light of 313 nm Induced 12(S)-Hydroxyeicosatetraenoic Acid Receptor Down-Regulation in Psoriatic Keratinocytes

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UV-light induces different changes in skin which may be shown by electron microscopic examination. DNA damage or apoptosis are well-known as disturbances caused by UV-radiation. On the cell surface, receptors could be also affected during treatment with artificial UV sources or during skin irradiation for cosmetic reasons. Because 12-hydroxyeicosatetraenoic acid (12-HETE) is considered to be the main epidermal eicosanoid, and is assumed to have both pathophysiological effects in inflammatory skin diseases such as psoriasis and atopic eczema as well as a physiological role in cutaneous biology, we decided to show the UV-light effect on 12-HETE cell surface receptors.

Therefore, the present work studied the effects of single and repeated irradiations with selected UV-B light of 313 nm from a Waldmann F 85/100 W – TL-01 bulb on the 12(S)-HETE receptors in psoriatic epidermal cells.

UV-light *in vivo* (0.5 J/m^2) and *in vitro* (50–150 J/m^2) induced a down-regulation of 12(S)HETE receptors in a dose-dependent manner. The above described effect occurred after a latency period of 6 h and reached its maximum at 7.5 h. *In vitro*, a single UV irradiation (150 J/m^2) or repeated irradiation (50 J/m^2) developed a 55% receptor down-regulation (Bmax); however, the receptor affinity remained unchanged.

The down-regulation of 12-HETE receptors on keratinocytes developed after the UV-B iradiation may contribute to the explanation of its effects in phototherapy or photoaging.

026

Congenital Cutaneous Smooth Muscle Hamartoma

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A two-year-old male infant presented with congenital, nonpainful, unspectacular lesions on the trunk. The slowly growing patches consisted of perifollicular papules without prominent hair and were not hyperpigmented. After excision, the lesions revealed the typical histological aspect of smooth muscle fibres interdigitating with collagen bundles and elastic fibres within the reticular dermis as well as ultrastructural smooth muscle differentiation with fusiform, characteristically lobulated cells, surrounded by a continuous, often multiplicated external lamina, dense bodies and blunt end nuclei with deep indentations; immunhistolchemistry further confirmed the findings by positive reactions with actin and desmin antibodies.

Therefore, the lesions turned out to be hamartomatous proliferations of smooth muscle cells. The features disclosing the diagnosis and histogenesis of this rare condition are described and discussed with respect to other cutaneous myogenic conditions.

027

3-Dimensional Reconstruction of the Free and Attached Gingiva of SSc-Patients

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Documented oral manifestations of systemic sclerosis (SSc) include impaired mouth opening with reduced interincisal distance, teleangiectasis, xerostomia, increased frequency of diseased, missing and filled teeth, periodontal disease, increased periodontal ligament width, and osseous resorption of the mandible.

The aim of this study was to compare the boundary between the oral epithelium and the underlying connective tissue both in SSc patients and patients with periodontitis but without SSc.

Marginal gingiva and gingival papilla were obtained from 13 scleroderma patients and 8 patients with periodontitis after routine tooth extraction and gingival curettage. The specimens were embedded in Technovit 7100 and studied by 3D-reconstructions concerning the height and width of the oral epithelium and the connective tissue papillae which are seperated from each other by epithelial ridges – so-called rete pegs.

In our study, a characteristic morphological feature of the investigated oral epthelium is the presence of rete pegs in the oral epithelium of patients with SSc and patients with periodontitis but without SSc. Focally, 4–5 connective tissue papillae confluence to an enlarged papillary body. However, in contrast to descriptions of SSc-derma, there are neither alterations of the oral epithelium nor the papillary bodies.

The pathological changes in the dermis of SSc patients are not transferable to the marginal gingiva.

028

The Ultrastructural Study of Squamous Cell Carcinoma and Keratoacanthoma

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The studied tumors (206 squamous cell carcinomas) and pseudotumors (45 keratoacanthomas) underwent histopathological examination in the light microscope (staining: HE; PAS). Additionally, specimens from 25 patients with squamous cell carcinoma and 12 patients with keratoacanthoma underwent examination in the transmission electron microscope (JEM – 1200 EX). The electron microscopy of squamous cell carcinoma revealed reduced number of desmosomes between the cells of spinous layer; occasionally the desmosomes were found within the cytoplasm of these cells. Numerous cytoplasmatic processes replaced desmosomes and hemidesmosomes on the surface of the cells in the spinous and basal layers. Lack of continuity was found in the basement membrane. Long cytoplasmatic processes of basal cells were penetrating through the basement membrane. Lymphocytes and mastocytes were found among the keratinocytes. The mastocytes were already degranulated or were loosing the granules. They were attached to the surrounding cells with numerous long processes. The amorphous tonofilament condensation or big amorphous structures – presumably the first phase of development of keratin pearls – were found within the cytoplasm of these cells.

Focal parakeratosis was found within the corneous layer in the electron microscopy study of keratoacanthoma. The spinous layer was hyperplastic and the intercellular spaces were dilated. The desmosomal junctions between cells were more frequently intact than in the squamous cell carcinoma. The morphological analogues of desmosomes were found within the cytoplasm of the spinous cells. Numerous cellular microvilli were localised on the surface of the spinous cells. Homogenous keratotic foci were found among the cells and within the cytoplasm. The cells with high degree of keratosis were filled up with homogenous corneous mass made of aggregated tonofilaments attached to the numerous ribosomes and vacuoles. Mastocytes were found among spinous cells and homogenous corneous masses; some of them were loosing the granules. The basement membrane was intact.

029

Autologous Minigrafting for Difficult Areas of Leukoderma

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Minigrafting is regarded as one of the treatments for leukoderma. Melanocyte transplantation for difficult localizations is not a common treatment and it has specific criteria.

We evaluated the efficacy of minigrafting in depigmentation of the eyelids, retroauricular areas, lips, nipples, fingers and male genitals.

Two test minigrafts of 2 mm were implanted in achromic lesions of patients with vitiligo focalis, segmentalis vulgaris and leukoderma after laser treatment. Patients were selected for pigment cell transplantation when the spread of pigment was observed within two months. Repigmentation was judged by the same dermatologist every month. Twenty-three patients (22 with vitiligo and 1 with leukoderma after laser treatment) were grafted with the minigraft test.

The results of the treatment were scored visually and with the use of photography. They showed 75–99% repigmentation in 16 patients (69%), 51–74% in 2 patients (9%), 25–50% in 3 patients (13%) and 0–24% in 2 patients (9%). The time of follow-up varied from 1 to 12 months after grafting. Except for two patients with vitiligo showing a positive Koebner phenomenon and developed depigmentation of the minigrafts, we did not observe undesirable effects such as scarring and infection at the donor site, or cobblestone-like texture and incomplete or spotty repigmentation at the acceptor site. The group of patients with stable leukoderma showed the best results in areas of nipples, face and male genitals.

Autologous minigrafting is a successful treatment for difficult areas in stable leukoderma.

030

Contact Dermatitis Due to Textile Dyes

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In respect of contact eczema-dermatitis, nonadjusted natural and artificial textile materials cause almost no problems in contact with the skin. The most frequent cause of contact dermatitis due to textile materials is the textile dyes. The typical effect is the localization of eczematous changes in the areas where clothes are in close contact with the skin, and where friction and perspiration is maximal.

Not many such cases are reported when compared with the large number of different dyes used in the textile industry.

The cases of two of our patients are described. In both of them, patch tests with the coloured material of their clothes led to a reaction of the contact allergic dermatitis type, while patch tests with TROLAB, the European Standard series of common contact allergens (produced by Hermal) yielded negative results.

The discussion is focused on the problems connected with determining the specific dyes used to colour the textile clothing materials.

031

Porphyria Cutanea Tarda – Case Report

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Porphyria cutanea tarda/PCT/is a chronic disorder in porphyrin metabolism, due to a deficiency of uroporphyrinogen III decarboxylase in the liver cell. Skin and liver findings are typical. The hepatotoxic factors are the starting factors of the acquired sporadic form. The liver cell damage appears to be the major basis for the pathogenesis of disease, activity of uroporphyrinogen III decarboxylase is reduced about 50%. High uroporphyrin excretion in the urine is typical. Alcohol is an important factor, participating both in manifestations of sporadic PCT and in the development of its exacerbations.

We report a 46-year-old man with skin fragility, trauma-induced blisters and erosions. The patient developed the lesions in Spring 2000, and they were localized in the sun-exposed areas/backs of the hands and fingers, face and neck. He usually drinks about 5 beers a day, sometimes a lot of vodka. During the first hospitalisation at another Department of Dermatology in Prague, the increase of porphyrins excretion in the urine was not detected. The first biopsy of lesional skin showed subepidermal vesicles and there was a suspicion of epidemolysis bullosa acquisita. (Many cases of PCT have been misdiagnosed as epidermolysis bullosa acquisita). The diagnosis of PCT was suggested following newly developed clinical symptoms and after taking biopsies for the second histological examination with direct immunofluorescence.

In our hospital, laboratory parameters showed highly elevated porhyrins in urine – uroporphyrin III/UP/, lower increase of coproporphyrin III/KP/lovel, and no porphyrin precursors. The following laboratory values were pathological: elevated transaminases, serum iron and ferritin levels. After phlebotomy, the patient was systemically treated wth hydroxychloroquine in a dose of 200 mg twice a week. Topical treatment included antiseptic bath treatment and ointment. This therapy was successful; the healing of skin lesions was followed by slight atrophy, scarring, hyperpigmentation and depigmentation. The rate of UP and KP in the urine had decreased to the normal value.

032

Evaluation of the Atopic Skin Residence by Staphylococcus Aureus Using an Impression Method, Effects of UVB 311 nm Phototherapy

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Eczema atopicum is strongly pruriginous, predominantly chronic inflammatory disease occuring mostly in children. In the vast majority of atopic patients, Staphylococcus aureus colonizes the skin, and its role in the etiopathogenesis in the course of another illness is essential.

An indirect impression method with the semiquantitive evaluation of the total number of pathogenic organisms on the determined area has been used by the authors for the evaluation of bacteriologic findings. Consequently, the effects of UVB 311 phototherapy on the residence of the Staphylococcus aureus in the skin have been observed by a comparison of the findings both before and after treatment. This method has been applied with 10 patients ranging in age from 5 to 15 years.

The impression method appears to be suitable for evaluating the effectiveness of the treatment of skin lesions. In the combination with UVB 311 phototherapy, it resulted in a reduction in the colonization of staphylococci in the skin. It can be successfully used as a supplement for antibacterial therapy, either overall or locally.

033

Autoimmune Reactions in Patients with Plaque Psoriasis

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The purpose of our work was to study cellular reactions to neurospecific protiens by sensitizing neutrophiles in patients suffering from psoriasis.

We examined and tested 22 patients, ranging in age from 25 to 55 years. All of them were diagnosed with Type II psoriasis, and their test indices were studied in the Neuroimmunology laboratory of the Neurosurgical Hospital.

All of our investigations showed that cell mediated sensitization was increased by more than twofold. However, the level of autoantibodies for tissue antigens was within the normal range.

In our opinion, determining the parameters of autoimmune reactions in every specific case may assist in evaluating the patient's immune response capability, and hence in determining the choice of appropriate, effective immunocorrective therapy.