Abstracts for the 17th Annual Meeting of The European Society for Dermatological Research

Amsterdam, The Netherlands—March 29–April 1, 1987

1

A DIFFERENCE IN DISTRIBUTION OF DR AND DQ ANTIGENS IN LANGERHANSCELLS, KERATINOCYTES AND TRANSFORMED B CELLS. B.J. Vermeer, A.H.M. Mommaas-Kienhuis, M.C. Wijsman, F. Koning, F.H.J. Claas, Depts. Dermatology, Electronmicroscopy, Immunohaematology, Univ. Medical center, Leiden, The Netherlands.

HLA-class II molecules are products of at least three different genetic loci (DR,DQ,DP), which may have different immunological functions. HLA-class II molecules are normally expressed on Langerhanscells but not on keratinocytes. In certain skin diseases and under experimental conditions however, HLA-class II antigens can be induced on the cellsurface of keratinocytes. Immunoelectronmicroscopical studies were performed to answer the question whether a difference in the intracellular distribution of DR and DQ exists in various cell types. For this purpose epidermal cell suspensions and EBV transformed B cell lines were fixed in 0,5% glutaraldehyde, 1% paraformaldehyde PH 7,4 and embedded in lowicryl K4M. Ultrathinsections were incubated with different monoclonal antibodies against DR and DQ, followed by a second step consisting of protein A gold 5 nm or 10 nm. In control experiments the first incubation step was normal mouse serum followed by protein A gold. These results show that DR molecules are more abundantly present compared to DQ both on the cell surface and inside the cell in both the B cell line and Langerhanscells. Moreover as shown by ultracryomicrotomy the DR molecules were predominantly present on membrane structures inside the B cells, whereas DQ was preferentially present in the cytoplasm. In the Langerhanscells, DR and DQ were never observed on Birbeck granules. The DR molecules were absent on the surface of keratinocytes but could be observed inside these cells. No DQ molecules could be observed inside the keratinocytes. These data show that: 1. DR and DQ have a different distribution in the intracellular compartments of keratinocytes, Langerhanscells, and transformed B cells. This fits well with the hypothesis that both molecules have a different function. 2. The Birbeck granules do not carry class II antigens and therefore presumably don't play a role in antigen presentation. 3. The absence of DR on the surface of keratinocytes does not exclude the possibility that DR molecules are present in the cytoplasm.

2

COMPARAISON OF PHENOTYPE AND ULTRASTRUCTURE OF HUMAN THYMIC DENDRITIC CELLS AND LANGERHANS CELLS. H. Barthélémy, D. Landry, S. Montplaisir, M. Pelletier, Department of Pathology, University of Montréal, Québec, Canada, and Department of Dermatology, CHRU St Etienne, France.

In man two dendritic cells have been extensively studied: thymic interdigitating cells and Langerhans cells. In order to analyse the relationships between these two populations we studied the morphology of human thymic dendritic cells (HTDCs) in vitro. Normal human thymus specimen were obtained from children undergoing cardiovascular surgery and were put in culture. After 7 days HTDCs could be easily identified and differenciated from the other thymic populations.

By transmission and scanning electron microscopy, HTDCs could be defined as cells with long and fine processes with an irregular nucleus. Their cytoplasm contained numerous mitochondria and dark homogeneous granules, but never displayed phagolysosomes and Birbeck granules. They presented with a strong membrane ATPase activity and were S100 positive. However, HTDCs were not stained by anti-lysosyme, anti-keratin antibodies and were esterase negative.

Using immunoelectron microscopy with a protein A-gold technique, HTDCs showed a strong positive surface reaction with both OKIa and OKT6 monoclonal antibodies. They were also stained weakly with OKT4 monoclonal antibody. Macrophages were only labeled with OKIa monoclonal antibody, and not with OKT3, OKT4, OKT6, OKT8 monoclonal antibodies. No labeling was observed on HTDCs with OKT3 and OKT8 monoclonal antibodies. The randomised distibution of Ia determinants was confirmed with a back scattered electron microscopy technique. The phenotype of HTDCs was similar to that observed on human Langerhans cells. A semi-quantitative analysis of antigenic sites (Ia, T4, T6) was performed. A strong expression of DR antigens as compared to T6 and T4 antigens was noticed. Two populations of HTDCs were defined by the quantitative expression of T6 antigens as it as been reported on cord blood dendritic cells.

These observations demonstrate that HTDCs can be isolated in vitro, and that they correspond to interdigitating cells of the thymus. Moreover, HTDCs share a number of markers with Langerhans cells, suggesting that they could belong to the same cellular lineage.

3

CD11 EXPRESSION BY LANGERHANS CELLS. Giuseppe De Panfilis, G.Carlo Manara, Corrado Ferrari, and Claudio Torresani, Departments of Dermatology and of Pathology, Parma University, Parma, Italy.

The cellular reactivity pattern of the D12 (anti-CD11) monoclonal antibody includes, together with other cell types, human peripheral blood monocytes. Since functional and phenotypical similarities do exist between monocytes and Langerhans cells (LC), we asked whether LC might be CD11 reactive.

(1) Epidermal sheets, (2) vertical cryostat tissue sections, and (3) freshly isolated epidermal cell suspensions, either untreated or enriched for LC by Ficoll -Hypaque sedimentation, were prepared from normal human skin. To allow the detection of CD11 positive cells, the following immunostaining procedures were performed. For (1) and (2), immunohistologic studies in light microscopy were carried out, using (i) three steps immunofluorescence procedures, (ii) four steps immunoperoxidase procedures, and (iii) double labellings for the simultaneous visualization of CD11 (tetramethylrhodamine) and CD1 or HLA-DR (fluorescein isothiocyanate) positive cells. For (3), immunoelectronmicroscopy studies were performed, using (i) a two layers immunoperoxidase technique, (ii) a two layers immunogold technique, and (iii) a double labelling immunogold technique to assess the coexpression on the same cell of two surface antigens (CD11 versus CD1 or HLA-DR).

CD11 reactivity was usually detected on dendritic cells of epidermal sheet preparations, cryostat skin sections, and suspended freshly isolated epidermal cells. Such CD11 positive dendritic cells shared CD1 (or HLA-DR) positivity, and displayed the ultrastructural features of LC.

The present investigation provides evidence that LC share with the cells of the monocyte-macrophage series even the CD11 reactivity. The exact functional significance of the CD11 expression by LC has still to be precisely defined at the present time. However, such a finding extends the known phenotypical profile of LC, and might perspectively yield an additional tool to further elucidate the origin, differentiation and functions of LC.

4

THE ADVANTAGES OF ADDING TANNIC ACID DURING LANGERHANS CELL FIXATION FOR ELECTRON MICROSCOPY. Didier A. Schmitt, Daniel Hanau, Michel Fabre, Roland Bury, and Jean-Pierre Cazenave, INSERM U. 311, Centre Régional de Transfusion Sanguine and Service Central de Microscopie Electronique, ULP, Strasbourg, France.

In epidermal Langerhans cells (LC) Birbeck granules (BG) can coexist with BG-like structures. They have apparently the same morphology and both of them result, though at a different stage, from receptor-ligand interactions. Tannic acid is known to be an excellent electron-dense stain for plasma membranes, internal membranes, cytoplasmic fibers and clathrin coat. In the present study we examined if adding tannic acid to LC, during the phase of fixation for electron microscopy, allowed morphological distinction of BG from BG-like structures. Suspensions of epidermal cells were obtained from normal human epidermis after trypsination. They were either immediately fixed for electron microscopy or first incubated with the monoclonal anti-T6 antibody BL6 in the presence, at the end of the incubation time, of 0.001 % digitonin (a known inductor of BG-like structures). The fixation for electron microscopy was done with 1.7 % glutaraldehyde-0.15 % picric acid-0.25 % tannic acid in a 0.1 M cacodylate buffer solution. After 1 h incubation at 4°C and two washes with the cacodylate buffer the cells were post-fixed with 2 % osmic acid in the cacodylate buffer (all the above-mentioned solutions were adjusted to 330 m Osmol with sucrose). The cells were then washed again in the cacodylate buffer, dehydrated in alcohol and embedded in Epon. Under the electron microscope, tannic acid reinforces the contrast of the dense external and internal layers of the LC trilaminar plasma membrane. Only the cytoplasmic surface of internal membranous systems (Golgi apparatus, reticulum) appears contrasted. This characteristic allows clear distinction of BG-like structures - which are connected to the plasma membrane and whose dense layers, as well as the internal striation, are reinforced - from intracytoplasmic BG. These BG only show reinforcement of their dense external, cytoplasmic layer. Tannic acid moreover clearly shows the tight links between BG and the microtubules which appear to be particulary dense around the BG and seem to fix to them. Lastly, tannic acid increases the density of the clathrin coat of coated pits and vesicles making their detection easier.

5

ANTIGENIC THYMUS-EPIDERMIS RELATIONSHIPS. REACTIVITY OF A PANEL OF ANTI-THYMIC CELL MONOCLONAL ANTIBODIES ON HUMAN KERATINOCYTES AND LANGERHANS CELLS. D. Schmitt, G. Zambruno, M.J. Staquet, C. Dezutter-Dambuyant, J. Thivolet. INSERM U.209, Hopital E. Herriot, Pavillon R., 69437 Lyon, Cedex 03, France.

We have investigated on human skin the reactivity of a panel of forty-two anti-thymus monoclonal antibodies (MCA) supplied by the Third International Workshop and Conference on Human Leucocyte Differentiation Antigens, Oxford, 1986.

MCA of the first cluster of differentiation (CD1) define a group of surface molecules expressed by cortical thymocytes. Some of them (OKT6, M241 and Nal/34) have been shown to react on normal human skin with the epidermal Langerhans cells (LC). Twenty-two CD1 MCA were investigated in the present study. On normal human skin, thirteen MCA reacted with LC in situ. This result confirms the heterogeneity of CD1 MCA. Some of them were shown to recognize biochemically different molecules and/or epitopes of thymocytes.

In addition, twenty anti-thymic epithelium MCA were tested on human skin. The MCA which only reacted with the thymic epithelial cell network (except the Hassall's corpuscles) decorated only the epidermal basal cell layer. The MCA which reacted with all the thymic epithelial cells (including the Hassall's corpuscles) decorated all the epidermal cell layers.

These results confirm the heterogeneity of the thymic epithelial microenvironment and underline the antigenic similarities between the thymic epithelial structures and the different epidermal cell layers. The existence of bone-marrow-derived CD1 positive cells (thymocytes or LC) in an epithelial cell network (respectively the thymus and the epidermis) focus the speculation around the immunological role of the epidermal basal cell layer in the leukocyte education and the exact lineage of the epidermal LC.

6

MODULATING EFFECTS OF RETINOIC ACID ON THE MORPHOLOGY OF NORMAL HUMAN KERATINOCYTES AND SQUAMOUS CARCINOMA CELLS CULTURED ON AIR-LIQUID INTERFACE. Maria Ponec, Johanna Kempenaar, Jan Gerrit van der Schroeff, Department of Dermatology University Hospital Leiden, The Netherlands.

Using a conventional (submerged) culture system both normal and malignant keratinocytes undergo differentiation, although to a degree which is lower than seen in

0022-202X/87/$03.50

the in vivo situation. When cultured at the air-liquid interface these cells were found to express morphological features of differentiation similar to those seen in vivo. Therefore, the latter culture system offers an attractive model to study the modulating effects of drugs such as retinoids on the proliferation and differentiation of malignant and normal cells.

In the present study the morphology of normal keratinocytes and three squamous carcinoma cell lines (SCC-4, SCC-15, and SCC-12F2) cultured on air-liquid interface, using dead de-epidermized dermis (DED) as substrate, was investigated in the presence or absence of retinoic acid (RA). Normal keratinocytes cultured in this system form a well organized epidermis-like structure, consisting of a basal layer, spinous layer, granular layer and an orthokeratotic horny layer. Addition of RA (2 uM) to the culture medium, however, induces an increase in the number of cell layers and also keratinization of individual cells. Furthermore, the granular layer is lost and the horny layer is transformed into a parakeratotic layer.

In contrast to normal keratinocytes, the SCC grown on DED form disorganized layers of cells showing atypical nuclei, individual cell keratinization and atypical mitotic figures. The SCC-12F2 cells showed some degree of stratification with the formation of a parakeratotic horny layer containing large nuclei. The SCC-15 cells showed a vary low degree of stratification, whereas the SCC-4 cells did not express this phenomenon at all. The differences between the SCC-12F2, SCC-15 and SCC-4 cells regarding the pattern of stratification reflect the differences in grade of differentiation of these cells as judged from their ability to form cornified envelopes. Addition of RA (2 uM) to the culture medium markedly reduced the number of cell layers for the three SCC lines studied. The morphology of these cells was not significantly altered.

The results of this study show that RA exerts distinctively different effects on proliferation and differentiation in cultured normal keratinocytes in contrast with SCC in culture, a phenomenon which is also found in the in vivo situation.

7

PLASMA TRANSPORT OF RETINOIDS USED IN HUMAN THERAPY. Georges Siegenthaler, Jean-Hilaire Saurat, Raymonde Hotz, Fabienne Jaunin. Clinique de Dermatologie, Hôpital Cantonal Universitaire, 1211 Genève 4. Suisse.

Previous reports have suggested that the major part of etretin (Ro 10-1670) the main metabolite of Tigason) and 13-cis-RA (acutane) was bound on serum albumin when patients were treated by these acidic retinoids. No information about the binding or analogs of RA on RBP is provided in the literature. We wondered whether retinoids used in human therapy could also be transported by plasma RBP. Indeed this might give some information about either pharmacological or side effects of these compounds.

We studied the binding of serveral retinoids by incubating them with delipidized human serum. The proteins were separated on a non-denaturating polyacrylamide gel electrophoresis (PAGE) in slab form (7.5%, 0.3M Tris/Cl, pH 8.8) or in horizontal isoelectrofocusing (IEF) (pH gradient 4-6.5). These techniques separate well the complex retinol-RBP (holo-RBP) from the RBP without its ligand (apo-RBP) (JID, 1987 in press). The separated proteins were then immunoblotted onto a nitrocellulose sheet and RBP bands revealed with an anti-human RBP serum. Holo-RBP reconstituted with retinol, 3-dehydroretinol (vitamin A_2) and retinaldehyde showed identical migration position on both PAGE and IEF. RA-RBP complexes migrated faster than apo- and holo-RBP in the PAGE but at the same pI than retinol and 3-dehydroretinol in IEF (indicating conformational changes of RBP upon RA binding). Incubation of delipidized serum with etretin, 13-cis-RA or arotinoid acid (Ro 13-7410) did not produce any modification of the electrophoretic mobility of apo-RBP.

This strongly suggests that these synthetic retinoids have no affinity for RBP and therefore:, cannot be: transported by RBP ; selectively delivered to target cells or compete with natural retinoids at this level. Some ocular side effects should therefore be due to another mechanism. In fact, in patients treated with 13-cis RA about 10% is found in the serum in the form of the trans isomer. Preliminary PAGE studies on the presence of all-trans-RA coupled to RBP in the serum of such patients were negative.

8

EPIDERMAL CELL PROLIFERATION AND THE EFFECTS OF RETINOIC ACID ON PATTERNS OF PROTEIN SYNTHESIS AND PHOSPHORYLATION IN CULTURE MEDIA WITH LOW AND HIGH CALCIUM ION CONCENTRATIONS Christopher P.F. Redfern, Department of Dermatology, University of Newcastle upon Tyne, England.

In many cell types all trans retinoic acid (RA) stimulates differentiation while inhibiting proliferation. It is generally agreed that epidermal cell differentiation is inhibited by RA but different studies have shown either stimulation or inhibition of proliferation. Stratifying primary cultures of neonatal rat keratinocytes responded to RA ($3x10^{-7}$) by an increased intensity of a Mr 23,200 (pp23) membrane-associated phosphoprotein and decreased intensities of Mr 26,500 (pp26, predominantly cytosolic) and 28,000 (pp28) phosphoproteins. These changes were also elicited by only a short 6 hour pulse of RA and were apparent within 24 hours. In separate experiments using media with low or high calcium ion concentrations, the incorporation of [^{3}H]thymidine into DNA was increased 16-24 hours after addition of RA to the culture medium. The pattern of phosphoproteins in RA treated cells was similar to that in proliferating monolayers in low [Ca^{2+}] medium. From these data, together with data from studies on phosphoprotein patterns in quiescent, proliferating and stratifying epidermal cells, we conclude that changes in the relative intensities of pp23 and pp26 correlate with epidermal cell proliferation and that RA stimulates epidermal cell proliferation under our culture conditions. We have also examined patterns of protein synthesis in stratifying cultures (high [Ca^{2+}]) and proliferating monolayers (low [Ca^{2+}]) in response to RA. In the absence of RA, patterns of protein synthesis on 2-dimensional polyacrylamide gels were similar in both types of culture with the exception of an acidic Mr 54,000 protein synthesised only in cultures grown in high [Ca^{2+}]. The synthesis or accumulation of this protein was reduced or abolished in response to RA, an effect detectable within 12 hours after addition of RA. We do not yet know whether the expression of this protein is specific to differentiating cells. RA induced the synthesis of a Mr 14,000 protein regardless of calcium ion concentration of the culture medium.

9

Increase of Human Epidermal CRABP after Topical Application of Etretin and Retinoic Acid. Suzanne Hirschel-Scholz, Georges Siegenthaler, Jean-Hilaire Saurat. Clinique de Dermatologie, Hôpital Cantonal Universitaire, 1211 Genève 4, Suisse.

We previously described an increase of epidermal cellular retinoid acid- binding protein (CRABP) in the non lesional skin of psoriatic patients after systemic administration of RO 10-1670 (etretin).

The aim of the present study was to investigate whether the increase of CRABP could be reproduced after topical application of etretin and whether it could also be induced by its natural ligand, i.e. retinoid acid (RA). Etretin and its solvent (ethanol + 10% DMSO) were applied on the right and left buttock of five healthy volonteers, covering about 2x2cm sqare each. The sites were put under occlusion and the same procedure was repeated during four days. On the fifth day skin samples were taken with a keratome set at 180µm and CRABP levels determined with a polyacrylamide gel electrophoresis. CRABP was signifcantly elevated on the etretin treated side (12.9+2.2 versus 4.6+0.9 pmol/mg protein on the non treated side; $p<0.025$,paired t-test). The same experiment was repeated with retinoic acid in 95% ethanol and there was also a significant increase of CRABP on the treated side (8.5+1.4 versus 2.5+0.5 pmol/mg protein $p< 0.025$; $n =4$).

These results show1) that etretin increases human epidermal CRABP levels also after topical application i.e. independently of systemic metabolisation; 2) that this effect is not restricted to etretin because a similar effect was observed after application of retinoic acid ; 3) and that elevation of CRABP by its ligands can also be observed in healthy subjects.

10

EFFECTS OF RETINOIDS AND HYDROCORTISONE ON DIFFERENTIATION, LIPID METABOLISM, EPIDERMAL GROWTH FACTOR (EGF) BINDING IN SQUAMOUS CARCINOMA CELLS. Maria Ponec, Arie Weerheim, and Johannes Boonstra*, Department of Dermatology, University Hospital Leiden and *Department of Molecular Cell Biology, University of Utrecht, The Netherlands.

Our recent studies showed that an inverse relationship exists between EGF receptor expression and the ability of normal and transformed keratinocytes to form cornified envelopes. This findings have led to the hypothesis that the plasma membrane may play an important role in keratinocyte differentiation, since changes in its properties have led to a modulation in differentiation capacity on one hand and to differences in receptor expression on the other. In order to obtain more insight into the interrelationships between keratinocyte differentiation, plasma membrane lipid composition, lipid synthesis and EGF binding characteristics, the effects of differentiation-modulating compounds (like retinoids and glucocorticoids) on these parameters were studied in SCC-12F2 cells. SCC-12F2 is an established cell line of squamous cell carcinoma with high degree of capability to form cornified envelopes.

The differentiation capacity of the SCC-12F2 cells, i.e. ionophore-induced cornified envelope formation, was inhibited by various retinoids and stimulated by hydrocortisone. Retinoids that caused a significant reduction of cornified envelope formation, i.e. all-trans and 13-cis retinoic acid, caused only minor changes in lipid synthesis and plasma membrane lipid composition, while arotinoid ethylsulfane, having minor effect on cornified envelope formation caused a drastic inhibition of cholesterol synthesis resulting in changes of the plasma membrane lipid composition. Hydrocortisone stimulated envelope formation but had only minor effects on lipid synthesis and plasma membrane composition. Of all retinoids tested, only arotinoid ethylsulfane caused a drastic increase of EGF binding, while hydrocortisone had no effect. These results clearly demonstrate that the plasma membrane lipid composition is not related to keratinocyte differentiation capacity, but most likely does determine EGF binding. Furthermore, EGF-binding does not determine keratinocyte differentiation capacity.

11

EPIDERMAL LYMPHOCYTE CHEMOTACTIC FACTOR IS SPECIFIC FOR OKT4 LYMPHOCYTES. Claus Zachariae, Viggo Nielsen*, Christian Grønhøj Larsen, Thomas Ternowitz and Kristian Thestrup-Pedersen. Department of Dermatology and *The Institute of Human Genetics, University of Aarhus, 8000 Aarhus C, Denmark.

We have previously shown that epidermis overlying a positive tuberculin reaction contains factor(s) specifically chemotactic for lymphocytes (JID 1986: 87; 613). The present study was performed in order to see, if epidermal lymphocyte chemotactic factor (ELCF) was specific for subsets of T lymphocytes. T cells were isolated from healthy persons using an E rosette technique and Isopaque-Ficoll. The cells were incubated with OKT4 or OKT8 monoclonal antibodies and separated using fluorescence activated cell sorting (FACS 2, Becton & Dickinson). The separated OKT4 and OKT8 lymphocytes as well as non-separated T cells were further incubated at 37 deg. C for two days prior to study of chemotaxis.

Chemotaxis was performed using a blind-well chamber assay measuring the passage of 51 Cr-labelled cells through a 5 µm filter. The chemoattractants were ELCF and LTB-4 (10^{-8} M). ELCF induced the following chemotaxis expressed as mean chemotactic index: Unseparated T cells 1.57 (1.37-1.84), OKT4 cells 1.75 (1.59-1.90), OKT8 cells 1.22 (1.10-1.33). LTB-4 induced chemotaxis: Unseparated T cells: 1.75 (1.65-1.94), OKT4 1.52 (1.41-1.74), OKT8 1.57 (1.22-1.82).

These studies show that ELCF exhibits a selective high chemotactic sti-

mulus for OKT4 cells, where LTB-4 induced a similar chemotactic response among all three groups of cells. The findings are possibly of importance for explaining the predominant infiltrate of OKT4 lymphocytes in many skin disorders.

12

HUMAN EPIDERMIS OVERLYING POSITIVE ALLERGIC PATCH TEST REACTIONS CONTAINS INCREASED ETAF/IL-1 LIKE ACTIVITY AS WELL AS LYMPHOCYTE CHEMOTACTIC ACTIVITY.
C. G. Larsen, T. Ternowitz, F. G. Larsen, K. Thestrup-Pedersen. Department of Dermatology, University of Aarhus, Denmark.

Fourteen patients with confirmed or suspected allergic contact dermatitis (nickel, benzocaine, naphtole, chromium) were patch tested. Suction blisters were performed over test area epidermis and control (non-test area) epidermis after 48 h. Activity of ETAF/IL-1 (ETAF) was determined (units/cm^2 epidermis). Additionally, in ten patients we have looked for the presence of epidermal lymphocyte chemotactic factor (ELCF) in epidermal sheets. Eleven responded positive to the patch test. Ten of these increased ETAF activity in test area epidermis (913 ± 489 units/cm2) compared to non-test epidermis (558 ± 518 units/cm^2), and one showed a decrease (980 vs. 1570 units/cm^2). One of three non-responding patients showed increase, one was unaltered, and one patient showed a decrease of ETAF activity in test area epidermis. ELCF activity was in general increased in test area (CI = 2.42 ± 0.74) compared to non-test epidermis (CI = 1.51 ± 0.32), including three non-responders. Five patients were in addition measured for ETAF and ELCF activities before applying tests: all showed increase of ETAF activity after 48 h in both non-test epidermis (656 ± 533 units/cm^2) and test area epidermis (696 ± 246 units/cm^2), compared to pre-test activities (287 ± 182 units/cm^2). One patient with earlier reactivity to chromium but present non-responding anyhow revealed increased activities in both non-test and test area epidermis. ELCF showed a similar pattern for all five patients.

In addition we have found increase of ETAF and ELCF activities in epidermis overlying positive tuberculin skin reactions. Increased ETAF activity and presence of ELCF could be detected 6 h after intradermal injection of 6 units tuberculin. Unresponsiveness did not result in activity increases after 48 h. In conclusion, ETAF and ELCF activity increase are early events in cutaneous type IV reaction and are observed both in non-test and test area epidermis following patch testing of sensitized patients. This indicates a generalized cutaneous immune reaction.

13

MAST CELL POPULATIONS IN HUMAN SKIN.
Andrew C Markey, D.M.MacDonald, Laboratory of Applied Dermatopathology, United Medical and Dental Schools of Guy's and St. Thomas' Hospitals, (Guy's campus), London.

On the basis of histochemical properties and fixative sensitivity, two populations of mast cells, termed "connective tissue" and "mucosal", have been reported to exist in human gut and lung. Evidence for a similar situation in human skin is conflicting, (Proc.Natl.Acad.Sci.USA,83:4464-4468,1987. Int.Archs. Allergy appl. Immun.79:332-334, 1986).

In rodents, mucosal mast cells are sensitive to standardly used formalin fixation and their demonstration requires the use of other fixatives such as Carnoy's medium. Moreover, mucosal mast cells and connective tissue mast cells are stained specifically by safranine O(saf) and alcian blue(ab) respectively.

Using both methods of fixation separately and staining with either ab/saf (J.Clin.Pathol.1981;34:851-858) or the chloroacetate esterase(ce) reaction we have examined 15 samples each of normal human skin and atopic dermatitis(AD) in which increased numbers of mast cells may be anticipated (intestine was used as a positive control for both mast cell populations).

Mast cells were counted blind at magnifications of X400 and X1000 to offset local variations in mast cell concentration and allow accurate assessment of granule staining respectively. Counts were expressed as mast cells per ten high power fields at both magnifications. Eight non-serial sections of each fixative/staining combination for every specimen were examined to minimise the effects on results of local variations.

In both normal skin and AD, only "connective tissue" mast cells were demonstrated except in occasional samples of normal skin where saf +ve cells were seen amounting to less than 3% of the cell population. Comparing paired Carnoy's and formal saline fixed specimens, the latter consistently showed a mean percentage reduction of mast cell numbers as follows; AD—ab/saf 22.6%, ce 41% and normal skin —ab/saf 31.7%, ce 36%.

Thus we were unable to demonstrate a population of saf+ve "mucosal" mast cells in cutaneous tissue but confirmed that formal saline fixation seriously underestimates mast cell numbers.

14

FUNCTIONAL ACTIVITY OF ISOLATED MAST CELLS FROM HUMAN SKIN
H.-J. Heßler, E.Christophers Dept. of Dermatology, University of Kiel

Human skin mast cells play an important role in allergic skin diseases. In previous studies skin slices were employed to investigate mediator reelease by these cells. Recently Benyon and coworkers (1986) (Int.Archs Allergy appl.Immun 79:332) described a method for the isolation of human mast cells from infant foreskin. We studied isolation of these cells and their susceptibility to receptor-mediated stimuli.

Mast cells were gained from human skin by a modification of the Benyon-method. Chopped human foreskin was incubated with type 1 collagenase and type 1 hyaluronidase for 1 hour at 37^0 C. Cells were separated from the supernatant by density centrifugation on Percoll and subsequently stimulated with C5a, formylated Tripeptide (F-M,M,M), Compound 48/80, anti-IgE (a-IgE) and calcium-ionophore (A-23187). Histamine-release was determined fluorimetrically after derivation with o-phthalaldehyde an separation of the fluorescent products by HPLC.

Our method yielded 7x10^5 mast cells/g wet tissue (8±0.7% of total dermal cell count) representing 40% of the total histamine content of the skin. Cell viability was >95% as assessed by trypan blue exclusion. Maximum net histamine release after incubation with C5a ranged from 0.2 to 6.2% of the total histamine content (as assessed after cell lysis). With F-MMM this was 0.1 to 6.1%, with a-IgE 12 to 69%, with Compund 48/80 2 to 53% and with A-23187 20 to 78%. Preincubation with Cytochalasin B (5 µg/ml) increased C5a- and a-IgE-mediated release. In contrast F-MMM, C 48/80 and A-23187 mediated histamine release remained unchanged.

Our results suggest that enzyme liberated human cutaneous mast cells may exhibit differential susceptibility towards receptor-mediated stimuli. This may cause limitations for practical use of the enzyme methodology.

15

IN VITRO STUDIES ON THE ROLE OF RAT MAST CELLS DURING THYMIDINE INCORPORATION OF SPLEEN LYMPHOCYTES.
Beate M. Czarnetzki, Miklos Csatò, Thomas Rosenbach, Imke Wüllenweber. Department of Dermatology, University Hospitals, MÜnster, F.R.G.

Mast cells are important effector cells of immediate type hypersensitivity. Their role in delayed type reactions is however only poorly understood. In the present investigation, we have examined the effect of mast cell-rich (10-12%) rat peritoneal exudate cells (MCC) on mitogen- or antigen-driven spleen cell thymidine incorporation. 1x10^5 spleen cells (SC) or MCC were incubated alone or combined in the presence of phytohemagglutinin (PHA) or concanavalin A (Con A), both at 3.2 and 0.8 µg/ml, ascaris antigen, 10 and 1 µg/ml, and buffer alone for 2 days, 37°C, and were pulsed with 0.5 µCi ^{3}H-thymidine for another day. In some experiments, cells from ascaris sensitized animals were used. Mastcell depleted rat peritoneal cells (MCD), sonicated MCC, histamine (10^{-4} to 10^{-10}M), heparin (100 to 6 units), chondroitin sulfate (10 to 1 mg/ml), hyaluronic acid (0.5 to 0.06 mg/ml) or compound 48/80, 5.0 to 0.1 µg/ml, were added to the cells in some experiments. The data showed a marked decrease of thymidine incorporation in combinations of SC with whole or sonicated MCC, but not with MCD. MCC or MCD were never stimulated by mitogens, but MCC responded to ascaris antigen and compound 48/80. Histamine, heparin and chondroitin sulfate inhibited control- and PHA-driven thymidine incorporation of SC whereas hyaluronic acid was stimulatory, and all four granule-associated mediators of MCC increased Con A stimulated thymidine incorporation. The data suggest that MCC or their mediators modulate the proliferative response of lymphocytes to mitogens and antigens and might thus be also involved in lymphocyte-dependent immune processes.

16

THE ROLE OF CALMODULIN AND CALMODULIN ANTAGONISTS ON PROLIFERATION OF HUMAN KERATINOCYTES IN-VITRO.
A.M. Al-Ani, S. MacNeil*, J. Lowry** and S.S.Bleehen, Departments of Dermatology, Medicine* and Virology**, University of Sheffield, Sheffield S10 2JF, U.K.

The level of calmodulin (CaM), an intracellular calcium binding protein, is elevated in the skin of psoriasis. We have studied the level of CaM in human keratinocytes grown in-vitro in high calcium medium 1.2mM/ml (HCM) and low calcium medium 0.15mM/ml (LCM) using a specific radioimmunoassay for CaM. Cell proliferation was studied using cell count and dual DNA flow cytometry with a flourescent activated cell sorter. Two CaM antagonists that block the cell cycle at late G_1 phase were examined, N-(6-aminohexyl)-5-chloro-1 napthalene sulfonomide (W_7) and a more potent and specific derivative 8-iodo W_7.

Keratinocytes responded to an increase in extracellular calcium with both an increase in the percentage of cells in S-phase and an increase in intracellular CaM content; the percentage of cells in S-phase was, for example, 9.3 ± 0.7 (Mean ± SEM) in one experiment for cells in LCM and 19.1 ± 0.8 for same cells in HCM. Similarly, CaM content increased by approximately 90% in HCM, for example, 0.37 ± 0.02ng/ug protein in LCM and 0.72 ± 0.12ng/ug protein in HCM (p<0.025). The CaM antagonists W_7 and 8-iodo W_7 decreased cell proliferation as determined by cell count and cell cycle analysis, these two drugs significantly increase the percentage of cells in G_1 phase, for example, 100uM/ml W_7 increased the percentage of cells in G_1 from 45.9 ± 1.5 to 55.7 ± 1.5. In all cases, the 8-iodo W_7 was more potent. These results show that CaM levels are related to the proliferative state of human keratinocytes in-vitro and the inhibitory effects of CaM antagonists in increasing the percentage of cells in G_1 phase of the cell cycle may be of use in the treatment of psoriasis.

17

THE APPLICATION OF BROMODEOXYURIDINE FOR KINETIC STUDIES IN EPIDERMIS.
F.W.Bauer, J.B.M.Boezeman, P.E.J.van Erp, J.J.Rijzewijk, Dept. of Dermatology Nijmegen, The Netherlands

Because of its greater versatility and potentials the thymidine analogue bromodeoxyuridine (BrdU) is rapidly replacing [^{3}H]-thymidine as the drug of choice for cell kinetic studies. Cells which have incorporated BrdU can be 'visualized' with an immunofluorescence or an immunoperoxidase technique using a monoclonal antibody against BrdU. This procedure, however, is critical, the main difficulty being to manipulate the nucleoprotein complex in such a way that the antibody can bind to its antigen. The overall technique has been optimised for use with various cell types, but the 'recipes' currently available are not suitable for epidermis.

We have now developed protocols for the application of BrdU in skin sections, for in situ staining of cultured keratinocytes on coverslips and for epidermal cell suspensions from skin and cultured keratinocytes. Critical parameters were found to be fixation, DNA denaturation and protein digestion. Acetone fixation was used for tissue cryostat sections and keratinocytes cultured on coverslips whereas 70% ethanol was essential for epidermal cell suspensions. The hydrolysis step had to be optimized for all three protocols being 4 N HCl for 15 minutes at room temperature for cryostat sections, 1 N HCl for 8 minutes at 60^0 C, and in the case of epidermal cell suspensions a combination of denaturation and proteolytic enzyme digestion was essential (0.2% pepsin in 2 N HCL for 30 minutes at room temperature). The results on sections and coverslips are comparable with those obtained with [^{3}H]-thymidine but can be derived much faster. In cell suspensions the use of BrdU allows detailed analysis of the cell cycle especially in flow cytometric studies if simultaneously the DNA content per cell is measured with propidium iodide.

Conclusion: The application of the BrdU-antiBrdU technique is very valuable and allows kinetic studies in epidermis beyond the present possibilities.

18

TWO ANTIGENIC BINDING SITES FOR KI67 RELATED TO QUIESCENT AND CYCLING CELLS IN HUMAN EPIDERMIS. J.J.Rijzewijk, H.Groenendal, P.E.J.van Erp, F.W.Bauer, Dept. of Dermatology Nijmegen, The Netherlands

In our search for tools to measure cell kinetic parameters in human epidermis we have studied the monoclonal antibody Ki67 which, at least in a number of human tissues, binds to a nuclear antigen expressed by cycling cells only.

We used an indirect immunohistochemical staining procedure for cryostat sections with a standard acetone fixation followed by incubations with Ki67, rabbit anti mouse peroxidase and its substrate amino-ethyl-carbazole. In squamous epithelia Ki67 also binds to the cytoplasm of the germinative layer. In our hands the cytoplasmic staining of normal human epidermis was so predominating that the nuclear staining was very difficult to evaluate. Using the same technique on epidermis in a hyperproliferative state, such as healthy skin 40 h after tape-stripping and psoriatic lesional epidermis, the cytoplasmic staining was diminished or completely gone so that the nuclei of the basal and suprabasal layers were visible and largely Ki67-positive. To be sure these observations were not incidental we investigated cryostat sections of biopsies of the marginal area of a psoriatic lesion and a tape-stripped skin area. In these sections the transition could be observed from predominantly cytoplasmic staining of the germinative layer in normal epidermis to almost exclusively nuclear staining of basal and suprabasal cells in the hyperproliferative epidermis. The normal human epidermis loses its basal cytoplasmic staining about 25 to 30 h after tape stripping in favour of the nuclear staining.

These results clearly indicate that Ki67 binds to the nuclei of cycling cells in human epidermis and that in normal epidermis cytoplasmic expression of a 'Ki67 antigen-like' substance occurs which decreases in hyperproliferative states. Apparently the difference between the cycling and G_0-state of cells is expressed in the nucleus as well as in the cytoplasm.

19

GROWTH FRACTION IN EPIDERMAL SKIN DISORDERS DETERMINED BY THE MONOCLONAL ANTIBODY KI67. P.E.J. van Erp, H. Groenendal, F.W. Bauer, J.J. Rijzewijk, Dept. of Dermatology Nijmegen, The Netherlands.

The monoclonal antibody Ki67 reacts with nuclei of cells in the proliferative phases (G_1, S, G_2 and M) of the cell cycle. This was used in an immunohistochemical labeling reaction to determine growth fractions in normal skin and lesions of skin disorders characterized by some degree of hyperproliferation and/or abnormal keratinization. Because only a proportion of germinative cells in the epidermis is actively cycling, at least in normal skin, the total germinative population was determined on serial cryostat sections using the anticytokeratin antibody Pab601. We have defined the growth fraction as the ratio of Ki67-positive cells to Pab601-positive cells.

Within the diseases we studied, the absolute numbers of ki67-positive nuclei in psoriasis and atopic dermatitis were much higher compared to normal skin. Quantitative measurements in normal skin can sometimes be difficult because of interference with cytoplasmic staining of the epidermal germinative layer. In psoriasis as well as in atopic dermatitis the growth fractions were also higher despite the gross increased in the fraction of germinative (Pab601-positive) cells. Whereas in psoriasis increased numbers of ki67-positive cells could be observed throughout the germinative layers of the lesion, in cryostat sections of lesions from patients with atopic dermatitis Ki67 staining was confined to certain areas, most often those areas underlying parakeratotic stratum corneum. In skin disorders characterized predominantly by abnormal keratinization (i.e. ichthyosis and Darier's disease) growth fractions seem much smaller, however, as in normal skin, quantitative measurements are sometimes difficult because of cytoplasmic staining of the epidermal germinative layer. Our data show that detection of Ki67 nuclear antigen by immunostaining may be a potential tool for easy and quick evaluation of growth fractions in normal and hyperproliferating human epidermis.

20

THE ROLE OF CYSTEINE PROTEINASE INHIBITORS IN THE CONTROL OF EPIDERMAL CELL PROLIFERATION. Väinö K. Hopsu-Havu, Iris A. Joronen, Ari Rinne*, and Mikko Järvinen*, Department of Dermatology, University of Turku, Turku, and Department of Pathology*, University of Oulu, Oulu, Finland.

Human epidermis and the cells of a continuous epidermal cell line are known to contain several cysteine proteinases (CP) and their inhibitors (CPI). We have asked whether these factors might play a role in the control of epidermal cell proliferation.

We have purified from epidermal cells four CPs and three CPIs and present evidence for the role of one of them, i.e. ACPI, in the cell proliferation. 1) CP is mainly located in the proliferating cell layer, while ACPI is located in the upper nonproliferating cells. 2) Malignant epidermal cells are known to produce CPs, while the concentration of ACPI in epidermal malignancies is greatly diminished. 3) ACPI added to epidermal cell cultures inhibits cell proliferation at low concentrations as well as it inhibits several of the CPs.

It thus appears that cell proliferation takes place at tissue sites and in culture conditions which are characterized by high activity of CPs and low activity of CPIs. CPs and CPIs may thus play a role in the control of epidermal cell proliferation.

21

INFLUENCE OF HISTAMINE UPON IN VITRO LYMPHOCYTE PROLIFERATION AND IgE SECRETION IN ATOPICS AND NORMALS. Johannes Ring, Peter Thomas, Peter Rieber, Dermatologische Klinik and Institut für Immunologie, Ludwig-Maximilians-Universität, Munich, Germany.

Increased IgE production is one of the characteristic features of atopic diseases. Nature and origin of this isotypic dysregulation are still largely unknown. Animal experiments and clinical studies have pointed to the role of T cell regulatory mechanisms involving a possible decreased function of T 8 suppressor cells. Altered histamine releasability is another finding of possible pathogenetic importance in atopy. Therefore we investigated the influence of histamine (H) upon invitro lymphocyte proliferation and IgE secretion in 21 patients with atopic diseases and 10 healthy non-atopic controls. Peripheral lymphocytes were isolated and kept in culture up to 7 days and stimulated with various mitogens with and without prior incubation with different concentrations of histamine. Mitogen-induced thymidine uptake was significantly reduced by histamine both in atopics and normals. When T 8 suppressor cells were depleted with monoclonal antibodies using a rosetting technique, this histamine effect was no longer demonstrable. Spontaneous in vitro IgE secretion was reduced by histamine in normals but not in atopics; this effect was no longer observed in T 8-depleted cultures. The results support the concept of a possible histamine-induceable suppression of lymphocyte reactions including IgE secretion in normal individuals, while this interaction might be impaired in atopic diseases.

22

THE IMMUNOPHENOTYPE OF DERMAL IgE BEARING CELLS IN ATOPIC DERMATITIS J. Ophir, JNWN Barker, VA Alegre and DM Mac Donald, Laboratory of Applied Dermatopathology, Guy's Hospital, London, UK.

It has been suggested that the lesions of atopic dermatitis (AD) may represent an overlap between type I and type IV immune responses. The recent finding of IgE on the surface of epidermal Langerhans cells AD (1), provides some evidence for this hypothesis. We have, however, observed that the majority of cutaneous IgE-bearing cells are present within the dermal inflammatory infiltrate. Using immunohistochemical methods we have attempted to identify the phenotype of these cells.

Twenty-two patients with AD were studied. The presence of IgE on twenty percent of dermal infiltrating cells was confirmed by an indirect immunoperoxidase assay using three monoclonal anti-human IgE antibodies. The large majority of these cells had a dendritic morphology. The phenotype of these cells were identified by a double indirect immunofluorescence technique. Skin sections were incubated with Rhodamine-labelled IgE, in parallel with fluorescein-labelled monoclonal antibodies: anti HLA-DR, OKT6, RFD1, OKT4, RFB7 and Leu 1. 80% of IgE positive cells also expressed cell surface HLA-DR, 35% expressed OKT6 and 30% RFD1. No double-labelling was seen with either T-cell or B-cell markers.

These results are compatible with the dermal IgE bearing cells being either Langerhans cells (OKT6 positive) or interdigitating cells (RFD1 positive), both of which are known to act as antigen presenting cells for the induction of a cell mediated immune response. The presence of IgE on these cell types may therefore provide a link between type I and type IV immune responses in the pathogenesis of AD, and also explain the positive patch test reaction to inhalant allergens seen in AD.

Reference

1. Bruynzeel-Koomen C, van Wichen KD, Toonstra J, Berrens L, Bruynzeel P. The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. Arch. Dermatol. Res. 278(3): 199-205, (1986).

23

COMPARATIVE STUDY OF IGE BEARING LANGERHANS CELLS AND SERUM IGE LEVEL IN ATOPIC DERMATITIS. Thomas BIEBER†, Johannes RING*, Peter RIEBER*, Department of Dermatology† and Institute of Immunology*, University of Munich, West Germany.

The presence of IgE molecules has recently been demonstrated on Langerhans cells (LC) from patients with atopic dermatitis (AD) and it was postulated that this disease could be a chronic delayed-type hypersensitivity reaction. The purpose of the present study was to determine whether all LC bear IgE molecules and the possible relationship between the proportion of IgE+ LC and the serum IgE level.

Frozen sections from skin lesions of 19 patients with AD were investigated with 2 monoclonal antibodies (Mcab) reacting with different epitopes of IgE molecules (IgE2 reacting with ε2; M-E567 reacting with a not yet defined epitope of IgE), and anti-CD1 (OKT 6) Mcab. The staining procedure was achieved with a highly sensitive alkaline phosphatase-mouse anti-alkaline phosphatase complex technique (APAAP). Serum IgE level was determined for each patient. The stained cells were counted by computer assisted morphometry and represented as the number of cells per mm^2 section surface. The ratio IgE2+/CD1+ expressed the proportion of IgE bearing LC for each

patient and was compared to the serum IgE level.
IgE positive cells with the typical dendritic pattern were found in the epidermal compartment in 10 patients. The percentage of IgE+ cells from these patients varied from 15% to 83% of CD1+ cells. whereas the serum IgE level varied from 302 to 45000 UI/ml. However. the findings seemed to be independant from each other and we could not notice any relationship between the percentage of IgE+ cells and the serum IgE level.
The results suggest that IgE molecules can be demonstrated on a variable fraction of LC from patients with atopic dermatitis and high serum IgE level. but that these findings do not seem to be strictly related.

24

ASSOCIATED EXPRESSION OF Fc-RECEPTOR FOR IgE AND T6 ANTIGEN ON EPIDERMAL LANGERHANS CELLS FROM PATIENTS WITH ATOPIC DERMATITIS. E.M.M.van der Donk*, C.A.F.M. Bruynzeel-Koomen**, G.C. Mudde*, M. Capron****, P.L.B. Bruynzeel*** and G.C. de Gast*, Departments of *Immunohaematology, **Dermatology ***Pulmonary Disease, State University Hospital Utrecht, The Netherlands, and ****Centre d'Immunology et de Biologie parasitaire, Institut Pasteur Lille, France.

Langerhans cell (LC) enriched epidermal cell suspensions from clinically uninvolved skin from patients with atopic dermatitis were analyzed for Fc receptors for IgE ($Fc_\varepsilon R$) by three different types of experiments. Firstly, cell-bound IgE was removed by acid elution and could be restored by a myeloma IgE protein (IgE_κ). Secondly, after pepsin treatment the number of LC staining with FITC conjugated anti-human kappa (K) and lambda (λ) light chain antibody significantly decreased in contrast to the number of LC staining with FITC conjugated anti-human epsilon (ε) heavy chain antibody. Thirdly, LC formed rosettes with fixed sheep erythrocytes coated with IgE (SRBC-IgE). LC from normal non-atopic controls did not form rosettes with SRBC-IgE. The SRBC-IgE rosettes were inhibited by IgE and by BB_{10} (MoAb against the $Fc_\varepsilon R$ on human eosinophils, platelets and macrophages), but also by IgG_κ, whereas the SRBC-IgG rosettes were neither inhibited by IgE_κ nor by BB_{10}. Both the SRBC-IgE and the SRBC-IgG rosette formation were inhibited by incubation of the LC with OKT6 antibody. Inhibition studies with OKT6 on the reconstitution of IgE on LC after acid elution revealed that OKT6 did not inhibit the restoration of the IgE-binding. After OKT6 preincubation the number of LC staining with anti-κ FITC antibody was in the same range as without OKT6 preincubation. However, the number of LC staining with anti-ε FITC antibody was significantly decreased. Preincubation with anti-HLA-DR antibody did not influence the number of LC staining with either anti-κ or with anti-ε antibody. After preincubation with BB_{10} both the number of LC staining with anti-κ and with anti-ε antibody was significantly decreased. The results of the inhibition studies with OKT6 strongly suggest an associated expression of the $Fc_\varepsilon R$ and the T6 antigen on LC from patients with atopic dermatitis.

25

ACTIVE PARTICIPATION OF EOSINOPHILS IN PATCH TEST REACTIONS TO INHALANT ALLERGENS IN PATIENTS WITH ATOPIC DERMATITIS. C.A.F.M. Bruynzeel-Koomen*, D.F. van Wichen*, Chr. Spry**, P. Venge*** and P. Bruynzeel****, Departments of *Dermatology, ****Pulmonary Disease, State University Hospital Utrecht, The Netherlands, **Immunology, Royal Postgraduate Medical School, Hammersmith Hospital London, U.K., and ***Clinical Chemistry, University Hospital Uppsala, Sweden.

Patch tests with house-dust mite (HM) and grass pollen (GP) allergens were performed in 15 patients with atopic dermatitis (AD). Thirteen out of 15 patients with an immediate type skin reaction to HM allergen showed a positive patch test reaction to HM allergen 24-48 h after testing. Patch test reactions at 20 min, 2 h and 6 h were also observed. In case of GP allergen only 5 out of 13 patients with an immediate type skin reaction to GP allergen showed a positive patch test reaction 24-48 h after testing. Normal non-atopic controls and atopics without AD showed no positive delayed in time patch test reactions to both HM and GP allergens. Analysis of the cellular infiltrate in the dermis (D) and epidermis (ED) demonstrated eosinophils in the D as early as 2 h after patch testing. This infiltration was followed by the appearance of Leu 1-, OKT6- and anti-IgE positive cells in the D. At 24-48 h after patch testing eosinophils also appeared in the ED. Electron microscopic studies revealed that eosinophils in the ED were frequently lying in close contact with Langerhans cells (LC), suggesting cell-cell interaction. Immunostaining with EG2 (monoclonal antibody against activated eosinophil cationic protein [ECP]) and R-a-ECP (polyclonal antibody recognizing activated and storage ECP) revealed that eosinophils lying in the D reacted with both antibodies, whereas eosinophils, lying in the ED at 24-48 h, were EG2 negative and R-a-ECP positive. These results show that in the course of the patch test reaction to HM and GP allergens in patients with AD, eosinophils appear early in the D and are readily in activated stage. This suggests an active role for eosinophils in the reaction mechanism. Furthermore, eosinophils appear in the ED 24-48 h after patch testing where they seem to contact LC. The significance of this contact between EG2 negative eosinophils and IgE bearing LC is not yet clear. Therefore it can only be speculated whether these eosinophils inhibit or stimulate the patch test reaction to HM and GP allergens.

26

NEUTRAL SERINE PROTEASE (NSP) INHIBITORS INFLUENCE EPIDERMALIZATION OF RECONSTRUCTED HUMAN SKIN (RHS)-THERAPEUTIC IMPLICATIONS FOR WOUND HEALING AND PSORIASIS. C.M.E. Rowland Payne, B. Bertaux, L. Michel, C. Prost, B. Coulomb, C. Lebreton and L. Dubertret. Laboratoire de Dermatologie (INSERM), Hopital Henri Mondor, Creteil, France.

NSP activity characterizes reepidermalization of normal skin after injury and is prolonged in psoriasis (Dubertret et al, Br J Dermatol, 1984, 110, 405). Using RHS, the effects of inhibitors of NSP on epidermal differentiation (EpD) and epidermal proliferation (EpP) were evaluated.

RHS was made (Coulomb et al, Br J Dermatol, 1986, 114, 91). Dermal equivalents were prepared in 90 mm bacteriological Petri dishes, each containing 12.75 ml concentrated medium containing antibiotics and foetal calf serum, 7.5ml rat tail collagen, 1.25ml 0.1M NaOH and 10^6 fibroblasts in 2.5ml of complete medium. The epidermal component developed from an implanted 1mm punch biopsy. At day 4, and then twice weekly, medium was changed. It contained epidermal growth factors with or without various NSP inhibitors. Experiments were performed in triplicate. At day 19, culture was arrested and the new epidermis separated off and analysed. EpD was assessed by the ratio DNA x 10^2 (µg/new epidermis)/planimetry (mm^2/new epidermis) and EpP by thymidine turnover (cpm/new epidermis)/planimetry (mm^2/new epidermis).

Phenylmethane sulphonide fluoride (PMSF), soy-bean trypsin inhibitor (SBTI), alpha-1-antitrypsin (A1AT) and aprotinin, at the concentrations tested, did not influence EpD and EpP, except that 1mM PMSF was toxic. N-alpha-tosyl-L-lysine chloromethyl ketone (TLCK) enhanced EpD and EpP. Dose-response relationships between TLCK and EpD and EpP were established. EpD and EpP were unaltered by concentrations up to 1 µM. With 5 mM TLCK EpD was maximal, 2.70 µg/mm^2 compared with a control of 1.96. With 10mM TLCK EpP was maximal, 36.39 cpm/mm^2 compared with 16.76. Concentrations of 50mM and above were toxic.

Using an in vitro model of epidermalization, we have shown that EpD and EpP are augmented by TLCK. Thus TLCK may provide a therapeutic means to enhance wound healing.

27

THE RELATION OF EPIDERMAL TERMINAL DIFFERENTIATION AND LDL RECEPTOR EXPRESSION. Anne-Marieke Mommaas-Kienhuis, Stephen Grayson, Marian C. Wijsman, Bert J. Vermeer and Peter M. Elias, Departments of Dermatology and Electron Microscopy, University Medical Center, Leiden, The Netherlands, and Dermatology Service, Veterans Administration Medical Center, San Francisco, USA.

A negative correlation between low density lipoprotein (LDL) receptor activity and epidermal terminal differentiation has been shown previously on cultured normal keratinocytes and squamous carcinoma cells. To determine the LDL receptor expression in situ, morphological studies were designed, using colloidal gold as an ultrastructural marker. Next to freshly isolated mouse and human epidermal cells, separate epidermal strata, obtained by using the staphylococcal epidermolytic toxin, were investigated. The results of the experiments on mouse and normal human epidermis show that cells with the morphological characteristics of basal cells, demonstrated binding and uptake of LDL-gold. No LDL-gold was found on Langerhans cells or highly differentiated keratinocytes. In contrast, in psoriatic skin, cells from the stratum spinosum, that show highly differentiated morphological characteristics, showed abundant LDL-gold binding and even on granular cells some LDL-gold association was found.
In conclusion, the present study enabled us to establish LDL-gold expression in mouse and human epidermis in situ. A reciprocal correlation was found between epidermal terminal differentiation and LDL-receptor expression, suggesting that LDL-gold can be used as a marker for differentiation.

28

LIPID COMPOSITION OF CULTURED HUMAN KERATINOCYTES. Maria Ponec, and Diederik H.Nugteren*, Department of Dermatology, University Hospital Leiden and *Unilever Research Laboratory Vlaardingen, the Netherlands.

Terminal epidermal differentiation has been shown to be accompanied by profound changes in lipid composition leading to a marked decrease in the phospholipid and an increase in the cholesterol and ceramide content. System of cultured human keratinocytes has been extensively used for studying the processes involved in the regulation of epidermal differentiation. Therefore, the present study was undertaken to determine whether this in vitro system is also a suitable model for studying changes in lipid composition in relation to keratinocyte differentiation. For this purpose the keratinocytes were cultured either (1) in submerged culture system on plastic and in the presence of feeder layer either under low Ca^{2+} (proliferating) or normal Ca^{2+} (differentiating) conditions or (2) in an air-liquid culture system using dead de-epidermized dermis as the substrate. The lipids were extracted and subsequently analyzed by two-dimensional TLC on silica-gel plates with chloroform/methanol/acetic acid/water (90:70:1:0.7, v/v) in the first direction and chloroform/methanol/25% (w/v) NH_3 (90:12:1.5, v/v) in the second direction.

No significant differences were observed in lipid composition between cells cultured under low and normal Ca^{2+} conditions in the submerged culture system. Under these conditions the presence of relatively large amounts of phospholipids and low quantity of ceramides suggests only minor keratinocyte maturation as compared to the in vivo conditions.

A quite different situation was found in the air-exposed culture, the morphology of which strongly resembles that of the epidermis under in vivo conditions. After 2 weeks of culture the presence of stratum corneum and of stratum

granulosum rich in keratohyalin and lamellar granules was observed. Also the lipid pattern was close to the in vivo one, since the lipid analysis revealed the presence of low amount of phospholipids and high amount of sterols and ceramides, especially of acylceramides. These results show the usefulness of the air-exposed culture model for the study of the lipogenesis related to water barrier and stratum corneum function.

29

ACTION OF STAPHYLOCOCCAL EPIDERMOLYTIC TOXIN ON HUMAN EPIDERMAL CELL CULTURES : EXPERIMENTAL STAPHYLOCOCCAL TOXIC EPIDERMAL NECROLYSIS (TEN). E. Gentilhomme*, M. Faure**,M.H. Dumaine***, F. Binder*, D. Marmet***, J. Fleurette***, J. Thivolet**.

*. C.E. Bouchet,

**. INSERM U.209, Pavillon R. Hop. E.-Herriot 69437 Lyon, Cedex 03, France.

***. Laboratoire de Bacteriologie, U.A. Carrel, Lyon.

Cultured human epithelia obtained from epidermal cells in vitro were used to assay the activity of staphylococcal epidermolytic toxin and develop an in vitro experimental model for the staphylococcal scalded skin syndrome. Human epidermal cells were grown from single epidermal cell suspensions obtained through trypsinization of adult normal skin into multilayered epithelia (with a basal cell layer, several intermediate and one or two upper layers) on mouse 3T3 feeder cells. First passage cultures were incubated with exfoliative toxin A from phage Group II staphylococci at various concentrations in DMEM. They were examined at various time intervals by direct microcopic and histological examination of respectively the culture plates or the epidermal sheets after their detachment from the plates with dispase grad II. A total exfoliation could be obtained at 18 and 24 hour at concentrations of 1mg and 500 µg/ML, only local areas of epidermolysis noted at 100 µg/ml. The intraepithelial separation was noted to occur between the basal layer and the lowest intermediate layer. No exfoliation could be observed at lower concentrations. Up to 4-5 hours few changes were evident, but at this time small areas of epidermolysis developed. With exfoliatin 100 µg/ml, intraepidermal blisters were clearly visible, occuring either between the basal cells and the lowest intermediate layer or between the first two intermediate layers. These data clearly indicate that human epidermal cell cultures, although their differentiation in culture only mimics what occurs in vivo, can be used as an in vitro model of the staphylococcal TEN to further investigate the site of action of such a toxin and the cellular mechanism responsible for the syndrome.

30

PROPERTIES OF ACID PHOSPHATASE IN HUMAN STRATUM CORNEUM. Jürg Ch. Meyer, Departement of Dermatology, Zürich, Switzerland.

It was claimed that negatively charged groups like sulfatase and phosphatase groups in stratum corneum influence the kohesion properties of corneocytes. To see whether acid phosphatase in human stratum corneum plays a role in diseases of hyperkeratinization we first investigated the properties of acid phosphatase in normal human stratum corneum. For this purpose sheets of stratum corneum were prepared from skin samples from the departement of plastic surgery, homogenized by a freeze press method and then fractionated into a sediment and a supernatant by high speed centrifugation.

The yield of acid phosphatase activity after fractionation as well as its relative distribution between sediment and supernatant (soluble fraction) were pH-dependant. If homogenization and fractionation were performed at ph 4.5, most of the activity remained in the pellet whereas at a more alkaline pH or in an unbuffered medium the bulk of the activity was found soluble. Unlike leukocyte acid phosphatase the stratum corneum activity was drastically inhibited by citric acid in a dose dependant manner. At pH 4.0 or lower the enzyme was found to be labile against freezing. Highperformance gel chromatography and isoelectric focusing yielded 2-3 activity peaks for acid phosphatase. Furthermore isoelectric focusing demonstrated that acid phosphatase from human stratum corneum was different from that of cultured skin fibroblasts.

From our experiments we conclude that human stratum corneum contains a tissue specific acid phosphatase which is different from other cell types like leukocytes or fibroblasts and seems to exist in 2 or 3 forms. Furthermore, its binding properties to corneocyte membranes are dependant on the acidity of the milieu within the stratum corneum.

31

SEQUENCE DATA AND mRNA LOCALIZATION OF A TYPE II 70Kd KERATIN OF MOUSE EPIDERMIS DISPLAYING POSTNATAL BODY SITE SPECIFICITY AND SENSITIVITY TO HYPERPROLIFERATION. M. Rentrop, R. Nischt, B. Knapp, H. Winter and J. Schweizer. German Cancer Research Center, Institute of Experimental Pathology, Im Neuenheimerfeld 280,69 Heidelberg.F.R.G.

The epidermis of adult mouse ear, footpad and tail contains large amounts of a typeII 70Kd keratin which is absent from any other body site. Its postnatal induction occurs during the first two weeks after birth and is first observed in tail, then in footpad and only rather late in the ear. Although in vitro translation experiments with polyA$^+$ RNA from tail and footpad epidermis consistently fail to reveal the 70Kd keratin among the translation products, we show by means of a specific cDNA clone that in vivo the protein is encoded by a discrete mRNA species. The respective cDNA clone contains sequence information for a typeII keratin subunit substantially larger than the mouse 67Kd keratin. Northern blot analysis with a specific 3'-fragment of the clone demonstrates a single 2.8±0. 1kb mRNA species exclusively in ear, footpad and tail epidermis. In situ hybridization with the same fragment reveals the presence of the 70Kd mRNA in both basal and suprabasal cells of ear and footpad epidermis and in the orthokeratinizing parts of tail epidermis, whereas in the epidermis covering the balls of the foot, the mRNA is restricted to suprabasal cells at the base of these nodular elevations. Treatment of adult tail or ear epidermis with hyperplasiogenic agents, i.e. TPA or retinoic acid leads to a gradual disappearance of the 70Kd keratin. We show by in situ hybridization that the loss of the 70Kd keratin is caused by a specific suppression of the transcription of its mRNA in non-committed basal cells. Therefore the topological restriction of the 70Kd keratin, its postnatal and time-dependent acquisition and its pronounced sensitivity to hyperproliferative stimuli make this keratin subunit an especially well suited candidate for studies concerning the regulation of differential keratin expresssion and morphogenesis.

32

DIRECT STIMULATION OF DISEASE-SPECIFIC T LYMPHOCYTES IN ALLERGIC NICKEL-CONTACT DERMATITIS IN MAN. M.L. Kapsenberg, J.D. Bos, A. Schootemeijer, Lab. for Histology and Cell Biology. Dept. of Dermatology, University of Amsterdam, The Netherlands.

Allergic contact dermatitis can be considered to result from an undampened response of inducer (helper) T lymphocytes. Here we will report on a detailed in vitro study on the activation of inducer T cells by contact allergen. Disease-specific T lymphocyte clones (TLC) were prepared from either lesional skin biopsies or from peripheral blood from patients with nickel-contact dermatitis. These TLC had the phenotype of inducer T cells and could proliferate in an in vitro response to nickel and not to other contact allergens. In spite of the dogma that inducer T cells can only recognize antigen (allergen) in association with class II MHC-encoded molecules on the cell surface of antigen presenting cells (APC), two nickel specific TLC were obtained that responded to nickel in absence of APC. Further experiments showed that the response of these two TLC was also not restricted by MHC-encoded molecules. The response of other nickel-specific TLC was also not restricted by MHC-encoded molecules. The response of other nickel-specific TLC required the presence of APC. However, these TLC were unusually restricted. In functional compatibility assays using APC from various healthy donors, nickel-specific TLC of each patient were found to be differently restricted, whereas only in a minority of the cases the restriction patterns could be correlated to serologically typing for MHC-encoded molecules. This remarkable heterogeneity of nickel-specific TLC may be explained by the capacity of nickel to induce complex formation between proteins. Nickel-specific T cell proliferation without participation of MHC-encoded molecules may result from direct nickel-induced cross-linking of antigen receptor molecules on T cells. Unusual genetic restriction patterns may be explained by cross-linking of MHC-encoded molecules of the cell surface of APC with antigen receptor molecules of T cells. It is tempting to speculate that the allergic reaction to simple haptens like nickel is induced by direct cross-linking of critical immune response molecules leading to a response of inducer T cells of such a broad repertoire of T cells, that it cannot be sufficiently dampened by suppressor circuits.

33

MONOCLONAL ANTIBODY GB3, A NEW TOOL FOR MOLECULAR CHARACTERIZATION OF BASEMENT MEMBRANES AND HEMIDESMOSOMES. Patrick VERRANDO*, Anne PISANI*, Bae Li HSI**, Chang Jing YEH**, Nathalie SERIEYS*, and Jean-Paul ORTONNE* - *Laboratoire de Recherches Dermatologiques, ** Unité INSERM 210, UER Médecine - NICE-FRANCE.

A monoclonal antibody, GB3, has been raised against human amnion. GB3 binds to the amnion epithelial basement membranes as well as to an antigenic structure expressed by epidermal and by some other human basement membranes. This antigen is synthesized (and excreted) by cultured normal human epidermal keratinocytes. Ultrastructural and biochemical studies have shown that the antigen recognized by GB3 is a non collagenous component found in the lamina lucida and in the lamina densa of epidermal basement membrane that is also associated with hemidesmosomes. From radioimmunoprecipitation studies performed on cultured human keratinocytes, the antigen is identified to be a complex protein consistuted by five polypeptides of 93.5 kD, 125 kD, 130 kD, 146 kD, and 150 kD. Biochemical and morphological data indicate that these peptides are quite different from the other known components of the epidermal basement membrane.

The in vitro expression of the antigen recognized by GB3 is impaired during cell transformation and is qualitatively modulated by retinoic acid, as demonstrated by radioimmunoprecipitation experiments carried out on cultured human keratinocytes.

By immunofluorescence, this antigen is not detected in the skin from patients with junctional epidermolysis bullosa (Herlitz syndrome) in which a dermo-epidermal spliting occurs at the level of the lamina lucida.

Thus, GB3 appears to be an interesting probe for : 1) The study of a new component of basement membrane ; 2) The understanding of hemidesmosome structure and function ; 3) Biochemical investigations on the molecular defect that occurs in lethal junctional epidermolysis bullosa.

34

A PROTEIN WITH A REMARKABLE STRUCTURE PRESENT IN HUMAN EPIDERMAL KERATINOCYTES, MYOEPITHELIAL AND SMOOTH MUSCLE CELLS. Tonja Kartasova,[1] Hilde M.L. van Pelt-Heerschap,[2] Goos N.P. van Muijen,[3] and Piet van de Putte,[1] Departments of Molecular Genetics[1] and Biochemistry,[2] University of Leiden, Wassenaarseweg 64 and Department of Pathology,[3] University Medical Centre, Leiden, The Netherlands.

Cultured epidermal keratinocytes derived from human foreskin were used as a model system to study effects of UV irradiation on human cells. We used nucleic acid hybridization and complementary DNA (cDNA) cloning techniques to isolate human sequences that respond to UV irradiation. Poly(A)RNA was isolated from UV irradiated keratinocytes to serve as template for the synthesis of cDNA. Subsequently, the cDNA was used for the construction of a cDNA library. This library was screened for sequences, corresponding to mRNAs, whose concentrations increased in the cytoplasm of keratinocytes after UV irradiation. Finally, forty clones were isolated and subjected to further analyses. A group of five related cDNA clones was identified by cross-hybridization and sequencing. The DNA sequence of these clones reveals an open reading frame for a protein of 89 amino acids. The protein is proline-rich (29%) and contains several repeats. A polypeptide of 30 amino acids, corresponding to the C-terminal region of the protein was synthesized chemically and used to raise antibodies in rabbit. Immunohistochemical staining of human skin sections using purified antibodies showed that this 89 amino acid long protein is expressed in epidermal keratinocytes, myoepithelial and smooth muscle cells.

35

GAP JUNCTIONAL CELL-TO-CELL COMMUNICATION : QUANTITATIVE ANALYSIS IN NORMAL AND PATHOLOGICAL HUMAN EPIDERMIS. Denis Salomon, *Paolo Méda, Jean-Hilaire Saurat. Clinique de Dermatologie, Hôpital Cantonal Universitaire et *Institut d'histologie et d'embryologie, Centre Médical Universitaire, 1211 Genève 4, Suisse.

We previously demonstrated the presence of gap junctions and of gap junction-mediated cell-to-cell communication (coupling) in normal human epidermis by electron microscopy and intercellular transfer of the membrane-impermeant tracer Lucifer Yellow (LY)respectively. We present now a *quantitative* analysis of dye coupling in 1)normal human skin (NHS) 2) nonlesional psoriatic epidermis (NLPE) 3) a case of lamellar ichtyosis,(LI) both before and during retinoid (Ro 13-7410) treatment and 4) in the nonlesional epidermis of non psoriatic patients (NLER) *during retinoid therapy* (Ro 13-7410). After a 15 min standardized microinjection of individual epidermal cells with LY, skin *fragments were fixed* araldit-embedded and serially sectioned. The extent of coupling was then determined, for each microinjected cell, by scoring, the number and arrangement of the LY containing cells on semithin sections cut serially every 10-14 µm.

This technique revealed qualitatively similar patterns of coupling under all conditions studied. Thus, when intercellular exchange of LY occurred (21 out of a total of 25 injections) the tracer diffused in *all directions around the injected cell and involved either* basal and spinous keratinocytes. If the injected cells is located in the upper epidermis coupling is present between spinous and granular keratinocytes. Uncoupling of intact cells was seen in 3 cases of basal cell injections, and one case of spinous cell injection.

By contrast, quantitative analysis revealed significant differences in the extent of coupling in the various conditions studied. Thus, while the average *number of coupled cells labelled by LY after each* microinjection was 30 ± 36 (n=6) in NHS and 34 ± 36 (n =6) in NLPE, it dramatically raised to 313 ± 32 (n = 3) cells in LI before therapy. After 6 weeks of systemic (Ro 13-7410)arotinoid acid (35 µg/day) treatment, an average coupling of 108 ± 119 (n = 4) cells was seen in the LI. Whereas the coupling in NLER during the same therapy was similar to that of NHS (40 ± 29, n = 6). This study (i) provides quantitative data on the extent of intercellular communication in *normal human epidermis* (ii) shows that the technique can dectect changes of junction communications in skin disorders (iii) *suggests that systemic retinoid treatment may* decrease the coupling when unusually extensive like in LI, but does not appear to affect this coupling in apparently normal epidermis.

36

ACTIVATION OF LYMPHOCYTES AND KERATINOCYTES IN ALOPECIA AREATA. Hartmut Kietzmann and Wolfram Sterry, Department of Dermatology, University of Kiel, D-2300 Kiel, Fed. Rep. of Germany.

The peribulbar infiltrate in alopecia areata (Aa) is predominantly composed of helper-T-cells. So far no data are available concerning the role of B-lymphocytes and macrophages, or the degree of T-cell stimulation. Therefore, we analyzed the dermal infiltrate in scalp lesions of ten patients with longstanding Aa using a panel of 40 monoclonal antibodies against various cell surface antigens. The following results were obtained:

In all specimens of Aa a perivascular and peribulbar infiltrate was found, consisting *predominantly of helper-T-cells*. The degree of stimulation was only moderate (10 - 20% transferrin receptor, 5 - 10% interleukin 2 receptor). There was a tendency towards an increase of stimulation in the peri- and intrabulbar portion of the infiltrate. All infiltrating lymphocytes expressed HLA-DR and -DQ antigens. The number of lymphocytes expressing Leu 8 antigen and CD7 showed considerable variability *from patient to patient (20 - 70%). Consistently, intraepidermal* helper-T-lymphocytes were present. Langerhans cells were increased in number both in epidermis and hair bulbs, and could account for up to 30% of the dermal infiltrate. In some specimens, focal nests of B-cells were present, sometimes together with dendritic reticulum cells. About 20% of the infiltrating cells belonged to the monocyte-macrophage series. Interestingly, keratinocytes expressed the following antigens in more than 5/10 cases: *HLA-DR, HLA-DQ, Leu 8, transferrin* receptor and complement receptor 2.

Our data show that apart from moderately stimulated helper-T-cells B-cells, langerhans cells and macrophages add to the infiltrate in Aa. The expression of several antigens, especially of Leu 8 on epidermal keratinocytes is not seen in banal inflammatory skin diseases and requires further investigation.

37

AN ANIMAL MODEL FOR ALOPECIA. K.A. Horne, C.A.B. Jahoda, *B.E. Johnson, H.J. Michie, and R.F. Oliver, Department of Biological Sciences and *Department of Dermatology, University of Dundee, Scotland.

A strain of hairless rat (DEBR), bred at the University of Dundee, has been evaluated as a model for determining the pathogenesis and causal factors involved in the development of alopecia in man. While the aetiology of alopecia remains unknown, there is evidence to support an autoimmune pathogenesis with an inherited predisposition to the disease. DEBR rats grow an apparently normal first coat of hair after birth and then become progressively hairless. As a first step in determining whether an autoimmune pathogenesis is responsible for the hairless condition, DEBR skin was examined histologically. This revealed that, in common with alopecia in man, hair follicles are present but show a marked peri- and intrafollicular lymphocytic infiltrate. To gain further insight into a possible immunological explanation for this phenomenon, three experimental procedures were performed: grafts of DEBR skin into nude (athymic) mice, which provide an immunologically "neutral" systemic environment; photochemotherapy and topical application of minoxidil. Both PUVA and minoxidil are known to modulate T lymphocyte-related immune function in skin.

Full-thickness, macroscopically hairless DEBR skin was grafted into the flank of nude mice. Within 2 weeks hairs emerged from the grafted skin to eventually produce tufts of hair 1 cm + in length. Photochemotherapy, using oral Psoralen and long wave ultraviolet radiation to measured areas of skin, resulted in hair growth restricted to the exposed areas. Minoxidil treatment (4 mg/ml applied to measured areas of hairless skin 3 times/week) also resulted in hair growth, but this occurred over the whole body to include all regions originally devoid of hairs.

These results provide evidence for a genetically inherited, autoimmune pathogenesis for the hairless condition in DEBR rats, rather than an inherited dysfunction of hair growth per se, and suggest that DEBR rats may provide an invaluable model for investigating the aetiology and pathogenesis of alopecia in man.

38

ULTRASTRUCTURAL DETECTION OF GRANULOCYTE ACTIVATION INDUCED BY MEDIATORS DERIVED FROM HUMAN MONOCYTES AND EPIDERMAL CELLS (M-GRAM/EC-GRAM). Gabriele Zeck-Kapp, Alexander Kapp and Urs Riede. Departments of Pathology and Dermatology, University of Freiburg, FRG.

As shown recently human monocytes and epidermal cells release mediators which are capable of inducing a long-lasting generation of reactive oxygen species (ROS) in human PMN (M-GRAM/EC-GRAM). To further evaluate subcellular mechanisms *of GRAM-induced PMN activation in the present study the effect* of these mediators on the ultrastructure of PMN was investigated by morphological criteria as well as by cytochemical detection of ROS, H_2O_2(1) and $^{\cdot}O_2^-$ (2). Phorbol-myristate-acetate (PMA) was used as a control stimulus. Similar to PMA (5 ng/ml) , but to a lower degree, M-GRAM and EC-GRAM induced in PMN the formation of *microvilli and small intracytoplasmic vesicles within 15 min.* Until 60 min the microvilli appear shortened. However, the vesicles did not increase in number and volume. In contrast to GRAM, upon stimulation with PMA microvilli were significantly longer as evidenced by morphometric criteria. This finding was paralleled by an volumetric increase of vacuoles during the time course of activation. *M-GRAM and EC-GRAM-induced generation of ROS was evidenced* by detection of the reaction product on the surface of the PMN and in the cytoplasmic vacuoles in the range of 30 to 60 min. Similar results were obtained upon stimulation with PMA even after 15 min. The data presented significantly demonstrate the metabolic activation of PMN by M-GRAM and EC-GRAM which were shown to induce a membrane-associated generation of ROS paralleled by *morphological signs of activation.* The results present further evidence for the mechanisms of interaction between epidermal cells and monocytes with PMN in inflammatory skin diseases.

(1) Briggs RT et al. J Cell Biol 67:566-586, 1975.
(2) Briggs RT et al. Histochem 84:371-378, 1986.

39

COMPARATIVE TOXICITY OF ANTIMICROBIAL AGENTS ON TRANSFORMED HUMAN KERATINOCYTES. F.M. Tatnall, I.M. Leigh and J.R. Gibson, Department of Dermatology, The London Hospital, London, E1 1BB, England.

Topical antibiotics and antiseptics are widely used to prevent and treat bacterial infections in wounds and ulcers. Antiseptics have been implicated local tissue injury and retardation of wound epithelialization. This prompted us to investigate the comparative cytotoxic

effects of antiseptics and antibiotics on human keratinocytes transformed by Simian virus 40 (SVK14 cells).

SVK14 cells were grown to semi-confluence by adding $6x10^4$ cells per petri dish. After 48 hours the cells were exposed to serial dilutions of the therapeutic concentrations (in parentheses) of each of the following agents: hydrogen peroxide (3%), cetrimide (1%), sodium hypochlorite (0.5%), povidone iodine (4%), neomycin (1%), bacitracin (50 units/ml), polymyxin B sulphate (10,000 units/ml). The cells were exposed to the drug for 15 minutes and then washed and incubated in the culture medium (RPMI 1640 plus 10% fetal calf serum) for 24 hours. Dead cells were then washed off and the viable adherent cells were trypsinised and counted in a Coulter counter.

At therapeutic concentrations none of the antibiotics were found to be cytotoxic whereas all of the antiseptics produced 100% killing of SVK14 cells. Dilutions of therapeutic concentrations of the antiseptics ranging between 1000= and 20,000-fold (depending on the agent) were needed to achieve no-effect levels. Calculations based on 100% killing values for the antiseptics indicate that their order of toxicity from highest to lowest is sodium hypochlorite, cetrimide, povidone iodine and hydrogen peroxide.

Whilst it is appreciated that more work is needed in order to assess the exact relevance of these findings to clinical practice, it should be noted that SVK14 cells share many of the characteristics of normal keratinocytes and that these preliminary results are broadly in line with in vitro, in vivo and human volunteer study findings made by other investigators studying antimicrobial toxicity profiles. We conclude that this cell line may be useful in studying the epithelial cytotoxicity of drugs in vitro and that care should be exercised in the selection of antimicrobial agents for use in wound treatment.

40

HETEROGENEITY OF BASAL LAYERS. KERATINOCYTES IN HYPERPAPILLOMATOUS HUMAN EPIDERMIS. R. Buidin+, J. Golstein*, D. Parent+ and M. Heenen+, +Department of Dermatology and *IRIBHN, School of Medecine, Brussels University Belgium.

Stratified squamous epithelia may be considered functionally to consist of two cell compartments, a basally positioned progenitor cell compartment from which cells move suprabasally into the differentiating cell compartment. Subdivision of the progenitor compartment is not possible from its histological appearance. A murine hybridoma secreting an IgG_1 monoclonal antibody (FB_1) was produced by cell fusion of PAIO myeloma cells with spleen cells from mice immunized with human fetal keratinocytes (17 weeks old). FB_1 selectively stained the basal layer of fetal and normal adult epidermis. The staining pattern was clearly cytoplasmic (phase 1 epidermis of Bullough, Br.J.Derm. 87,347,1972). In normal thick epidermis (phase 2 epidermis of Bullough) staining was heterogenous. At the bottom of the rete pegs most of the basal cells exhibit a strong positive fluorescence but in the thin portion of epidermis, overlying the dermal papilla, basal cells were negative. In psoriatic epidermis, we observed a selective staining of basal cells in the lower part of the rete pegs. This suggests the existence in hyperplastic epidermis of a stem cell subpopulation at the base of the pegs and cell flow along the basement membrane with amplification by dividing transit divisions. In psoriasis, the staining pattern indicates that probably the increased size of the germinative population is due to an increased number of amplification divisions.

41

VITAMIN E AND COENZYME Q_{10} CONTENT IN HUMAN EPIDERMIS AND BASAL CELL EPITHELIOMA. Clara De Simone°Luigi Rusciani°Antonio Venier°Filippo M.Larussa°Gian Paolo Littarru*Alessandro Oradei**Silvio Lippa**and Ferdinando Serri°.Department of Dermatology°,Department of Physiology**,Catholic University,Rome;*Department of Biochemistry, University of Ancona, Italy.

Vitamin E (Vit.E) and Coenzyme Q_{10}(CoQ_{10}) are small antioxidant molecules that represent a cellular defense mechanism against free radicals (F.R.) mediated damage.Their main protective function is to prevent lipid peroxidation of biological membranes by scavenging the highly reactive toxic species.As F.R.are thought to be involved in the process of aging and in the development of cancer,evaluation of these two antioxidant compounds in the epidermis is particularly suitable because this tissue is exposed to enviromental physical and chemical agents capable of generating F.R.The aim of this study was to evaluate the content of both Vit.E and CoQ_{10} in samples from sun-exposed and covered healthy epidermis as well as in samples from the most common epidermal neoplasm,the basal cell epithelioma. The measurements were performed on thin slices from ten biopsy specimens for each group of samples.The method used to evaluate Vit.E and CoQ_{10} includes several steps (homogenization of frozen tissue,extraction of both the substances from homogenate, purification of extract on silica gel G),the last of which is HPLC.

Healthy covered epidermis revealed lower values of Vit.E(181 ± 50 ng/mg of protein) and CoQ_{10}(212 ± 64 ng/mg of protein) with respect to both exposed epidermis (Vit.E=675 ± 273 ng/mg of protein;CoQ_{10}=637 ± 397 ng/mg of protein)and basal cell epithelioma (Vit.E=2437 ± 1070 ng/mg of protein;CoQ_{10}=585 ± 211 ng/mg of protein).Regarding the first comparison the difference could be related to the different level of risk of oxidative damage between covered and exposed epidermis.The increased content of both the protective substances in basal cell carcinoma could compensate for a possible defect in the enzymatic antioxidative system.It could also be responsible for a certain resistance of neoplastic tissue to the body's defense mechanism.

42

HEXADECANE INDUCED SKIN HYPERPLASIA IN THE HAIRLESS RAT : TIME COURSE OF HISTOLOGICAL AND BIOCHEMICAL EVENTS RELATED TO THE SYNTHESIS OF POLYAMINES AND DNA. M. Bouclier, C.N. Hensby, G. Milano,* J. Ferracin, A. Chatelus and B. Shroot. Centre International de Recherches Dermatologiques (CIRD), Sophia-Antipolis, 06560 Valbonne. *Centre Lacassagne, 06000 Nice, France.

Epidermal hyperplasia, was induced in the male hairless rat by three successive topical application of 200 µl of pure n-hexadecane on the back (about 8 cm^2 surface area) at 4 h intervals. Two biochemical parameters of epidermal hyperplasia (ornithine decarboxylase (ODC) activity and levels of polyamines) were measured in the epidermis. The levels of ODC activity and polyamines were found to be increased 12 h after the first application of n-hexadecane.

	Control	12h after first treatment
ODC (nmol µg $DNA^{-1}h^{-1}$)	10	70 ***
Putrescine (nmol mg DNA^{-1})	8	16 **
Spermidine (nmol mg DNA^{-1})	75	225 **
Spermine (nmol mg DNA^{-1})	40	110 **

** $p<0.005$ *** $p<0.001$

At 24 h the peak of DNA biosynthesis, as measured by ^3H thymidine incorporation was observed. At this time, histological evaluation revealed a significant cellular oedema whereas at 72 h a distinct epidermal hyperplasia was histologically observed.

These data support the view that ODC activation and increased polyamines biosynthesis are related to the synthesis of DNA and epidermal cell hyperproliferation. This technique may be a useful model for studying skin hyperplasia, its biochemical events and the effects of pharmacological agents.

43

HISTOCHEMICAL ANALYSIS OF TRANSGLUTAMINASE PROFILES IN DIFFERENT TYPES OF BOVINE EPITHELIA. Daniël Broekaert, Ann Lagaisse, Peter Reyniers, Paul Coucke, Jean De Bersaques and E. Gillis. Laboratory of Physiological Chemistry and Department of Dermatology, State Univ. Ghent, Ghent, Belgium.

A histochemical study was performed to elucidate the role of transglutaminase (Tgase) in the differentiation of different types of bovine epithelia. It was our purpose to provide additional evidence for the presence in internal epithelia of soft keratinization markers, other than cytokeratins. Three independent approaches were followed and appropriate controls were systematically performed.(1). The Tgase activity test, based on the covalent attachment to tissue glutamine acceptor sites of monodansylcadaverine. (2). Localization, after irreversible inactivation of the endogeneous Tgase, of all potential acyldonor substrates, reached by subsequently added exogeneous epidermal Tgase and monodansylcadaverine. (3). Immunohistochemical localization of determinants homologous to epidermal Tgase by the indirect peroxidase technique, using a polyclonal serum raised in rabbits (K193 : anti 71-74kD epidermal Tgase).

In cornified stratified squamous epithelia (tongue and oesophagus), the dual nature of Tgase sites could unequivocally be revealed during terminal stages of keratinization, respectively in the cytoplasm and at the cornifying membrane, in accordance with epidermal tissues. The Tgase activity is restricted to cytoplasmic terminal sites in noncornified stratified squamous epithelia (conjunctiva and cornea), (pseudo)stratified columnar epithelia (trachea, bronchi) and transitional epithelia (ureter). Transitional epithelia (urinary bladder and urethra) and simple epithelia (lung, liver parenchyme and gall ducti, gall bladder, small and large intestine) express an overall moderate to weak cytoplasmic Tgase activity. In all samples, the cytoplasmic Tgase activity profile corresponds to the distributional pattern of epidermal Tgase homologous determinants. Moreover, epidermal Tgase determinants are observed near the cell membrane in late stages of keratinization in noncornified stratified squamous epithelia and in (pseudo)stratified columnar epithelia, probably corresponding to an inactive form of Tgase.

The presence of acyldonors was not concomitant with Tgase sites, but generally extended throughout the entire epithelial tissue.

The present study suggests that all noncornified epithelia display a simplified Tgase distribution, reminiscent of the less complex cytokeratin sets.

44

COMPETITION OF TOPICAL SPIRONOLACTONE FOR DIHYDROTESTERONE RECEPTORS IN HUMAN SEBACEOUS GLANDS: AN AUTORADIOGRAPHIC STUDY. Enzo Berardesca, Paola Gabba, Giovanni Ucci*, Giovanni Borroni, Giacomo Rabbiosi, Department of Dermatology and *Department of Medicine II, University of Pavia, IRCCS Policlinico S. Matteo, Pavia, Italy.

Topical spironolactone has been demonstrated to be an effective antiandrogenic compound in animal models. It acts as an antiandrogen by competing for the androgen receptor. The aim of this study is to demonstrate the penetration of topically applied spironolactone into human sebaceous glands and its binding to dihydrotestosterone receptors. 6 male volunteer subjects affected by acne vulgaris were treated with 5%-spironolactone cream for 48 hours. The drug was applied on an area of 25 sq. cms. of the dorsum of the patient at a concentration of 100 mgs. per sq. cm. Two 4 mm. punch biopsies were taken at the end of the treatment

respectively on treated and untreated skin. The slices were incubated at 4°C for 2 hours in a medium containing 20 ul of tritiated dihydrotestosterone (specific activity 60 Curies mm/mol.), then fixed and processed for autoradiography. In sebaceous glands of the untreated side were detected 24.1 grains per area unit whereas the treated side presented 5.9 grains per area unit. Student's t-test showed a significance of 9.95 ($P<0.0005$). Topical spironolactone has been demonstrated to reduce the size and activity of the sebaceous glands of hamster's flank organ. Our data reveals both the penetration of the drug in the human sebaceous glands and the binding to the androgen receptor and thus demonstrates that spironolactone has antiandrogenic properties in man as well as animals.

45

CELL ENVELOPE PRODUCTION IN ACNE. H Thomas Rupniak*, David M Turner*, William J Cunliffe+, Rita Dhuna*, Michael R West*. *Department of Cell Biology, Glaxo Group Research, Greenford, Middlesex, U.K. and + Leeds Dermatological Research Foundation, Dermatology Department, The General Infirmary at Leeds, Leeds, U.K.

We have recently developed a monoclonal antibody, using envelopes from cultured epidermal cells as immunogen, which specifically recognises a particular class of epidermal cell envelope. From immunoblotting and immunolocalisation studies, the anti-envelope monoclonal specifically recognises an antigenic determinant on the cross-linked envelope structure but not on non cross-linked envelope components, such as involucrin or plasma-membrane proteins. The epitope is not present, or not accessible, in normal epidermal envelopes but is present in psoriatic epidermis (J Invest Dermatol 87:164A, 1986). In indirect immunofluorescence studies on sections of normal human epidermis, the monoclonal recognises only the cell envelopes produced in the upper regions of the intra-follicular areas. In normal epidermis, a relatively sharp boundary exists between the positive intra- and the negative inter-follicular regions. Non-inflamed, whitehead acne lesions have an increased thickness of intra-follicular epidermis possessing cell envelopes concomitant with the epidermal hyperplasia observed in these lesions. In addition, the inter-follicular epidermis neighbouring the involved hair follicle now also demonstrates positive staining extending out to a variable distance away from the actual acne lesion. This acquisition of antibody positivity in the normally negative inter-follicular epidermis bears considerable resemblance to the development of positivity in the inter-follicular epidermis of psoriatic lesions and normal epidermis placed into organ or tissue culture. Thus, the envelopes of cultured epidermal cells show structural similarity with the envelopes produced by intra-follicular epidermis and the inter-follicular epidermis of psoriatic lesions and organ-cultured skin. In contrast, the inter-follicular epidermis of normal skin produces cell envelopes of a structurally different type. These findings appear to correlate with studies demonstrating the appearance of an envelope type in psoriatic lesions and wounded skin which is morphologically different to the type of envelope found in normal epidermis when characterised by Nomarski contrast microscopy (J Invest Dermatol 87:156A, 1986).

46

ANTIBODIES TO P. ACNES EXOCELLULAR ENZYMES IN THE NORMAL POPULATION AND ACNE PATIENTS. E. Ingham and W.J. Cunliffe,* University Departments of Immunology and Dermatology*, Leeds, U.K.

Antibodies to P. acnes and its products have been implicated in inflammatory acne, however, antibodies to P. acnes exocellular enzymes have not been previously demonstrated in individuals. Standard techniques were used to measure neutralising antibodies to P. acnes lipase (L), hyaluronate lyase (HL) and acid phosphatase (AP) in an age-related study of 89 normal sera, in severe acne patients and controls.

Neutralising antibodies to L were present in 20% of 13-17 year olds (mean $\log_2$ titre ± 95% confidence limits of positives = 5.5 ± 2.1), 17% (4.0 ± 4.3) of 18-25 year olds; 42% (3.4 ± 0.6) of 25-33 year olds and 20% (3.2 ± 0.5) of 33-65 year olds, but undetectable in children below 13. Anti-HL antibodies were absent in children and teenagers with 17% (3.0 ± 0), 8% (3.0 ± 0) and 4% (3.0 ± 0) of 18-25, 25-33 and 33-65 year olds positive. Anti-AP antibodies were undetectable in the sera. There was no significant difference in the prevalence of, or titre of when present, antibodies to the enzymes in severe acne patients compared to controls (see table).

Anti-enzyme antibodies in acne patients and controls

Group	No.	Age ± 95% CL	% with antibodies to:- L	HL	AP
Con.	30	19.2 ± 1.1	30 (3.7 ± .4)*	7 (3 ± 0)	0
Acne	30	19.1 ± 1.1	30 (3.6 ± .4)	10 (3 ± 0)	0

( )* mean $\log_2$ titre ± 95% confidence limits (CL)

Thus, following puberty, some individuals produce anti-L and anti-H antibodies and there is a greater prevalence of antibodies to L than H in normal teenagers and adults, however the presence of these antibodies is unrelated to acne. This is in contrast to levels of antibodies to P. acnes cells, which increase in severe acne.

47

THE DISTRIBUTION OF *PITYROSPORUM (MALASSEZIA)* SPECIES ON NORMAL HUMAN SKIN. J.P. Leeming, F.H. Notman, K.T. Holland, and W.J. Cunliffe*, Departments of Microbiology and Dermatology*, University of Leeds, Leeds U.K.

Yeasts of the genus *Pityrosporum (Malassezia)* are known to constitute a major proportion of the saprophytic microflora of normal human skin and are associated with a number of disease conditions including pityriasis (tinea) versicolor, folloculitis, seborrhoeic dermatitis, dandruff, acne vulgaris and systemic infections of compromised patients (particularly infants). However investigations into their distribution on both healthy and diseased skin have been limited by deficiencies in methods available for isolation and enumeration of these yeasts. The purpose of this study was to exploit recent advances in isolation techniques to determine the distribution of *Pityrosporum* on normal human skin.

Twenty sites on the head, trunk and limbs of 10 young adults (5 males, 5 females) were sampled by the scrub-wash technique where possible (areas of flat, glabrous skin) or by swabbing. Viable counts were performed by surface-drop methods on a previously described medium (*Proc. XIV Int. Cong. Microbiol. 1986:* P.M8-2) and incubated aerobically for 14 days at 34°C. Viable counts of both aerobic and anaerobic bacteria in each sample were also performed using standard bacteriological procedures.

Geometric mean *Pityrosporum* counts were highest on the chest and upper back (both approximately $10^4/cm^2$ and the outer ear (2×10^4/swab). Other sites on the head and lower trunk had moderate counts ($2x10^2$-$2x10^3/cm^2$ or swab). Skin of the limbs yielded lower counts which decreased as the extremities were approached. Counts were considerably higher than have been previously reported using other media. The distribution of *Pityrosporum* was similar to that of propionibacteria (although there were some distinct differences), but not to aerobic cocci or coryneforms). This probably reflects the lipophylic nature of these yeasts. A number of colony variants were noted which differed in their relative abundancies at different body sites. The ability to recognise different types of *Pityrosporum* and to determine their distribution on normal and diseased skin should facilitate much-needed investigations into their role in the ecology and pathology of skin.

48

ANTISEPTIC INCORPORATED BIOLOGICAL DRESSINGS J.N. Kearney, T.Arain* and K.T. Holland* Yorkshire Regional Tissue Bank, Pinderfields Hospital, Wakefield and *Department of Microbiology, University of Leeds, Leeds. U.K.

Biological dressings possess many advantages for use on cutaneous wounds, however, a lack of antimicrobial activity precludes their use on contaminated/infected wounds e.g.leg ulcers, burns. We have determined the binding and release characteristics of three antiseptics to biological dressings using bioassays against 3 disparate but common wound microorganisms, in a simple wound model. Human skin and amnio-chorion, $1cm^1$ in size were soaked for 24 hours at 4°C in 0.5% and 2% solutions of Povidone iodine (P), Silver nitrate (S) and Chlorhexidine acetate (C), and subsequently sterilised with Ethylene Oxide following lyophilisation. Clinical isolates of Ps. aeruginosa, Staph. aureus and Candida albicans, were used to prepare indicator cultures on isosensitest medium. Zones of inhibition of growth around (5 replicate) pieces of the antiseptic incorporated dressing (and controls) were measured after 24 hours incubation. Each was subsequently transferred to a fresh indicator culture and re-incubated. This was repeated until all activity had been lost. Strips of dressings material placed at 90° to one another, were used to evaluate any synergy/antogonism between the antiseptics. Both tissues demonstrated binding of an effective concentration of (C) and (S) and release over a clinically relevant period (Table 1). The release curve was exponential for (C) whereas linear for (S). No evidence of resistant colonies was seen. (C) and (S) did not demonstrate either synergy or antagonism toward any of the microorganisms in this system. We conclude that (C) incorporated biological dressings provide excellent broad spectrum activity over a prolonged period. (S) dressings demonstrated high initial activity (1-5 days) against Pseudomonas and should be retained for this indication.

Table 1 Mean No. transfers (days) before loss of activity (SKIN)

	(C)0.5%	(C)2%	(S)0.5%	(S)2%	(P))0.5%	(P)2%
Pseudomonas	12.3	16.5	9.1	9.6	0	0
Staphylococcus	25.4	40.7	6.0	8.0	0	0
Candida	13.8	19.0	3.1	4.0	0	0

Similar results were obtained using AMNIO-CHORION

49

PRODUCTION OF TOXIC SHOCK SYNDROME TOXIN-1 : CONTROL BY GROWTH RATE OR MAGNESIUM CONCENTRATION ? D. Taylor, and K. T. Holland, Department of Microbiology, University of Leeds, Leeds, U.K.

Toxic Shock Syndrome (TSS) is a potentially fatal illness with symptoms of sudden onset, high fever and delayed desquamation of the palms and soles during convalescence. In many cases there is progression to multi-system involvement. Investigations have shown TSS to be associated with both *Staphylococcus aureus* and the use of tampons during menstruation. Tampons consisting of new high absorbent synthetic materials have been particularly implicated in the disease. Lack of bacteraemia in most cases of TSS has suggested a role for soluble products produced by *S. aureus*. One such important extracellular protein is Toxic Shock Syndrome Toxin-1 (TSST-1). It is probable that toxin production by the organism will be directly effected by the environment. Mills *et al.*(J. Inf. Dis. 151: 1158. 1985.) have reported that the concentration of Mg^{2+} in the culture medium controls production of TSST-1 by the organism and that the greatest concentration of toxin occurs when the concentration of Mg^{2+} is low. The aim of this study was to test this hypothesis using more sophisticated experimentation than previously employed. Continuous culture provides a means of investigating the physiology of *S. aureus*. The many variables present in batch culture studies can be finely controlled in continuous culture and hence meaningful results obtained. By using the concept of relative specific growth rate (μ_{rel}) it is also possible to divorce the effects of growth rate on cell physiology from the effects of other environmental parameters e.g. Mg^{2+} concentration. A TSS isolate of *S. aureus* was grown in a chemostat under constant conditions of pH 7.4 and temperature 37°C. The growth rate of the organism was controlled by varying the dilution rate of the defined synthetic medium supplying the culture. High levels of TSST-1 relative to cell dry weight occurred at 0.2 μ_{max} (μ_{max} = maximum specific growth rate) and 0.81 μ_{max}, with other growth rates tested yielding low levels. In a subsequent experiment cells were grown at 0.33 μ_{max} in conditions of high or limiting Mg^{2+} concentration. Production of TSST-1 relative to biomass was around twenty-fold lower in the Mg^{2+} limited culture when compared with the Mg^{2+} replete cells. These results indicate that limiting Mg^{2+} concentrations do not increase TSST-1 production in *S. aureus*. However consequential reduction in specific growth rate as a result of decreased Mg^{2+} concentration may explain other workers' findings.

50

THE GROWTH AND DIFFERENTIATION OF VARIOUS CELL TYPES ON A RECONSTITUTED BASEMENT MEMBRANE. Michael Edward, Jean A. Gold and Rona M. MacKie, Department of Dermatology, University of Glasgow, Glasgow, Scotland.

Extracellular matrices contribute to the physical properties of tissues, but also provide a unique substrate for the resident cells. Changes in the composition and structure of the matrix greatly influence the migration, growth and differentiation of the resident cells. A major limitation of conventional tissue culture techniques is that cells grown on plastic lack a proper polarity and may lose the ability to express differentiated functions. We have examined the growth and morphology of various cell types on a reconstituted basement membrane termed matrigel, derived from a 2M urea extract of the Engelbreth-Holm-Swarm sarcoma. The matrigel contains laminin, type IV collagen, heparan sulphate proteoglycan, nidogen and entactin.

Normal human skin melanocytes, fibroblasts, dispersed epidermal cells and plucked hair bulbs, murine melanoma cells and bovine aortic endothelial cells were seeded on matrigel, collagen gel and plastic and their growth and morphology examined. A characteristic feature of the cells grown on the matrigel was an increased cell-cell adhesiveness. Melanocytes formed clumps with dendritic processes, with increasing numbers of single cells appearing up to four weeks after

seeding. The melanoma cells formed interconnected network-like colonies similar to those observed in endothelial cell cultures on matrigel, which in addition formed capillary-like structures. Fibroblasts also formed interconnected clumps, and brought about a rapid reorganisation of the matrigel, but outgrowth was more rapid on the collagen substrate, while similar rates of epidermal outgrowth were observed from plucked hair bulbs on both substrates. The melanocytes, melanoma cells, fibroblasts and endothelial cells rapidly formed monolayers on plastic and collagen gels. Fibroblasts embedded within the matrigel remained as rounded single cells, while endothelial cells grew slowly and formed dendritic-like interconnecting processes.

The matrigel appears to promote cellular differentiation and may provide a more suitable substrate for the in vitro study of epidermal and other cell types.

51

BINDING OF SKIN FIBROBLASTS TO COLLAGEN IS MEDIATED BY SPECIFIC MEMBRANE PROTEINS. C.Mauch,M.Borchert,M.Pfäffle,K.v.d.Mark,T.Krieg, Dermatologische Klinik der LMU München, MPI für Biochemie, Martinsried.

A prerequesite for the normal functionning of connective tissue is a strong and highly specific interaction of the cells with their surrounding extracellular matrix (ECM), which influences differentiation and phenotype of the cells. This interaction was shown to be mediated by specific cell receptors, which recognize distinct proteins of the ECM, eg. laminin and fibronectin. In recent years also collagen receptors have been charcterized in platelets, endodermal cells and chondrocytes. It is the aim of the present study to identify receptors on skin fibroblasts which mediate the cellular interaction with collagen in the dermis in order to develop systems allowing to study altered cell matrix interaction in disease processes eg. wound healing and fibrosis.

Membrane vesicles from fibroblasts attaching preferable to collagen type I were prepared and found to bind ^{131}J-labeled type I collagen. After characterizing membrane proteins on slab gel electrophoresis which were then blotted to nitrocellulose paper and incubated with radioactively labeled collagen, the collagen binding activity could be localized to a protein with a molecular weight of 35 kd. In order to obtain information whether this collagen binding protein in fibroblasts is related to anchorin described as a type II receptor in chondrocytes, antibodies were used for immunofluorescence microscopy showing a punctate cell surface localization. This was further supported by immunoprecipitation of metabolically labeled membrane proteins, which demonstrated the presence of a 35 and 38 kd protein in fibroblasts. In addition similar cell membrane proteins were found when dermal extracts were investigated by immunoblotting. It is assumed that these molecules represent a class of collagen binding proteins in fibroblasts, which are closely related to anchorin C II, and further experiments are designed to elucidate their role in disease processes.

52

THE EFFECT OF UVB ON OXY- AND DEOXYHEMOGLOOBIN.
N. Kollias, A. Baqer, Physics Department, Kuwait Univ. and Dermatology Department, Al Sabah Hospital, Kuwait.

In studying diffuse reflectance spectra from human skin in vivo, post-UV irradiation, we can identify spectroscopically methemoglobin. The purpose of the present investigation was to determine whether oxy- and deoxy-hemoglobin can be transformed to methemoglobin by UV radiation. And whether intact erythrocytes are affected by UVB.

Hemoglobin was obtained by hemolysing human erythrocytes. The resulting solution, was diluted so that the 415nm absorption resonance of oxyhemoglobin did not exceed 0.9. The absorption spectrum of the solution was measured on a spectrophotometer (H-P, mod 8450A). It was then irradiated by a bank of UVB fluorescent lamps (Philips TL20W/12UV) with doses ranging from 10 to 500 mJ/cm^2 assessing the absorbance in between exposures. Deoxyhemoglobin samples were tested in a similar fashion. Erythrocytes were diluted with physiological buffer until a spectrum could be recorded, they were then irradiated with similar doses of UVB radiation.

We find that oxyhemoglobin is converted entirely to methemoglobin in what appears to be a direct transformation. Deoxyhemoglobin is also transformed entirely to methemoglobin at similar doses of UVB by an indirect transformation. Similar transformations have been obtained for intact human erythrocytes. In preliminary action spectra we find that the wavelength that is most effective is 285nm with the rate decreasing at least by two orders of magnitude by 320nm.

We thus find that both oxy- and deoxy-hemoglobin will be converted to met-hemoglobin upon irradiation with UVB radiation, at doses similar to those used for the treatment of psoriasis.

53

SKIN SENSITIVITY IN ATOPIC ECZEMA (AE) BEFORE AND AFTER UVA PHOTOTHERAPY - EVALUATION BY VISUAL ASSESSMENT, EVAPORIMETER AND LASER DOPPLER FLOWMETER
Rainer Gollhausen, Konrad Göttsberger, Hanno Winter, Bernhard Przybilla and Johannes Ring, Department of Dermatology, University of Munich, FRG

Phototherapy of AE has gained increasing interest in recent years. However, its mode of action in this condition has not yet been elucidated. We evaluated skin sensitivity to a chemical irritant and to ultraviolet B (UVB) in patients with AE before and after ultraviolet A (UVA) phototherapy.

8 patients with AE (6 males, 2 females; aged 19 - 45 years) were treated with UVA (UVAPUR; Saalmann) 3 to 4 times weekly for 6 weeks (102 ± 25 J/cm^2 total UVA dose). Before and after this period skin sensitivity to sodium lauryl sulfate (SLS) and to UVB was measured with visual assessment of erythema, laser doppler flowmeter and evaporimeter: On day 0 (D0) the volar forearms were exposed to 1 % aqueous SLS in Finn chambers for 24 hours. Reactions were evaluated on D1, D2, D4 and D7. - After determination of the individual minimal erythema dose (MED) to polychromatic UVB each subject was irradiated on the lower back with a single dose of 3 MEDs. On D1, D3 and D6 the reaction was assessed. - 6 non-atopic individuals served as controls.

Nonlesional skin of patients with AE was more sensitive to SLS and UVB before than after phototherapy. Furthermore, before UVA treatment patients with AE were more sensitive than healthy controls. However, significant differences ($P < 0.05$) were demonstrable only by measuring the skin vapour loss with the evaporimeter and by assessing the cutaneous blood flow with the laser doppler flowmeter. The scores of erythema assessed visually did not significantly differ neither for SLS nor for UVB.

In patients with manifest AE nonlesional skin is more sensitive to irritating stimuli than skin of non-atopic individuals. By UVA phototherapy skin resistance is restored at least to some extent. Laser doppler flowmeter and evaporimeter are more appropriate instruments to measure this effect than is visual assessment of erythema.

54

DERMAL ALTERATIONS OF HAIRLESS MICE INDUCED BY INCREASING DOSES OF UV-B IRRADIATION : HISTOLOGY, IMMUNOCHEMISTRY AND ULTRASTRUCTURE. C. Berrebi, F. Fiat, A. Fourtanier, N. Horisszny, D. Rouers-Pelé and T. Vila. Laboratoire de Recherche L'Oréal - 93600 Aulney-Sous-Bois - France

In a recent publication of Chen et al (J.I.D. 87 : 334-337, 1986), the nature of elastotic material in sun-damaged human skin was investigated by indirect immunofluorescence and was found to consist predominantly of elastin, microfibrillar protein and fibronectin, with the interstitial collagens present to a lesser extent. Using the same technique and electron microscopy, we show a similar cutaneous response of the animal, to UV-B irradiation. Irradiation of MF1/hr hairless mice is accomplished with a xenon solar simulator, 5 days per week. Initial daily dose of 0.9 M.E.D. is incremented by 20 % every week during 7 and 12 weeks, according to I. Willis et al (J.I.D. 80 : 416-419, 1983).

Techniques used in this study are : 1/ routine histology : formal fixation, paraffin embedding, specific staining of elastic fibers (Luna), collagen (Van Gieson) and glycosaminoglycans (Mowry). 2/ indirect immunofluorescence on cryostat sections, using guinea-pig antisera against bovine types I and III collagens, rabbit antiserum against rat elastin and rabbit antiserum against human factor VIII for blood vessels. 3/ electron microscopy : glutaraldehyde fixation, osmium tetroxide post-fixation, Epon embedding, staining of thin sections with uranylacetate and lead citrate, and measurement of transverse sections diameter of collagen fibers with a quantimet Q10 from Cambridge Instruments.

Alterations of connective tissue appear at 7 weeks of irradiation and become more severe at 12 weeks. Histological changes are mainly elastosis and increased deposition of GAG at the dermal-epidermal junction and between altered collagen bundles in all the dermis. Immunohistochemistry shows thickened and broken elastic fibers in the upper papillary dermis and lack, in some places, of elastin fibers in reticular dermis, loss of collagen I and increase of collagen III in the papillary dermis, leading to an increase of coll. III/I ratio, as recently found by biochemical studies (S.R. Platow et al. E.S.D.R. 1986).

Electron microscopy reveals a predominance of microfibrillar component in elastin masses, which appears often mothed and intermingled with collagen fibers. Marked degradation of collagen is characterized by disorganized and very short fibers, loss of tinctorial affinity and amorphous aspect. Transverse sections show and important variability of diameters (9 classes of size from 27 to 95 nm, in comparison to 2 classes > 95 nm in the control).

These results will be discussed in the light of earlier publications.

55

EPIDERMODYSPLASIA VERRUCIFORMIS: LANGERHANS CELLS, IMMUNOLOGIC EFFECT OF RETINOID TREATMENT AND CYTOGENETICS. P.C. van Voorst Vader, M.C.J.M. de Jong, C.G.M. Kallenberg* and J.M.J.C. Scheres**, Departments of Dermatology and Clinical Immunology*, University Hospital, Groningen and Anthropogenetic Institute**, Catholic University, Nijmegen, the Netherlands.

A case study is presented of a 44-year old negroid male with epidermodysplasia verruciformis (EV), cutaneous carcinomas and impaired cell mediated immunity (CMI), treated with the retinoid etretinate, 1mg/kg/day, during 8 days. Analysis was made of: 1) T6+ and HLA-DR+ Langerhans cells (LCs) by immunoperoxidase staining in the epidermis of lesional and clinically normal skin on forehead and back before and during retinoid treatment; 2)the effect of the retinoid treatment on CMI in vivo (DNCB skin test) and in vitro (mitogen-induced lymphoproliferative response) and on the Th/s-cell ratio of peripheral blood lymphocytes (PBLs); 3) the rate of spontaneous and UV-light induced chromosomal aberrations and the frequency of sister chromatid exchanges (SCE) in cultured PBLs.

The results showed: 1) the virtual absence of T6+ and HLA-DR+ LCs in koilocytic areas of epidermis histologically involved with EV, clinically normal skin on the forehead also harbouring foci with these abnormalities, with a decrease of LCs/mm and LC/mm2, particularly on the forehead, after retinoid treatment; 2) before retinoid treatment a DNCB skin test score of 1 (controls: 10.4±1.6), a Th/s-cell ratio of 0.91 (controls: 1.6±0.2) - a decrease due to a decrease of Leu-3a+ PBLs - and a PHA-induced lymphoproliferative response in vitro below the normal range, while the Con A- and PWM-induced response was in the lower range of normal, retinoid treatment not markedly affecting these parameters except for a slightly increased PHA- and Con A-induced response after treatment; 3) a percentage of PBLs grown in medium 199 with spontaneous chromosomal breakage of 14.9% (controls: 5-20%), no structural aberrations including breakage in PBLs cultured in RPMI 1640 medium, 12% of these cells showing aberrations after UV-light irradiation (control: 14%) and a frequency of SCE of 5.2/cell (normal range: 4-12/cell). Thus, no evidence was found of spontaneous or UV-light induced chromosomal instability.

The absence of epidermal T6+ and HLA-DR+ LCs in EV involved skin may indicate a deficient role of epidermal LCs in the human papillomavirus(HPV)-specific CMI response in this EV patient. The impairment of the non-HPV-specific CMI response in this EV patient was not markedly affected by short-term retinoid treatment.

56

DECREASE OF MEMBRANE LIPID FLUIDITY BY RETINOIC ACID IN HUMAN EPIDERMAL CELLS. B. Bonnekoh[1], B. Thiele[1], G.R.F. Krüger[2], and G. Mahrle[1], Dpts. of [1]Dermatology and [2]Pathology, University of Cologne, FRG.

We recently introduced fluorescence polarization technique with lipophilic 1,6-diphenyl-1,3,5-hexatriene (DPH) as an assay for the study of membrane lipid fluidity (MLF) of epidermal cells. MLF of epidermal cells was relatively low and stable to changes of the temperature in comparison to fibroblasts and fibrosarcoma cells. It has been shown by other authors that retinoic acid decreased MLF in embryonal carcinoma cells and increased MLF in 3T6 cells. Moreover there was evidence that tocopherol interfered with the retinoid effect on MLF. In the present study we therefore focussed on the influence of all-trans-retinoic acid (RA) and d-α-tocopherol acetate (TA) on the MLF in normal human epidermal cells.

Cells of the stratum basale and spinosum were isolated from normal skin of 5 adults by trypsinization. The cells were incubated in McCoy's 5A medium containing 2 or 20 µM RA or 100 µM TA in DMSO (final concentration 1%) at 37 °C for 2.5 h. After incubation with 1 µM DPH solution for 1 h, the cells were processed to spec-

trofluorometry. The degree of fluorescence polarization (P-value) was determined at a concentration of 550,000 cells/ml at 25 °C.
The mean P-values were 0.280 ± 0.008 for the control with pure medium and 0.280 ± 0.007 for the control with 1% DMSO. The mean P-value increased to 0.302 ± 0.011 for 2 µM RA and to 0.320 ± 0.009 for 20 µM RA. Statistical analysis (Scheffé-test, $p = 0.05$) revealed a significant dose-dependent decrease of MLF under the influence of RA. The mean P-value for epidermal cells incubated with TA was 0.288 ± 0.006 and did not significantly differ from the control values.
In conclusion RA induced "hardening" of cell membranes in human epidermal cells. This effect can be interpreted as an acceleration of the maturation of cell membranes.

57

RETINOIDS ENHANCE MONOCYTE/ENDOTHELIUM INTERACTION. ASJ Barkley, PC Bather & BR Allen. Department of Dermatology, University Hospital, Nottingham, UK.

Using an in vitro model we have studied the effects of a variety of inflammation modifying drugs, including etretinate and isotretinoin, on the early activation of circulating monocytes at the endothelial cell surface.
Human umbilical vein endothelial cells (EC) were grown to confluence on fibronectin treated plate wells in supplemented medium RPMI 1640. Mononuclear cells (>93% monocytes by esterase staining), or in some experiments, purified polymorphonuclear leukocytes (PMN), were extracted from the blood of healthy subjects by gradient centrifugation and incubated for 2 hours with serial dilutions of anti-inflammatory agents. 10^5 viable cells were applied to the EC monolayers in dark conditions and incubated at 37°. After 1 hour, non-adherent cells were removed by plate inversion and standard agitation. Attached leukocytes were counted by phase contrast microscopy. Adherence (mean of 10 high power fields, triplicate experiments) was expressed as percentage variation from controls.
The retinoids increased monocyte/EC adhesion over the concentration range 10^{-4}M - 10^{-7}M. Maximum changes were +191% ±45 SE for etretinate at 10^{-6}M and +130% ±64 for isotretinoin at 10^{-5}M. This activity was reduced if plastic surfaces were substituted for EC - etretinate ±96% +22, 10^{-5}M; isotretinoin 96% ±17, 10^{-4}M - and absent if instead EC were preincubated with with either compound. There was also no similar effect on PMN/EC binding. In contrast, indomethacin, benoxaprofen and thalidomide reduced monocyte/EC adherence. Dexamethasone was without effect except on PMN/EC binding (-300% ±33, 10^{-6}M).
These retinoids thus appear to possess a specific monocyte mediated activity not shared with other unrelated anti-inflammatory agents. We have reproduced this activity only with Interleukin-1 (IL-1) which increases the EC binding of all types of inflammatory cells. If, as reported, retinoids can potentiate monocyte IL-1 release these in-vitro observations might explain the apparent pro-inflammatory action of these drugs which often complicates their clinical use.

58

CAULIFLOWER COLLAGEN FIBRILS IN DERMATOSPARAXIS AND EHLERS-DANLOS SYNDROME TYPE I. G.E. Piérard, J.L. Lé, J.F. Hermanns, B. Nusgens and Ch. M. Lapière, Dept. of Dermatology, University of Liège, Belgium.

The Ehlers-Danlos syndromes (EDS) encompass distinct entities (type I to XI) including dermatosparaxis (DX). In these disorders, a variable proportion of the cross-sections of the collagen fibrils have a cauliflower shape. We have compared by computerized morphometry in transmission electron microscopy the size and shape of the cauliflower collagen fibrils (CCF) in DX and EDS type I, as well as the volumetric relationship between the extrafibrillar space and the CCF within bundles of the mid reticular dermis. Samples were collected from 5 DX and 5 normal age-matched calves, as well as in 3 patients with EDS I and 5 normal age-matched individuals. More than 20,000 measurements were made. Statistical analysis was performed by using the Kolmogoroff-Smirnow test and the U test.
The structure of CCF appeared obviously heterogeneous in each disease and different in the two disorders. Two main aspects of the cross-section of DX-CCF were recognized : spider-like CCF made of ribbons of procollagen polymers and serrated CCF with a dense central core. Both types of DX-CCF had similar volumes and the perimeter of spider-like DX-CCF was almost twice that of serrated DX-CCF. The volume of DX-CCF was similar to that of normal fibrils, while EDS I-CCF were significantly thicker than normal. The CCF shape was evaluated by the form factor (4 area divided by π x max. diam. x min. diam.). This parameter was significantly lower in spider-like DX-CFF (< 0.6) (i.e. fibrils are more corrugated) than in serrated DX-CCF (0.7 to 0.95) and EDS I - CCF (0.8 to 0.95). The volume of the interfibrillar space within the bundles was twice as large in DX than in normal or EDS I.
It therefore appears that DX-CCF are due to a defect in the cohesion between the constitutive polymers of the fibrils, but with persistance of a correct control of their ultimate size. This is associated with an excess of non collagenous material embedding the DX-CCF. Conversely, the size control of EDS I-CCF is lost, eventuating in an increased amount of collagen polymers forming the fibrils.

59

REORGANIZATION OF COLLAGEN LATTICES IN THE PRESENCE OF HUMAN DERMAL FIBROBLASTS
P.J.E. ROWLING+, M.J. RAXWORTHY+, E.J. WOOD+, J.N. KEARNEY° and W.J. CUNLIFFE*
+Department of Biochemistry, University of Leeds, Leeds LS2 9JT; °Yorkshire *Regional Tissue Bank, Pinderfields General Hospital, Wakefield, WF1 4DG; Department of Dermatology, Leeds General Infirmary, Leeds LS1 3EX, U.K.

The incorporation of fibroblasts into a collagen gel results in gel contraction and collagen reorganization. This may lead to stabilization of the gel lattice producing a dermal equivalent suitable for skin grafting since dissolution of the dermal equivalent on the wound bed may be inhibited. Eventually the heterologous dermal equivalent should be replaced by autologous tissue in vivo. We have investigated the reorganization of collagen lattices in the presence of fibroblasts using light and electron microscopy, resistance to collagenase digestion and SDS-PAGE. Collagen gels were formed by mixing 7.7mg acetic acid-solubilized rat-tail tendon collagen, 2 x 10^5 human dermal fibroblasts, Dulbecco's modified Eagle's medium and 5% newborn calf serum in 13.5ml. Addition of NaOH induced fibrillogenesis of the collagen which was complete within 30 min.
Over 25 days of incubation at 37°C the collagen lattice became increasingly resistant to digestion by 0.05% clostridial collagenase as assessed by the protein content of aliquots taken at intervals following incubation of the gel with collagenase. Newly-cast gels were completely hydrolysed in 3h, whereas after 25 days in culture, digestion required over 24h. Analysis by SDS-PAGE showed that the initial collagen preparation contained larger amounts of the monomer (α band) than the dimer (β band) or the high M_r (γ band) (α,β,γ band ratio) 8.4:4.3:1.0, determined by densitometric scanning). After 25 days in culture there was an increase in the amount of the γ band (band ratio 2.8:2.4:1.0). Upon reduction of the 25 day-cultured samples with 2-mercaptoethanol there was only a slight increase in the amount of the α band indicating that the collagen had become cross-linked by non-reducible covalent bonds.
These results indicate that a dermal equivalent better suited for grafting may be produced after 25 days of culture as described. Presumably the increased resistance to collagenase results from increased collagen cross-linking. However, the precise role of fibroblasts in the reorganization process remains to be elucidated.

60

EFFECT OF A THERAPEUTIC CONCENTRATION OF DITHRANOL IN THE MOUSE TAIL TEST. Mechtild Hofbauer, Pauline M. Dowd, Martin Whitefield and Malcolm W. Greaves, Institute of Dermatology, St. Thomas's Hospital, London SE1 7EH

The adult mouse tail epidermis exhibits alternating areas of parakeratosis where the granular layer is absent ('scale' areas) and orthokeratosis overlying areas where the granular layer is present. The ability of topical formulations to induce a granular layer and orthokeratosis in the scale areas has been used as a screening test for antipsoriatic activity. However, a previous assessment of a 10% dithranol ointment in this test yielded a negative result (1). In view of the definite antipsoriatic activity of dithranol in psoriasis we considered it worthwhile to assess the effect of a therapeutic concentration of dithranol in the mouse tail test.
Three matched groups of 10 adult mice were treated daily for 7 days, as described by Wrench (2). In the first group, 0.2% dithranol in white soft paraffin (WSP) was applied to the tails. In the second group WSP was applied only. In the third group the tail was rubbed twice without any ointment application. Mice were sacrificed 24 hours after the last treatment, the tail skin removed and processed for light microscopy as described by Jarret and Spearman (3). Histological examination revealed that 7 of 10 dithranol treated mice showed restoration of the granular layer and orthokeratosis in scale areas of epidermis. These effects were not produced by application of WSP alone or the action of rubbing the tail skin. These results indicate that the mouse tail test can be used to demonstrate the antipsoriatic activity of a therapeutic concentration of dithranol and may be useful to evaluate its analogues.

Ref: (1) Wrench, R., Britten, A.Z. (1975) Brit.J.Derm. 93, 75.
(2) Wrench, R. (1985) in: Models in Dermatology (Ed. by H.I. Maibach and N.J. Lowe), Vol.2, p.76.
(3) Jarret, A., Spearman, R.I.C. (1964), Histochemistry of the skin - Psoriasis, Engl.Univ.Press, London.

61

COMPARATIVE STUDIES ON SKIN LIPIDS PEROXIDATION INDUCED BY PHYSICAL AND BIOLOGICAL AGENTS. Nazzaro-Porro M., Picardo M., Finotti E., Passi S., Istituto Dermatologico San Gallicano, Roma, Italy.

It has been shown that both the fungus Pityrosporum and UV rays are capable of peroxidating unsaturated lipids. We have studied the peroxidation of skin surface lipids (SSL) following irradiation with UVA or UVB in comparison with that induced by the lipoxigenase activity of Pityrosporum. Lipid fractions of SSL, i.e. squalene (SQ), triglycerides (TG), free fatty acids (FFA), cholesterol esters (CE), cholesterol (CH) and wax esters (WE) obtained from 50 healthy human volunteers were separated by preparative TLC. 5 mg of each fraction were irradiated with UVA (0.5 mWatt/cm^2 at the distance of 20 cm) up to 8 hours, or UVB (0.5 mWatt/cm^2 at the distance of 50 cm) up to 1 hour, or added to cultures of Pityrosporum, strain 4701 and 4702, obtained from patients affected by Pityriasis versicolor, supplemented with 1% Tween 40 as lipid source; the culture were grown aerobically on a gyro-rotary shaker (Nazzaro-Porro et al., JID 87,108,1986). The lipoperoxidation was evaluated by the direct N,N-diethyl-1,4-phenylene-diamonium sulfate (DEPD) test. The decomposition of the unsaturated lipids was measured by GLC. Both UVA and UVB were capable of peroxidating each lipid fraction examined, mainly SQ and CH, with formation of numerous by-products . However following exposure to UVB the rate of lipoperoxidation was more rapid and levels of lipoperoxides more conspicuous than those observed with UVA. In culture Pityrosporum was capable of peroxidating, to different extents SQ, CH, TG and FFA. CH and WE did not undergo lipoperoxidation probably because of the inability of the fungus to hydrolize these esters. In the peroxidated lipid fractions (examined by TLC and GLC), a clear similarity was found between the intermediates formed by the lipoxigenase activity of Pityrosporum and those induced by UV irradiation, indicating a similar mechanism for physical and biological peroxidation of SSL

62

NON INVASIVE EVALUATION OF FUNCTIONAL CHANGES INDUCED BY SHORT TERM APPLICATION OF SODIUM LAURYLSULFATE ON HUMAN SKIN. Dominique J. Van Neste D., Jesus de la Cuadra, Jean L. Antoine. Unit for Occupational and Environmental Dermatology, Louvain University, Brussels, Belgium.

Functional changes induced by short term application of sodium laurylsulfate (SLS) to the surface of human skin are reported.
We investigated the effect of aqueous solutions of SLS (10%,5%,1%; control: water) left under occlusion for periods of time ranging from 4 to 24 hours. Cutaneous changes were monitored by using the following non invasive methods: transepidermal water loss (TEWL, Evaporimeter, Servomed, S), cutaneous blood flow values (CBFV, Periflux, PF1, Perimed, S), dielectrical constant of the skin surface in contact with a condenser (COND, Corneometer, CM420, Schwarzhaupt, D), and surface lipids (SLIP, Sebumeter, Schwarzhaupt, D).
TEWL and CBFV were significantly correlated ($r = .86$) and closely reflected incipient damage associated with topical application of SLS even in the absence of clinical changes. When TEWL and COND were measured within minutes after removal of the patches, water desorption was monitored. 50% desorption times (DT50) of 10 and 5 min were observed with TEWL and COND respectively. DT50 were independent of SLS concentration and time of occlusion. Contrasting with COND values which reached normal levels within 30 min, TEWL remained increased over a period of time depending from SLS concentration and occlusion time.
SLIP values were decreased by non specific adsorption on the patch material.

From these non invasive follow-up studies we conclude that
1/ TEWL and CBFV are appropriate monitors of SLS induced skin alterations.

Indeed, both are positively correlated with each other and related to the concentration of SLS and occlusion times.
They also reflect epidermal repair (TEWL) and regression of skin inflammation (CBFV) after SLS insult.
2/ SLIP and COND do not reflect the intensity of the skin damage but are closely associated with changes occuring at the skin surface. Hence, the relevance of COND values in clinical research and its biological significance in pathological skin remains to be ascertained.

63

DIFFERENTIAL EXPRESSION OF CLASS II MHC ANTIGENS ON KERATINOCYTES IN CUTANEOUS DISEASES. Dr. JNWN Barker, Dr. J. Ophir and Dr. DM MacDonald, Laboratory of Applied Dermatopathology, Guy's Hospital, London, UK.

Aberrant Class II MHC expression has been described on epithelial cells from different organs in certain disease states. While it is known that keratinocytes express HLA-DR in a number of cutaneous disorders little is known about expression of the other Class I MHC subregions, HLA-DQ and -DP. This may be of some functional importance as each subregion is thought to have a different immunoregulatory function. Now that monoclonal antibodies to each subregion are available for use on cutaneous tissue we have assessed keratinocyte expression of each Class II MHC subregion in a variety of cutaneous disorders.

Conditions studied included: allergic contact dermatitis (CD), lichen planus, cutaneous T cell lymphoma, psoriasis, sarcoidosis, discoid lupus erythematosus and squamous cell carcinoma (SCC). Standard indirect immunoperoxidase and the more sensitive peroxidase anti-peroxidase (PAP) methods were employed as appropriate. A panel of monoclonal antibodies including anti-HLA-DR, Leu 10, B7/21, DA6/231 recognizing each subregion were used as the primary antibody together with OKT6 to differentiate Langerhan's cells from the epidermal labelling observed.

HLA-Dr was observed on keratinocytes in many diseases. Interestingly, keratinocytes were found to express HLA-DR in SCC but not in keratoacanthoma. In contact dermatitis at 48 hours keratinocytes expressed HLA-DR and -DQ, but not -DP, while at 72 hours all three subregions were expressed. In the other diseases studied where keratinocytes expressed HLA-DR, expression of -DQ or -DP was absent although all three subregions were expressed on epidermal Langerhan's cells and some dermal infiltrating cells. Positive keratinocyte labelling with either -DQ or -DP but absent -DR was not observed in any of the disorders studied

These results show that keratinocytes differentially express Class II MHC subregion antigens in a wide range of cutaneous disorders. In only CD did we observe expression of -DQ and -DP. HLA-D antigens are probably synthesized on keratinocytes in response to gamma-interferon released from T-lymphocytes in the dermal infiltrate. As local tissue levels of gamma-interferon appear to be important in stimulating production of HLA-D antigens, it is possible that levels are insufficient to stimulate HLA-DQ or -DP.

64

THE EFFECTS OF 5-METHOXYPSORALEN ON ENDOCRINE FUNCTION IN HUMANS. Eric SOUETRE*, Edouard SALVATI*, Bernard KREBS*, Jean-Paul ORTONNE** Guy DARCOURT*. * Department of Psychiatry, **Department of Dermatology, University School of Medicine, NICE-FRANCE.

The aim of this study was to evaluate the effects of 5-Methoxypsoralen (5-MOP) on endocrine function in humans, especially the melatonin secretion without U.V. irradiation.

11 normal male volonteers were investigated before (drug-free), and after 6-day periods of treatment with oral 5-MOP (40 mg) first daily administred at 9 a.m., and after a 1-week free interval, daily administred at 9 p.m. Under nyctemeral conditions, the plasma levels, melatonin, cortisol, thyrotropin (TSH), prolactine (PRL) and Growth Hormone (GH) were evaluated over a 24-h period on each session by hourly blood samples and radioimmunoassay. The sensitivity of retina to light was also evaluated by means of electroretinography performed at 11 a.m. before and after a morning administration of 5-MOP.

Plasma levels of melatonin were significantly increased from the second hour after 5-MOP administration with a peak 6 hours and a decrease to baseline values 15 hours after administration. This stimulating effect of 5-MOP melatonin level was more pronounced during the dark period. The cortisol, TSH, PRL and GH circadian rythms remained unchanged after either morning or evening administration.

Besides, 5-MOP increased the sensivity of the retina to light under photopic conditions and in the early stages of the dark adaptation period, as observed under scotopic conditions.

These results suggest that 5-MOP alone may have specific stimulating effects on the melatonin secretion in humans.

65

In vitro photosensitizing properties of Khellin: P.Morliere*, G.Huppe*, B.Ortel*, S.Averbeck†, D.Averbeck†, R.Santus‡, H.Honigsmann* and L.Dubertret*.*Lab.Recherche Bioclinique en Dermatologie, INSERM U.312, Hop. H. Mondor, 94010 CRETEIL, France*Dpt.Dermatology, Univ.Vienna, VIENNA, Austria† Inst.Radium, Sect.Biologie, 75231 PARIS,France ‡Lab.Physicochimie de l'Adaptation Biologique, CNRS U.A. 481, 43 rue Cuvier, 75231 PARIS,France.

Khellin is currently used as a coronary vasodilator and has been recently reported to exhibit beneficial skin photosensitizing properties, in treating vitiligo for example. Its chemical structure immediatly appears to closely resemble the psoralens structure and therefore one might expect khellin to exhibit some in vitro photosensitizing properties of psoralens. In aqueous solution, khellin is much more soluble than most psoralens but exhibits lower absorption properties. In aqueous medium, UVA maximum is found at 337 nm with a molar extinction coefficient of about 4200 $M^{-1}cm^{-1}$. Dark binding to DNA was investigated by equilibrium dialyses and absorption spectroscopy. It is shown that khellin binds to DNA with an affinity constant of 2000 M^{-1} and 3 binding sites per 100 nucleotides, which is lower than that found for 5-Methoxypsoralen (5MOP), for example. Crosslinking to DNA was investigated by measuring, with respect to DNA alone, the amount of non-renaturating fraction after thermal denaturation of irradiated DNA-khellin complexes or irradiated DNA-5MOP complexes. Our results clearly show that khellin does not behave as a bifunctional agent, in contrast to what is observed for 5MOP. Type II and type I photodynamic properties were investigated with His and Trp as substrates respectively. The His photosensitized oxidation quantum yield is about $8x10^{-5}$ while it raises $1x10^{-3}$, $4x10^{-3}$ and $1.8x10^{-2}$ for 5MOP, 8MOP and psoralen respectively. This illustrates that khellin is a poor type II photosensitizer. The Trp photosensitized oxidation quantum yield is about $1x10^{-4}$ which is low compared to that obtained with 5MOP as photosensitizer, e.g. $5x10^{-3}$. Therefore, Khellin is also a poor type I photosensitizer.

In conclusion, in view of its skin photosensitizing properties and its very poor in vitro photosensitizing properties, khellin appears to be a very interesting chromophore. It may be thought to offer peculiar mechanisms for its skin photosensitizing action.

66

EFFECTS OF SKIN SURFACE LIPOPEROXIDES AND REACTIVE RADICAL SPECIES ON HUMAN NATURAL KILLER ACTIVITY. M. Picardo, C. Zompetta*, C. De Luca, M. Nazzaro-Porro, S. Passi. Ist. Dermatol. San Gallicano, * Ist. Patologia Generales, Roma, Italy.

It has been demonstrated that UVB irradiation inhibits delay-type hypersensitivity in mice and suppresses natural killer activity (NK) of human peripheral blood lymphocytes (PBL). These phenomenons are likely to involve the production of reactive species such as oxygen radicals and trans to cis isomerization of urocanic acid. The aim of this investigation was to evaluate the effect on NK activity of human PBL of other reactive species, in particular those formed following UVB irradiation of surface lipids of human skin. PBL, in saline, were exposed for different periods (5,10,20,30,60 min.) to: 1) UVB peroxidated (50-500 mJ/cm^2) fractions of skin surface lipids (SSL), i.e. free and esterified fatty acids (FFA, TG), squalene (SQ) and cholesterol (CH) (o.1-0.5 mg); 2) 9,10-epoxy-stearate, obtained by epoxidation of oleic acid (1-50 ug); 3) rapidly autoxidable triphenols, which generate oxygen radicals, i.e. 6-OH dopa and 6-OH dopamine (10^{-7} - 10^{-4} M). The rate of lipid peroxidation was evaluated by diethyl p-phenilendiammine (DEPD) test and by GLC analysis, and the rate of autoxidation of triphenols by HPLC. Results showed that: 1) among SSL, UVB peroxidated SQ and TG suppressed 20-50 % NK activity, without affecting cell viability, depending upon the period of PBL exposure, concentration and time of irradiation; the percentage of inhibition was well correlated with the degree of peroxidation achieved. 2) Low concentration (1-10 ug) epoxyde increased (20-60 %) NK activity, while concentrations over 20 ug decreased (20-40 %) NK. 3) From 10^{-6} to 10^{-4} M 6-OH dopa or 6-OH dopamine inhibited (20-80 %) NK activity depending upon the concentration and the time of PBL exposure to triphenols. These results suggest that UVB-dependent inhibition of NK activity may be partly mediated by peroxidation of some fractions of SSL and/or the generation of radical species.

67

TREATMENT OF ACTINIC PRURIGO WITH PUVA - MECHANISM OF ACTION P.M. Farr & B.L. Diffey*, Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne and *Regional Medical Physics Department, Dryburn Hospital, Durham, UK.

Topical indomethacin augments the erythemal response to ultraviolet radiation (UVR) in actinic prurigo suggesting that lipoxygenase products of arachidonic acid metabolism are involved in the mechanism of photosensitivity [1]. This action of indomethacin has allowed us to study the mechanism of the therapeutic effect of PUVA in this disease.

Five females (aged 30-39 years) with actinic prurigo were studied who had photosensitivity since early childhood, excoriated erythematous plaques on exposed skin and worsening of symptoms within hours of sun exposure. All patients were treated twice weekly with PUVA using oral 8-methoxypsoralen (mean number of treatments 29; mean cumulative UV-A dose 58J/cm^2). One area on the back of each patient was protected from UV-A exposure throughout the treatment period. The action spectrum for delayed (24 h) ultraviolet erythema was determined before and after the course of PUVA, both on treated and protected skin. The effect of 1% indomethacin gel (0.1mg/cm^2) on the intensity of UV-B erythema was measured with a reflectance instrument before and after the course of PUVA, both on treated and protected skin.

Before PUVA all patients showed increased erythemal sensitivity to UVR and after PUVA there was no change in the doses required for minimal erythema on protected skin. By contrast, on PUVA treated skin the doses of UVR required for minimal erythema were within the normal range in all patients. UV-B erythema was augmented by topical indomethacin in four patients, and this abnormal response was still present after the course of PUVA, both on treated and protected skin.

These results show that in actinic prurigo PUVA: (1) normalises the response to UVR measured as the minimal erythema dose; (2) has only a local action; (3) does not change the underlying mechanism of photosensitivity. Therefore, the effect of PUVA in actinic prurigo is likely to be due to photoprotection from increased melanin pigmentation and epidermal hyperplasia.

1. P.M.Farr & B.L. Diffey. Paper presented to the British Society for Investigative Dermatology, September 1986.

68

EFFECT OF TOPICAL PUVA THERAPY ON THE COLLAGEN METABOLISM AND PROLIFERATION OF SKIN FIBROBLASTS

Aarne Oikarinen, Minna Tamminen, Jaakko Karvonen, Matti Hannuksela. Collagen Research Unit, University of Oulu, Departments of Dermatology and Medical Biochemistry, University of Oulu, Oulu, Finland

The synthesis and degradation of collagen and proliferation of skin fibroblasts were studied in fibroblasts derived from six patients who had received topical trioxalen PUVA therapy. The mean cumulative dose of UVA was 481 J/cm^2. As a control, skin fibroblasts from four healthy subjects were studied. The [^{3}H]thymidine incorporation in PUVA fibroblasts was 8.3 ± 1.9 and in control fibroblasts 14.7 ± 4.6 DPM x 10^{-3} per ug DNA (mean ± S.D.). The relative increase in cellular DNA on six days was 2.9 ± 1.4 in PUVA fibroblasts and 2.6 ± 0.6 in control cells. For the measurement of total protein and collagen synthesis the cells were labelled with [^{14}C]proline and total nondialyzable radioactivity and [^{14}C]hydroxyproline, a collagen specific amino acid, were then assayed. The total protein synthesis was 136.5 ± 90.9 in control cells and 191.9 ± 50.1 DPM x 10^{-3} in PUVA treated cells and collagen synthesis 33.7 ± 23.1 and 48.7 ± 12.3 DPM x 10^{-3} per plate, respectively. The ratio of hydroxyproline to total radioactivity was 0.247 in control cells and 0.254 in PUVA cells, indicating that the relative collagen synthesis was similar in both cell lines. In order to study the synthesis of genetically distinct collagen types the medium proteins were analyzed by using interrupted gel electrophoresis. The ratio of type III to type I collagen was 0.17 ± 0.07 in PUVA cells and 0.21 ± 0.04 in control cells, indicating that PUVA treatment in vivo does not change the relative synthesis of type I and type III collagen. The activity of collagenase was measured from cell culture media by using proline labelled type I collagen as the substrate. The activity of collagenase in PUVA cells was 3.48 ± 1.74 and in control cells 5.36 ± 3.78 DPM x 10^{-2}/ug DNA. Thus the results indicate that long term PUVA treatment in vivo decreases slightly the thymidine incorporation in vitro. In contrast the collagen synthesis and collagenase activity are slightly increased in vitro. However, it seems that even the long term local PUVA therapy does not affect markedly the gene expression of distinct collagen types.

69

THE MECLR IS THE MOST SIGNIFICANT PROGNOSTIC FACTOR FOR MANAGEMENT AND TREATMENT OF PATIENTS GRAFTED WITH HLA-IDENTICAL BONE MARROW.

M. Bagot, M. Heslan, J.Y. Mary, C. Cordonnier, M. Kuentz, J.P. Levy and L. Dubertret INSERM U312 and Bone Marrow Graft Unit, Hopital Henri Mondor, 94010 Creteil, France, INSERM U263, 2 place Jussieu, Paris, INSERM U152, Hopital Cochin, Paris.

We have previously shown in preliminary studies that mixed epidermal cell-lymphocyte reactions (MECLR) could be positive between HLA-identical bone marrow graft donors and recipients, and that the intensity of the proliferation observed before graft in MECLR was correlated with later incidence of acute and chronic graft-versus-host disease (GVHD). In the present study, we performed statistical analyses in a series of 58 recipients of HLA-identical bone marrow transplants, 33 grafted with non depleted marrow and 25 with T-cell depleted marrow. We evaluated 8 factors for their influence on acute and chronic GVHD, in order to precisely assess the value of the MECLR among other possible prognostic factors. Donor peripheral blood lymphocytes (PBL) were cultured in 0.2 ml microtiter plates with either epidermal cells (EC) or irradiated PBL of the recipient. Tritiated thymidine incorporation was measured after 6 days. For each donor/recipient pair, the index of the mean cpm in MECLR divided by the mean cpm in mixed lymphocyte reaction (MLR) was calculated. Similarly, recipient reactivity against donor was evaluated by coculture of recipient PBL with donor EC or PBL. Statistical analyses were performed, using Kruskall-Wallis or Chi square as univariate test and linear multiple discrimination as multivariate method.

Results obtained in the group of 33 patients grafted with non depleted marrow showed that incidence of GVHD was not related to recipient age and sex, donor age and sex, and sex-mismatch between donor and recipient. Three factors were associated with significantly increased risk of GVHD. Donor parity was associated with a greater incidence of acute GVHD ($p<0.03$) with a relative risk of 2.4 when the donor had experienced at least one pregnancy. Diagnosis of hematological malignancy also influenced the risk of acute GVHD ($p<0.03$) with a greater incidence of GVHD among patients with chronic myelogenous leukemia and acute myeloblastic leukemia than in patients with acute lymphoblastic leukemia. The MECLR/MLR index was found to be the most significant prognostic factor for acute ($p<0.005$) and chronic GVHD ($p<0.03$). Relative risk of acute GVHD was 3.03 with an index greater than 3. In contrast, no GVHD was observed with an index lower than 1. Combining these three prognostic factors (diagnosis, donor parity, and MECLR/MLR index) with coefficients calculated according to their importance, we could correctly classify 30/33 patients in the two groups with severe GVHD or with no GVHD. This method, allowing to precisely evaluate the risk for a given recipient to present severe GVHD, has been further validated on 10 additional patients. A multicenter study is currently in progress to test the possible application of these findings in larger series and in other bone marrow graft units.

Incidence of GVHD was extremely low in the 25 patients grafted with T-cell depleted marrow, since only 2/21 evaluable recipients presented GVHD. However, failure of engraftment or graft rejection occurred in 6/25 (24%) patients, followed in all cases by increased immunosuppression and death. In these cases, the MECLR/MLR index evaluating donor reactivity against recipient was significantly lower than in the group of patients who did not reject their graft ($p<0.04$) whereas the reactivity of the recipient against the donor is often high.

The MECLR/MLR index is therefore the most significant prognostic factor for management and treatment of bone marrow graft recipients, and is actually the only available test to evaluate the degree of non-HLA histocompatibility antigen disparity between HLA-identical siblings. MECLR is thus of major importance to point out recipients at high risk of either severe GVHD or graft rejection, and to better define appropriate indications of T-cell depletion of increased GVHD prophylaxis.

70

EXPRESSION OF INTERLEUKIN-1 mRNA IS REGULATED BY UVB IN NORMAL HUMAN SKIN.

Liliane Didierjean, Jean-Hilaire Saurat, C. Ucla*, B. Mach*, Jean-Michel Dayer**. Clinique de Dermatologie and**Division d'Immunologie et d'Allergologie, Département de Médecine, Hôpital Cantonal Universitaire, *Département de Microbiologie, Centre Médical Universitaire, 1211 Genève 4, Suisse.

Interleukin-1 (IL-1)α and ß mRNAs have been found in cultured normal human keratinocytes in the absence of identifiable stimulation (1) ; UVB exposure of transformed murine keratinocytes cultures (PAM 212 cell line) enhanced the amount of mRNA encoding for murine IL-1 (2). As we previously found large amount of IL-1 biological activities in normal unstimulated human epidermis (3), the present study was designed to address two questions : (i) is normal human epidermis able to express IL-1 mRNAs ? (ii) do UVB modulate this expression ?

Two volunteers received 40 mJ UVB on 6 cm^2 area of buttocks. Four hours after irradiation, the irradiated and 6 cm^2 area of adjacent unirradiated skin were taken using a dermatome (180 um set = epidermis+ superficial dermal papillae). Total RNA was extrated by the guanidine thiocyanate method. After denaturation with glyoxal, 5µg of RNA obtained from each sample were fractioned on 1.2% agarose gels and transfered by blotting on nylon Biodyne membranes. The RNA blots were hybridized with ^{32}p- labeled IL-1ß or IL-1α single stranded RNAs in vitro transcripts (riboprobes) and autoradiographied.

In the two experiments, IL-1ß mRNA was detected with the IL-1ß single-stranded RNA transcript. The signal was enhanced in samples derived from UVB irradiated skin. No hybridization was observed with the IL-1α probe while RNA of stimulated U937 monocytic cells hybridized in the same conditions.

This study shows that normal human skin expresses in vivo mRNA encoding for IL-1ß and that UVB increases this expression whereas no mRNA encoding for IL-1α was detectable in either conditions. As immunoreactive IL-1α is detectable in normal human epidermis by immunohistochemistry and protein blot analysis (submitted), the lack of detectable IL-1α mRNA expression in the conditions of hybridization used, suggests that either the amount is too low to be detected or epidermal IL-1 α originates from an other source than epidermis.

(1) J. Exp. Med. in press. (2) J. Invest. Dermatol. 87: 155 (abst) 1986. (3) J. Immunol. 136:3317-3323, 1986.

71

INDUCER T CELL SUBPOPULATIONS IN INFLAMMATORY DERMATOSES AND IN NORMAL SKIN.

J. D. Bos, Chr. Hagenaars, M. R. H. Brautigam, P. K. Das, M. L. Kapsenberg. Departments of Dermatology, Pathology & Histology and Cell Biology, University of Amsterdam, Academisch Medisch Centrum, 1105 AZ Amsterdam, The Netherlands.

The complexity of cells associated with immune responses in human skin was recently reasserted as the skin immune system (SIS). Its infiltrating T cell subsets were evaluated quantitatively in a variety of inflammatory dermatoses using monoclonal antibodies to leukocyte differentiation antigens and compared to those in normal human skin.

In normal skin, the majority (over 90%) of T cells were found perivascularly and they were evenly distributed over CD4+ inducer and CD8+ suppressor / cytotoxic T cell subsets. Among CD4+ inducer T cells, most were phenotyped as CD4+ 4B4+ helper inducer types, wheras CD4+ 2H4+ suppressor inducer types were found to be relatively rare. Intra-epidermal, directly subepidermal and other ('free') lymphocytes were mostly of the CD8+ suppressor/cytotoxic T cell subpopulation and accounted for less than 2% of the total mean T cell number.

In untreated psoriasis, nummular dermatitis, lichen ruber planus, atopic dermatitis, and pityriasis rosea, perivascular CD4/CD8 ratio's were almost always greater than 1. In these conditions, CD4+ cells were found to be almost exclusively of the CD4+ 4B4+ helper inducer subpopulation. Compared to normal skin, absolute numbers of the helper inducer T cell subpopulation were found to be increased up to 25 fold, wheras CD8+ suppressor/cytotoxic T lymphocytes were increased to a lesser extent, up to 8 fold. Our results indicate that CD4+ 2H4+ suppressor inducer T cells are relatively scarce both in normal human skin as well as in the benign inflammatory dermatoses studied here.

Since the CD4+, 4B4+, 2H4- helper inducer subpopulation proliferates preferentially to soluble antigens, we predict that the predominant presence in SIS of the helper inducer T cell subpopulation both in diseased and normal skin can be explained on the basis of selective immigration and subsequent proliferation of CD4+, 4B4+, 2H4- T cells in response to soluble antigens which need to be identified.

72

IN VITRO STIMULATION OF LYMPHOCYTES BY NICKEL - RELIABILITY IN DETECTING IN VITRO SENSITIVITY AND COMPARISON OF MONOCYTES AND LANGERHANS CELLS IN ANTIGEN PRESENTATION.

A C Chu M Shaw J Elliot
Unit of Dermatology, Royal Postgraduate Medical School, Hammersmith Hospital, London.

We have studied the in vitro stimulation of lymphocytes by Nickel (Ni) in 5 Ni sensitive and 5 control individuals. Initial studies used peripheral blood mononuclear cells (PBM). A dose response curve for Ni using Ni sulphate with foetal calf serum in a 6-day incubation showed maximal stimulation at 0.01% and 0.001% Ni. Using these concentrations in both groups, considerable variation in stimulation index (SI) was seen. A SI of >2 was regarded as significant. 2 of the control group showed SI of >2 and one of the Ni sensitive subjects showed low reactivity. 14 days post patch testing with Ni showed less variation with control showing SI of 1.0 to 2.4 (mean 2) and positive subjects a range of 6 to

23 (mean 19.1). Volunteers were then subjected to suction blisters and single cell suspensions were produced by overnight trypsinisation. Langerhans cells were enumerated on cytospin preparations using an APAAP method and OKT6. The epidermal cells were then used in the antigen stimulation assay with autologous T cells purified by E rosetting, at a ratio of T to Langerhans cells of 60:1 to 200:1. Controls in these experiments once again showed considerable variation, with SI in PBM of 1 to 3.7. In controls with SI >2 for, PBMs T/Langerhans cells showed SIs of up to 45. In the Ni positive subjects, one failed to respond with PBM and also with T/Langerhans cells. Others showed a SI with T/Langerhans cells of up to 5 x that of PBM.
The results show that in vitro testing for Ni sensitivity shows a high degree of both false positive and negative results. In vitro testing following patch testing with Ni, shows more consistent and reliable results. Substituting Langerhans cells for monocytes in the in vitro test showed the same false negative and positive results. However, Langerhans cells showed much greater SIs with Ni than autologous PBMs. Studies are underway to investigate the carrier protein for Ni to try to improve the reliability of this test.

73

COMPARISON OF THE FUNCTIONAL ACTIVITY OF CIRCULATING MONOCYTES SEPARATED BY IMMUNE ADHERENCE OF DENSITY GRADIENTS AND LANGERHANS CELLS IN PRESENTING RECALL ANTIGEN TO AUTOLOGOUS T-CELLS.

J McLelland J Morris A C Chu. Unit of Dermatology, Royal Postgraduate Medical School, Hammersmith Hospital, London.

We have compared the antigen presenting properties of circulating monocytes separated by immune adherrence, monocytes separated by density gradient on Nycodenz and epidermal Langerhans cells. The antigen selected was Tuberculin PPD. 4 volunteers showing positive mantoux tests were selected. Mononuclear cells were separated from venous blood on ficol hypaque were used in a standard antigen stimulation assay in flat-bottomed plates with 5 days incubation to establish the dose response curve for PPD. Maximal stimulation showed a range of 5-20 $\mu g\ ml^{-1}$ in the 4 volunteers. Heparinised venous blood was separated on ficol hypaque and cell subpopulations were purified - T-cells by E rosetting, monocytes by immune adherrence on foetal calf serum coated plates overnight (MA). Venous blood taken in potassium EDTA was used to separate monocytes on Nycodenz grandients (MD). Using OKT3 and Dako macrophage monoclonal antibodies, cell purity was 90-100%. Suction blisters were raised and 1.5cm blister roofs were trypsinised overnight at 4°C to produce a single cell suspension. Langerhans cells were enumerated on cytospin preparations using an APAAP method and OKT6. Using 3 concentrations of PPD - 5, 10 & 20 µg/ml, T-cells and MA, MD or epidermal cells were combined at varying ratios in an antigen stimulations assay. MA showed maximal stimulation at a T-cells:MA ratio of 80:1 to 20:1 and was 43± 10% of the response of unseparated mononuclear cells. MD showed maximal stimulation at a T-cell:MD ratio of 40:1 to 80:1, but was 7± 2% of the response of unseparated mononuclear cells. Epidermal cells showed maximal T-cells:Langerhans cells at a ratio of 60:1 to 200:1, but was 12± 2% of the response of unseparated mononuclear cells. This study confirms that Langerhans cells can present complex recall antigen to purified autologous T-cells, and shows that the efficiency with which they do so is only 30% of monocytes separated by immune adherence but is 70% more efficient than Nycodenz separated monocytes. The explanation for this discrepancy may be the activation of the monocytes by adherence during their separation. It may also be that the 2 methods of monocyte separation may be selecting out different subpopulations of monocytes. This is under investigation at present.

74

LACK OF A GENETIC EQUILIBRIUM IS NOT COMPATIBLE WITH AUTOSOMAL DOMINANT INHERITANCE OF DYSPLASTIC NEVI

Heiko Traupe, Egon Macher, Henning Hamm and Rudolf Happle
Departments of Dermatology, Münster, FRG, and Nijmegen, The Netherlands

The association of dysplastic nevi and familial melanoma has so far been considered an autosomal dominant trait.
To test this hypothesis we calculated the mutation rate indirectly according to the Haldane formula ($\mu = 0.5\ (1-f)\ X$; μ = mutation rate, f = reproductive fitness, X = incidence of familial occurence) and directly according to the formula μ = n-sp/2n-p (n-sp = number of sporadic cases; n-p = number of cases examined = size of population) using available data on the sporadic occurence of this trait (1.8%-5%).
The indirect calculation yielded a mutation rate of $\mu = 0.02\%$, whereas the mutation rate obtained directly was $\mu = 0.9\%-2.5\%$. According to the directly derived mutation rate new mutations would outnumber old ones by at least 10 : 1 revealing the absence of a genetic equilibrium between old and new mutations. The lack of a genetic equilibrium explains the discrepancies between the result of the directly and indirectly calculated mutation rate and violates a basic law of genetics. The absence of this genetic equilibrium is not compatible with autosomal dominant inheritance of dysplastic nevi. The simplistic concept of Mendelian inheritance of this trait should be replaced by a polygenic model.

75

SUPPRESSION OF HLA-CLASS II EXPRESSION IN HUMAN MELANOMA CELLS XENOGRAFTED TO NUDE MICE.

M.R.M. van den Brink, D.J. Ruiter, G.N.P. van Muijen, R.E. Brouwer, P.I. Schrier, and S. Ferrone. Department of Pathology, Nijmegen University, Nijmegen, The Netherlands, Departments of Pathology and Clinical Oncology, Leiden University, Leiden, The Netherlands, and Department of Immunology and Microbiology, New York Medical College, Valhalla, NY 10595, USA.

Antigen expression of human melanoma cells after xenografting to nude mice was studied by direct and indirect immunoperoxidase techniques using a panel of monoclonal antibodies including anti-HLA-A,B,C, anti-HLA-DR, anti-HLA-DQ, anti-HLA-DP, and using PAL-M1 and M2, NKI/beteb, AMF7 and 763.74T, which recognize melanoma-associated antigens (MAA's). The five different human melanoma cell lines that were used (Clone IF6, Clone II, 0Mel, Mel 57, and BRO) showed in vitro a highly variable expression of HLA class I and class II antigens and a more consistent expression of MAA's. Melanoma cells (10^6 cells) were subcutaneously injected into Balb/c nude mice; the tumor take rate varied between 33% and 88%. Xenografts were excised at different intervals after inoculation and snap frozen. The expression of HLA-A,B,C and MAA's in the xenografts and in early pulmonary metastases (of BRO melanoma cells) showed a similar pattern as in the melanoma cell lines. However, HLA class II antigens could not be demonstrated, neither in xenografts nor in metastatic lesions, including those melanoma cell lines that in vitro had a marked HLA class II expression. Cell lines of xenografts regained their original HLA class II expressions. These findings suggest a selective suppression of HLA class II antigen expression in melanoma cells xenografted to nude mice.

76

AN INVESTIGATION OF THE INTRACELLULAR MESSENGER SYSTEMS INVOLVED IN MELANOGENESIS IN B16 MELANOMA.

Sarah Hill*, Stanley S. Bleehen**, and Sheila Mac Neil*, Department of Medicine, Clinical Sciences Centre, Northern General Hospital, Sheffield S5 7AU, U.K.* and Department of Dermatology, Royal Hallamshire Hospital, Sheffield S10 2JF, U.K.**

Melanogenesis is a differentiated function of epidermal melanocytes which many diverse agents are reported to induce; for example UV irradiation, Vitamin D_3, MSH and, as we found some years ago, calmodulin antagonists (Mac Neil et al. 1984 J. Invest. Dermatol. 83, 15-19). The purpose of this study is to examine how these various agents interact with the cell to try to determine the intracellular criteria necessary for melanogenesis.

Melanin production (O.D. at 400 nM), cell proliferation (^3H thymidine incorporation into DNA), cyclic AMP accumulation and membrane phosphatidylinositol (PI) metabolism (^3H inositol incorporation into water soluble inositol phosphates) were measured in B16 melanoma cells cultured in Medium 199 plus 10% foetal calf serum.

Melanogenesis was significantly increased within 3 days by the calmodulin antagonists W7 (N-(6-aminohexyl)-5-chloro-1-naphthalene sulphonamide) and a more potent and specific analogue of W7 (N-(6-aminooctyl)-5-iodo-1-naphthalene sulphonamide). For example, melanin synthesis was increased 9-fold with 100 uM of the latter. The calmodulin antagonists also caused a decrease in cell proliferation but had little effect on cyclic AMP or PI metabolism. Melanogenesis was not, however, obtained with MSH (which elevated intracellular cyclic AMP without affecting cell proliferation or PI metabolism) or Vitamin D_3 or 1,25 $(OH)_2$ Vitamin D_3 (which had slight inhibitory effects on cell proliferation and MSH-stimulated cyclic AMP accumulation) or UVB irradiation (which significantly inhibited cell proliferation and markedly decreased MSH-stimulated cyclic AMP accumulation and PI metabolism) in experiments lasting up to 13 days in some cases.

In conclusion, our data suggests that the intracellular production of melanin is unlikely to be under simple control e.g. induced solely by an elevation in intracellular cyclic AMP. Further study of the melanogenic response to calmodulin antagonists may reveal how this differentiated cell function is induced.

77

MELANOCYTE GROWTH STIMULATION BY LETHALLY IRRADIATED FIBROBLASTS IN VITRO.

M. Benathan and E. Frenk. University Department of Dermatology, Lausanne, Switzerland.

The mechanisms by which epidermal melanocyte growth is regulated in vivo remain largely unknown. In order to evaluate the role of fibroblasts in this process, we have examined the in vitro interaction of human epidermal melanocytes co-cultured with lethally irradiated fibroblasts.

Melanocyte cultures were established from human foreskin in medium supplemented with phorbol 12-myristate 13-acetate and cholera toxin. Growth experiments were performed with pigmented cell lines, 22 to 45 weeks old. As source of fibroblasts, mouse embryonic 3T3-J2 cells and human dermal fibroblasts irradiated with 4000 RAD were used.

An ever increasing melanocyte proliferation rate was observed between 12.5 and 50% confluency of 3T3-J2 cells. At 50% 3T3-J2 confluency, the mean doubling-time of the melanocyte population was half of the control value (2.0 vs 4.3 days). However, the distribution of the proliferative activity appeared to be uneven with the melanocyte density being directly related to the local 3T3-J2 density. In addition, the melanocytes showed morphological changes depending on the proximity of the fibroblasts. When they were in close contact, melanocytes developped numerous dendrites and some pigment was transferred to the fibroblasts. Enhanced proliferative activity was also observed in similar experiments with human dermal fibroblasts.

Our data suggest that fibroblasts could release factors with melanocyte growth-promoting activity. Due to molecular diffusion in the culture medium, their availability would be optimal for those melanocytes immediately adjacent to fibroblasts.

78

INCREASED ANTHRALIN IRRITANCE RESPONSE IN VITILIGINOUS SKIN. Wiete Westerhof, Jan D. Bos, Anwar H.Siddiqui and Rudi H.Cormane. Department of Dermatology, Academisch Medisch Centrum, University of Amsterdam, Amsterdam, The Netherlands.

Various physically and (immuno)chemically induced erythematous responses have been evaluated in vitiligo, being a good in vivo model to assess the role of melanocytes or melanin in inflammatory reaction.

We studied the irritance response by anthralin with the test chamber technique in 17 patients with vitiligo. Concentrations of 0.1%, 0.5%, 1.0% and 5.0% in lanette wax were applied for 24 hours to vitiliginous and nearby pigmented skin. Reading of the erythematous response was done on the 2nd day after application. In the visual assessment of the paired anthralin patches the erythema was more intense in the pigmented skin than in the vitiliginous skin in 15 out of 17 patients. Chromameter readings, however, indicated that the erythematous response was stronger in vitiliginous skin compared to pigmented skin, proving that the human eye is not accurate in the quantitative assessment of mixed colours (red of erythema and red of the composite brown colour of the melanin pigment).

These results were compared with those of infiltrate studies of patch-tested vitiliginous and pigmented skin. Monocytes, granulocytes, Langerhans cells, pan T-cells, T-helper cells, T-suppressor cells, and mast cells were counted microscopically making use of the immune peroxidase technique and specific monoclonal antibodies. The difference in inflammatory cells in vitiliginous (V) and pigmented skin (P) after anthralin patch-testing is shown in the next table:

infiltration	$\bar{d}$(V-P) ±	P
monocytes	218 ± 131	< 0.005
granulocytes	87 ± 137	0.03
Langerhans cells	39 ± 72	0.07 N.S.
mast cells	14 ± 32	0.42 N.S.
pan T-cells	82 ± 384	0.46 N.S.
T-suppressor cells	83 ± 230	0.27 N.S.
T-helper cells	4 ± 333	0.94 N.S.

Granulocytes and especially monocytes are apparently the first cell types to be attracted by mediators generated via the action of radical species from the oxidation of anthralin. In pigmented skin the development of this acute inflammation is mitigated probably by the radical scavenging properties of melanin.

79

CLONAL REARRANGEMENTS OF THE BETA AND GAMMA T-CELL RECEPTOR GENES IN CUTANEOUS LYMPHOMAS. S.J. Whittaker, L. Foroni, L. Luzzatto, A.C. Chu, R. Russell Jones. Departments of Dermatology and Haematology, Royal Postgraduate Medical School, Hammersmith Hospital, London England.

The classification of T-cell lymphomas in the skin is currently based on clinicopathological criteria. Immunohistochemical studies are of some value, but diagnostic difficulties arise where a pleomorphic infiltrate is associated with an atypical clinical presentation.

Three such patients who presented with self healing nodules were studied using immunoglobulin heavy chain joining (JH) probe, a T-cell receptor constant (C) and joining (J) probe. High molecular weight DNA was prepared by homogenization of cutaneous tissue and from granulocytes, prepared from normal donors, to be used as a control. The purified DNA was digested with bacterial restriction enzymes ECORI, HIND III, BAM H1 AND Bgl II. DNA fragments were separated by gel electrophoresis and transferred onto nylon membranes which were then hybridized with the specific radiolabelled DNA probes described.

T-cell receptor (C) chain gene analysis in all 3 patients revealed the presence, in ECORI digests, of discrete rearranged bands of differing sizes. Analysis of DNA using T-cell receptor (J) probe in BAM H1 digests also revealed the presence of rearranged bands in 2 patients. Analysis of DNA using an immunoglobulin heavy chain joining (JH) probe against both BG1 II and HIND III DNA digests.revealed a germ line configuration.

Our results reveal rearrangements of the Beta and Gamma T-cell receptor genes in cutaneous tissue, confirming the presence of a clonal T-cell proliferation. These studies indicate the value of T-cell receptor gene analysis in the diagnosis of cutaneous T-cell lymphomas.

80

Transferrin and Interleukin-2 Receptors in Cutaneous T-Cell Lymphomas. K. Meissner C. Mathis, K. Michaelis, R. Arndt, Th. Loening, A. Rehpenning. Dept. of Dermatology, Dept. of Pathology, Dept. of Mathematics in Medicine, University of Hamburg, Hamburg; Lab. of Immunology, Hamburg, F.R.G..

Cutaneous T-cell lymphomas (CTCL) represent a malignant proliferative disease of helper T-cells. Interleukin-2 (IL2R) and transferrin (TR) receptors are markers of T-cell activation and proliferation. TR^+ cells have been found in the large majority of CTCL in contrast to large plaque parapsoriasis. IL2R expression has been reported to be very variable in CTCL. This prompted us to examine the expression of both receptors in CTCL and in a control group of benign dermatoses and to address the question if one of these markers could be used in the differential diagnosis of CTCL. Up to now, cryostat sections of 16 cases with CTCL (stage Ia:3, Ib:2, IIb:2, III:1, IVa:6, IVb:2) and of 11 benign dermatoses (large plaque parapsoriasis:3, mucinosis follicularis:1, psoriatic erythrodermia:2, psoriasis vulgaris:2, contact dermatitis:1, atopic dermatitis:2) were examined as well as the peripheral blood mononuclear cells of 7 cases with CTCL (stage Ia:1, Ib:1, IVa:4, IVb:1). OKT9 and Clonab IL2R were used as monoclonal antibodies. Antigen binding was visualized either by a 3-step or, in the case of peripheral blood mononuclear cells, by a 2-step immunoperoxidase technique. Positive skin cells, counted in 25 high power fields (x400), were calculated as numbers per mm^2 of tissue section or, in the case of peripheral blood examination, positive cells were calculated as numbers per nl. Statistical analysis was performed with Spearman's test and with the step by step analysis of discrimination.- Both groups exhibited positive IL2R as well as positive TR cells. In both groups the expression of IL2R was significantly correlated ($p<0,01$) with the expression of TR in the skin. Additionally, a statistically significant correlation between $IL2R^+$ cells in the skin and in the peripheral blood could be observed ($p<0,005$). In CTCL, the numbers of both TR^+ ($\bar{x}$:315,4) and $IL2R^+$ ($\bar{x}$: 228,3) were significantly increased ($p<0,01$) in comparison with the control group ($\bar{x}$ (TR^+ cells): 52,5; $\bar{x}$ ($IL2R^+$ cells): 82). Nevertheless, step by step analysis revealed that neither IL2R nor TR can be used as a clear cut marker for the discrimination between CTCL and benign dermatoses. In conclusion, these results point out that (1) all cases of CTCL examined exhibited $IL2R^+$ and TR^+ cells, (2) there exist a significant correlation between $IL2R^+$ and TR^+ cells in the skin of CTCL and between $IL2R^+$ cells in skin and the peripheral blood.

81

IMMUNOARCHITECTURE OF CUTANEOUS PSEUDOLYMPHOMAS-DELINEATION OF DISTINCT PATTERNS. Josef Smolle, Ramon Torne, and Helmut Kerl, Department of Dermatology, University of Graz, Austria.

Because of the broad spectrum of the clinical and histological features, cutaneous pseudolymphomas are difficult to classify. In order to delineate objective criteria for the classification, we investigated the immuno- architecture of 50 causes of pseudolymphoma.

Frozen sections were stained by a panel of 20 monoclonal antibodies using a three-step immunoperoxidase technique. The immunohistochemical results were semi-quantitatively assessed according to a four-graded scale.

24 cases were classified as T-cell pseudolymphomas. The immunohistologic characteristics were the absence of B zones, the predominance of T-helper/inducer cells and the presence of Langerhans cells/indeterminate cells. Disseminated pseudolymphoma, lymphocytic infiltration of the skin, lymphomatoid papulosis, drug reactions, persistent scabies, and lymphomatoid contact dermatitis were found in this group.

16 cases represented B cell pseudolymphomas containing a nodular arrangement of B lymphocytes. In 12 lesions, the B cell clusters were associated with dendritic reticulum cells and a typical expression of IgM and IgD, thus forming fully developed germinal centers (B cell pseudolymphoma, follicular type). In 4 lesions, the B cell aggregates lacked the association with dendritic reticulum cells (B cell pseudolymphoma, non follicular type). The B cell clusters were always surrounded by distinct T zones. These B cells patterns were present in lymphadenosis benigna cutis, large cell lymphocytoma and occasionally in arthropod bite reactions.

Our results provide the basis for a simple and reliable immunohistological classification of cutaneous pseudolymphomas. Despite the marked clinical and histological heterogeneity, the immunoarchitecture of pseudolymphomas can be attributed to three distinct patterns.

82

AIDS AND LANGERHANS CELLS: CD1, CD4 AND HLA CLASS II ANTIGEN EXPRESSION. V. Gielen, D. Schmitt, C. Dezutter-Dambuyant, J.F. Nicolas, J. Thivolet. INSERM U.209, Pavillon R., Hopital E.-Herriot, 69437 Lyon, Cedex 03, France.

Recent studies have suggested that Langerhans cells (LC) are involved in AIDS pathological process. Conflicting results have been reported in the immunohistochemical investigation of LC surface antigens. In patients with AIDS, we have studied the LC markers (CD1, CD4 and HLA class II) using specific monoclonal antibodies (MCA) (respectively L544, L161, 4A76, D47 against CD1 antigens; BL2 and an anti-DQ MCA for class II antigens and Leu 3a against CD4 antigens). We investigated clinically unaffected skin and skin overlying Kaposi's lesions. Normal skin of healthy donors was used as controls. Using indirect immunofluorescence, the relative number of LC, the labelling intensity and the LC localization were estimated on frozen tissue sections. When compared with controls, the relative density of CD4+ and DQ+ cells was increased in AIDS patients' normal skin. No significant modifications were observed with anti-CD1 and anti-DR MCA. In the skin overlying old Kaposi's lesions, we observed a dramatic reduction of LC number whereas no changes were found in recent lesions. For each considered antigen, labelling intensity was similar in controls and AIDS unaffected skin. Epidermal LC were more superficially situated and more dendritic in patients as compared to controls. We conclude that LC, CD1 antigens (differentiation antigens) do not seem to be modified. In contrast, CD4 and HLA-DQ antigens which are involved in the immune antigen presentation are increased. In addition, LC superficial location and strong dendritic aspect are in favor of a stimulation of this cell population. In old Kaposi's lesions, the drastic reduction of labelled LC can be explained by either the loss of surface antigens or cell disappearance in the epidermis. Immunocytochemical investigations at ultrastructural level are in progress in order to quantify CD4 and DQ antigen density on LC of patients with AIDS. In addition, we try to correlate these surface antigen modifications with the presence of viral particles.

83

INTERFERON THERAPY OF MYCOSIS FUNGOIDES. Kristian Thestrup-Pedersen, Hugh Zachariae & Rud Hammer. Department of Dermatology, University of Aarhus, Marselisborg Hospital, 8000 Aarhus C, Denmark.

Recombinant alfa-interferon (Ro-feron^{R}) was used as treatment in 11 patients with mycosis fungoides. Five patients were in stage II of the disease, four in stage IVa and two in stage IVb. The therapy was started either following primary diagnosis of the disease or following a relapse. The interferon was given as daily subcutaneous injections at a starting dosage of 3 mill. units and increasing the dosage to a maximum of 36 mill. units daily for three months, then three times weekly until complete remission or one year.

One patient in stage II has completed a one-year therapy and is in complete remission for now 7 months. The remaining four patients in stage II are receiving therapy except one, who stopped due to side effects following one months therapy. One patient with stage IVb completed a one-year treatment with good result, but this disease relapsed after seven months without therapy. He has been re-started on interferon with partial remission following four months therapy. The remaining patient suffered from end-stage disease and the therapy did not prevent a fatal course of his disease.

All patients had side effects such as flu-like symptoms, fever and leucopenia. Some had side effects such as "pneumonia" or lung infiltrates on X-ray and gastro-intestinal complaints. All but one of the patients who had a beneficial effect showed an increased polyclonal increase of immunoglobulin in serum and the patient who cleared and is still in complete remission developed antibodies in serum with reactivity for a viral antigen.

84

15-HYDROXY-EICOSATETRAENOIC ACID (15-HETE) SPECIFICALLY INHIBITS LTB_4-INDUCED CHEMOTAXIS OF PMNs. Knud Kragballe, Thomas Ternowitz, Karsten Fogh, Department of Dermatology, Marselisborg Hospital, University of Aarhus, Denmark.

Leukotriene B_4 (LTB_4) is a potent chemoattractant toward leukocytes and has been shown to mediate inflammation of human skin. 15-hydroxyeicosatetraenoic acid (15-HETE), another lipoxygenase product of arachidonic acid, has the potential to inhibit the formation of LTB_4 in certain cell types. In the present study the effect of 15-HETE on LTB_4-induced leukocytes chemotaxis was investigated. 15-HETE was formed by oxygenation of arachidonic acid by soybean lipoxygenase, purified by reversed phase high performance liquid chromatography (RP-HPLC), and identified by mass spectrometric analysis. Chemotaxis was assayed with ^{51}Cr-labelled leukocytes in a Boyden chamber using LTB_4, FMLP and C5a as chemoattractans. Purified polymorphonuclear leukocytes (PMNs) and monocytes were used as effector cells. LTB_4-induced chemotaxis of PMNs was inhibited in a dose-dependent manner by 15-HETE. Maximal inhibition (51%) occurred at a 15-HETE concentration of 10^{-5} M. 15-HEPE, identical to 15-HETE except for 5 double bonds instead of 4 double bonds, was approximately 10 times less potent in inhibiting LTB_4-induced PMN chemotaxis. Using FMLP and C5a as chemoattractants, 15-HETE did not change PMN chemotaxis. Furthermore, LTB_4-induced chemotaxis of monocytes was unaffected by 15-HETE. The present results demonstrate that 15-HETE is a specific and selective inhibitor of LTB_4-induced chemotaxis of PMNs. By inhibiting not only the formation, but also the effect of LTB_4, 15-HETE may have a profound effect on skin inflammation elicited by LTB_4.

85

STUDIES ON ARACHIDONATE 15-LIPOXIGENASE ACTIVITIES OF HUMAN EOSINOPHILS AND NEUTROPHILS. E. Morita, J.M. Schroder and E. Christophers, Dept. of Dermatology, Kiel, Schittenhelmstr. 7, 2300 Kiel, FRG

Eosinophils (Eos) play a role in a variety of cutaneous inflammatory diseases. Previous studies have shown that Eos predominantly form 15-HETE and other 15-lipoxigenase (15-LO)-products with antiinflammatory properties. We were interested in mechanisms activating arachidonate-15-LO of human Eos as well as neutrophils (PMN), which are supposed to contain 5-LO and 15-LOs. Human Eos or PMN were purified from healthy donor blood by Ficoll- and Percoll-gradient-centrifugation. Cell purities for eosinophils were 90-98 % whereas PMN were more than 99 % pure. Eos were incubated with exogeneous arachidonic acid (AA, 10^{-6} -10^{-4} M) in the presence or absence of stimuli (Ca-ionophore A 23187 (5 µM), C5a (10^{-8} M), FMLP (10^{-6} M) or PAF (10^{-6} M)) for 10 min. at 37°C in PBS. AA-metabolites were separated by reversed phase-HPLC and 15-HETE was quantitated by integration of peak areas. As a result using $4x10^{-5}$ M AA the amounts of 15-HETE produced in the presence or absence of stimuli were in a similar order indicating that the 15-LO of Eos is in an active state. The amounts of 15-HETE formed by Eos were 1.3pg/cell, which shows that these cells have an extremely high capacity to form 15-HETE.

When Eo/PMN mixtures (1:10, 10^7 cells) were incubated with 10^{-4} M AA in the presence or absence of 5 µM Ca-ionophore, large amounts of 15-HETE were seen. These, however did not exceed that formed by 10^6 Eos alone indicating that PMN apparently did not produce 15-HETE. To verify this, highly purified PMN were incubated with 10^{-4} M AA in the presence or absence of Ca-ionophore. Now we found only very low amounts of 15-HETE (0.01pg/cell) which we attribute to contaminating eosinophils (~1 %).

We conclude that human Eos contain a potent 15-LO which does not need to be activated. In contrast this enzyme is apparently absent in human PMN.

86

LONAPALENE (RS-43179)-A SPECIFIC 5-LIPOXYGENASE INHIBITOR INCREASES 15-HETE FORMATION IN HUMAN NEUTROPHILS. Karsten Fogh, Knud Kragballe, Department of Dermatology, Marselisborg Hospital, University of Aarhus, Denmark.

Leukotriene B_4 (LTB_4) and other 5-lipoxygenase (5-LO) products of arachidonic acid are present at biologically active concentrations in psoriatic skin. LTB_4 may play a pathogenic role in psoriasis, because treatment with the 5-LO inhibitor Lonapalene (RS-43179) is accompanied by a improvement of the disease. The ability of Lonapalene to inhibit 5-LO activity is shared with the 15-LO product 15-hydroxyeicosatetraenoic acid (15-HETE). The purpose of the present study was to determine the effect of Lonapalene on 15-HETE formation in polymorphonuclear leukocytes (PMNs). Purified PMNs were incubated in phosphate buffered saline containing 0.87 mM calcium-chloride in the presence of the A 23187 (5µM) and arachidonic acid (25 µM). Incubation was carried out with and without RS 43179 (1-100µM) for 30 min. at 37°. Extracted lipids were subjected to reversed phase high performance liquid chromatography using 70% methanol/30% water as the mobile phase. Chromatographic peaks were identified by their coalition with authentic standards and by UV-spectrometry. Compounds purified by RP-HPLC were assayed for immunoreactivity by RIA. Quantitation was made by integrated optical density. It was found that RS 43179 in a dose-dependent way inhibited the formation of LTB_4 (IC_{50} = 20µM). Concomitantly we found a dose related increase in the formation of 15-HETE. Maximal increase of 15-HETE formation (13 fold) was observed at 25µM RS 43179. The stimulation of 15-HETE formation was not associated with cell damage as assessed by LDH release. These results indicate Lonapalene in vitro is a potent stimulator of 15-HETE formation. Because 15-HETE itself inhibits 5-LO activity, the stimulatory effect of RS 43179 on 15-HETE formation may partly explain its therapeutic effect in psoriasis.

87

5(S),12(R)-DIHYDROXY-(6E,8Z,10E,14Z)-EICOSATETRAENOIC ACID - A NEW CHEMOTACTIC LEUKOTRIENE FORMED BY INTERACTION OF HUMAN NEUTROPHILS WITH 12(R)-HETE. J.-M. Schröder, Dept. of Dermatology, Kiel, FRG

Recently stereochemical analysis of psoriatic scale derived 12-HETE has shown to be 12(R)-HETE and not, however, 12(S)-HETE, which is known to be platelet-derived. Because 12(S)-HETE serves as substrate for neutrophil-5-lipoxigenase with the formation of the cell-cell-interaction product 5(S),12(S)DiHETE, we wanted to know whether epidermis-derived 12(R)-HETE may similarly serve as substrate for activated neutrophils forming a new eicosanoid. 12(R)-HETE was synthesized by air-oxidation of arachidonic acid and separated by thin-layer chromatography, chiralHPLC and RP-HPLC.

$2x10^7$ human neutrophils (PMN) were incubated (10 min.) with 10 µg 12(R)-HETE in the presence of Ca-ionophore 23187 or 10^{-6}M FMLP. As a result two newly detected peaks absorbing at 270 nm were found in the RP-HPLC-chromatogram. These new products eluted next to ω-OH-LTB_4 and LTB_4, respectively, showing slightly different elution times. Purification of the peak eluting next to LTB_4 by straight phase- and RP-HPLC gave a single component with the typical leukotriene- UV-pattern. This newly detected leukotriene is supposed to be structurally identical with 5(S),12(R)-Dihydroxy-(6E,8Z,10E,14Z) eicosatetraenoic acid (5(S),12(R)-DiHETE).

Chemotaxis experiments using human neutrophils and the Boyden chamber system demonstrated inherent chemotactic activity with an EC_{50} of 20 ng/ml.

Our results show that this newly detected leukotriene seems to be the most potent chemotaxin of the eicosanoid family apart from LTB_4 and ω-OH-LTB_4. This could be of importance in skin inflammation, especially psoriasis where both, 12-lipoxygenases and 5-lipoxigenase are active. In addition, several similarities of this new leukotriene with LTB_4 indicate, that care must be taken in discussing chemotaxisdata as well as LTB_4-RIA-data of "LTB_4-like" material in inflammatory skin material.

88

THE METABOLISM OF LEUKOTRIENE B4 BY PERIPHERAL BLOOD POLYMORPHONUCLEAR LEUKOCYTES IN PSORIASIS. P.D.L. Maurice, R.D.R. Camp and B.R. Allen. Institute of Dermatology London; Department of Dermatology, University Hospital, Nottingham, UK.

Polymorphonuclear leukocytes (PMNL) may contribute to the elevated leukotriene B4 (LTB4) levels observed in psoriatic skin. An earlier study showed no difference in LTB4 levels after incubation of psoriatic and control PMNL for 5 min, but an efficient PMNL ω-oxidation system may metabolise an increased LTB4 load so that levels measured at one point in time remain unchanged. We have therefore measured the formation of total LTB4 ω-oxidation products (ω-ox) and compared the kinetics

of the ω-oxidation enzyme LTB4 20-hydroxylase in psoriatic and control PMNL in vitro.

PMNL suspensions from patients with chronic plaque psoriasis and healthy controls were incubated for varying times at 37°C with A23187 (3.8 μM.) The products were extracted, separated by HPLC and quantitated by measurement of peak area. Further psoriatic and control PMNL suspensions were incubated for 2 min with 5 different concentrations of LTB4 (0.1-10 μM), premixed with (^{3}H)-LTB4. HPLC fractions containing LTB4 and ω-ox were collected for measurement of radioactivity. From the percentage conversion of LTB4 at each substrate concentration, the apparent Km and Vmax of the 20-hydroxylase reaction was calculated.

Psoriatic and control PMNL suspensions incubated with A23187 for 5 min produced median levels of ω-ox of 1.28 (0.83-1.94, n=14) and 0.95 (0.50-1.27, n=13) nmole per $1.5x10^7$ cells respectively ($p<0.05$ Mann-Whitney U-test.) With 4 psoriatic and 3 control suspensions, incubations with A23187 were terminated after 0.5, 2, 5 8 and 20 min. Time courses of LTB4 levels in psoriatic samples did not differ significantly from those in controls whereas ω-ox levels in psoriatic samples were consistently elevated ($p<0.05$, analysis of variance and covariance with repeated measures). The mean apparent Km values for LTB4 20-hydroxylase in psoriatic (n=3) and control (n=3) PMNL were 0.8 and 1.3 μM respectively; the corresponding apparent Vmax values were 94 and 140 pmole/10^7 cells/min. Neither of these differences was statistically significant (unpaired t-test.)

Psoriatic PMNL produced increased amounts of endogenous ω-ox under the conditions described. Since no inherent abnormality of the 20-hydroxylase enzyme could be demonstrated in the psoriatic cells by kinetic studies, it is likely that the increased formation of ω-ox is secondary to increased synthesis of LTB4.

89

GROWTH OF APOCRINE CELLS IN TISSUE CULTURE BJ Harrison, DL Jones, LE Hughes, Department of Surgery, University of Wales College of Medicine, Cardiff, Wales.

Inflammation hampers accurate quantification of androgen metabolism in apocrine sweat glands of patients with hidradenitis suppurativa. We have therefore developed a method for purification and culture of apocrine epithelial cells.

Subcutaneous axillary fat (500-1000 mg) was incubated with collagenase, and a pure suspension of apocrine glands obtained by centrifugation of the digest on a discontinuous Percoll gradient. Washed apocrine gland fragments were plated into tissue culture flasks containing RPMI 1640 culture medium, cholera toxin (100 ng/ml), epidermal growth factor (100 ng/ml) and 10% foetal calf serum.

Cellular outgrowth was observed after 72 hours, confluent epithelial cultures were achieved at approximately 14 days. Contaminating fibroblasts required selective removal with EDTA-Trypsin. This technique has been used successfully for recovery of apocrine glands and subsequent cell culture in 12 patients.

Both purified apocrine glands and cultured cells stain positively with known apocrine markers, including P.A.S., Non Specific Esterase and the monoclonal antibody HMFGII. Cultured cells exhibited high 5 alpha reductase activity, which is consistent with apocrine sweat gland metabolic activity.

We are not aware of any previous report of in vitro culture of apocrine sweat glands. It is proposed to utilize this simple technique to investigate target organ androgen metabolism in patients with hidradenitis suppurativa.

90

ISOTRETINOIN AND ANDROGEN METABOLISM IN HUMAN BACK SKIN. Nick Simpson, D. Brown-Douglas, M. B. Hoggins* Departments of Dermatology, Royal Infirmary and The University*, Glasgow, Scotland.

Previous studies have shown that most of the metabolism of androgens in whole skin resides in sebaceous glands. An increase in conversion of testosterone (T) to dihydrotestosterone (DHT) by the enzyme 5α - reductase has been suggested as a factor in the aetiology of acne, hirsutism and pattern alopecia. However, it is important to determine the extent to which changes in skin 5α- reductase activity result from changes in sebaceous gland size. Isotretinoin provides a useful probe to investigate this relationship through non-hormonal suppression of sebaceous glands. Young adults (16-25yr) with moderately severe acne were used in the study. 15 (14M:1F) received isotretinoin (1mg.Kg body $wt^{-1}.dey^{-1}$.) and 21 (16M:5F) served as untreated controls. Before and after 1 month of therapy 4mm punch biopsies were taken from clinically normal back skin, snap frozen and sectioned in a cryostat. 5 x 16μ thick alternate sections were used for histometric analysis of sebaceous gland volume and enzyme assay. 5α- reductase activity was measured by incubating homogenates of cryostat sections with ^{3}H Testosterone 33 nM, ^{14}C Dihydrotestosterone 664 nM and NADPH 3.4 mM in 0.1ml 0.05M TRIS/HCl buffer pH 7.2 for 14 min at 37°C. Separation of reaction products was by TLC and quantitation by liquid scintillation counting.

There was a high correlation between sebaceous gland volume and both ^{3}H-DHT production ($r=0.78$) and total 5α- reduced products ($r=0.80$). This relationship was unchanged in repeat biopsies on the control group after one month. Following isotretinoin there was a 10- to100-fold reduction in sebaceous gland volume and a similar reduction in ^{3}H-DHT production. After treatment there was a lower correlation between sebaceous gland volume and enzyme activity ($r=0.57$); suggesting a decrease in the proportion of 5α - reductase activity originating in sebaceous glands. These studies have established a quantitative relationship between 5α - reductase activity and sebaceous gland volume in back skin. They provide evidence that changes in 5α - reductase activity may be secondary to changes in sebaceous gland size.

91

MULTIPLY RESISTANT STAPHYLOCOCCI FROM ANTIBIOTIC-TREATED ACNE PATIENTS: A CAUSE FOR CONCERN? E.Anne Eady, J.H.Cove, K.T.Holland and *W.J.Cunliffe, Dept. of Microbiology, Univ. of Leeds and *Dept. of Dermatology, Leeds General Infirmary.

Antibiotic therapy for acne rapidly selects for a predominantly resistant staphylococcal skin flora. What is not known is whether resistance is single (only to the administered antibiotic) or multiple. Infections due to multiply resistant staphylococci have presented therapeutic problems for many years. However, there is now increasing awareness of the practices likely to lead to the generation of such strains. The purpose of this study was to determine whether dermatological use of long term antibiotic therapy for acne is associated with the development of potentially hazardous multiply resistant staphylococci. Staphylococcal isolates were obtained from the skin surface of 55 antibiotic-treated patients and 24 untreated controls by the detergent scrub technique of Williamson and Kligman. Single resistances were detected by plating wash fluid directly onto antibiotic-containing media. Multiple resistance was identified by screening individual isolates for growth in the presence of supra M.I.C. levels of 18 different antibiotics. Resistant strains comprised over 10% of the total staphylococcal flora of the majority (87%) of patients but of a minority (29%) of untreated controls ($p<0.001$). Multiply resistant isolates were obtained from 89% of patients compared with 33% of controls ($p<0.001$). The maximum number of resistances carried by any one isolate was seven but strains with four or five resistances were common. Individuals usually harboured several different resistant strains. Resistant S.aureus were isolated from two patients. Amongst patients, the most frequently encountered resistances were to tetracycline (96%), erythromycin (76%) and penicillin (93%). Aminoglycoside resistance was less frequently encountered (20%) and methicillin resistance was rare (7%). All isolates were sensitive to rifampicin and vancomycin. Overall the study has shown that antibiotic therapy for acne encourages the development of a multiply resistant staphylococcal skin flora. However, resistant isolates are rarely S.aureus and are infrequently aminoglycoside or methicillin resistant. Thus, although their creation must be regarded as undesirable, they do not constitute a reservoir of strains which would present therapeutic problems were they to become associated with infection. It is likely that the emergence of multiply resistant strains could be minimised if patients were not switched from one antibiotic to another unless absolutely necessary.

92

THE MICROBIOLOGY OF INFLAMMED ACNE VULGARIS LESIONS. K. T. Holland, J. P. Leeming, and W. J. Cunliffe*, Departments of Microbiology and Dermatology*, University of Leeds, Leeds, U.K.

Although micro-organisms, particularly *Propionibacterium acnes*, are often imputed in the pathogenesis of inflammatory acne vulgaris, few studies have attempted to quantify and identify the microbial contents of inflamed lesions. This is probably due in part to the difficulty in interpreting data obtained from lesions subject to immunological activity (particularly in pustules or long-standing lesions). This investigation was undertaken to define the microbial profile of papules in their early stages of development.

The progress of acne lesions was monitored by tracing an area of the upper back onto a transparent acetate sheet. This template was used the next day , and in some cases after three days, to identify lesions of less than 1 day and 2-3 days duration. These were biopsied and entire pilosebaceous units (52 "one day" and 19 "three day" lesions) were isolated by calcium chloride-mediated micro-dissection. These were homogenised and examined for microbial presence by cultural and microscopical methods.

Propionibacteria colonised 68% of 1 day lesions and 79% of 3 day lesions; staphylococci colonised 19% and 37% respectively and *Pityrosporum* (*Malassezia*) were found in 52% and 68%. Although the prevalence of each microbial group was higher in the more chronic lesions, these differences did not achieve statistical significance. The microbial profile of inflamed lesions was similar both qualitatively and quantitatively to non-inflamed lesions studied previously. These results call into question the assertion that micro-organisms are (always) the source of the mediators responsible for initiating inflammation in acne lesions.

93

AZELAIC ACID UPTAKE BY *STAPHYLOCOCCUS EPIDERMIDIS*. R.A. Bojar, J.P. Leeming, K.T. Holland and W.J. Cunliffe*. Microbiology Department, Leeds University and * The Leeds Foundation for Dermatological Research, The General Infirmary at Leeds, Leeds, U.K.

Topically applied azelaic acid is a useful therapy for acne (Br.J.Dermatol. *109*,45.1983; Br.J.Dermatol. *114*,493.1986). During treatment the cutaneous flora is reduced (Br.J.Dermatol. *114*,493.1986) and *in vitro* both staphylococci and propionibacteria are either killed or growth inhibited depending on the nutritional state of the environment (Br.J.Dermatol. *115*,551.1986). This investigation is an extension of these findings and reports on the uptake of azelaic acid by *Staphylococcus epidermidis*.

The cells were grown in a defined medium of glucose, arginine, proline, glycine, histidine, valine, cystine, nicotinamide, thiamine, biotin and salts, washed free of nutrients and resuspended in buffer with radiolabelled ^{14}C azelaic acid. Uptake was measured over a period of 30 minutes. Cells in exponential, late exponential and stationary phase had relative uptake of 1:2.04:2.68. The pH of the environment greatly altered azelaic acid uptake by cells in the stationary phase. If pH 4.0 arbitrarily represented 100% uptake then other pHs gave 4.4,57%; 4.8,47%; 5.2,42%; 5.6,36% and 6.0,10%. Similar results were obtained for exponential phase cells. To determine whether the azelaic acid was transported actively into the cell inhibitors of energy transduction, nigercin (ΔpH dissipation), valinomycin (ΔΨ dissipation) and carbonylcyanide -m- chlorophenylhydrazone (ΔpH and ΔΨ dissipation) were used in uptake experiments. Only inhibitors affecting cytoplasmic membrane ΔpH inhibited uptake. Consequently, azelaic acid uptake is energy dependent and relies on a significant ΔpH. Also uptake is affected by cell state and environmental pH. These results may explain the variablity of cell sensitivity to azelaic acid and indicate better formulation for its topical use.

94

T CELL EXPOSURE TO ALLOGENEIC LANGERHANS CELLS FREE EPIDERMAL CELLS INHIBIT THEIR SUBSEQUENT ALLOREACTIVITY IN VITRO AND INDUCES THE SUPPRESSION OF T CELL RESPONSES.
A. Demiden, M. Faure, J. Thivolet. INSERM U.209, Pavillon R., Hopital E. Herriot, 69437 Lyon, Cedex 03, France.

Previous works have shown that cultured human epithelia devoid of Langerhans cells and MHC-class II antigens are not rejected when used as epidermal allografts in normal recipients with superficial dermal wounds. Primary and secondary type in vitro Mixed Epidermal Cell Lymphocytes Reactions (MELR) were conducted to analyze the mechanisms of immune tolerance noted in cultured epidermal allograft recipients and to investigate the possibility of a T cell suppressive activity generated after contact with these epidermal allografts. T cell from normal adults were induced to proliferate in 6 day or 3 day MELR with either crude epidermal cells-EC (2-4% of Langerhans cells) or Langerhans cell and class II MHC antigen free cultured EC (keratinocyte) suspensions; cultured keratinocytes were obtained from confluent first passage EC cultures performed on 3T3 feeder cells and were not able to stimulate allogeneic T cell in primary MELR. A secondary type allogeneic response was noted when T cells after primary stimulation by allogeneic EC were induced to proliferate by EC from the same origin (stimulation index -SI = 24 $\pm$ 5). This response to EC suspensions was abolished when T cells were first incubated with cultured keratinocytes devoid of class II antigens, a phenomenon which was noted to be allo specific. After exposure in primary MELR to cultured keratinocytes a low response (SI = 3 $\pm$ 0.5) was noted when cells were restimulated by cultured keratinocytes from the same origin. Moreover, when T cells after exposition in primary MELR to cultured keratinocytes but not to Langerhans cell undepleted crude EC suspensions were added to allogeneic MELR consisting of unprimed T cells and EC from the same origin as cultured keratinocytes, an inhibition of the usual allogeneic primary response could be noted. These data evidence that in the absence of Langerhans cells and class II determinants, keratinocytes induce an inhibition of T cell alloreactivity in subsequent MELR. They suggest that under these conditions T cells with suppressive activity may be generated and induced to proliferate after restimulation by cultured keratinocytes expressing class I and minor histocompatibility allo-antigens. The present work supports the hypothesis of clonal activation of T cells with suppressive activity in the absence of class II antigens as an important mechanism in induction of tolerance to allo-class I antigens.

95

IMMUNOREACTIVE INTERLEUKIN 1 (IL-1) α AND β IN NORMAL HUMAN SKIN.
Y. Mérot, MD, L. Didierjean, Ph D, JM Dayer', MD, C. Fournier, P. Carraux, JH Saurat, MD.
Clinique de Dermatologie, Division d'Immunologie et d'Allergologie', Département de Médecine, Hôpital Cantonal Universitaire, GENEVA, SWITZERLAND.

Human epidermis and stratum corneum (SC) contain high amounts of preformed IL-1-like biological activity in the absence of apparent stimulation. We have now studied the localization of the two IL-1 species IL-1α and β within epidermal compartments as well as the MW of the immunoreactive proteins. A rabbit polyclonal antiserum (hr IL-1β pcl as) and a monoclonal antibody (hr IL-1β mAb) directed against human recombinant IL-1β and a rabbit polyclonal antiserum against human recombinant IL-1α (hr IL-1α pcl as) were used in an avidin-biotin immunoperoxidase technique on frozen normal skin specimens obtained from abdomen, breast and buttocks. Proper controls included specific absorptions with hr IL-1α or hr IL-1β, pre-immune sera and ascitic fluid.

Protein blot analysis of epidermal extracts showed that both antisera and the mAb reacted with proteins with apparent MW 17 and 31 kD and with a 52 kD band, supposed to be either aggregates and/or associations with other proteins. This 52 kD form was not found in human liver, placenta, uterus and heart extracts. In all the specimens studied, staining of the epidermis was observed. The bulk of IL-1β immnoreactivity was localized to the stratum granulosum (SG) including those of the eccrin acrosyringium and the hair follicle infundibulum. The SC was consistently not reactive. The staining pattern was a network either intercellular or membranous (hr IL-1β pcl antiserum) or cytoplasmic (hr IL-1β mab, major band at 31 kD in protein blots). The bulk of IL-1α immunoreactivity was localized to the first lowest cell layers of the SC, sparing the SG and the upper SC. These immunostainings were abolished when the antisera or mAb were preabsorbed with hr IL-1α or hr IL-1β. In none of the samples analyzed did Langerhans cells show IL-1 immunoreactivity. These observations : (i) show that the previously detected IL-1 like biological activities in the normal unstimulated human epidermis correlate with the presence of immunoreactive IL-1 species, (ii) demonstrate that both IL-1 α and β are detectable in roughtly similar amounts, but slightly distinct distributions, (iii) localize in the upper part of the epidermis the bulk of immunoreactive IL-1 species. The significance of this distribution and of the 52 kD immunoreactive protein which appears to be unique for the epidermis should be of importance for the understanding of the biological function of preformed epidermal IL1 pool.

96

STELWAGON PRIZE PRESENTATION

GENETIC AND BIOCHEMICAL ANALYSIS OF A MUTANT RAS ONCOGENE WHOSE TRANSFORMING ACTIVITY IS TEMPERATURE SENSITIVE.
William S. Sawchuk and Douglas R. Lowy, Laboratory of Cellular Oncology, NCI Bethesda MD 20892 USA.

Activated ras oncogenes have been found in many histological types of spontaneous and experimentally induced tumors, including several forms of benign and malignant cutaneous neoplasms. All ras proteins bind guanosine nucleotides (GTP and GDP) with high affinity and share other features with regulatory proteins and also bind guanosine nucleotides (such as those of the adenylate cyclase system). In an attempt to study the mechanism of ras induced cellular transformation we are examining genetic and biochemical aspects of a mutant of Kirsten murine sarcoma virus whose k-ras encoded 21 kd protein (p21) is temperature sensitive for transformation.

The mutant k-ras gene was molecularly cloned and transfected into NIH 3T3 cells. When grown at 34°, the cells were morphologically transformed in that they lost contact inhibition and grew efficiently in low concentrations of serum. When grown at 39° the cells reverted to the normal phenotype. In parallel studies, no such temperature dependence was seen with the wild type k-ras gene; the cells were transformed at both temperatures.

Nucleotide sequence analysis of the mutant k-ras gene, whose protein product is 189 amino acids in length, revealed one significant point mutation : in the codon encoding amino acids 119. The mutation at codon 119 changes this amino acid from Asp to Asn. Thus, the temperature sensitive phenotype of this protein appears to arise from the single amino acid substitution at position 119.

Results obtained in our laboratory, as well as by other investigators, have suggested that amino acid 119 may play a critical role in guanosine nucleotide binding to p21. We have therefore introduced the mutant k-ras gene into a bacterial expression vector in order to synthesize a sufficient amount of mutant p21 ras protein to compare its biochemical properties (such as its ability to bind nucleotides at different temperatures) with those of the wild type version of the p21 protein.

97

FAST ISOLATION OF HUMAN EPIDERMAL LANGERHANS CELLS BY IMMUNOMAGNETIC PARTICLES.
Daniel Hanau, Didier A. Schmitt, Michel Fabre, Roland Bury, and Jean-Pierre Cazenave, INSERM U.311, Centre Régional de Transfusion Sanguine and Service Central de Microscopie Electronique, ULP, Strasbourg, France.

In order to study the function and pharmacology of human epidermal Langerhans cells (LC) or to obtain LC-free keratinocyte cultures, it would be helpful to have a simple method to isolate rapidly and selectively LC under sterile conditions and to maintain their viability. Immunomagnetic direct or indirect depletion procedures have been recently described for the isolation of T cell subsets, using monodisperse polymer particles, with a magnetic core, conjuguated to anti-mouse antibody (indirect method) or to anti-human antibody (direct method). We have adapted these techniques to the isolation of human epidermal LC. Suspensions of epidermal cells were obtained from normal human epidermis after trypsinisation. They were incubated, for 20 min at 4°C, with Dynabeads M-450 coated with sheep anti-mouse IgG1 (Dynal-SHAM) (Dynal, Oslo, Norway), used in either a direct or an indirect technique. In the direct technique the mouse monoclonal anti-T6 antibody BL6 (IgG1 subclass) was bound to the Dynal-SHAM before incubation with the epidermal cell suspension ; in the indirect technique the cells were pretreated with BL6 before being mixed with Dynal-SHAM. The target cells rosetted with Dynal-SHAM through BL6 were then easily isolated by applying a Dynal magnetic particle concentrator. The rosetted and rosette depleted cell fractions were characterized by light microscopy, immunofluorescence and electron microscopy. Viability and function of the rosette cells were tested by electron immunogold staining using BL6 conjugated to gold particles. Under light microscopy, the cell fraction isolated by the magnet shows a very high percentage of rosettes (95%), whereas the epidermal cells, not retained by the magnet, are free of rosettes. Fluorescence studies show that the cells situated at the centre of the rosettes are all $T6^+$ and confirms the absence of $T6^+$ cells in the cell fraction depleted of rosettes. Electron microscopy confirms the Langerhans nature of the cells in the centre of the rosettes. Since these cells internalize gold-labeled BL6 by receptor-mediated endocytosis, isolated LC are viable and functional. Such a technique should permit by successive use of two antibodies isolation of cell subpopulations from a single epidermal cell suspension.

98

COLLAGEN GENE EXPRESSION IN SCLERODERMA - DETECTION OF COLLAGEN mRNA IN SITU.
Scharffetter K., Lankat-Buttgereit B., Krieg T, Dermatologische Klinik und Poliklinik der LMU München, FRG

Although the primary cause of scleroderma is not yet clarified excessive accumulation of collagen represents the final step determining the prognosis of the disease. Recent data indicate that the transcriptional control plays a major role in regulation of collagen metabolism. However, most of the biosynthetic studies have been carried out using fibroblasts in monolayer cultures and there is considerable doubt that this system reflects the in vivo situation in all aspects. Therefore we have developed a technique which allows a detection of specific mRNA coding for collagens on a cellular level within histological sections in scleroderma. For in situ hybridization cDNA clones for collagens I and III were subcloned into Gem-vectors. The plasmids were lineralized and used in an in vitro transcription system in the presence of S^{35} labelled UTP. Frozen sections from normal and affected skin were incubated with these probes and following autoradiography the sections were stained. Specificity of the various collagen probes was prooved by northern blot analysis, cDNA clones for human keratins were used as internal controls Whereas hybridization to 1 type I collagen mRNA shows a distinct but weak labelling of only some fibroblasts within normal dermis, there was a heavy accumulation of grains in many fibroblastic cells in section of scleroderma. These cells were usually located in the deep dermis and in the subcutaneous fat tissue or around blood vessels in the reticular layer of the dermis. Here the labelled cells were often surrounded by mononuclear cells. In addition to extensively labelled groups of fibroblasts, single fibroblastic cells with a distinct cytoplasmic labelling were detected. These results support the idea that scleroderma starts in the deep layers and the subcutaneous fat tissue of the skin. Moreover our data are in agreement with the previously proposed concept that collagen synthesis of fibroblasts can be activated by inflammatory and immunocompetent cells. The in situ hybridization seems to be a valuable tool to investigate collagen gene expression on a cellular level and provides insight in the pathogenesis of scleroderma under in vivo conditions.

99

CRYOPRESERVATION OF ALLOGENEIC SKIN. J.N. Kearney and D. Harnby, Regional Tissue Bank, Pinderfields General Hospital, Wakefield, U.K.

Recent clinical success with skin allografts following either Langerhans cell suppression or use of Cyclosporin A indicates great potential for future use, thus requiring techniques for long term viable allograft storage. We have evaluated cryogenic storage of human and murine skin using tetrazolium reductase activity as an index of viability.

The technique consists of 5 phases: - (1) initial immunomodulation (2) use of cryoprotective agents (3) controlled rate freezing (4) rate of thawing (5) post-thaw deterioration/stabilization. All except phase (4) were evaluated and optimized. Phase 1: - High dosage UVB (600 mJ. cm^{-2}) resulted in a 30% decline in tetrazolium reductase activity whereas 150 mJ. cm^{-2} had little effect. Results were the same when UVB was applied to either the epidermal or dermal surface. Use of the 150 mJ. cm^{-2} UVB did not enhance freeze-induced damage during subsequent cryopreservation. Phase 2: - Use of 15% glycerol (2hrs, 4 C) as a cryo protectant was significantly beneficial regardless of the basal medium ($p<0.001$). An independent effect on basal medium was also detected ($p<0.001$). Phase 3: - Use of freezing rates between 0.25 to > 60° C. min^{-1} did not significantly differ in their effect on viability of murine or human skin. This contradicts published recommendations for slow cooling based largely on freezing of single cell suspensions. Phase 4: -Skin was thawed rapidly at 37 C. Phase 5: - The rapid deterioration in activity following thawing was halted by using various preparations (e.g. serum) or environmental conditions (e.g. pH) known to inhibit protease enzymes. We suggest that proteases released from freeze damaged skin precipitate further damage. In conclusion, optimization and simplification of cryo-preservation and post-thaw recovery protocols for skin, including its prior treatment with UVB, have revealed simple techniques suitable for establishing an allogeneic skin bank.

100

CELL CYCLE ANALYSIS AND BROMODEOXYURIDINE UPTAKE OF HeLa CELLS AFTER TREATMENT WITH DITHRANOL (ANTHRALIN) AND 10-BUTYRYL DITHRANOL. Anita Göransson, Eero Lehtonen, Arja-Leena Kariniemi and Stig Nordling, Departments of Dermatology and Pathology, University of Helsinki, Finland.

The effect of two antipsoriatic drugs dithranol (anthralin) and butantrone (10-butyryl dithranol) on the proliferation of HeLa cells was studied. Non-confluent monolayers of HeLa cells were treated with various concentrations (10^{-5} - 10^{-8}) of dithranol or butantrone for 45 min. Thereafter the cells were cultivated for various times. 30 minutes before harvesting the cells were treated with 10µM bromodeoxyuridine (BrdU) and 10µM fluorodeoxyuridine. The number of cells was monitored with a Coulter cell counter. To determine the cell cycle and BrdU-uptake cells were fixed with paraformaldehyde, permeabilized and treated with anti-BrdU antibody followed by a fluoroisothiocyanate labelled second antibody and propidium iodide. The cells were analyzed with FACS IV flow cytometer. In one experiment the recovery was studied by cultivation of the cells up to three weeks. Both drugs, at the two highest concentrations 10^{-5}M and 10^{-6}M, inhibited the uptake of BrdU almost immediately. After 2 h this effect was more pronounced and the proportion of cells in S-phase had decreased. After one day the number of cells had decreased, the BrdU uptake was small and few cells were in S-phase. The effect of dithranol started at a lower concentration than that of butantrone, but at higher concentrations the effect of butantrone was more pronounced and after one day most cells had detached from the culture plate. According to our study dithranol and butantrone at the concentrations 10^{-5}M and 10^{-6}M, which are below those used in therapy, arrest the proliferation of HeLa cells. The effect of dithranol is more linear whereas that of butantrone is more abrupt. This finding suggests a smaller therapeutic width of butantrone found in clinical trials.

101

EFFECT OF DIFFERENT THERAPEUTIC REGIMES ON EPIDERMAL KERATINS IN PSORIASIS. D.B. Holland[1], E.J. Wood[2], W.J. Cunliffe[1], M.R. West[3], and D.M. Turner[3].

[1]Department of Dermatology, The General Infirmary at Leeds, Leeds, LS1 3EX, [2]Department of Biochemistry, University of Leeds, Leeds, LS2 9JT, and [3]Department of Cell Biology, Glaxo Group Research Ltd., Greenford UB6 OHE, U.K.

Keratinocyes of psoriatic epidermis fail to undergo normal terminal differentiation and the electrophoretic profile of their keratin polypeptides is modified, with a reduction in the heaviest polypeptides (keratins 1,2: 67,65 kD) and the appearance of additional low Mr polypeptides (6,16,18?: 56,48,45kD). We have investigated quantitative changes (i.e. normalisation) of the keratins during therapies promoting clinical resolution of psoriatic lesions. Keratotome shavings (0.1mm) were taken from lesions of patients on four different treatment regimes (dithranol (5), PUVA(5), etretinate(4), and hydroxyurea (3)) before and during therapy over 3-4 months. Simultaneously clinical photographs were taken. Keratins were extracted in pH 2.65 buffer, analysed by SDS/polyacrylamide gel electrophoresis, and the amounts of polypeptides were quantified by scanning densitometry. Samples of epidermis from clinically normal individuals from a variety of anatomical sites were analysed similarly for comparison.

The initial low levels of keratin1 were unchanged during PUVA treatment although lesion resolution occurred after 9-12 weeks. However, increases in keratin1 to levels comparable with those in normal epidermis occurred during dithranol and hydroxyurea therapy, with an apparent overproduction of this keratin during etretinate therapy. The amounts of keratin2 normalised during dithranol PUVA and hydroxyurea treatments, but only minor increases in levels were observed during etretinate therapy for up to 28 weeks. All four treatment regimes reduced the levels of keratins 16 and 18 but the rate at which these changes occurred differed with different therapies. This suggests that resolution of lesions as judged by clinical criteria can occur without normalisation of the electrophoretic profile. Possibly the most reliable marker of clinical resolution was the reduction in keratin 16, since treatment effects on the differentiation keratins 1 and 2 were different.

102

ESTABLISHMENT OF HUMAN CUTANEOUS NEURO-ENDOCRINE CARCINOMA (CNEC) CELL LINE: MAINTENANCE OF HISTOLOGICAL, ULTRASTRUCTURAL, IMMUNOLOGICAL AND BIOCHEMICAL CHARACTERS AFTER TRANSPLANTATION INTO THE NUDE MOUSE AND IN VITRO CULTURE. M. Demarchez*, L. Lemieux*, A. Pisani**, J. Bailly*, F. Bernerd*, M. Darmon*, and J.P. Ortonne**, *Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, 06565 Valbonne, FRANCE, **Laboratoire de Recherches Dermatologiques, Faculte de Medecine, Nice, FRANCE.

Cutaneous neuroendocrine carcinoma cells are difficult to establish in vitro, and no cell line is presently available. As an attempt to establish a human CNEC cell line, a surgical sample of such a tumor was transplanted into athymic nude mice. Serially transplantable tumors were obtained in these animals, and a cell line could be established by culturing explants from nude mice tumors on 3T3 feeder layers.

The original CNEC was a large tumor of the right temporal region. Neuro-secretory granules positive for the uranaffin reaction were recognized by electron microscopy. Indirect immunofluorescence and gel electrophoresis revealed that CNEC cells contain both keratins (mainly of the simple epithelial types) and neurofilaments. Moreover, they showed immunoreactivities for neurone-specific enolase and vasointestinal peptide (VIP).

Up to now, three passages of the tumor have been performed in nude mice. On the other hand, cells have been maintained in culture for over six months. In both cases, CNEC cells retained their original characteristics. This observation shows that this cell line (called $CNEC_1$) could be a good model to study the physiology of cutaneous neuroendocrine tumors and their possible Merkel cell origin.

103

RELATIONSHIPS BETWEEN MERKEL CELLS (MC) AND NERVE ENDINGS (NE) DURING EMBRYOGENESIS IN THE MOUSE EPIDERMIS. Florence Pasche, Yves Mérot, Jean-Hilaire Saurat. Clinique de Dermatologie, Hôpital Cantonal Universitaire, 1211 Genève 4, Switzerland.

Close relationships between MC and NE exist in the adult mouse. We found in a previous study that about 95% of MC are already in close contact with NE in the newborn mouse. As MC may serve as a target for the NE during embryogenesis, the purpose of the present study was to precise the relationships between MC and NE during embryogenesis.

NMRI mouse embryos 12, 13, 14, 15, 16, 17 days of gestational age and double-labelling indirect immunofluorescence (IIF) on frozen material of mouse whisker pad were used with the cytokeratin monoclonal antibody RGE_{53} and a neurofilament rabbit anti-serum R_{39}, against the 3 neurofilament subunits : 70 kd, 150 kd and 220 kd. At day 12, no MC was observed by IIF. From day 13 to 17 the number of MC progressively increased.

gestational age (days)	total number of MC	% of NE associated MC: Isthmic	Parafollicular	Interfollicular
12	0	0	0	0
13	530	70%	43%	31%
14	475	42	18	22
15	1680	81%	70%	55%
16	540	90%	83%	72%
17	750	95%	100%	91%

The results were confirmed by EM observations. On the back the same relationships between MC and NE were observed but delayed in the course of embryogenesis. These observations :

1. establish the time course of MC and NE contacts during embryogenesis.
2. show that MC are present in the epidermis and appendages before NE reach the epithelium and
3. support the hypothesis that MC could act as a target for the growing NE.

104

T6, A DEDIFFERENTIATION ANTIGEN IN CUTANEOUS T CELL LYMPHOMA. Brigitte Dreno, Brigitte Milpied, Gerard Guillet, Jean-François Stalder, Pierre Litoux. Department of Dermatology, Centre Hospitalier Universitaire, Hotel-Dieu, 44035 Nantes, France.

In cutaneous T cell lymphoma, T6 reactive cells are considered as Langerhans cells in epidermis, but their nature in dermis is controversial.

For some authors, these dermal T6+ cells are thought to be Langerhans cells, whereas others suppose that T6 represents a differentiation antigen of lymphocyte infiltrate. In this context, we studied the dermal infiltrate of 5 cutaneous T cell lymphoma at stage I (TNM classification) and then 2 years later with an aggressive disease (stage IV). For each cutaneous section, 2 immunological techniques (immunofluorescence and immunoperoxydase) were used with the same series of monoclonal antibodies (IOT1, IOT4, IOT6, IOT8 and HLA-DR). At stage I, the 5 dermal infiltrates showed the same phenotype (T1+, T4+, DR+, T6-), but at stage IV, many cells of dermal infiltrate expressed T6 antigen. In order to specify the nature of these T6 cells, we performed a double-labeling technique (Leu3 phycoerythrin-IOT6 fluorescein and IOT1 rhodamine-IOT6 fluorescein). This study revealed that 50 to 70% of T1+, T4+ cells expressed T6 antigen. It is concluded that T6 antigen may be expressed by lymphocyte cells in T cell lymphoma dermal infiltrate and that it is probably a dedifferentiation antigen since it appears at an aggressive stage of the disease.

105

A UNIQUE TYPE OF CUTANEOUS LYMPHOMA SOLELY COMPOSED OF KI-1 POSITIVE TUMOR CELLS. Peter Kaudewitz, Fritjof Eckert, Roland Schwarting, Gunther Burg and Harald Stein. Department of Dermatology University of Munich, Department of Pathology Free University of Berlin FRG

The Ki-1 antigen defined by the monoclonal antibodies Ki-1 and Ber-H2, has been demonstrated to be expressed on activated lymphoid cells. Our purpose is to describe a distinct lymphoproliferative disorder solely composed of tumor cells derived from such activated lymphoid cells. Histologically, the neoplasia could not be attributed to one of the entities defined by the Kiel classification and, in most cases was categorized as true histiocytic malignancy.

Eight patients were included in the present study. Clinically, the patients presented with several rapidly growing tumors . At the time of diagnosis no systemic involvement was detectable and follow up ranging from 6 months to 3 years revealed no systemic spread, arguing against a high grade malignancy. Immunologically, the tumor cells in all cases were positive for CD2, CD4, HLA-DR, Ki-1 and Ber-H2 antigens, expressed the Il-2 receptor, and were negative for macrophage associated antigens Leu-M1 and OKM1 and lysozyme. By using the monoclonal antibody Ber-H2 working on paraffin sections it was possible to directly assess the morphology of the Ki-1 positive cells. All tumor cells were Ber-H2 positive. They were large, sometimes multinucleated cells with prominent nucleoli and abundant cytoplasm, and were morphologically identical to the the tumor cells found in the so-called true histiocytic sarcomas. According to our results, in some cases these may in fact represent Ki-1 positive large cell T lymphomas.

The lymphoproliferative disorder described here may be termed primary Ki-1 lymphoma of the skin to be distinguished from peripheral T cell lymphomas which develop into large cell neoplasias with varying numbers of Ki-1 positive cells. Though the natural history of the described primary Ki-1 positive large cell lymphomas needs further study, they should be recognized as a distinct type of cutaneous T cell lymphoma.

106

RELEVANCE OF MONOCLONAL ANTIBODY BT5.9 AND DIPEPTIDYL PEPTIDASE IV (DP IV) ACTIVITY FOR THE DIAGNOSIS OF SEZARY SYNDROME. Maria Grazia Bernengo, Massimo Meregalli, Mauro Novelli, Roberta Massobrio, Antonietta De Matteis, Gian Carlo Doveil, Dermatologic Clinic, University of Turin, Turin, Italy.

BT5.9 is a monoclonal antibody reported to be reactive with 15-20% of peripheral human T lymphocytes. Flow cytometry and the two color immunofluorescence technique, with phycoerythrin (PE) as the second dye in a single laser system, were employed to demonstrate that: 1) BT5.9 is expressed on a larger amount of normal peripheral blood mononuclear cells (PBMC) (62.9±11.4%, N=18) than that observed on fluorescent microscope (FM) counts (24.5±11.5), because the antigen is weakly expressed on a large proportion of cells. 2) DP IV activity of PBMC is significantly correlated to their BT5.9 positivity. In cytospins prepared from BT5.9 FACS sorted cells, 81.2±10.7% cells showed a DP IV activity; on the other hand only 9.4±6.5% BT5.9- cells were DP IV+. 82% PE-CD4+ sorted cells coexpressed BT5.9 antigen. In 5 experiments FACS sorted BT5.9- cells were tested with FITC conjugated CD19, CD4, CD8, SK11/TQ1 and CD7: 34.6±12.4% cells were CD4+ and 88.4±5.2% CD7+. BT5.9- cells were stained with PE-CD4 and FITC-CD7 antibodies: 41.8± 10.1% cells were CD4+/CD7+, 11.8±3.5% CD4-/CD7-, 47.4±5.1 CD4-/CD7+ and 4.8±2.4% CD4+/CD7-. DP IV activity was present in 51±10% of BT5.9-/CD4+/CD7+ cells and in <2% of the BT5.9-/CD4+/CD7- subset. From these data we calculated that the BT5.9-/CD4+/CD7-/DP IV- subset is equivalent to the .5-2% of normal PBMC. 3) FACS analysis of PBMC from 13 patients with Sézary syndrome (SS) revealed a proportion of BT5.9 cells (5.1±3.8%) significantly lower ($p < .001$) than in normal subjects. DP IV activity was present in 4.7±4% cells. In all patients SS cells were BT5.9 and DP IV negative. Morphological and immunocytochemical analysis of BT5.9+ sorted cells from these patients revealed that they were not neoplastic cells. Two subgroups can be identified by CD7 MoAb: CD4+/BT5.9-/DP IV-/CD7+ and CD4+/BT5.9-/DP IV-/CD7-. These subtypes could therefore represent the expansion of two small subsets present in normal T lymphocytes. BT5.9 and DP IV reactivity of infiltrating T cells in cryostatic sections was tested in 62 skin biopsy specimens from 50 patients with cutaneous lymphomas and benign inflammatory diseases. Both BT5.9 and DP IV were negative in 8/8 SS patients. A deficiency was observed in 12/14 mycosis fungoides (MF). Two MF cases evolved in high grade malignant lymphoma and 4/4 cases of TdT+ T cell lymphomas were BT5.9 and DP IV positive. 2/2 lymphomatoid papulosis were BT5.9 and DP IV negative, while the majority of T cells in 12/12 benign inflammatory diseases were positive. B cells in 10/10 B cell lymphomas were negative.

107

HLA-MARKERS, C4 AND 21-HYDROXYLASE GENES IN PATIENTS WITH SUBACUTE CUTANEOUS LUPUS ERYTHEMATOSUS (SCLE)

Partanen, J*, Koskimies S*, and Johansson E**.
Finnish Red Cross Blood Tranfusion Service,* Tissue Typing Laboratory*, Department of Dermatology, University Central Hospital**, Helsinki, Finland

Lupus erythematosus (LE) is characterized by abnormal production of a wide variety of autoantibodies and immuno-complexes but the etiology and pathogenesis of LE is unknown. Genetic studies have revealed that LE is associated with certain major histocompatibility complex (MHC) markers especially haplotype HLA B8 DR3 has been reported to be over-represented. On the other hand defects in the early components of the classic complement pathway C1, C2 and C4 predispose to LE. In order to evaluate the role of the HLA markers and complement genes in LE we combined the protein level typings of the MHC class I (HLA-A,B,C) class II (HLA DR) and class III (Bf,C4) with DNA level analysis of the C4 gene region. To avoid clinical and obvious genetic heterogeneity in LE we restricted our analysis on 13 patients with subacute cutaneous LE, (SCLE) which represents a quite homogenous subgroup of LE. The HLA and Bf-typings did not bring up any obvious markers for SCLE in this series but altogether 83 % (10/12) of the patients had at least one C4 null allele, whereas this frequency was 50 % (112/254; $p \leq 0.025$) in the controls. By combining the DNA level and protein level analyses one can expect to be able to obtain more accurate determination of the C4 null alleles, because variations in patients' C4 levels make the evaluation difficult solely on the basis of protein typing. In four of the nine patients studied the DNA level analysis showed that at least one of the C4 and closely linked steroid 21-hydroxylase (21-OH) genes was was deleted. Furthermore, two novel polymorphisms with the restriction enzyme Xba I in the C4 genes were observed. Our results suggest an important role for C4 null alleles and gene re-arrangements in SCLE.

108

PLETHYSMOGRAPHICALLY RECORDED DIGITAL BLOOD FLOW SHOW DIAGNOSTIC PATTERNS in PRIMARY RAYNAUD and SLERODERMA PATIENTS G.Wagner, A.Jageneau, H.Mensing
Department of Dermatology, University of Hamburg FRG
Janssen Research Laboratories, Beerse, Belgium

Raynaud's phenomenon is one of the most important early signs of the so called connective tissue disorders. About 5 - 10% of patients suffering from this phenomenon later on develop scleroderma (PSS).
Therefore differentiation of primary Raynaud (RP) and secondary Raynaud as precursor of PSS is helpful for the estimation of the individual prognosis.

50 patients (32 PSS, 18 RP) and 20 healthy volunteers were investigated using a semi-continuously working ECG-triggered plethysmograph (Periflow, Janssen). Measurements were performed under various environmental temperature conditions. Digital arterial blood flow, calculated as the change of finger volume after fast venous occlusion during one systole, were used to characterize the different types of reaction patterns.
Figures from the recorded finger flow variations measured under various temperature conditions demonstrate typical vector curves for each of the investigated groups: Of the 20 healthy volunteers 19 showed a similar temperature dependent increase and decrease of the vector, followed by a spontaneous rewarming. In 14 of the 18 RP patients a strong falling-off after cooling was noted, the rewarming after stop of cooling typically was protracted. In 24 of the 32 PSS patients a delayed falling-off of the finger flow was measured. Closing of vessels took place in lower temperatures than in the RP patients. The vector curves of 3 of the remaining 8 PSS patients (early stage of disease) resemble to the Raynaud type. 5 could not be classified.
In further studies 15 PSS-patients were treated with ketanserin, a selective antagonist of serotonin in order to ameliorate Raynaud attacks. After a 3 - 6 month treatment intervall 11 of 15 patients declared a subjective improvement. This could be confirmed in all cases using this plethysmographic measurement, whereas patients without improvement didn't show any change in their vector curve.

109

PLATELET SECRETION AND THROMBIN ACTIVITY IN PATIENTS WITH PROGRESSIVE SCLEROSIS (PSS) G. Goerz[2], P. Kind[2], V. Burstedee[1], R.E. Scharf[1] Department of Internal Medicine[1], Department of Dermatology[2], University of Düsseldorf, FRG

Scleroderma is the third frequent rheumatic disorder. Although its etiology and pathogenesis are unknown, the primary event is postulated to be endothelial cell injury in small blood vessels. Since it is conceivable that activation of platelets and coagulation may be involved in the pathogenesis of PSS, the present pilot study was designed to investigate blood platelet secretion and thrombin activity in vivo in patients suffering from PSS. Reliable indices of these parameters are plasma and platelet concentrations of platelet specific proteins B-thromboglobulin (BTG) and platelet factor 4 (PF4) and plasma levels of fibrinopeptide A (FPA), a thrombin-induced fibrinogen metabolite.

According to the extension of skin lesions, PSS can be staged into degrees ranging from I to III (I: hands involved only; II: additional proximal lesions on the arms; III: skin lesions spread to involve the upper part of the chest, abdomen or back). Using this clinical score, we studied II patients with PSS (10 female, 1 male; age ranging from 34 to 64 years). Three of them were classified of having stage I, eight had stage II. BTG, PF4 and FPA were evaluated by radioimmunoassays. Twenty age-matched healthy volunteers served as controls.

The mean plasma levels of platelet specific proteins were slightly increased in patients with PSS (BTG: mean 36,3 ng/ml, range 22-70 ng/ml; PF4: mean 7,2 ng/ml, range 4-13 ng/ml) in comparison to healthy subjects (BTG: mean 19 ng/ml, range 5-30 ng/ml; PF4: mean 3,6 ng/ml, range 0-6 ng/ml). Six (54%) of the II patients revealed BTG plasma concentrations greater than 30 ng/ml. Evaluation of intraplatelet concentrations of the alpha granular constituents showed reduced levels in patients (BTG: mean 32 ug/10^9 pla., range 23-41 ug/10^9 pla., PF4: mean 13,4 ug/10^9 pla., range 9-18 ug/10^9 pla.; normal ranges: BTG: mean 57 ug/10^9 pla., range 40-92 ug/10^9 pla.; PF4: mean 18 ug/10^9 pla., range 13-32 ug/10^9 pla).

The mean plasma level of FPA (5,6 ng/ml, range 1,7-12,8 ng/ml) was elevated in patients with PSS compared to normal subjects (mean 1,5 ng/ml, range 0-2,5 ng/ml), whereby 9 (82%) of the patients showed FPA plasma concentrations above 4 ng/ml.

This pilot study indicates that platelet secretion and thrombin activity are increased in the majority of patients with PSS at stage I and II. Thus, our data support the hypothesis that activation of platelets and coagulation is involved during the course of PSS. However, it remains to be assessed whether or not abnormal hemostasis is an epiphenomenon in PSS. At the present, one can not exclude that activation of platelets and/or coagulation add to vascular injury and vessel wall proliferation in the pathogenesis and during progression of systemic sclerosis.

110

IN VITRO STUDIES ON THE ROLE OF PEPTIDO-LEUKOTRIENES IN EXPERIMENTAL MURINE CONTACT DERMATITIS. Thomas Rosenbach, Miklos Csato, and Beate M. Czarnetzki. Dept. of Dermatology, University Hospitals, Munster, F.R.G.

Leukotrienes have been demonstrated in various inflammatory dermatoses, including contact dermatitis (CD). Their significance in the induction or maintenance of CD is however unclear. In the present investigation, we have studied a very potent peptidoleukotriene receptor antagonist, Ro 23-3544, for its effect on irritant and allergic CD in mice. Balb/c mice were sensitized twice with 15 µl of 0.5% DNFB in acetone/olive oil, 1/4 on the abdomen and were challenged two days later on both ears. Irritant dermatitis was induced with a single painting of 0.5% croton oil in acetone/olive oil. Ro 23-3544 was dissolved in PEG 400 or acetone/olive oil, 1/4, with similar final results. Concentrations of 0.1, 0.5, 1.0 and 5.0% were applied one week before elicitation of CD or daily for up to 5 days on one of the elicited ears. Normal untreated or Ro 23-3544 treated ears served as additional controls. Ear thickness was measured with a spring loaded micrometer. Pretreatment with Ro 23-3544 caused a significant decrease ($p < 0.05$) of allergic, but not of irritant CD. The data suggest that peptidoleukotrienes play a role in the initiation of allergic, but not of irritant CD, but their inhibitors are unable to suppress an established CD.

111

METABOLISM OF 12-HYDROXYEICOSATETRAENOIC ACID (12-HETE) BY HUMAN SKIN IN VIVO AND IN VITRO. Julia A. Newton, Robert M. Barr, Anthony I. Mallet and *William R. Otto. The Institute of Dermatology, St. Thomas's Hospital, London, UK. and *The Department of Histopathology, The Royal Postgraduate Medical School, Hammersmith Hospital, London, UK.

Lesional psoriatic skin contains raised levels of 12-HETE which may result from increased skin 12-lipoxygenase activity, increased arachidonic acid substrate concentration or decreased metabolism. Metabolism of 12-HETE has not been extensively studied but it is known that polymorphonuclear cells can make 12,20-dihydroxyeicosatetraenoic acid (12,20-diHETE). In this study we investigated the synthesis of 12,20-diHETE by human skin in vivo and by epidermal cells in vitro.

Scale from psoriatic skin was obtained by gentle abrasion of lesions with a scalpel blade. Skin exudates were collected into phosphate buffered saline in plastic chambers fixed to the abraded skin for 30 min. Samples were extracted and purified by HPLC before identification and semi-quantitative analysis by gas chromatography-mass spectrometry (gc-ms). Fresh epidermal cell suspensions and 2nd passage keratinocytes, prepared from neonatal foreskins, were incubated with (^{3}H)12(S)-HETE for periods from 10 min to 2hr. The culture medium and cells were analysed for 12,20-diHETE by HPLC and liquid scintillation counting.

12,20-diHETE was detected by gc-ms in scale from lesional skin and in exudates from lesional and uninvolved psoriatic skin. The measured levels (not corrected for recovery) were about 8 ng/100mg scale, 60 and 125 pg/ml for exudates from lesional and uninvolved skin respectively, (n=3). In comparison, 12-HETE has been measured in scale at levels >1ug/100mg and in exudates at about 40 and 15 ng/ml for lesional and uninvolved skin respectively. Although (^{3}H)12-HETE was metabolised to a number of products in vitro, there was no evidence for conversion to 12,20-diHETE.

In conclusion, the raised levels of 12-HETE found in lesional psoriatic skin are unlikely to be due to decreased metabolism to 12,20-diHETE although we cannot exclude decreased metabolism by some other pathway.

112

ENHANCEMENT OF PROSTAGLANDIN (PG) D_2 SYNTHESIS AND APPEARANCE OF ENDOGENOUS PEROXIDASE (PO) IN BONE-MARROW DERIVED MAST CELLS (BMMC) TREATED WITH DEXAMETHASONE (DM)

MICHEL Laurence*, PITTON Christina*, PROST Catherine*, MENCIA-HUERTA Jean-Michel*, BENVENISTE Jacques* and DUBERTRET Louis*. *INSERM U 312, Hôp. H. Mondor, 94010 Créteil, FRANCE.* INSERM U 200, 32 rue des Carnets, 92140 Clamart, FRANCE.

Mouse BMMC, derived from culture of bone marrow precursors in presence of concanavalin A-conditioned medium, have been shown to be ultrastructurally and functionnally analogous to mucosal mast cells (MC). Particularly, immunologically-stimulated BMMC, as well as mucosal MC, generate very low amounts of PGD_2. Enhancement of PGD_2 synthesis is induced in BMMC by treatment with 1µM DM for 24 h.

PO, an endogenous enzyme destroyed by cell fixation, has been associated with PG synthesis in human platelets and monocytes. PO has been localized in the perinuclear envelope (NE) and in the endoplasmic reticulum (ER) of connective tissue MC.

We examined the synthesis of PGD_2 and the appearance of PO in BMMC treated with 1 µM DM for 24h and up to 14 days.

After IgE sensitization and specific antigen stimulation, BMMC treated with DM for 24h and 14 days released respectively 2.6 and 7.6 times more PGD_2 than untreated cells, reaching 58.03 ± 6.15 ng/10^6 cells after 14 days of DM-treatment.

When the diaminobenzine (DAB) reaction was carried out prior to the cell fixation, PO was localized in NE of 24h-treated BMMC and in both NE and ER of 14 day-treated BMMC, whereas no PO was detected in untreated cells. When the cell fixation was carried out prior the DAB reaction, no PO was detected in NE or ER of both treated and untreated BMMC.

In conclusion, short- and long-term treatment of BMMC with DM induced the enhancement of immunologically-induced PGD_2 release and the appearance of PO in NE and ER. Thus, DM-treated BMMC share some of the characteristics of mature connective tissue-type MC. Moreover, our results suggest that endogeneous PO, destroyed by cell fixation, could be involved in PG biosynthesis in DM-treated BMMC.

113

THE EFFECTS OF EPIDERMAL GROWTH FACTOR (EGF) ON ARACHIDONIC ACID METABOLISM IN HUMAN SKIN IN VIVO. R. Camp and A. Mallet, Institute of Dermatology, London, England.

EGF (up to 0.1 µg per ml) has been reported to stimulate the release of free arachidonic acid (AA) from pig epidermis during 5-30 min. incubations in vitro (Aoyagi et al., J. Invest. Dermatol. 84 168-171, 1985). In an investigation of the mechanism of activation of AA metabolism in inflammatory skin diseases such as psoriasis, we have studied the capacity of EGF to stimulate the release of free AA from human skin in vivo.

In six healthy male volunteers aged 21-41, the skin of the flexor aspect of each forearm was scraped with a scalpel blade to give 3.5 cm^2 abrasions to which plastic chambers were fixed with cyanoacrylate adhesive. Following washing with 1 ml phosphate buffered saline for 5 min., a sterile solution of murine submaxillary gland EGF (25µg) in 1 ml 80 mM phosphate buffer, pH 7.2, containing 2% mannitol and 0.025% human albumin, and control solution without EGF, were placed in the paired chambers of each volunteer under double blind conditions. After 1 h, chamber fluid was removed, placed in tubes containing 100 ng [$^2H_8$]-AA internal standard, acidified and extracted into ethyl acetate. Following evaporation of the organic phase, residues were purified on a Nucleosil 5 C18 analytical HPLC column eluted with methanol/water/acetic acid (93:7:0.01) at 1 ml per min. Appropriate fractions (8.6-10 min) were collected, evaporated and derivated to yield tertbutyldimethylsilyl (tBDMS) esters. The derivatives were subjected to gas chromatography-mass spectrometry with monitoring of ions characteristic of the tBDMS esters of AA and the deuterated internal standard (m/z 361 and 369 respectively). The amount of AA in the samples was quantified by reference to a standard calibration curve.

The assay gave AA levels of 62 ± 25 ng per ml in EGF containing chamber fluid and 81 ± 34 ng per ml in control chamber fluid (mean ± S.D., n=6). These results therefore do not support a role for EGF in the activation of AA metabolism in human skin in vivo.

114

A PSORIATIC SCALE-DERIVED CHEMOTACTIC PEPTIDE (ANAP) IS A SPECIFIC ACTIVATOR OF NEUTROPHIL FUNCTIONS. U.Mrowietz,* J.-M.Schröder,* E.Morita* T.Ternowitz,* E.Christophers* Depts. of Dermatology, Universities of Kiel,* Kiel, FRG, and Aarhus*, Aarhus, Denmark

One of the most prominent histological features of psoriasis is the presence of neutrophil granulocytes (PMN) in the epidermis. However the process of PMN accumulation remains unknown.

In search for epidermis derived leukotactic substances a 12.5/15 kD anionic peptide (ANAP) could recently be identified in psoriatic scales which differs from interleukin 1 in various aspects. This peptide can activate a number of PMN functions in vitro, i.e. chemotaxis, lysosomal enzyme release and superoxide-anion (O_2^-)-production, whereby ANAP elicited chemotaxis was found to be the most sensitive. To further characterize the activating potency of ANAP (partially purified from psoriatic scales by ion-exchange followed by exclusion - and reversed phase HPLC) we investigated the chemotactic activities in response to ANAP in highly purified monocytes, eosinophils as well as B- and T-lymphocytes in comparison to PMN. Additionally, monocytes were tested for ANAP-induced ß-glucuronidase release and O_2^--generation and compared with PMN functional activities. The chemotaxins f-met-leu-phe as well as complement splitproduct C5a served as positive controls for all four cell types studied.

As a result, ANAP showed strong PMN activating potency (pool-dilution 1:1 to 1:128) with a halfmaximal response at a pool-dilution of 1:32 for chemotaxis of the preparation used. In contrast, monocytes, eosinophils as well as B- and T-lymphocytes showed no chemotactic responsiveness towards ANAP. Also, there was no ANAP-induced ß-glucuronidase release as well as O_2^--production from monocytes. In comparison, monocytes, eosinophils and B- and T-lymphocytes showed a normal activation pattern when stimulated with f-met-leu-phe or C5a.

The results of our study indicate, that ANAP can be regarded as a highly specific activator of neutrophil functions of which chemotactic migration is the most sensitive. Thus, ANAP could be of great inportance for the elicitation of neutrophilic tissue responses.

115

EXPRESSION OF GAMMA INTERFERON INDUCED 96K ANTIGEN AND HLA-DR, HLA-DQ AND HLA-DP ANTIGENS IN PRIMARY AND METASTATIC MELANOMA.
H. van Vreeswijk, D.J. Ruiter, E-B. Broecker, K. Welvaart and S. Ferrone. Departments of Pathology (H.v.V., D.J.R.) and Surgery (K.W.), University Medical Center, Leiden, The Netherlands, Department of Pathology, University of Nijmegen, Nijmegen, The Netherlands (D.J.R.) Department of Dermatology, Wilhelms University of Münster, FRG (E-B.B) and Department of Immunology and Microbiology, New York Medical College, Valhalla, NY, USA. (S.F.).

To investigate the role of immune interferon (IFN-γ)in the in vivo expression of HLA class II antigens by melanoma cells, 55 primary and 33 metastatic surgically removed melanoma lesions were stained in indirect immunoperoxidase with anti HLA-DR, DQ and DP monoclonal antibodies and with the monoclonal antibody CL 203.4 to a 96K melanoma associated antigen (MAA). The latter represents a useful marker to monitor local production of IFN-γ and susceptibility of melanoma cells to modulation by IFN-γ, since it is barely expressed by control melanoma cells, but is highly susceptible to induction by IFN-γ. In the primary melanomas staining for HLA-DR, HLA-DQ and HLA-DP antigens was found in 67%, 49% and 33% of the lesions studied respectively, and for the 96K MAA in 71%. A high degree of concordance in the reactivity pattern of individual lesions stained for HLA-DR antigens and for the 96K MAA was found. In addition, a statistically significant association ($p<0.05$) was demonstrated between the degree of intratumoral lymphocytic infiltrate and the expression of HLA-DR and HLA-DQ antigens. In metastases 94%, 73%, 84% and 100% of the lesions tested were stained by anti HLA-DR, DQ, DP and 96K MAA monoclonal antibodies, respectively. Therefore, this investigation has confirmed that HLA class II antigens are expressed in a higher percentage of metastatic than of primary lesions and that the gene products of the HLA-D region are differentially expressed both in primary and in metastatic lesions. Furthermore, the present investigation suggests that the regulatory mechanisms which control the expression of HLA class II antigens in primary and metastatic melanoma lesions are different; and that IFN-γ may play a role in the expression of HLA class II antigens in primary lesions, but not in metastatic lesions.

116

THE CYTOTOXIC EFFECTS OF 4-HYDROXYANISOLE IN B16 MELANOMA CELL LINES.
I. Oliver, G.V. Sherbet*, and A.J. Thody, Department of Dermatology and *Cancer Research Unit, University of Newcastle upon Tyne, England.

It has been shown that 4-hydroxyanisole (4-OHA) has melanocytotoxic effects and anti-tumor activity in mice bearing transplantable Harding-Passey melanomas (Dewey, Butcher & Galpine [1977] J. Pathol. 122,117). Its mechanism of action is, however, not yet clear. According to one view the phenol is converted, under the influence of tyrosinase, to toxic intermediates and it is these that bring about cell death (Riley [ed] Hydroxyanisole: Recent advances in anti-melanoma therapy. IRL Press, Oxford). In the present study we have examined the cytotoxicity of 4-OHA on three different B16 melanoma cell lines that show differences in tyrosinase expression.

B16F1, B16F10 and B16BL6 melanoma cells were cultured in Eagles MEM with Earles Salts, 10% fetal calf serum, antibiotics, non-essential amino acids, sodium pyruvate, sodium bicarbonate, L-glutamine and added vitamins for up to 4 days. Under these conditions all cells expressed tyrosinase activity although the F1 cells displayed twice as much activity as the other two lines. The cytotoxicity of 4-OHA was assessed by growing the cells in varying concentrations of the drug (10^{-3} - 10^{-6} M) and comparing the number of viable cells remaining after 4 days with that in control dishes containing no 4-OHA.

The lower concentrations (10^{-6} and 10^{-5} M) of 4-OHA had little cytotoxic activity. At 10^{-4} M 4-OHA however, survival was decreased to 10 and 15% in the E1 and F10 cells and to only 5% in BL6 cells. At 10^{-3} M 4-OHA survival was approximately 1% for all three cell lines. The presence of α-MSH (10^{-7} M), which increased tyrosinase activity in all three cell lines, had no effect upon the cytotoxicity of 4-OHA.

These findings confirm that 4-OHA has cytotoxic properties in melanoma cells. However, they do not support the view that the cytotoxicity of 4-OHA is related to the tyrosinase activity of the cell.

117

MONITORING OF DRUG SENSITIVITY BY FLOW CYTOMETRY. A COMPARATIVE ANALYSIS OF MELANOMA CELL CULTURES AND XENOGRAFTS.
W. Tilgen, B. Hennes, M. Weigand, D. Haag*. Universitäts-Hautklinik und *Pathologisches Institut der Universität Heidelberg, FRG.

Despite a wealth of information about preclinical testing of drug sensitivity of tumor cells, none of the experimental models have been introduced into clinical routine investigation.

To elucidate difficulties preventing the clinical application, we analyzed the proliferate behavior of three melanoma cell lines with different origin, morphology, growth rates, DNA-stem lines, and derived xenografts after treatment with 3 cytostatic agents-DTIC, THP (Doxorubicin), ACM (Aclaplastin) to flow cytometry. Additionally, cell lines were tested for viability by recultivation. Volume reduction of xenografts was followed by caliper measurements in two dimensions.

Testing different cell lines with the same drug, DNA-histograms exhibited different reaction patterns: No change, cell arrest in the S-phase or accumulation in the G_2M-phase.

Testing the same cell line with different cytostatics out of the anthracycline group, divergent effects have been observed, too.

Even when testing the same cytostatic on the same cell line but on different subcultures changing effects resulted.

When comparing cell lines to the respective xenografts in nude mice, again different results were obtained.

In all cases, however, changes of the DNA-distribution pattern were dose dependent.

Thus, flow cytometry cannot classify potentially useful cytostatic drugs in this predictive assay. On the other hand it may reflect the inter- and intra-individual heterogeneity of malignant melanomas and the unpredictable clinical course of disease.

118

LARGE SCALE CULTIVATION OF HUMAN MELANOCYTES IN A MICROCARRIER CULTURE SYSTEM
Nico Smit, Wiete Westerhof, Shaffy S.Asghar, Stan Pavel, Anwar H.Siddiqui, Rudi H.Cormane. Department of Dermatology. Academisch Medisch Centrum. Univ. of Amsterdam. The Netherlands.

To culture human melanocytes in sufficient numbers for in vitro studies we investigated the possibilities to grow melanocytes in a microcarrier system. Pure melanocyte monolayers were obtained from split skin newborn fore skin following the method of Eisinger et al (1). The problem of fibroblast contamination was overcome by treatment with the antibiotic geneticin (2) and by using Falcon Primaria T-flasks. We checked for purity of our melanocyte culture by DOPA-staining and staining with leucine aminopeptidase. Several monoclonal antibodies specific to cytoplasmic and membrane proteins of melanoma cells were tested for reactivity with melanocyte antigens. Replating of cells was done after short EDTA treatment. Clustered cells were seeded in wells to form colonies.

Single cell suspensions from these colonies showed preferential attachment to cytodex 3 in comparison to cytodex 1 microcarriers. When cytodex 1 and cytodex 3 microcarriers were seeded into a monolayer of melanocytes only migration of melanocytes onto cytodex 3 was observed.

In a ratio of 10 to 1 melanocytes and cytodex 3 microcarriers were then dispersed into plastic tubes with culture medium and kept in suspension on a rotating turn-table (10 r.p.m.). After 20 hours all cells adhered to the microcarriers. These results showed the possibility of culturing melanocytes in an automatic system, in which environmental conditions such as 0_2, CO_2, temperature, etc. are controlled to the optimal level (3).

Large scale production of melanocytes (10^9 cells) will enable us to perform studies on melanin synthesis which were sofar done in vivo (4).

1) Eisinger M., Marco O. Proc. Natl. Acad. Sci. USA 1982;79:2018
2) Halaban R., Alfano F.D. In Vitro 1984;20:447
3) van Wezel A.L. J.Chem.Tech.Biotechnol. 1982;32:318
4) Westerhof W., Pavel S., Kammeyer A., Beuseberg F., Cormane R.H. J. of Invest. Dermatol. 1986;87:174

119

IMMUNOELECTRON MICROSCOPIC CHARACTERIZATION OF HUMAN NATURAL KILLER CELLS RELATED TO THE EXPRESSION OF CD16 AND LEU-19 ANTIGENS.
Gian Carlo Manara, Corrado Ferrari, Paolo Sansoni, and Giuseppe De Panfilis, Departments of Dermatology, Pathology, and Medicine, Parma University, Parma, Italy.

The relevance of natural killer (NK) cells in various cutaneous deseases has been underlined in recent investigations. NK cells are a population of lymphoid cells that are able to lyse certain tumor cells and virally infected cells without major histocompatibility complex restriction. Most NK cells express the CD16 (Leu-11) antigen, which is associated with the Fc receptor for IgG. Recently, a new antigen, named NKH-1 (Leu-19), has been identified on the surface of a subset of NK cells. The present study was intended to investigate the correlated expression of CD16 and Leu-19 antigens on peripheral blood NK cells, and to characterize the ultrastructural features of Leu-19$^+$ cells.

Mononuclear cells, from normal human peripheral blood, were isolated by using Ficoll-Hypaque. Monocytes were depleted by adherence to plastic tissue culture flasks. B lymphocytes and residual adherent cells were removed by passing the cells through nylon wool. E rosetting and non-E rosetting cells were isolated by a single step rosetting method by using AET-treated sheep red blood cells. Null cells were subsequently incubated with: 1°) anti-Leu-19 MAb; 2°) goat anti-mouse IgG antibody coupled to colloidal gold particles; 3°) mouse purified IgG; 4°) anti-Leu-11b MAb; 5°) goat anti-mouse μ chain antibody coupled to peroxidase. Moreover, both CD16$^+$ and Leu-19$^+$ panning enriched cell fractions have been functionally characterized.

More than 90% of CD16$^+$ cells expressed the Leu-19 antigen, whilst a small proportion of CD16$^+$ cells lacked expression of Leu-19 antigen. Within the CD16$^+$ cells both Leu-19$^+$ and Leu-19$^-$ cells showed ultrastructural features characterized by a low nuclear/cytoplasmic ratio, many cytoplasmic organelles, electron dense granules and an irregular cell surface with cytoplasmic extensions. Both CD16$^+$ and Leu-19$^+$ panning enriched cell fractions mediated high levels of cytotoxic capability.

This is the first report in which the ultrastructural features of CD16$^+$ - Leu-19$^+$ cells have been delineated and correlated to the one of CD16$^+$ - Leu-19$^-$ cells. We demonstrated that more than 90% of CD16$^+$ NK cells coexpress the Leu-19 antigen. On the basis of both morphological and functional characterization of Leu-19$^+$ cells, we concluded that Leu-19 antigen may be considered a useful marker in the detection of NK cells.

120

A METHOD FOR THE POST-EMBEDDED LABELLING OF T LYMPHOCYTES IN CUTANEOUS INFILTRATES BY LIGHT AND ELECTRON MICROSCOPY.
Carolyn M. Willis and John D. Wilkinson, Department of Dermatology, Wycombe General Hospital, High Wycombe, Bucks. HP11 2TT, U.K.

Identification of T cells in skin infiltrates relies on the use of immunohistochemical techniques. Unfortunately, conventional tissue processing for light and electron microscopy destroys the immunoreactivity of T cell surface antigens. As a result, immunolabelling for the light microscope is generally performed on frozen tissue, leading to poor morphology and problems of tissue storage. Pre-embedding methods, which are unreliable and technically difficult, are mainly used for electron microscopy. Recent improvements in tissue processing have enabled T cells to be visualized in paraffin and glycol methacrylate embedded tissue, however these media are unstable in the electron microscope. This study was designed to develop a post-embedded labelling technique which could be applied to both light and electron microscopy.

Using the avidin-biotin peroxidase system on 1μm sections, a series of fixatives, dehydrating agents and embedding resins were tested for their ability to retain antigens recognised by a panel of T cell markers including OKT3, anti-Leu 1, anti-Leu 4, UCHL1, DAKO-T2, RFT-1, MAS 092 and anti-leucocyte common antigen (a LCA).

Successful immunolabelling was achieved with anti-Leu 4, UCHL1 and a LCA on sections processed as follows: fixation for 4 hours at room temperature in either periodate lysine paraformaldehyde or 4% paraformaldehyde + 0.05% monomeric glutaraldehyde; dehydration in acetone at 4°C; embedding in Taab Premix resin polymerized at 55°C. Etching of sections with alcoholic sodium hydroxide prior to labelling was essential, but proteolytic enzyme pretreatment was not required. These antigens could also be detected in the same tissue sample, at the ultrastructural level, using the immunogold labelling technique.

This post-embedded labelling method should provide a useful tool for the study of T lymphocytes in the skin.

121

CD1 ANTIGENS: IMMUNOHISTOCHEMICAL REACTIVITY IN NORMAL AND PATHOLOGICAL TISSUES. Emilio Berti, Simona Muratori, Amilcare Cerri, Dario Tomasini, and Ruggero Caputo, 1st Department of Dermatology of the University of Milan.

Non-class I molecules consisting of a cell surface glycoprotein heavy chain of approx 45 Kd associated with a 12 kd β2 microglobulin have been defined in human thymocytes using monoclonal and polyclonal antibodies. MAbs directed against these antigens are referred as CD1, according to the 1st, 2nd, and 3rd International Workshop on Leucocyte Differentiation Antigens. Recently Calabi and Milstein using the MAbs Nal/34, NuT2, M241 (1) demonstrated that exist at least three different molecules recognized by antibodies belonging to the group CD1 respectively 49kd, 45kd, 43kd MW. We have tested 23 CD1 MAbs (L119, L404, L544, BL6, Nal/34, T6, L232, VIT6, VIT6b, VIT6d, 10D12, 10H39, D47, OKT6, T2/23.1, 7C4, 4A76, NuT2, WM25, L161, 10C3, M241, 7C6) on normal, activated and neoplastic cells from various tissues. Skin biopsies from normal skin and from damaged skin of patients suffering from Contact Dermatitis, Atopic Dermatitis, Mycosis Fungoides in early stage, Histiocytosis X (Letterer - Siwe and Hand-Schüller-Christian) and Self Healing Histiocytosis have been studied. In addition normal thymus and lymph nodes have been examined too. For immunological studies on fresh material skin biopsies were frozen in freon 22, cooled in liquid nitrogen, and stored at -80°. Five micron frozen sections were air dried for 12-24 hours. Immediately before staining the slides were fixed for 10 minutes in acetone at room temperature and air dried. APAAP techniques and MAbs were used to stain the sections. Three different groups of MAbs have been identified on the ground of the immunohistochemical reactivity in normal tissue corresponding to the CD1a, b, and c groups defined by Milstein (1). CD1a MAbs (Nal/34, ...) stained cortical thymocytes, interdigitating cells of lymph nodes, Langerhans cells, dendritic dermic cells. CD1b MAbs (NuT2, ...) were positive in cortical thymocytes, interdigitating cells and scattered dendritic cells in the dermis. CD1c group (M241, ...) consists of MAbs reacting with cortical thymocytes, subpopulation of Langerhans cells, all dendritic dermic cells and mantle zone B lymphocytes of the follicle. The number of cells recognized by antibodies of the CD1a group, in Contact Dermatitis and Mycosis Fungoides, is surely increased both in the dermis and in the epidermis; in addition the number of cells recognized into the epidermis by CD1c group antibodies (activated Langerhans cells?) reaches the 50-70%. The expression of the CD1b antigens in the dermis was higher in the patterns of damaged skin of patients suffering from Contact Dermatitis or Mycosis Fungoides in the early stage showing perivascular dendritic cells assemblages in apposition with T lymphocytes and a few stained cells into the epidermis with dendritic or round morphology. Histiocytosis X cells and the infiltrate of Self Healing Histiocytosis react for CD1a and CD1c MAbs, while only a few scattered cells labelled CD1b MAbs. These data permit to hypothesize the possibility of recognizing subsets of dendritic cells both in the dermis and in the epidermis. In addition the MAbs of CD1b group resulted to be negative in the cases of Histiocytosis X and in the case of Self Healing Histiocytosis, suggesting a common cellular derivation of these two diseases. Further studies are needed to clarify the role of CD1a-b-c in cell activation and differentiation. Immunoelectron microscopic studies are in progress to demonstrate the presence of Birbeck Granules in the three different groups of Dendritic Cells.

References

1. Calabi F, Milstein C: Nature 1986, Vol. 321

122

EFFECT OF EGF ON HUMAN SKIN FIBROBLASTS IN MONOLAYER AND IN COLLAGEN LATTICE. Alain Colige, Bety Nusgens and Charles M. Lapière, Department of Experimental Dermatology, Liège University, Liège, Belgium.

Receptors for epidermal growth factor (EGF) are present on the membrane of human skin fibroblasts. Their expression and function might be regulated through interactions of the cells with their support.

Fibroblasts were cultured on plastic, on polymeric type I collagen (Ip), at the surface of a hydrated type I collagen gel (Ig) and within a three-dimensional collagen lattice. The monolayer cultures were labeled with 3H-proline just before subconfluence and the lattices after 18 h, 42 h and 14 days of culture. Protein (NCP) synthesis was determined as non-dialyzable 3H-proteins and collagen synthesis from 3H-hydroxyproline measurements. As compared to cells on plastic on Ip, a specific inhibition of collagen synthesis was observed (- 31 %) without significant modification of the non collagen protein (NCP) synthesis. On Ig, the overall collagen synthesis was not significantly modified (+ 9 %) but the deposition of the newly synthesized collagen on the hydrated support (+ 113 %) and degradation (+ 99 %) were increased. In the lattice, NCP and collagen synthesis were drastically repressed already after 18 h of culture (respectively - 79 % and - 71 %), after 42 h (- 81 % and - 81 %) and after 14 days (- 87 % and - 83 %). The addition of EGF (5 ng/ml) to fibroblasts on plastic induced a specific inhibition of collagen synthesis (- 34 %). On Ip and Ig, an additional inhibition of the biosynthetic activity was observed in presence of EGF (Ip: NCP - 11 %, collagen - 38 %; Ig: NCP - 4 %, collagen - 19 %). In each case the cell multiplication was slightly stimulated. In contrast, the addition of EGF to fibroblasts embedded in a collagen lattice resulted in a significant increase of the biosynthetic activity mainly of the NCP (18 h : + 9 %, 42 h : + 51 % and 14 days : + 75 %). The opposite effect of EGF on biosynthetic activity of fibroblasts in monolayer and in the lattice does not seem to be related to the number of EGF receptors per cell neither to their Kd which were similar under these culture conditions. These results suggest that the regulation of biosynthetic activity of fibroblasts by EGF can be modulated by the extracellular matrix.

123

REGULATION OF METABOLISM OF SKIN FIBROBLASTS BY MECHANICAL TENSION. Pierre Delvoye, Betty Nusgens and Charles M. Lapière, Department of Experimental Dermatology, Liège University, Liège, Belgium.

Skin fibroblasts (F) seeded in a three-dimensional freely floating collagen gel reduce progressively the dimension of this matrix (isotonic retraction). When the reduction in size of the gel is hindered by fixing its extremities (isometric retraction), F develop mechanical forces on the aligned collagen bundles. Total protein and collagen synthesis were measured in both conditions by incorporation of 3H-proline according with JUVA and PROCKOP (Anal. Biochem., 15, 77, 1966). Collagen synthesis was increased by a factor of 2, 10 and 7 respectively on days 2, 4 and 7 in isometric as compared to isotonic conditions. An optimal stimulation was observed for the collagen deposited in the lattice on day 2, secreted in the medium on day 4 in the medium and the lattice on day 7. The proportion of collagen degradation was high on day 2 (73 % in isometric, 83 % in isotonic conditions) and decreased on day 4 (38 %) and 7 (20 %) for both conditions. The analysis of the collagen polypeptides secreted in the medium by SDS-PAGE electrophoresis revealed that the processing of the pN-collagen type I and type III was 1.5 more effective under isometric than isotonic condition. The synthesis of proteins was increased to a smaller extent than collagen in isometric compared to isotonic condition.

In isotonic condition, the reduction of length of the gel was 62 % (day 2), 66 % (day 4) and 76 % (day 7) of the initial length, while the collagen concentration was respectively increased by a factor of 27, 37 and 55. In isometric condition the increase of collagen concentration was 1.5, 1.3 and 1.2 times lower than in isotonic condition respectively on days 2, 4 and 7.

The stimulation of collagen and protein synthesis in isometric compared to isotonic conditions might be in part explained by the lower density of collagen in isometric gels. However the larger extent of the qualitative and quantitative differences observed between the two conditions suggest that other factors and mainly a mechanical stimulation is involved in the control of the biosynthetic activity of fibroblasts.

124

DERMO-EPIDERMAL SEPARATION USING THERMOLYSIN: IMPROVEMENT IN COLONY-FORMING EFFICIENCY OF HUMAN KERATINOCYTES IN CULTURE. M.J. Raxworthy, W.J. Cunliffe & E.J. Wood. Department of Biochemistry, University of Leeds, Leeds LS2 9JT and *Dermatology, Leeds General Infirmary, Leeds LS1 3EX, U.K.

Most methods for the isolation of human keratinocytes for growth in vitro employ incubation with trypsin (TR) to disaggregate cells. Because prolonged exposure to TR may be deleterious to keratinocytes we have limited this by using the proteases thermolysin (TL) and Dispase (DP) to separate dermis and epidermis prior to disaggregation of epidermis with TR and compared the cell yield and

colony-forming efficiency (CFE) or keratinocytes in culture.

Human foreskins were obtained by routine surgical circumcision from donors aged 5 months, 16 and 26 years. An estimate of surface area was made and the samples were divided into three portions, each of which was cut into 1 x 10 mm strips and placed in 5 ml of the appropriate protease solution, namely TR (1:250, Difco) 0.25% in phosphate buffered saline, DP (neutral protease, Grade II, Boehringer) 2 mg/ml in culture medium, or TL (protease type X, Sigma) 0.5 mg/ml in culture medium. All were incubated at 4°C for 18-20h and after removal from the protease solution, the epidermis was gently stripped from the dermis. The epidermal strips were incubated for 1h at 37°C in 0.1% TR plus 0.1% glucose in phosphate buffered saline. Keratinocyte cultures were established and maintained for 10 days. After fixing and staining colonies containing five or more cells were counted and the CFE was determined.

The ease of dermo-epidermal separation was greatly increased when TL or DP rather than TR was used. This is reflected by the yield of keratinocytes per cm^2 of tissue following separation and disaggregation of cells (TL 13.4 x 10^5, DP 10.5 x 10^5, TR 5.1 x 10^5). The mean CFEs were TL 0.41% DP 0.32%, TR 0.25% (n = 9 for each protease). The CFE obtained with TL differed significantly from that obtained with TR at the $P < 0.1$ level. The results indicate that the use of TL to effect dermo-epidermal separation is advantageous, compared with TR, as assessed by the cell yield and subsequent CFE of keratinocytes in culture. The effects may be due to the greater specificity of TL and to the protective effects of serum-containing culture medium during incubation.

125

EFFECT OF Ca^{++} ANTAGONISTS AND - AGONISTS ON CULTURED EPIDERMAL CELLS UNDER LOW AND HIGH Ca^{++} CONDITIONS. B. Thiele, B. Bonnekoh, H. Merk, C. Mahrle, Department of Dermatology, University of Cologne, FRG.

Ca^{++} plays an important role in epidermal cells, e.g. proliferation and differentiation. Ca^{++} antagonists have been shown to influence the function of heart and smooth muscle cells and fibroblasts. They protect heart muscle cells from the overload with Ca^{++} and cell necrosis. On the other hand, Ca^{++} agonists affect endothelial cells and heart muscle cells by enhancing the Ca^{++} influx into the cells. There is no information of the effect of Ca^{++} modulators on epidermal cells.

In the present study guinea pig epidermal cells were cultured under low (1.0mM) and high (2.7, 3.6mM) conditions for 7 days. They were continuously exposed to the Ca^{++} antagonists nifedipine and verapamil (0.2, 0.5, 1.0µg/ml) or to the Ca^{++} agonist BAY K8644 (0.2, 0.5, 1.0µg/ml). Cell cultures were monitored by H^3-thymidine incorporation, trypan blue staining and cell counting. The cell-bound Ca^{++} was measured in the cell homogenisates by photometry.

Under low Ca^{++} conditions nifedipine and BAY K8644 dose-dependently reduced DNA-synthesis and the cell number, whereas verapamil had no effect compared to untreated controls. The cell-bound Ca^{++} was diminished only by nifedipine (50%) but not by verapamil or BAY K8644. High Ca^{++} concentrations inhibited cell spreading, reduced the cell number and the cells showed vacuolization. Nifedipine protected the cells and increased the cell number. Verapamil and BAY K8644 were ineffective under high Ca^{++} concentrations.

From the three tested drugs apparently only nifedipine had an inhibited cell proliferation in vito under low Ca^{++} conditions and protected the cells under high Ca^{++} conditions.

126

CHARACTERIZATION OF NEONATAL RAT EPIDERMAL CELLS SEPARATED BY DENSITY GRADIENT CENTRIFUGATION. Geoffrey Allan and Christopher P.F. Redfern, Department of Dermatology, University of Newcastle upon Tyne, England.

Some aspects of epidermal differentiation can be studied by isolating cell subpopulations at successive stages of maturation. The differentiation of keratocytes in accompanied by changes in morphology, and a decrease in cellular buoyant density. The aim of this study was to fractionate neonatal rat epidermal cells by density gradient centrifugation, and to describe some of their biochemical and cellular properties. Epidermal cell suspensions from 1 day old Wistar rats were separated into four fractions, F4 (most dense), F3, F2 and F1 (least dense) by isopycnic centrifugation on discontinuous density gradients of Percoll. Cells in the less dense fractions were progressively larger and more irregularly shaped than F4 (basal) cells. 80% of F1 cells gave cornified envelopes while less than 1% of cells in the other fractions did so. Particulate transglutaminase activity per viable cell was in the ratio F4:F3:F2:F1 of 1:2:3:10, i.e. an approximately 3 fold rise between F4 and F2, and between F2 and F1. Cytosolic transglutminase activity showed only a 1.5 fold increase between F4 and F1. Examination of SDS soluble proteins by 2-D electrophoresis demonstrated a greater diversity of keratins in F1 and F2 compared with F3 and F4. The 53.5 and 66 kdalton keratins characteristic of terminally differentiated cells were present only in F1 and F2. Protein synthesis in cells of different fractions was examined by [^{35}S]-methionine labelling. Incorporation of label into total protein per cell decreased from F4 to F1 but a greater proportion was incorporated into water insoluble proteins. Patterns of protein synthesis as shown by 2-D gel electrophoresis, varied between the fractions, particularly between F3 and F2.

127

OBJECTIVE ASSESSMENT OF DYSPLASIA. S.P. Barton, A.D. Pearse and R. Marks, Department of Medicine, University of Wales College of Medicine, Cardiff, U.K.

Dysplasia - a disordered pattern of growth - is taken to represent an early stage in the neoplastic process. In such lesions as solar keratoses, the characteristics of dysplasia are normally assessed subjectively by pathologists. Objective quantification of the degree of dysplasia would improve this assessment and permit the process of neoplasia to be more adequately studied. To this end we have used Image Analysis of histological sections to derive measurements of epidermal thickness, nuclear fraction (%), mean and standard deviation of nuclear profile area and a nuclear shape factor.

In 12 patients presenting with histologically confirmed solar keratosis, skin from the lesion and unaffected paralesional skin was formalin fixed and sectioned, together with distal unaffected (normal) skin from unexposed sites in 10 of these individuals. Assessment, made blindly, of the degree of dysplasia was measured by these objective criteria and compared with subjective assessment made independently under the same conditions by one of us (RM) using a visual analogue scale (VAS). A dysplasia index (DI)* formulated from the above measurements was compared to the subjective assessment and found to correlate (Spearman's rank correlation rho = 0.67, $p<0.001$). In addition, the DI was significantly different between the normal and lesional skin ($p<0.001$ Kruskal-Wallis analysis of variance/Mann-Whitney U test). These results show that objective assessment is capable of measuring dysplasia. This allows the clinician greater insight into the nature of the lesion, increasing diagnostic and prognostic information.

*DI = Epidermal thickness + mean nuclear area + SD nuclear area + shape factor Nuclear fraction

TABLE:

	Dysplastic Index derived from:		
	NS	PS	L
Mean = S.D.	57.40 = 9.78	63.96 = 17.73	93.07 = 22.12

NS = Normal skin
PS = Paralesional skin
L = Lesion

128

RECONSTRUCTION OF SPECIFIC TUMOR ARCHITECTURE BY A HUMAN SQUAMOUS CARCINOMA CELL LINE GROWN ON DE-EPIDERMIZED DERMIS. M. Régnier and M. Darmon, Cell Biology Department, Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, F-06565 VALBONNE, FRANCE.

TR146, a continuous cell line established several years ago from a human squamous cell carcinoma (Rupniak, H.T.R., J. Natl. Cancer Inst., in press), exhibits limited morphological differentiation when grown on conventional plastic dishes. However, when TR146 cells are seeded on human de-epidermized dermis, they acquire the morphology of squamous cell tumors with typical nodule and horn pearl formation. The distribution in such "reconstructed tumors" of differentiation markers, such as bullous pemphigoid antigen, 67kD suprabasal keratin, 56kD "hyperproliferative" keratin, involucrin, membrane-bound transglutaminase, and filaggrin, was found to be very similar to their distribution in squamous carcinoma specimens. Moreover, the degree of differentiation of TR146 cells was extremely sensitive to culture conditions such as retinoic acid concentration, emersion of the cultures, etc. ...

These results show that an established cell line is able to recover its morphogenetic potential when cultured in an environment close to the in vivo situation.

129

EPITHELIAL ANTIGEN ALTERATIONS IN CUTANEOUS AND MUCOSAL PAPILLOMAS. J. Viac, Y. Chardonnet, H. Gilgenkrantz and J. Thivolet. INSERM U.209, CNRS UA.601, Pavillon R., Hopital E. Herriot, 69437 Lyon, Cedex 03, France.

Human papillomaviruses (HPV) induce epithelial proliferations with alterations of cell differentiation process depending on viral expression and virus type. The incidence of HPV infection (virus capsid antigen or DNA) on epithelial antigen expression in human papillomas was studied by indirect immunofluorescence on frozen sections of 50 cutaneous and mucosal lesions with histological features of papillomas, using a panel of specific antibodies to membrane and cytoplasmic antigens of epithelial cells.

In most lesions, differentiation antigens (keratins, involucrin, desmosome related antigens) and HLA class I antigen (β2-microglobulin) were modified: - slight modifications of basal cells indicated a more advanced state of Keratin differentiation: - involucrin, a precursor of cross-linked envelop was considerably increased: - desmosome related antigens were modified in granular layers of cutaneous lesions: - β2-microglobulin was preserved in the lower part of the papilloma epithelium but disappear in the granular layer.

Viral antigen was detected with a polyclonal rabbit antiserum raised to heated SDS-purified virus and HPV DNA sequences by in situ hybridization with biotinylated probes. Viral DNA was more frequently detected (80%) than viral capsid antigen (40%). Interestingly the epithelial cellular alterations described here were found in papillomas with viral DNA in absence of virus capsid antigen.

Taken together these results show that the more drastic modifications were observed in cutaneous warts with severe cytopathic effect. The alterations of most markers were observed in upper layer cells whereas basal cells exhibited only advanced program of keratinization.

130

EFFECT OF EGF AND RETINOIDS ON THE PROLIFERATION OF SQUAMOUS CARCINOMA CELLS. Maria Ponec, Johanna Kempenaar, Arie Weerheim, Department of Dermatology, University Hospital, Leiden, The Netherlands.

Using a number of cultured squamous carcinoma cell (SCC) lines with a varying capability to differentiate we could recently demonstrate that an inverse relationship exists between the EGF receptor expression and the ability of cells to differentiate. The EGF binding capacity was highest in poorly differentiating SCC-4 cells, medium in moderately differentiating SCC-15 cells and low in well differentiating SCC-12F2. In all types of cells the induced decrease of differentiating capacity (by culturing cells under low Ca^{2+} conditions) led to a marked increase in EGF binding capacity.

EGF is known to be a potent mitrogenic modulator in a great variety of cells. Therefore, the present study was undertaken to investigate the possible effects of EGF on the proliferation of SCC cells and to correlate these effects with the EGF_2 binding capacity. For this purpose the cells were grown either under low Ca^{2+} (0.06 mM) or normal Ca^{2+} (1.6 mM) conditions in the presence of increasing amounts of EGF (0 to 20 ng/ml). A marked inhibition of cell proliferation has been observed for all types of cell and under both culture conditions at EGF concentrations above 0.1 ng/ml.

Since retinoids were found to modulate the EGF binding, experiments were also performed in which the effects of various retinoids all-trans retinoic acid (RA), 13-cis retinoic acid (Ro 4-3780), etretinate (Ro 10-3959), etretin (RO 10-1670), arotinoid acid (Ro 13-7410) and arotinoid ethylsulfane (RO 15-1570) in the absence or presence of EGF on the proliferation of SCC cells were studied. All retinoids used were found to inhibit the proliferation of all SCC lines used in a dose-dependent fashion. The extent of inhibition occurred in the following rank order: Ro 10-1670 = Ro 10-9359 < Ro 4-3780 < RA < Ro 15-1570 = Ro 13-7410. The extent of inhibition was found to be more pronounced when EGF was also added.

These results suggest that no correlation exits between the number of EGF binding sites and the effects of EGF on proliferation in SCC lines studied. Furthermore, the modulating effects of EGF and retinoids on the proliferation of SCC cells were found to be partially additive.

131

EXPRESSION OF EGF RECEPTOR, INVOLUCRIN AND CYTOKERATINS IN BASAL CELL AND SQUAMOUS CELL CARCINOMAS. Sjan Lavrijsen, Linda M. Tieben, Maria Ponec, Jan Gerrit van der Schroeff and Goos van Muijen*, Departments of Dermatology and Pathology*, University Hospital Leiden, The Netherlands.

The distribution of several markers of keratinoctye differentiation was studied in normal epidermis, basal cell carcinomas (BCC) and squamous cell carcinomas (SCC), using an immunoperoxidase technique on frozen sections of punch biopsies. As markers we used a large panel of chain-specific monoclonal antibodies (MoA) directed against cytokeratins 4,8, 10, 13, 18 and 19 as well as a MoA against the epidermal growth factor (EGF) receptor. Furthermore, we used antiserum against involucrin, a precursor of the cross-linked envelope protein. This antiserum was kindly provided by Dr. H. Green. Expression of EGF receptor was found in the basal cell layers of the normal epidermis, in all cells of BCC's and in the peripheral layers of SCC's. Involucrin was expressed in the upper layers of normal epidermis and in the center of SCC's. No expression was seen in BCC's except for some squamous horn cysts.

Staining with the MoA's against cytokeratins was positive for numbers 8 and 10. Cytokeratin 8 was seen in almost all BCC's but in none of the cell layers of the adult epidermis and cytokeratin 10 was found in SCC's as well as in the suprabasal layers of normal epidermis. MoA's against the other cytokeratins showed no positive staining.

In conclusion, our study reveals differences in staining pattern between normal epidermis, BCC's and SCC's. We confirmed that involucrin and cytokeratin 10 are markers for differentiating keratinocytes. On the other hand, EGF receptor stains undifferentiated keratinocytes. The presence of cytokeratin 8 in BCC and its absence in normal epidermis may be an indication of dedifferentiation, also because it is known to be present in early fetal epidermis. Studying markers of keratinocyte differentiation may lead to a future cell-type related subclassification of various skin tumours.

132

IMMUNOGOLD LABELLING OF BASEMENT MEMBRANE ANTIGENS AFTER FREEZE SUBSTITUTION. Robin A.J. Eady, Andrew R. Kennedy, Adrian H.M. Heagerty, Institute of Dermatology, United Medical and Dental Schools, University of London, London, U.K.

We have previously shown that low-temperature post-embedding immunogold techniques can provide useful information on the ultrastructural localization of epidermal basement membrane (EBM) associated antigens in human skin. Because of the liability of bullous pemphigoid (BP) antigen, labelling with BP antisera is consistently poor in fixed tissue. We are now reporting our preliminary immunoelectron microscopic data on labelling of various EBM components after using freeze substitution (FS) instead of chemical fixation.

Very small (< 1mm diam.) samples of unfixed normal skin were cryoprotected with 20% glycerol in PBS. They were then plunge frozen in liquid propane at -190°C, substituted with 97% methanol at -85°C for 36hrs in a Reichert CS auto machine and embedded in Lowicryl K11M which was then polymerized under UVR at -65° C. Thin sections were incubated with antibodies to laminin (LN) and type IV collagen (IVC) and with BP antisera. Anti-rabbit or anti-human IgG-5nm gold conjugates (Janssen Pharmaceutica) were used in the second step. Our results confirmed our previous findings on the distribution of LN and IVC in both lamina lucida and lamina densa of the EBM with both antigens localizing mainly to the lamina densa. BP antigen was associated with hemidesmosomes at both intra- and extra-cellular sites. The cellular preservation was generally good and the cell membranes intact and clearly visible.

We conclude that FS offers an excellent preparative method for the localization of certain antigens that are affected by chemical fixation. Ultrastructural preservation compares favorably with that obtained with (para) formaldehyde and other fixatives routinely used for immunoelectron microscopy.

133

DIAGNOSIS AND ASSESSMENT OF SMALL SKIN TUMOURS USING PULSED A-SCAN ULTRASOUND. M. Al-Aboosi, C. Edwards and R. Marks, Department of Medicine, University of Wales College of Medicine, Cardiff, UK.

In recent years several ultrasound techniques have been applied to the study of skin. Initially the A-scan technique was used for skin thickness measurement, but more recently attempts have been made to image skin using commercial high frequency B-scanners. However, the resolution of these latter images is still at best approximately 0.3 mm in the depth axis and approximately 1 mm in the lateral axis. Therefore fine detail of the structure of tumours is not presented.

In order to move the use of ultrasound techniques into the diagnostic field, we have used an A-scan system with high resolution (0.05mm in depth, <0.5mm in the lateral dimension) to characterise and distinguish between small skin tumours The echo trace from skin contains information on the structure, physical nature and dimensions of the interrogated tissue. By carefully recording and analysing the echoes from various tumours and correlating these data with clinical and histological information we have been able to identify the type of tissue comprising the tumours from the echographic traces. The sonographic morphologies of 20 basal cell carcinomas (BCC) were analysed in terms of their echo pattern and their histological appearance. Similar analyses were performed for dermatofibromas and keloids, and from pigmented lesions of the melanocytic type, lesions of Bowen's disease, and cysts. The echo characteristics of these tumour types were sufficiently distinctive to allow differential diagnosis between BCC and the above mentioned tumours. The BCC A-scan shows an area of low amplitude with regularly spaced signals from the tumour mass, and clear signals from underlying tissues. Scar tissue (fibrosis) A-scans also show low amplitude echoes from the lesion, but their spacing is highly irregular. Keloids are easily distinguished by the large reflections from their thick walls, surrounding an echolucent zone within the lesion. This paper will describe in detail the ultrasonic diagnostic

discriminants of BCC tumours and will illustrate the differences with respect to other small tumours which exhibit similar clinical appearances.

This application, plus the existing method of measuring depths of invasion of malignant melanomas, establishes that the A-scan technique is an advance in the non-invasive diagnosis and assessment of skin tumours.

134

COMPARISON BETWEEN LIGHT AND ELECTRON MICROSCOPY USING HIGH-RESOLUTION IMAGE AND MULTIVARIATE ANALYSIS. Wilhelm Stolz *, Wolfgang Abmayr **, Christian Schmoeckel *, and Susanne Korherr **, * Department of Dermatology, University of Munich and ** Gesellschaft f. Strahlen- und Umweltforschung mbH Munich, FRG

We recently investigated the ability of high-resolution image analysis to distinguish between nuclei of malignant melanomas (MM) and nevocytic nevi (NN) in electron microscopy. We found that by using chromatin features alone 86.7 % of the individual nuclei, and 100 % of the cases were correctly classified as benign or malignant.

The aim of this study was to test the correlation between light and electron microscopy using 3 karyometric (area, perimeter, and shape factor), and 18 chromatin structure (CS) features derived from the difference between the scanned image and the median smoothed image describing the relationship of hetero- and euchromatin. In addition, 6 features were derived from the co-occurence matrix (Co-oc). Briefly, the Co-oc features describe the amount of homogeneity of the chromatin structure by evaluating the statistical differences between the intensity of a given pixel and those adjacent to it.

Semithin and thin sections of 163 intraepidermal nuclei of NN and 76 of MM were specially prepared so that the identical nuclei could be investigated in EM and LM. Multivariate analysis was performed.

The best cell by cell correlation was found for the karyometric features (0.97 for the nuclear area, 0.87 for the perimeter, and 0.84 for the shape factor) indicating, that the identical cells were measured in LM and EM. The correlation of the Co-oc features between LM and EM varied between 0.53 - 0.62 for MM and 0.46- 0.53 for NN. The 0.001 significance level tested against zero for MM was 0.38 and for NN was 0.27. The correlation coefficients for the CS features were not significant (0.18- 0.26).

In conclusion, there is a significant correlation between LM and EM for the Co-oc features. Therefore, these features might be useful in light microscopy for distinguishing between MM and NN in both the individual nuclei as well as the lesions.

135

PMNS MIGRATION THROUGH DENUDED SKIN AREAS : A NON INVASIVE AND QUANTITATIVE METHOD TO STUDY EPIDERMAL CICATRIZATION IN VIVO IN MAN. P. Saiag, C. Lebreton C. Gely, L. Dubertret, INSERM U 312, Hôpital Henri Mondor - 94010 CRETEIL - FRANCE

In order to obtain a quantitative method for studying epidermal wound healing *in vivo* in man, we have studied the decrease of PMNs migration into skin chambers stuck over purely epidermal defects obtained by suction.

Reproductible purely epidermal wounds were produced by mild suction (-500 g/cm^2). After removal of the blister rooves, sterile skin chambers were stuck over the denuded areas and filled with 1 ml of sterile collecting medium. The migrating PMNs were counted every 24 h and the collecting media replaced by fresh ones (thus providing constant chemotactic stimuli throughout the experiment) until cell migration became undetectable. To study the reproductibility of the measurements, 2 suction blisters of 12.5 mm^2 were performed on each forearm of 10 individuals (age: 21-25). Hank's medium (HM) and autologous serum (AS) were used to fill the first and second chamber on each forearm. To compare the healing of different sized wounded areas, suction blisters of 12.5 and 38.5 mm^2 were performed simultaneoulsy on a forearm of 9 volunteers (age: 20-27) (collecting medium:M 199).

A progressive decrease of PMNs migration was observed, suggesting a progressive decrease of the area available for PMNs migration. Skin biopsies, performed when PMNs migration had stopped, showed total epidermal healing. After wounds of 12.5 mm^2, a complete cicatrization was achieved in 5.5 ± 0.6 days (m ± sd) with HM as collecting medium and in 5.8 ± 0.7 days with AS (no significant difference, Student t test for paired values). The coefficient of variation of the measurements between the 2 homologous skin chambers filled with the same medium was 11 ± 6%, assessing the reproductibility of the measurements. In the second set of experiments, total epidermal healing was achieved in respectively 7.6 ± 0.5 and 6.3 ± 0.4 days after wounds of 38.5 and 12.5 mm^2 ($p < 0.01$, Student t test for paired values).

The reproductibility of the measurements allowed us to develop a mathemathical model of epidermal wound healing. For same sized wounded areas, the total cumulative number of migrating PMNS depended on individuals and on the collecting medium used (PMNS were much more numerous if AS was used). Assessing that, in a given experiment, N (Si, t) (cumulative number of PMNs collected at time t after an initial wound Si) and S (Si, t) (surface of the wounded area at time t) vary according only to t (time) and Si (initial surface of the wound) and that d N (Si, t) /d t is a linear function of S (Si, t), the following formulas were developed for the stationary state of cicatrization : $N(Si, t) = \frac{kSi}{\lambda}(1-e^{1-\lambda t}) \leftrightarrow n(t) = \frac{N(Si, t)}{N(Si, \infty)} = 1 - e^{-\lambda t}$.

In these formulas, λ is a constant of cicatrization rate, characteristic of an individual in a given experiment and k is a constant reflecting PMNs migration speed. Numerous tests were performed to validate this model using the datas obtained from the second set of experiments. 1) At t =24 h. (when keratinocyte migration has not begun), the amount of PMNs collected is proportional to Si. 2) No significant difference (Student t test for paired values) was observed in λ calculated for a same individual after wounds of 38.5 (.049±.003) and 12.5 mm2 (.056 ±.005)(t expressed in hours, N (Si, t) in millions of cells). 3) Logarithmic regressions of the datas gave a linear pattern.

This non invasive technique gave quantitative and reproductible results. That could be used for studying in vivo the consequences of drugs on human epidermal wound healing.

136

SPECIFIC INDUCTION OF METALLOTHIONEIN IN HAIRLESS MOUSE SKIN BY ZINC AND DEXAMETHASONE

P. Kind°, G. Goerz°, C. Mullin+, G. Prings°, J. Abel+
° Department of Dermatology, University of Düsseldorf FRG
+ Institute of Environmental Hygiene Düsseldorf FRG

Metallathioneins (MT) are metal binding proteins that are widely distributed in mammals, invertebrates, plants and microorganismus. MT's can be induced by various heavy metals and MT is also transcriptionally controlled by glucocorticosteroids. Although much is known about MT, its exact physiological function remains to be elucidated. We studied increased MT m RNA accumulation and MT synthesis in mouse skin after intraperitoneal administration of Zn and Dexamethasone.

Hairless mice (HR/HR Henkel) were injected i.p. at different concentrations. 6 mm punch biopsies were taken and MT was quantified using the Cd saturation assay. Specific metallothionein m RNA induction was determined by Northern analysis. A significant increase of MT mRNA was already seen after 4 hours when compared to controls.

The dose response for dexamethasone and Zn was conducted at 18 hours. A maximum response was seen between 50-100 mg/Kg dexamethasone and 10 mg Zn^{++}/kg. There was a dose dependant increase in MT.

mouse	Dexamethasone 1 mg/mouse (25g)	Zn 0,25 mg/mouse (25g)	Control
12	2,60±0,58	2,30±0,66	1,00±0,25
18	2,40±0,47	1,64±0,28	0,96±0,12
24	1,16±0,21	1,40±0,47	1,12±0,12
48	1,20±0,18	1,50±0,23	1,26±0,06

The data cleary demonstrate that MT is present in mouse skin and can be specifically induced by dexamethasone and Zn.

137

ONCOGENE EXPRESSION IN CULTURED FIBROBLASTS IN PSORIASIS. Nicole Basset, Jean-Marie Blanchard, Alain Vie, and Jean-Jacques Guilhou, Clinique de Dermatologie Hôpital Saint-Charles, et Laboratoire de Biologie Moléculaire USTL, Montpellier, France.

An abnormal high rate of proliferation has been described in cultutred fibroblasts from involved (I) and uninvolved (U) psoriatic skin and the capacity for psoriatic fibroblasts to stimulate the growth of normal keratinocytes has recently been demonstrated. These data could result from inappropriate expression of cellular oncogens (c onc) since they carry out functions that are critical to cellular growth and differenciation.

Four c onc (c-Myc, c-Myb, Erb-B, N Ras) were studied in cultured fibroblasts from I and U psoriatic skin (N = 6) and compared with normal subjects (N = 3). RNA was analysed by hybridization with nick-translated cloned human DNA probes after extraction by the guanidinium thiocyanate/LiCl procedure, electrophoresis and transfer on nitrocellulose. An invariant house keeping gene was used as internal control to take into account possible variations in the amount of RNA loaded into the gel.

No differences in c-Myc and c-Myb expression could be detected in I and U psoriatic skin compared with controls. N Ras did not give a detectable signal and c Erb-B exhibited individual variations which were not linked to the disease.

In this work only quantitative modifications of normal c onc were investigated. Consequently the results do not rule out subtle qualitative changes of these genes. Moreover, an abnormal expression of other c onc remains possible in psoriasis.

138

RETINOID TREATMENT OF HUMAN PSORIATIC FIBROBLASTS INDUCES AN INCREASE IN CYCLIC AMP DEPENDENT PROTEIN KINASE ACTIVITY. Françoise Raynaud Wayne B. Anderson, Danièle Evain Brion, Unité INSERM 188, 74 avenue Denfert Rochereau, 75014 Paris, France.

We recently showed a deficiency of cAMP dependent protein kinases in different cells of psoriatic subjects (PNAS, 83, 5272-5276). Retinoïds have been used with success as therapy in psoriasis ; these compounds have been shown to increase cyclic AMP dependent protein kinases during differentiation of tumoral cells. Therefore in this work the effects of retinoïds on cAMP dependent protein kinases of fibroblasts from seven normal subjects and seven psoriatic patients were studied. The levels of RI and RII present in control and retinoic acid treated cells were quantitated by photoaffinity labeling with 8-azido-P^{32} cAMP. In psoriatic fibroblasts the levels of RII are decreased or undetectable compared with that of normal fibroblasts both in the cytosolic and membrane fractions. The amount of RI was normal in the cytosol of fibroblasts of five out of seven patients and decreased in two. Membrane associated levels of RI were decreased in five patients and normal in two. Retinoic acid treatment induces an increase in the amount of RI and RII regulatory subunits when they are deficient in the cytosolic and membrane fractions of psoriatic fibroblasts. Retinoic acid has no effect on RI and RII in normal fibroblasts. In addition with in vitro retinoic acid treatment the cAMP dependent protein kinase activity, measured in the fibroblasts of four psoriatic patients, was increased in the cytosol in two patients and in the membranes in all four patients. In these studies, comparable results were obtained with fibroblasts cultured from involved and uninvolved skin. This in vitro effect of retinoïds on cAMP dependent protein kinases in psoriatic fibroblasts may help to explain some of the in vivo therapeutic effects of retinoïds.

139

ACTIVATED MACROPHAGES IN PUSTULAR PSORIASIS. C.Schubert and E.Christophers, Dept. of Dermatology, University of Kiel, Kiel FRG

In previous studies earliest changes seen in initial psoriasis consisted in activated macrophages in the upper dermis. In addition macrophages were noted to be the first inflammatory cells migrating into the epidermis. In association with intraepidermal macrophages there was a loss of desmosome tonofilament complexes as well as intercellular widening (spongiosis). We have now investigated the role of macrophages in fully developed lesions of pustular psoriasis (Barber type). Histologically these lesions are characterised by the presence of large numbers of intraepidermal polymorphonuclear leukocytes (PMN) as the predominant inflammatory cell type. Electronmicroscopically there were no signs of degranulation of PMN, but when these cells are studied in long standing pustules signs of necrosis were present. Interestingly high numbers of intraepidermal macrophages were noted especially between the corneocytes above the pustules. These cells showed all signs of enhanced secretory activity. This is in contrast to the ultrastructural features of intraepidermal macrophages in the lower spinous layer or to macrophages seen in initial psoriasis. These cells contain mitochondria, free ribosomes, a small amount of rough endoplasmatic reticulum as well as some lysosomes but only few secretory vesicles. On the other hand in macrophages surrounding the pustules cell organelles signaling secretory activity predominate. Besides a rather extensive rough endoplasmatic reticulum, bundles of microfilaments and a variable number of mitochondria the cells exhibited a well developed Golgi-area with coated pits and coated vesicles as well as numerous secretory granules on its trans-face. These secretory vesicles arising in the Golgi-area are known to export secretory

products to extracellular destinations. It seems likely that secretory activity of macrophages is linked to the subsequent transepidermal migration of PMN and therefore essential for the development of skin lesions in pustular psoriasis.

140

ENZYMATIC QUANTIFICATION OF POLYMORPHONUCLEAR LEUKOCYTES IN NORMAL AND PSORIATIC SKIN FOLLOWING STANDARDIZED INJURY. A. Chang, P.C.M. van de Kerkhof, G.J. de Jongh, P.D. Mier, Department of Dermatology, University of Nijmegen, The Netherlands.

Histological studies following sellotape stripping have revealed only discrete PMN exocytosis in some patients with unstable psoriasis (Hautarzt 1981; 32; 169-172). Recently an ultrasensitive method for the quantification of PMN in skin infiltrates has been developed: the fluorometric measurement of PMN elastase (Brit J Dermatol 1986; 115; 181-186). Using this method we tried to answer the following questions: (i) What is the dynamics of PMN accumulation in normal skin following sellotape stripping? (ii) Is there a difference in this respect between healthy controls and the clinically uninvolved skin of psoriatic patients?

In total 12 healthy controls and 12 psoriatics participated in this study. At 2, 4, 8, 16, 24, 48, 72 and 168 hours following sellotape stripping razorblade biopsies were taken and processed for the measurement of elastase. In all biopsies the endogenous inhibition of elastase was measured, using a standard preparation of blood derived PMN.

We made the following observations: (i) As early as 2 hours following sellotape stripping PMN elastase activity could be shown and its presence persisted for at least 24 hours in all 24 subjects. PMN accumulation was followed in all subjects by the induction of inhibitory activity for elastase. (ii) Elastase activities or inhibitor induction in the clinically uninvolved skin of psoriatics were not significantly different from the values in healthy controls. However patients with unstable psoriasis tended to much higher elastase activities. These results suggest that the degree of PMN exocytosis following surface trauma is essentially normal in stable chronic plaque psoriasis but increases in unstable phases of the disease.

141

PROLIFERATION AND COMPARTMENTATION OF EPIDERMAL CELLS FROM NORMAL AND PSORIATIC SKIN IN VITRO. Hans-Joachim Schulze and Gustav Mahrle, Department of Dermatology, University of Cologne, Cologne, West Germany.

For a clear cut separation of acanthotic epidermis from the dermis and the isolation of a relatively pure fraction from the germinative cell pool we recently introduced a two step separation technique combined with a microassay for culturing of small quantities of epidermal cells.

0.6 cm punch biopsies of normal human skin (NS), of lesional (LPS) and non-lesional areas of psoriatic skin (NPS) were incubated in dispase (1.5 U/ml) at 4°C for 6 h followed by 0.3% trypsin at 37°C for 2 min. Isolated cell suspensions were seeded on collagen coated microtiter plates at a density of 2×10^5 cells per dish (ø 0.4 cm) and grown in MEM, containing antibiotics and 10% FCS. In order to study cell proliferation in relation to compartmentation of epidermal cells in vitro, cell cultures were monitored by immunoautoradiography at day 0, 1, 3, and 5 after seeding, combining pulse H^3-thymidine labeling for 1 h and immunostaining with an antibody (VM-2; Mörhenn VB et al, J Clin Invest 76:1978-1983, 1985) which exclusively stained basal cells of normal and psoriatic epidermis.

Immediately after isolation, keratinocytes were negative for H^3-thymidine labeling, and equal compartments of basal and suprabasal cells were demonstrated for NS, NPS, and LPS (VM-2 positive vs. VM-2 negative = 1 : 1). 24 h after seeding, the total number of attached cells was decreased to approximately 70% in cultures of NS and NPS and to 55% in LPS. Keratinocytes from NPS grew almost identically to cells from NS and reached seeding density at day 5 whereas cells from LPS only reached 80% of seeding density at this time. The relation of basal to suprabasal cells changed to 3 : 2 in NS and NPS at day 5 whereas in cultures of LPS VM-2 positive cells never exceeded 45% of attached cells. On the other hand, the total H^3-thymidine labeling index of cells from NS and NPS increased more rapidly and was slightly higher at day 3 and 5 than that of cells from LPS. With respect to the compartmentation with VM-2, the H^3-thymidine labeling index of VM-2 negative cells significantly decreased in keratinocytes of NS and NPS after day 1 of seeding whereas it stayed high in cell cultures of LPS.

The present study introduced immunoautoradiography as a more precise definition of cell proliferation in vitro. It showed that isolated epidermal cells from LPS had a different compartmentation of proliferating cells in comparison to NS or NPS.

142

TISSUE DISTRIBUTION OF TYPE VII COLLAGEN, THE MAJOR STRUCTURAL COMPONENT OF THE ANCHORING FIBRILS. L. Bruckner-Tuderman, B. Odermatt & U.W. Schnyder, Departments of Dermatology and Pathology, University Hospital of Zurich, Zurich, Switzerland.

Type VII collagen is a major structural component of the anchoring fibrils in human skin. However, the initial molecular characterization of this protein was done with molecules isolated from the foetal membranes which are not known to contain fibrillar structures similar to the anchoring fibrils. Therefore, type VII collagen must represent a connective tissue component of wider distribution and possibly other functions.

In this study, the tissue localization of human type VII collagen was investigated. 20 different tissues were stained with immunofluorescence technique using affinity absorbed polyclonal antibodies. Type VII collagen could only be found in tissues of ectodermal origin. It is always localized in a subepithelial zone in a narrow band-like pattern, especially under epithelia exposed to mechanical stress. Tissues showing a strong fluorescence were skin, mucous membranes (oral, nasal, vaginal, bronchial), transitional epithelia of the bladder, chorio-amniotic membranes and cornea. Exocrine glands like salivary glands and sweat glands of the skin showed a weak staining in the basement membrane region of the acini but more pronounced staining around secretory ducts. Type VII collagen as tested by immunofluorescence was absent in nerves, muscle, liver, spleen, kidney, blood vessels (aorta, arteries, veins, capillaries), villous placenta, thyroid and in the basement membrane zone of gastrointestinal epithelia.

There was no clear co-distribution with type IV collagen, the major basement membrane collagen. For instance, kidney and villous placenta which are rich in basement membranes and type IV collagen do not contain any measurable amounts of type VII collagen.

In summary, type VII collagen represents a structural component of the subepithelial zone in ectodermally derived tissue. An interesting open question remains its function in other tissues than skin and its defects in pathological conditions.

143

A NEW SKIN-EQUIVALENT MODEL: GROWTH OF HUMAN EPITHELIA FROM EPIDERMAL CELLS ON DERMAL EQUIVALENTS RETRACTED IN ONLY ONE DIMENSION. E. Tinois*, M. Faure*, C. N'Guyen**, J. Kanitakis*, M. Tardy**, J.L. Tavot, J. Thivolet*, *INSERM U.209, Hopital E. Herriot, Lyon, France. **Institut Merieux, Marcy l'Etoile, France.

In order to improve the attachment of cultured human epithelia grown from normal keratinocytes on surgical wounds, we recently developed a new model of skin-equivalent. It consists of a dermal equivalent made up of human fibroblasts in a collagen matrix that is only contracted in thickness, and of an "epidermis" developing from keratinocytes plated on its surface. Human fibroblasts (10^5 and 5×10^4 cells/ml) in DMEM with 1-2 mg/ml of human type III + I collagen supplemented with 10% FCS were added in culture dishes on human fibroblast monolayers. Within four days that lead to the formation of thin translucid dermal equivalents adhering to the bottom of the plates and on which keratinocytes in suspension could be seeded. These epidermal cells were plated before and after retraction of the lattice. Human keratinocytes obtained in suspension through trypsinization of confluent primary cell cultures (cells grown on 3T3 feeder layers) were cultured in a 3:1 mixture of DMEM + HAM F12 with 10% FCS, Adenine, hydrocortisone, insulin, T3, transferrin, cholera toxin and EDF. Epithelia on the collagen lattices were mechanically detached from the plates and examined by conventional microscopy, immunofluorescence and electron microscopy. They corresponded to multilayered epidermal equivalents with keratinocytes possessing various markers of differentiation (desmosomes, tonofilaments, keratohyalin granules). Although condensations of the basal cell membrane were occasionally observed, neither true hemidesmosomes nor a lamina densa were clearly visible.

These data show that a skin-equivalent with a dermal lattice retracted in only one dimension can be easily reconstituted in vitro. The adhesion between its two components is similar to that obtained in previous models of artificial skin. The advantages of this new model have to be evaluated in grafting experiments.

144

INFLUENCE OF DERMAL FIBROBLASTS ON EPIDERMALIZATION - Bernard Coulomb, Corinne Lebreton and Louis Dubertret - Laboratoire de Dermatologie, Hôpital Henri Mondor, 94010 Créteil, France.

The human living skin equivalent culture model, composed as in vivo of a dermis and an epidermis, provides a way to study the influence of normal dermal fibroblasts on epidermalization.

Human dermal fibroblasts are combined with collagen, serum and culture medium. The collagen have been extracted by acetic acid from rat tail tendons. Almost immediately a gel forms which the fibroblasts begin to contract. Within a few days, the contraction is stabilized and a tissue of firm consistency is formed : the dermal equivalent (1). In this three dimensional collagen matrix, fibroblasts are in a state of differentiation similar to that observed in vivo (2). This dermal equivalent (DE) was shown to be a good substrat for epidermalization and the implantation of a skin biopsy permits quantitative evaluation of the proliferation and differentiation of the newly formed epidermis (3).

We used this dynamic tissue culture system to study the influence of the fibroblasts on the epidermalization. At different steps of the rearrangement of the collagen matrix by fibroblasts, cells are killed by an osmotic shock. So, substrats of different consistency can be made, in which fibroblasts can be alive or dead. Epidermalization is then initialized by sticking on these substrats either very thin 1 mm in diameter skin punch biopsies (Epidermis and superficial dermis) or 2mm in diameter biopsies made in succion blister roof (Epidermis alone).

-The epidermalization is better on a collagen matrix that have been previously reorganized by the fibroblasts, than on a simple collagen gel.

-The presence of the fibroblasts of the biopsy promotes the epidermalization when no living fibroblasts are in the DE, but this effect is masked when fibroblasts are alive in the DE.

-For a same collagen texture, the epidermalization is promoted when the fibroblasts of the dermal equivalent are alive.

These results underline the importance of the dermal fibroblasts on the dermal-epidermal interactions. Fibroblasts not only remodel the extracellular matrix, but also secrete growth factors.

References : (1) Bell, E., Ivarsson, B., Merrill, C. Proc. Natl. Acad. Sci. USA, 76 : 1274-1278, (1979) - (2) Coulomb, B., Dubertret, L., Merrill, C., Touraine, R., Bell, E. Brit. J. of Dermatol., 111, suppl. 27, 83-87, (1984) - (3) Coulomb, B., Saiag, Ph., Bell, E., Breitburd, F., Lebreton, C., Heslan, M., Dubertret, L. Br. J. of Dermatol., 114, 91-101, (1986).

145

MODULATION OF THE BIOSYNTHETIC ACTIVITY OF HUMAN SKIN FIBROBLASTS BY TUMOR CELLS. Agnès Noel*, Betty Nusgens*, Jean-Michel Foidart# and Charles M. Lapière*, Department of Experimental Dermatology* and of Gynecology#, Liège University, Liège, Belgium.

During invasion and metastasis, tumor cells come into contact with interstitial stroma. Interactions between neoplastic cells and stromal cells could induce a modulation of the biosynthetic activity of both types of cells. In the present work, we studied the effect of tumor cells on collagen synthesis by human skin fibroblasts. MCF 7 (human invasive breast adenocarcinoma cell line) and normal skin fibroblasts were cultured either separately (monoculture) or cocultured at a cell ratio of 1:1. Eighteen hours after seeding, cells were labeled for 24 hours by 5-3H-proline (10 µc/ml) in DMEM supplemented with 10 % FCS and ascorbic acid (50 µg/ml). Total non-dialyzable 3H-radioactivity was considered as protein synthesis while collagen synthesis and degradation were calculated respectively from non dialyzable and dialyzable 3H-hyroxyproline.

MCF 7 cells in monoculture did not synthesize collagen while in monoculture of fibroblasts, 17 % of the proteins secreted in the medium was collagen. The collagen deposited in the pericellular matrix represented only 1 % of the insoluble proteins. In coculture with MCF 7 collagen synthesis by fibroblasts measured between 24 and 48 hours of contact was 1,5 to 2 times higher than in monoculture. The type of collagen polypeptides synthesized in the presence of tumor cells was not modified. Non collagen protein synthesis was unchanged. The proportion of newly synthesized collagen deposited in the pericellular matrix was also unchanged.

Tumor cells may therefore influence extracellular matrix metabolism of the surrounding stroma cells.

146

CORRECTION OF EXCESSIVE COLLAGEN SYNTHESIS OF SCLERODERMA FIBROBLASTS IN VITRO BY FACTOR XIII (FXIIIa). Marc Paye, Pierre Delvoye, Betty Nusgens and Charles M. Lapière, Department of Experimental Dermatology, Liège University, Liège, Belgium.

Fibroblasts collected from lesions of progressive systemic acrosclerosis (PSS) display, in some cases, an excessive collagen production. This observation has been related to fibrosis of the skin and internal organs. Administration of FXIIIa of blood coagulation has been reported to be beneficial to some of the PSS patients. Our study aimed at investigating the effect of FXIIIa on the biosynthetic activity of skin fibroblasts from normal and PSS patients.

Fibroblasts were cultured in monolayer on plastic and in a three-dimensional collagen lattice. Total protein and collagen synthesis by reticular (R) and papillary (P) dermal fibroblasts from an active lesion (forearm, a) and from an uninvolved area of the skin (abdomen, u) of a PSS patient and from the whole dermis of a normal subject (N) were determined by metabolic labeling for 24 h with 3H-proline in presence or absence of FXIIIa. Pa and Ra fibroblasts displayed a slight (in monolayer) or unsignificant (in lattice) increase in protein synthesis while a large increase in collagen synthesis was observed for these two cell strains under both culture conditions. The addition of FXIIIa (1 U/ml) in monolayer repressed collagen synthesis of Ra and Pa to the level observed for N, Ru and Pu cells in absence of FXIIIa. No effect on collagen degradation was noted. A similar repression of collagen synthesis was demonstrated for Pa and Ra in the lattice. For all cell strains (a, u and N) the presence of FXIIIa in the lattice largely increased the degradation of newly synthesized collagen.

Our results demonstrate that the excessive collagen production by scleroderma fibroblasts can be reduced to normal by FXIIIa in vitro through inhibition of collagen biosynthesis. This observation represents a biochemical support to the therapeutical effect of FXIIIa in scleroderma patients described by Thivolet et al. (La Nouvelle Presse Médicale, 4, nr.39, 2779-2782, 1975).

147

THE EFFECT OF L-652,343 ON THE CYCLO-OXYGENASE AND 5-LIPOXYGENASE ARACHIDONATE PATHWAYS IN LESIONAL PSORIATIC SKIN IN VIVO. Anne Kobza Black, Pauline M. Dowd, Robert M. Barr, Osamu Koro, Kay Mistry and Malcolm W. Greaves, Institute of Dermatology, St. Thomas's Hospital, London SE1 7EH.

3-hydroxy-5-trifluoromethyl-N-[2-(2-thienyl)-2-phenyl-ethenyl]-benzo (B) thiophene-2-carboxamide (L-652,343) is a potent 5-lipoxygenase and cyclo-oxygenase inhibitor in vitro with an IC50 of 0.3 µM for inhibition of LTB_4 synthesis by isolated human and rat polymorphonuclear cells and 1.5 µM for inhibition of rat seminal vesicle cyclo-oxygenase activity. However, in other studies leukotrine B_4 (LTB_4) release from whole blood stimulated with ionophore was not inhibited. L-652,343 suppressed carrageenan induced paw oedema (20 mg/kg) and adjuvant arthritis (<3 mg/kg) in the rat.

In psoriasis there is elevated 5-lipoxygenase activity in the lesional skin which may be important in the pathogenesis of the disease. We have measured the effect of orally administered L-652,343 on LTB_4 and prostaglandin (PG) inhibition in the lesional skin.

Eight patients with stable chronic plaque psoriasis received 500 and 250 mg of L-652,343 in 2 doses, 12 hours apart. A skin chamber technique was used to collect duplicate exudate samples from abraded plaques before drug and at 4, 24 and 48 h after the first dose. Exudates were analyzed for LTB_4 by a neutrophil chemokinesis assay and for PGE_2 and PGD_2 by RIA.

LTB_4 levels were not affected by L-652,343. Values (mean ± S.D., pg/ml exudate) were 100 ± 37, 83 ± 29, 97 ± 81, 91 ± 54 at 0, 4, 24 and 48 hr respectively. Prostaglandin release was significantly reduced. Values for PGE_2 (mean ± S.D., pg/ml) were 19.5 ± 8.2, 8 ± 2.5, 13.7 ± 7.6, 18.7 ± 6.6 respectively. PGD_2 behaved similarly.

L652,343 therefore acts as an effective cyclo-oxygenase inhibitor in vivo and in vitro, but 5-lipoxygenase inhibition was not demonstrated in vivo. This indicates that in vitro assays of 5-lipoxygenase inhibitors have to be interpreted with caution in the absence of corroborative in vivo measurement.

148

METHOTREXATE INHIBITS THE HUMAN C5a INDUCED SKIN RESPONSE IN PSORIASIS PATIENTS. Thomas Ternowitz, Peter Bjerring, Peter H. Andersen, Jens-M. Schröder*, and Knud Kragballe. Department of Dermatology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark, and *Department of Dermatology, University of Kiel, Kiel, FRG.

In this study we examined the effects of intradermal injections of human complement split product C5a in 10 psoriasis patients in long-term treatment with methotrexate (MTX). The C5a was injected at the end of the weekly MTX-cycle just before the intake of the first MTX-dose and 3 hours after the second of the three doses. The C5a-induced skin response was evaluated by measuring the diameter of the wheal and the area of the flare and by erythema index, which was determined objectively by reflectance spectrophotometry. In all patients the skin response was significantly depressed when C5a was injected after MTX intake. The decrease of wheal, flare and erythema index averaged 61.6%, 71.1% and 57.5% respectively, when all parameters were obtained at maximal skin response. The in vitro chemotaxis of peripheral blood neutrophils and monocytes from the patients towards C5a was markedly inhibited after intake of MTX ($p < 0.01$). The skin biopsies obtained after C5a injection before intake of MTX revealed a perivascular inflammatory infiltrate and considerable dermal edema. After MTX intake the number of infiltrating leukocytes and the degree of dermal edema was markedly reduced. This study indicates that MTX is a potent inhibitor of C5a-induced skin inflammation, and that this inhibition may be caused by a direct effect on circulating neutrophils and monocytes. The results obtained in this work support the idea that anti-inflammatory effects of MTX may be partially responsible for its anti-psoriatic effect.

149

COMPARISON OF AQUEOUS AND ORGANIC EXTRACTS OF PSORIATIC SCALES ON THE RESPIRATORY BURST OF PHAGOCYTES. Rudolf E. Schopf, Thomas Klaas, Peter Benes, Konrad Bork, Bernd Morsches, Univ.-Hautklinik, D-6500 Mainz, West Germany.

We tested the effects of aqueous and ether extracts of scales from psoriatic lesions and extracts of callus from normal individuals on the respiratory burst of phagocytes.

Aqueous extracts of freeze-dried scales from 14 patients with active psoriasis and callus from 15 healthy controls each were adjusted to 1 mg/ml protein. These extracts were also subjected to separation by fast protein liquid chromatography (FPLC) according to their molecular weight. Ether extracts (pH 3.0) were prepared from 300 mg freeze-dried material each. Dilutions of extracts were incubated with normal polymorphonuclear leukocytes (PMN) and monocytes (Mø) both in absence and presence of zymosan. Luminol-enhanced chemiluminescence served to monitor the respiratory burst.

Aqueous scale extracts as a whole induced an activation of the respiratory burst in PMN ($P < 0.05$, compared to callus). Mø were stimulated markedly by the highest concentration of scale extracts used ($P < 0.0003$, compared to callus). Scale extracts also potentiated the zymosan-induced chemiluminescence of PMN ($P < 0.01$, compared to callus) indicating opsonization, but not of Mø. Ether extracts of scales were highly stimulatory both for Mø and PMN ($P < 0.0001$, compared to callus), no opsonization of the zymosan response was found. FPLC profiles from callus were nearly all identical, by contrast, psoriatic scale extracts eluted all in a different pattern. Chemiluminescence with fractions of scales tended to exhibit the highest activity at molecular weights of < 1,000 and in the range of 200,000.

Our results indicate that aqueous and organic extracts of psoriatic scales differ in their potential to elicit oxidative mechanisms of inflammation, the organic extracts being more active. Furthermore, as psoriatic scales were composed differently in all patients examined, pooling of such scales will not allow insight into pathogenesis in individual patients.

150

PSORIASIS: IDENTIFICATION OF NEUTROPHIL ACTIVATING PEPTIDES IN SKIN ELUATES. O.Wiedow, J.M.Schröder, E.Christophers, Dept. of Dermatology, of Kiel, Kiel FRG

Although salt water bathing is commonly used in the treatment of various dermatoses, the effects of saturated salt solutions as compared to water baths in normal skin as well as in different disease states have not been studied in detail. In order to overcome the

problems involved in small volume eluates we developed a method which enables us to evaluate biologically active peptides after whole body immersion. We investigated 12 psoriatic patients. 200 l bathing water containing different NaCl-concentration between 0 and32% were used for bathing (30 min. at 37°C). The solution was concentrated 10000-fold by Amicon hollow fiber concentration combined with ultrafiltration cells (Amicon) using YM5-filters. 10-20 kD fractions obtained by subsequent G75-chromatography revealed neutrophil activating substances (as assessed by chemotaxis or ß-glucuronidase release of polymorphonuclear leukocytes, PMN). These were present in the bathing solution of 4 out of 6 patients studied at the beginning. In order to improve recovery the patients (after keratolytic pretreatment with 3% salicylic acid) were immersed in a polyethylene foil bath tube containing 2 l bathing solution. 7 experiments in 5 psoriatic patients now showed a high yield of PMN activating substances (YM5-concentration, G75-chromatography, ß-glucuronidase release of PMN). One could be shown to be C5a desarg and a further factor distinct from formylated peptides was also detected. This was identical to the previously described anionic neutrophil activating peptide (ANAP). In 3 healthy controls and 1 atopic patient no or small amounts of PMN activating substances were detectable. Furthermore in 1 psoriatic patient tap water and 27% NaCl-solution were compared. The 27% NaCl eluate showed increased chemotactic activity of PMN in contrast to tap water. We conclude that salt water bathing of psoriatic skin eluates neutrophil activating substances which are ANAP as well as the complement split product C5a desarg.

151

NEUTROPHIL CHEMOKINETIC AND INTERLEUKIN 1-LIKE ACTIVITY IN PSORIATIC SCALE: FURTHER CHARACTERISATION. N. Fincham, R. Camp, F. Cunningham and *A. Gearing, Institute of Dermatology and *National Institute for Biological Standards and Control, London, England.

We have recently described the presence of 10-30kD neutrophil chemokinetic activity which is distinct from C5a des arg and which coelutes with thymocyte co-stimulating activity on reversed phase (RP)-HPLC of lesional psoriatic scale extracts.

To further characterise this activity, extracts of bulked psoriatic scale (500mg) were ultrafiltered through Amicon YM30 membranes, purified on Sep-Pak C18 minicolumns and concentrated on YM10 membranes. The YM10 retentates were then purified by RP-HPLC and fractions assayed for chemokinetic activity by an agarose microdroplet method. Selected fractions were repurified on a TSK DEAE 5PW column eluted with a gradient of 20mM Tris acetate pH 8 to 20mM Tris acetate pH 4 over 32 min. Dialysed fractions (1 min) were assayed for chemokinetic and interleukin 1 (IL 1) activity, the latter being determined by measurement of thymidine incorporation in an IL 1-specific CTLL/EL-4 NOB-1 cell assay. The activity of native, affinity purified human monocyte IL 1 (predominantly IL 1ß) and recombinant human IL 1α were also determined in the neutrophil chemokinesis assay.

RP-HPLC of bulked scale extracts consistently revealed a peak of chemokinetic activity which was distinct from C5a des arg (n=4). Repurification of this activity by anion exchange HPLC showed several peaks of neutrophil chemokinetic material, four peaks being consistently seen in fractions 2 or 3, 17 or 18, 20 or 21, and 24 or 25 (n=4). Assay of the same fractions by the EL-4 NOB-1 method showed a number of broader peaks of IL 1-like activity , with apices usually seen in fractions 4 or 5, 17, 19 or 20, and 22 or 23 (n=4). Maximal chemokinetic activity observed in response to the two IL 1 standards was no greater than 33% of the maximal responses seen with potent chemokinetic agents such as leukotriene B_4 and zymosan activated plasma.

These results indicate that multiple neutral and acidic forms of neutrophil chemokinetic and IL 1-like 10-30kD material exist in lesional psoriatic scale extracts. The lack of precise co-purification on anion exchange HPLC suggests that the neutrophil chemokinetic and IL 1-like activities in psoriatic scale extracts are in some instances generated by different molecules. The weak chemokinetic responses to the IL 1 standards also suggest that the material producing the major chemokinetic peaks on anion exchange HPLC is likely to be different from these standards. The identity of these biologically active compounds and their importance in the pathogenesis of psoriasis and other inflammatory diseases remain to be determined.

152

THE EFFECT OF SERA FROM PATIENTS WITH RAYNAUD'S PHENOMENON ON ENDOTHELIAL CELL PGI_2 AND PGE_2 PRODUCTION. Malcolm H.A. Rustin*, Helen A. Bull#, Samuel J. Machin#, Pauline M. Dowd*, Departments of Dermatology* and Haematology#, The Middlesex Hospital, Mortimer Street, London W1N 8AA.

An inability to produce appropriate concentrations of the vasodilator prostanoids PGI_2 and PGE_2 by endothelial cells may be a factor in the pathogenesis of the digital vasospasm experienced by patients with Raynaud's phenomenon (RP). The effect of sera from normal subjects, patients with 1° RP and patients with RP in association with systemic sclerosis (SS) on the production of PGI_2 and PGE_2 by cultured human endothelial cells was investigated.

Endothelial cells were obtained from human umbilical cord veins and were cultured in medium 199 containing 20% pooled human sera. Confluent primary cultures were washed once with M199 and then were incubated with either 1, 5, 10 or 20% serum from the patients or controls for 24 hours. The cells were washed with M199 and following stimulation with the calcium ionophore A23187, PGI_2 and PGE_2 formation in the supernatant were measured by radioimmunoassay.

All sera produced a dose dependent inhibition of 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) but both the 10 and 20% sera from patients with RP and SS produced a significantly greater inhibition than the same concentrations of control sera. The mean production of 6-keto-$PGF_{1\alpha}$ expressed in ng/10^4 cells was 2.278 (normal), 1.9311 (RP) and 2.1824 (RP and SS) after incubation with 1% serum for 24 hours. This decreased to 1.3647, 0.5927 and 0.4171 respectively following incubation with 20% sera for 24 hours. This represented a 44% (normal), 76% (RP) and 83% (SS) inhibition of 6-keto-$PGF_{1\alpha}$ production compared to serum free media. Similar results were obtained after 1 hour incubation experiments. There was a non-significant decrease in mean PGE_2 production following similar incubations with 1% and 20% sera for 24 hours. Sera also were centrifuged to remove immune complexes and the same level of inhibition as untreated sera resulted.

To assess whether these findings are specific for patients with RP, the effect of sera from 15 patients with systemic lupus erythematosus were also examined.

The results suggest that factor(s) present in the sera of patients with RP may reduce the ability of endothelial cells to synthesize or release the vasodilator and antiaggregatory prostanoid PGI_2.

153

THE EFFECT OF CAPTOPRIL ON CUTANEOUS BLOOD FLOW IN PATIENTS WITH RAYNAUD'S PHENOMENON. Pauline M. Dowd*, Malcolm H.A. Rustin*, Nick E. Almond#, Julie Beacham#, Ernest D. Cooke#, Department of Dermatology*, The Middlesex Hospital, Mortimer Street, London W1N 8AA and Department of Medical Electronics#, St. Bartholomew's Hospital, West Smithfield, London EC1A 7BE.

Captopril is a potent vasodilator and acts by competitively inhibiting angiotensin II formation and the catabolism of kinins. The purpose of this study was to determine whether oral captopril increases the cutaneous blood flow in patients with Raynaud's phenomenon.

Thirteen patients with primary Raynaud's phenomenon were randomized to receive either captopril 25mg or placebo, three times daily for six weeks each, in a double blind cross-over study. Measurements of digital cutaneous blood flow by thermography (THG), laser Doppler flowmetry (LDF) and photoplethysmography (PPG) were performed at the end of a 15 minute equilibration (E) and after a 20°C cold water stress (St) at baseline and at the end of each treatment period. The frequency and severity of attacks of Raynaud's phenomenon and the ambient temperature at which they occurred were recorded in diary cards.

There was a significant improvement in cutaneous blood flow during treatment with captopril when the active treatment periods were compared to the baseline readings. LDF (E) increased by 0.473 ± 0.17 volts ($p < 0.02$) and (St) by 0.343 ± 0.142 ($p < 0.04$). PPG amplitude also was significantly increased (0.865 ± 0.372, $p < 0.04$). There was no significant change in the THG (E) during the active treatment period but THG (St) rose by 20.1 ± 7.61 integrated units ($p < 0.03$). Comparison between the placebo and baseline blood flow measurements showed no significant difference. When the active treatment periods were compared to placebo, PPG and LDF (St) were increased (1.087 ± 0.404, $p < 0.03$ and 0.308 ± 0.101, $p < 0.02$ respectively). There was no significant change in the subjective data.

These results demonstrate that captopril increases the cutaneous blood flow in patients with Raynaud's phenomenon but does not alter the frequency or severity of attacks. Thus a therapy which increases the cutaneous blood flow in these patients does not necessarily influence the triggering factors provoking vasospasm.

154

THE EFFECT OF NIFEDIPINE ON CALCIUM INFLUX INTO RED BLOOD CELLS IN PATIENTS WITH SYSTEMIC SCLEROSIS. Rademaker M, Meyrick Thomas R H, Kirby J D T, Kovacs I, Department of Dermatology, St Bartholomew's Hospital, West Smithfield, London UK.

Several studies have demonstrated significant alterations in the behavior of red blood cells (eg deformability, adhesion, etc) in patients with systemic sclerosis. These alterations may be related to an increase in intracellular calcium levels. A previous study has demonstrated that sera from patients with systemic sclerosis increases the calcium influx into normal red blood cells.

To investigate this further we have examined the effect of nifedipine on calcium influx in 9 patients with systemic sclerosis and 16 normal volunteers. Serum was collected before and ninety minutes after oral ingestion of 10 mg nifedipine. Calcium influx was then assessed by the measurement of chemiluminescence produced by the addition of either ionophore A23187 or triton X-100 to photoprotein (aequorin) loaded red blood cells which had been pre-incubated with sera for 24 hours.

Ionophore induced calcium influx decreased significantly (37.1 ± 2.1 to 29.5 ± 2.2, $p = 0.002$) following nifedipine in normal volunteers. It also fell

in patients with systemic sclerosis although did not reach statistical significance (32.1 ± 3.0 to 23.4 ± 3.8, p = 0.07). Nifedipine had no effect on spontaneous calcium influx, as measured by the total triton X-100 releasable intracellular calcium, in either controls or patients with systemic sclerosis.

Inhibition of calcium influx into red blood cells by nifedipine improves their rheological behavior and could account for the improved blood flow seen in clinical trials.

155

EPIDERMAL EXPRESSION OF HLA-DR ON KERATINOCYTES AND MONONUCLEAR SUBSETS IN TOXIC EPIDERMAL NECROLYSIS. Th. Clerici*, J.C. Roujeau**, J. Wechsler*, G. Villada*, Ph. Saiag**, Y. Pinaudeau*, *Pathology Department-**Dermatology Department, CHU Henri Mondor 94010 Creteil, France.

The Pathogenesis of Toxic Epidermal Necrolysis (T.E.N.) remain unknown. However, some clinical data, common histological features and biological abnormalities have suggested similarities between TEN and graft-versus-host reaction.

In search of additional data supporting an immune cell mediated mechanism in TEN, 7 patients were investigated for HLA-DR expression on keratinocytes and phenotypes of inflammatory cells in lesional skin.

Sections from frozen skin biopsies taken within an early erythematous prenecrotic lesions were studied with 11 different mouse monoclonal antibodies using a 3 step immunohistochemical technique (A.P.A.A.P.). In addition, immunofluorescence double staining techniques were performed.

Our studies yielded several major findings:

1) Keratinocytes of lesional non-blistering skin expressed HLA-DR antigens but none other class II HLA antigen.
2) Most infiltrating cells in dermis expressed a leu 4^+, Leu $3a^+$, HLA DR^+ phenotype and were observed in the superficial dermis or at the dermal epidermal junction.
3) Few Leu 4^+ Leu $2a^+$ cells were scattered in the dermis but this population was more abundant along epidermal basement membrane and within the epidermis.
4) Some monocytes/macrophages (Leu $M3^+$, Leu $M5^+$) were observed in epidermis near necrotic areas. Very few cells reactive with Leu 7 or with anti-B cell reagents were seen.

These results support the hypothesis that cell mediated immune mechanism could be involved in the pathogenis of T.E.N.

156

DRUGS AND MIXED LYMPHOCYTE EPIDERMAL CELL CULTURES (MLEC) IN TOXIC EPIDERMAL NECROLYSIS (TEN).

J-C Roujeau. Département d'Immunologie et Service de Dermatologie, Hôpital Henri Mondor, Créteil, France.

Most cases of TEN are clearly induced by drugs. An hypersensitivity reaction is usually postulated but direct evidence is still lacking. The hypothesis of a cell mediated reaction against drugs combined to epidermal cells ("altered self") was tested by MLEC in presence of the suspect drug.

Lymphocytes from patients and normal controls (10^6/ml) were tested in primary (7 days) and secondary (7 + 3 days) cultures with 2.5 10^5/ml autologous epidermal cells (EC) derived from the roof of succion blisters (done on normal skin weeks or months after TEN). Variable concentrations of drugs were added to the cultures. Proliferation of lymphocytes was estimated by the amount of H^3thymidine incorporated over 16 hours.

Primary cultures, performed in 10 patients, showed no stimulation of lymphocytes by autologous EC with or without addition of drugs (mean stimulation indexes 0.95 and 1.12). Secondary cultures, performed in 8 patients and 8 controls, demonstrated a high secondary response to autologous EC both for patients (mean 16729 cpm) and for controls (mean 20546 cpm). In presence of drugs the secondary response was slightly increased in controls (mean 22599 cpm) and decreased in patients (mean 12961 cpm). Anyhow the differences were not significant. In 3 cases we tested also one metabolite of the drug with similar negative results.

Drugs responsible for TEN added in vitro to epidermal cells did not induce proliferation of autologous lymphocytes. If TEN is an immune reaction to altered EC, these negative results suggest that such alteration is not induced by the drug itself.

157

12-HYDROXYEICOSATETRAENOIC ACID (12-HETE) DOES NOT PROMOTE GROWTH OF HUMAN KERATINOCYTES IN VITRO. William R. Otto, Robert M. Barr*, Pauline M. Dowd*, Nicholas A. Wright, Malcolm W. Greaves*, Department of Histopathology, Royal Postgraduate Medical School, and (*)Institute of Dermatology, UMDS St. Thomas Hospital, London.

A sensitive growth assay was developed, which did not rely on single observations of ^{3}H-Thymidine (^{3}H-Tdr) incorporation, to test the recent claim that 12-HETE promotes the growth of human keratinocytes in vitro (Arch. Dermatol. Res. 278 449). Human foreskin keratinocytes (2nd passage) were grown without feeder cells or growth factors in 24-well plates (Falcon) from moderate seeding densities (18000 cells/cm^2). Twenty four hours after plating in Dulbecco's modified Eagle medium (DMEM) + 10% foetal calf serum (FCS) cultures were washed and grown in DMEM + 0.5% FCS plus added ethanolic solutions of 12(RS)- or 12(S)-HETE in concentrations of 0 (vehicle), 10^{-10} to 10^{-6}M. Positive controls were an important addition, and comprised cells treated with DMEM + 0.5% FCS + 3 growth factors (cholera toxin, CT, 10^{-10}M, human epidermal growth factor, EGF, 10ng/ml, hydrocortisone, HC, 0.4 µg/ml) or DMEM +10% FCS. Cultures were fed every 2nd day for up to 2 weeks. Fibroblast contamination did not occur. Cell growth was determined at several times by a sensitive (10ng/ml) and specific fluorometric DNA assay, and by ^{3}H-Tdr incorporation by liquid scintillation counting or autoradiography.

No changes in DNA or ^{3}H-Tdr incorporation were seen at any concentration or time with cells grown in 0.5% FCS plus added 12-HETE. Stimulated growth was seen with the positive control cultures (10% FCS, or 0.5% FCS + CT + EGF + HC). Experiments on confluent cells grown in 10% human AB serum also showed no stimulation of growth after a single 72hr exposure to the compounds.

We have been unable to confirm previous data that 12-HETE stimulates growth of keratinocytes, despite using a variety of experimental conditions. A rigorous approach to the assay of in vitro keratinocyte proliferation is necessary in assessing putative growth factors, and should use sensitive DNA measurements over a time course, without reliance on ^{3}H-Tdr incorporation.

158

BIOLOGIC ACTIVITIES OF EICOSANOIDS ON FIBROBLASTS IN CULTURE-Regulation of chemotactic activity and collagen synthesis-. Georg Rieger, Rudiger Hein, Thomas Ruzicka, Franz Vach, *Bernhard Adelmann-Grill and Thomas Krieg, Dermatologische Klinik u. Poliklinik der LMU München, * MPI f. Biochemie, Martinsried.

Metabolism of fibroblasts plays an important role in wound healing and can be modulated by mediators produced by other cell types e.g. platelets, lymphocytes and epidermal cells. Apart from other substances, most of these cells secrete products of arachidonic acid metabolism, which have been shown in previous studies to exhibit chemotactic activity for fibroblasts. It was the aim of the present study to investigate this effect of eicosanoids on a molecular level and to determine, whether additional parameters of fibroblast metabolism can be altered by these compounds.

Chemotactic activity of fibroblasts was investigated with a blind well Boyden chamber, in which different eicosanoids were used as chemoattractans. Proliferation of normal human fibroblasts in the presence of eicosanoids was measured by cell counts and biosynthetic capacities were characterized by labelling the cells with 3H-Proline for 24 hrs. For measuring synthesis of collagen and non-collagenous proteins the newly synthesized material was dialyzed, hydrolyzed and then analyzed on an automated amino acid analyzer. In addition to LTB4, 5S- and 12S-HETE were found to be strongly chemotactic for fibroblasts. Further analyses of the structural requirements for this activity indicated that the position of hydroxylation on the C5 and C12 OH-groups was essential for chemotactic activity. Furthermore, preincubation of the cells with 12S- HETE reduced the chemotactic response to 5S-HETE and LTB4, whereas chemotaxis to other chemoattractans e.g. fibronectin and PDGF was unaltered, indicating that all eicosanoids utilize the same receptor on cell surface. Protein synthesis was not influenced by any of the eicosanoids tested. Except that, specific decrease of collagen synthesis was noted when the fibroblasts were incubated in the presence of 12S-HETE.

These data suggest that eicosanoids can play a role in the regulation of fibroblast metabolism during chronic inflammation and in fibrotic processes. They also indicate that the chemotactic response of fibroblasts to eicosanoids depends on the specific interaction of certain active sites of these components with defined receptors on the fibroblast surface.

159

A POSSIBLE FUNCTION OF GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) IN HUMAN SKIN: DIRECT STIMULATION OF TOXIC OXYGEN RADICALS BY HUMAN NEUTROPHILS. M.Danner*, A.Kapp**, T.A.Luger**, *Department of Dermatology II and LBI for Dermatovenerological Serodiagnosis, Laboratory for Cellbiology, University of Vienna and **Department of Dermatology, University of Freiburg, FRG.

The neutrophilic granulocyte through the release of toxic oxygen radicals is one of the major early host defense cells. The production of reactive oxygen species by granulocytes has alos been shown to be regulated by cytokines and GM-CSF is believed to be identical with neutrophil migration inhibiting factor (NIF). Although the production of GM-CSF by human epidermal cells has not yet been reported, it recently has been published that interleukin 1 (IL 1) stimulates fibroblasts to release GM-CSF (J. Clin.Invest. 77, p 1867, 1986). Moreover tumor necrosis factor type α (TNFα), which shares many of the biological characteristics with IL 1, also stimulates human endothelial cells to release GM-CSF (PNAS 83, p7467, 1986). The following study was performed to investigate whether human GM-CSF has the capacity to stimulate directly frehsly isolated human polymorphonuclear leukocytes (PMN). For the detection of the granulocyte oxygenating activity a Lucigenin-dependent chemiluminescence assay was used. Semipurified preparations of native human GM-CSF and highly purified recombinant human GM-CSF (rh-GM-CSF) both were active on freshly isolated human PMN,

whereby rh-GM-CSF was able to stimulate the oxygen radical production up to a dilution of 1:10.000 (20 CFU's/ml). Oxygen radical release was detectable within 15-20 min after incubation and had a maximum at 60-90 min within an intensity comparable to 5 ng/ml phorbol myristate acetate (PMA). Activation lasted more than 3 hr and led to a complete unsusceptibitity of the granulocytes to an additional GM-CSF stimulation. Native GM-CSF and rh-GM-CSF eluted of RP-HPLC at 40-50% acetonitrile exhibiting granulocyte stimulating activity as well as colony forming activity. Cytokines such as h-G-CSF, interferons (α,γ), TNFα, IL 1 (α,β) did not induce neutrophil oxygen radical release. Therefore GM-CSF released upon stimulation with keratinocyte derived IL 1 from fibroblasts and endothelial cells appears to be a major candidate for neutrophil activation in the skin, and thereby may play a crucial role in inflammatory skin diseases where neutrophil infiltration is a prominent feature.

160

CELLSURFACE GLYCOPROTEINS DEFINE DIFFERENTIATION OF HUMAN KERATINOCYTES; REGULATION OF BIOSYNTHESIS IN FRESH EPIDERMIS AND SHORT TERM CULTURES. Carlos Cordon-Cardo*, C. Eberhard Klein*#, Rolf Soehnchen*, Lutz Weber#, Magdalena Eisinger*, Lloyd J. Old*, From the Memorial Sloan Kettering Cancer Center*, New York, N.Y., and Department of Dermatology#, University Ulm, FRG.

The epidermis provides a well defined model system to study cellular differentiation. To extend current knowledge about cell surface antigens expressed at various stages of epidermal differentiation, we have tested a panel of more than 100 monoclonal antibodies (mAbs) generated against surface antigens of human cancer cells. Six mAbs defining distinct glycoproteins were selected for a detailed study on human epidermal cells. Antigen expression was assessed using avidin-biotin immunoperoxidase and indirect immunofluorescence tests. In tests on tissue sections, three of the glycoproteins (J143 (gp140/30); T43 (gp85/36); H99 (gp38)), were expressed in the basal cell layer of the epidermis, while the other three glycoproteins (T179 (gp140/95); T16 (gp40/50); BT15 (gp80)) were preferentially expressed in maturing keratinocytes above the basal layer. We also compared synthesis of these glycoproteins in fresh epidermis and in primary epidermal short term cultures using (^{35}S)methionine for metabolic labeling (six hours) in radioimmunoprecipitation assays. Synthesis of J143 was 8- to 20-fold higher and synthesis of T43 was 4- to 10-fold lower in cultured cells as compared to fresh epidermis. BT15, an antigen strongly expressed on terminally differentiating keratinocytes, was synthesized at 5- to 15-fold higher levels in fresh epidermis than in cultured cells. Biosynthesis levels of H99, T179 and T16 did not change in cultured epidermal cells.

Based on our findings, we propose a model of surface antigenic changes that occur during keratinocyte differentiation in vivo.

161

BULLOUS PEMPHIGOID: ANALYSIS OF HAEMOPOIETIC PRECURSOR CELLS IN BONE MARROW AND HAEMOPOIETIC GROWTH FACTORS/DIFFERENTIATION FACTORS IN SERA AND BLISTER FLUIDS. Peter Kern, and Wilhelm Meigel, Clinical Department, Bernhard-Nocht-Institut, and Department of Dermatology, Allgemeines Krankenhaus St. Georg, Hamburg.

The early accumulation of eosinophil granulocytes is a characteristic feature of bullous pemphigoid lesions. The blister fluids are known to be enriched in mast cell products as well as in haemopoietic growth factors. The aim of our study was to determine the concentration of those factors in blister fluids and sera from patients with bullous pemphigoid in addition to the characterization of patients' haemopoietic precursor cells.

We used the semi-solid agar culture assay as well as a suspension culture technique and analyzed growth and differentiation of cells and colonies (granulocyte/macrophage colony-forming cells, GM-CFC, eosinophil colony-forming cells, Eo-CFC). Patients' bone marrow precursor cells could be grown in the presence of standard colony-stimulating factors (GM-CSF), e.g. derived from human placenta-conditioned medium. A normal differentiation pattern was found, but the buoyant density of the cells indicated heterogeneous subpopulations of GM-CFC as well as Eo-CFC. A variable concentration of GM-CSF was found in blister fluids from 5 patients. No specific enrichment of eosinophil colony-stimulating factor (Eo-CSF) was found in contrast to eosinophil differentiation factor assayed in the liquid suspension culture system. Thus, our findings indicate a different composition of haemopoietic precursor cells during the acute disease stage. Besides low concentrations of GM-CSF, regulatory molecules inducing differentiation into the eosinophil series are enriched in the blister fluid of patients.

162

INHIBITION OF CLASS II ANTIGEN EXPRESSION IN VIVO BY ANTIBODIES AGAINST γ-INTERFERON. Curt Skoglund and Annika Scheynius, Institute of Clinical Bacteriology, University of Uppsala, Uppsala, Sweden.

During the delayed type hypersensitivity (DTH) reaction in the skin, class II antigens are expressed on the keratinocytes. This induction is attributed to the action of γ-interferon(IFN-γ). We have now studied the influence of antibodies against IFN-γ on the DTH-reaction.

The shaved abdomens of Lewis rats were sensitized by application of 20 ul 0.5% DNFB in acetone:olive oil for two consecutive days. On the fifth day all rats were challenged with 20 ul 0.2% DNFB on the ears. Immediately thereafter one group (n=8) was given 1 mg of mouse monoclonal antibodies (mMoAb) against rat IFN-γ, denoted DB-1, intraperitoneally (i.p.) and another group (n=8), 1 mg of an irrelevant mMoAb. The ear thickness was measured with a micrometer, before challenge and after 24,48 and 72 hours. At 72 hrs all rats were sacrificed, the ears were snap frozen and stained with immunoperoxidase using mMoAb OX 6 (Sera-Lab, Cambridge, U.K.), directed against rat Ia-antigen. The class II antigen expression on keratinocytes was classified into four groups, ranging from 0 to +++, where + is weak and patchy, ++ is continuous but only covering the basal layer and +++ is strong and reaching through the whole epidermis.

We found that the group treated with DB-1 showed class II antigen expression on Langerhans cells, but almost none on keratinocytes (0/+), while the control animals gave a ++/+++-graded expression. Furthermore ear increment tended to be greater in the DB-1 treated animals.

It is concluded that DB-1 can inhibit class II antigen expression on keratinocytes during the DTH-reaction and might also enhance the local reaction.

163

RE-EXPRESSION OF LAMELLAR ICHTHYOSIS AFTER GRAFTING OF EPIDERMAL SHEETS OBTAINED BY PATHOLOGICAL KERATINOCYTE CULTURE TO "NUDE" MICE. A. Joubaud, M. Haftek, G. Zambruno, M. Faure, J. Thivolet, CNRS UA 601, INSERM U.209, Dept. of Dermatology, Hopital E. Herriot, 69437 Lyon, France.

Previous studies on autosomal recessive type Lamellar ichthyosis (LI) proved purely epidermal expression of this disease. However, immerged keratinocyte cultures failed to reproduce the disease in this "in vitro" system. We have used a model of epidermal sheet graft to congenitally athymic mice for further studies on pathological keratinocytes maintained "in vivo" but out of their human host. Lesional skin of LI was biopsed and after trypsinization (0.3%) collected epidermal cells were seeded (10^6 per 25 cm^2 plate) on irradiated mouse 3T3 fibroblast feeder layer and cultured in DMEM/HAM F12 medium at 37°C. Secondary 15-day cultures were detached from their support with dispase (2.4 U/ml) and grafted to the "nude" BALB/C mice. Grafts of normal human keratinocyte culture and non-grafted spontaneous cicatrization sites were used as controls in this study. Animals were sacrified every week during 1 month after grafting and the graft sites were excised with a margin of normal mouse skin. Routine histopathology, standard electron microscopy and immunofluorescent studies were performed on the initial biopsies, cultured epidermal sheets, and grafts. Photonic and electron microscopy helped to evaluate histopathological characteristics of the tissue. Anti-involucrin antibody was used as a marker of human keratinocytes and anti-H2 monoclonal antibody stained cells of mouse origin permitting determination of limits between the grafted and cicatrical host epidermis. The KM48 antibody labelled desmosome-related antigen, expression of which depended on a stage of keratinocyte differentiation. All keratinocyte cultures were KM48 negative and no sign of LI morphology could be observed before grafting. The epidermal grafts could be maintained on mice throughout the time of experiment. Human epidermal sheets re-expressed histopathological features characteristic of the original LI biopsies from the first week after grafting on. This coincided with reappearance of the differentiation-related antigen (KM48). Gradual replacement of grafted human cells by mouse cicatrical epidermis could be observed.

The model of epidermal culture grafted to athymic mice can be used for studies on several other dematoses but such experiments would require regular and reliable control of the human graft persistence.

164

ACID HYDROLASE ACTIVITY IN THE LESIONS OF CERTAIN GENETICALLY-DETERMINED DYSKERATOSES. T. Jansen, P.D. Mier, P.v.d. Kerkhof, R. Happle, Dept. of Dermatology, University of Nijmegan, The Netherlands.

It is probable that epidermal acid hydrolases are necessary for the degradation of glycoprotein and other

macromolecules during terminal differentiation. We have therefore investigated patients with certain genetically-determined dyskeratoses to establish whether individual diseases may be characterized by abnormal levels of these enzymes. Shave biopsies of about 5 mm diameter were homogenized in 0.1% Triton X-100 and soluble (S) and particulate (P) fractions prepared by centrifugation. Six enzymes were measured by fluorimetric analysis: β-galactosidase, N-acetylglucosaminidase, α-fucosidase, α-mannosidase, arylsulphatase A/B and sialidase.

Our findings include the following (a) lesions of x-linked ichthyosis showed reduced levels of arylsulphatase (S and P). (b) Lesions of lamellar ichthyosis contained increased amounts of both arylsulphatase (S and P) and sialidase (S and P). (c) Lesions of certain dominant disorders, including Darier s disease, showed normal total levels of all enzymes but a grossly reduced ratio S/P. (d) All specimens of clinically uninvolved skin showed consistently normal enzyme patterns. The changes described above are sufficiently clear-cut to provide reliable diagnostic criteria, and may throw some light on the pathogenetic processes involved.

165

HLA DR AND DQ SEROLOGICAL AND DNA POLYMORPHISMS IN PATIENTS WITH LINEAR IgA DISEASE AND DERMATITIS HERPETIFORMIS. J A Sachs, J N Leonard, G A Hitman, J Awad, A V Powles, C E M Griffiths and Lionel Fry, Department of Immunology & Medical Unit, The London Hospital Medical College and the Dermatology Department, St. Mary's Hospital, London, England.

There is a strong association of the Class I antigens A1 and B8 with dermatitis herpetiformis (DH) and a weak association with linear IgA disease (LAD). In DH the association with the Class II HLA-DR3 antigen (in linkage disequilibrium with HLA-A1 and B8) is high in all reports. There have been no studies to date on the incidence of Class II antigens in LAD. This study has (i) tested for the presence of the Class II region DR and DQ serologically defined products and restriction fragment polymorphism (RFLP) derived from Southern blots with DQ alpha DNA probes in 23 patients with LAD and (ii) compared their frquencies in patients with LAD with those with DH as well as healthy controls. In DH, HLA-B8 and DR3 were highly increased compared with controls - 81% vs 21% and 88% vs 26% respectively (significant at $p < 0.001$). In LAD both antigens were less significantly increased at 55% vs 21% for B8 ($p < 0.025$) and 53% vs 26% for DR3 ($p < 0.05$). In both diseases DR2 (DQw1 associated) was non-significantly increased. However, after correcting for the increase of DR3, DR2 was significantly increased in DH but not in LAD; after a similar correction the frequency of DRw6 (also DQw1 associated) was significantly increased in LAD but not DH. In addition, there was an increase of the allelic DQ&6·8 kb and 6·2 kb fragments (both DQw1 associated) in LAD when compared with DH and controls ($p < 0.025$ 3x2 contingency tables). Thus in DH there is a large increase of DR3 and slight increase of DR2 and in LAD a slight increase of both DR3 and the DQw1 associated DRw6 and 6·8 kb and 6·2 kb RFLP. The differences in DR3 frequencies and the importance of DQw1 rather than DR2 in LAD may indicate that different susceptibility genes operate in the two diseases.

166

CULTIVATED PATHOLOGICAL KERATINOCYTES FROM DARIER'S DISEASE, LAMELLAR ICHTHYOSIS AND ICHTHYOSIS VULGARIS ARE INSUFFICIENTLY DIFFERENTIATED TO EXPRESS DISEASE-CHARACTERISTIC FEATURES. M. Haftek, G. Zambruno, A. Reano, M. Faure, J. Thivolet. CNRS UA 601, INSERM U.209, Dept. of Dermatology, Hopital E. Herriot, 69437 Lyon, France.

Immerged epidermal cell(EC) cultures were performed for in vitro studies on growth and differentiation of pathological keratinocytes isolated from influences of the host's systemic (circulating humoral factors, infiltrate, immunocompetent cells) and dermal (fibroblasts, endothelial cells) components. Three genetically transmissible keratinization disorders: Darier's disease, autosomal recessive Lamellar ichthyosis and Ichthyosis vulgaris were selected for the present study. The three diseases are believed to be of purely epidermal origin and the aim of this work was to re-produce the respective disease features in vitro, in epidermal sheets generated from lesional keratinocytes. Two types of pure keratinocyte cultures were performed: on irradiated mouse 3T3 fibroblast feeder layer and on human collagene type III + I (Merieux). EC isolated from epidermal lesions by tripsinization (0.3%) were seeded (10^6 cells per 25 cm^2) and cultured in the enriched DMEM/HAM F12 (3:1) medium at 37°C (10% CO^2). Confluent and stratified secondary cultures were harvested on days 15 and 25, and studied using following methods : 1) routine histopathology on hematoxilin-eosin stained paraffin sections; 2) standard electron microsopy; 3) Immunofluorescence staining on frozen tissue sections with differentiation-related antibodies (KL1 =antikeratin 56.5 KD, EM 48 =antidesmosome, GP 37 =antifillagrin, and polyclonal antiinvolucrin Ab). The results were compared with these obtained in normal human keratinocyte cultures and with the initial, in situ, pictures.

Immerged cultures of pathological keratinocytes from Darier's disease, Lamellar ichthyosis and Ichthyosis vulgaris failed to clearly reproduce stigmates of the original diseases. Immunofluorescent studies indicated low-grade differentiation of immerged keratinocyte cultures (both normal and pathologic). Slight increase of the differenitation obtained by introduction of a lipid-free medium was insufficient for re-expression in vitro of keratinization disorders studied. It is likely that higer degree of epidermal maturation would be necessary to achieve this goal. In this context beneficial effect of retinoids in the menagement of keratinization disorders may be discussed.

167

A 130 KD CELL SURFACE GLYCOPROTEIN IS GROWTH-REGULATED IN NORMAL FETAL FIBROBLASTS AND HIGHLY INDUCED IN TRANSFORMED FIBROBLASTS AND KERATINOCYTES. C.E. Klein°*, H.L. Ozer#, F. Traganos°, L. Weber* and L.J. Old°, from the Memorial Sloan Kettering Cancer Center°, New York, NY; Hunter College#, New York, NY and the Department of Dermatology*/University Ulm, FRG.

Gene products associated with malignant transformation of cells represent a major focus of biological research. We have studied the expression of cell surface antigens defined by monoclonal antibodies in normal and malignant cells using the fluorescence activated cell sorter, serological and comparative radioimmunoprecipitation assays (metabolic labeling with (^{35}S) methionine). Human fibroblasts were compared with their malignant counterparts derived by transformation with SV 40 virus DNA (Hs74 → CL39) or with the chemical 4-Nitroquinolineoxide (KD → HuT12,-14). Furthermore, normal fibroblasts and keratinocytes were compared with cell lines established from squamous cell carcinomas and fibrosarcomas. In fibroblasts, biosynthesis and expression of a 130 KD cell surface glycoprotein (Q14) was more than 10-fold higher after both viral and chemical transformation, when compared with the untransformed parent cell lines. In addition, 3 out of 3 fibrosarcoma cell lines (HT1080, 8387, SW684) also expressed high levels of Q14. Furthermore, Q14 was not found on cultured keratinocytes, but was strongly expressed on a cell line derived from a facial squamous cell carcinoma (SCL1). To further investigate its regulation Q14 expression was studied in normal fetal fibroblasts (F135-60-86 p6-8; F135-60-18 p8-10) under various growth conditions. Cultures supplemented with 20% fetal calf serum (FCS) and parallel cultures additionally treated with 1.5 mM Hydroxyurea for 3 days showed significant higher biosynthesis of Q14 than parallel cultures that were serumstarved for six days (0.5% FCS). A simultaneous analysis of surface immunofluorescence and DNA-content demonstrated that Q14 was preferentially expressed on cells progressing through the cell cycle (G1-, S- and G2-phase), but was not detectable or in very low levels only on serum-starved cells in G0-phase.

This glycoprotein, growth regulated in normal fibroblasts and highly induced after transformation, is controlled differently than the transferrin receptor protein that was studied in parallel and may also have an important role for cell growth of normal and transformed cells.

168

ANTI-C1-INHIBITOR AUTOANTIBODY-MEDIATED ACQUIRED C1-INHIBITOR (C1-INH) DEFICIENCY IN PATIENTS WITH RECURRENT ANGIOEDEMA. Konrad Bork*, Jochem Alsenz**, Rudolf E. Schopf*, and Michael Loos**, Dermatological Clinic* and Institute of Medical Microbiology**, Johannes Gutenberg-University, D-6500 Mainz, F.R.G.

Anti-C1-INH antibodies (IgG) were found in two patients (G.H, W.K.) with the acquired form of C1-INH deficiency but without other associated diseases. Both patients had 50-80% of normal, however functionally inactive C1-INH protein with a 96 KDa C1-INH molecule in SDS PAGE analysis (normal C1-INH: 105 KDa). The titers of antibodies directed against C1-INH were 8 to 10 times higher in G.H. than in W.K.. Since the antibodies blocked the binding of isolated C1-INH to $C1\bar{s}$ and did not recognize preformed $C1\bar{s}$-C1-INH complexes, these antibodies bind to the $C1\bar{s}$-binding site of C1-INH or close to it. Complexes consisting of anti-C1-INH antibodies and C1-INH were present in large numbers in W.K.'s serum, whereas G.H.'s serum contained only few. Catabolic studies of C1-INH were carried out by incubating purified ^{125}I-labeled C1-INH with the different sera. After 24 hours very low amounts (<1%) of the labeled 105 KDa C1-INH molecule were cleaved into a 96 KDa molecule in normal human serum. In the patients sera, however, this cleavage was accelerated more than 100 times with rates 5 to 10 times faster in G.H. than in W.K.. Other HAE sera (9 patients) tested revealed the presence of a critical level of functional C1-INH for the cleavage of the 105 KDa into the 96 KDa molecule: sera with functional C1-INH concentrations higher than 30-35% of normal showed hardly any cleavage, whereas sera with less than 30% showed a rapid cleavage into the 96 KDa molecule. Gelfiltration studies with the patients sera, inhibition experiments and cleavage studies with $C1\bar{s}$, plasmin and kallikrein make it likely that $C1\bar{s}$ is the enzyme in the patients sera responsible for C1-INH cleavage. The complete (G.H.) or partial (W.K.) resistance of the patients to replacement therapy with C1-INH might therefore base on two events: blockade of the functional activities of C1-INH by the anti-C1-INH antibodies and subsequent inactivation of C1-INH by the now uncontrolled enzyme $C1\bar{s}$.

169

RECOMBINANT HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (CSF-H) STIMULATES THE RELEASE OF TOXIC OXYGEN RADICALS BY HUMAN PMN. Alexander Kapp, Martin Danner, Thomas A. Luger and Erwin Schöpf. Department of Dermatology, University of Freiburg, FRG and 2nd Department of Dematology, University of Vienna, Austria.

Activation of PMN is an essential requirement for the defense against invading microbes. Defective PMN functions in most cases lead to severe and recurrent infectious skin diseases which are often prolonged in spite of appropriate antimicrobial therapy. It recently was demonstrated that CSF-H modulates different PMN functions such as phagocytosis and killing of parasites as well as the antibody-dependent cytotoxicity. Since release of oxygen radicals represents a measure of the potential microbicidal and tissue destruction power of PMN we investigated the direct stimulatory effect of recombinant human CSF-H on the oxidative metabolism of isolated human PMN. Release of reactive oxygen species (ROS) was assessed by a lucigenin-dependent chemiluminescence (CL). CSF-H stimulated the production of ROS up to a concentration of 20 CFU/ml. CL response was observed within 5-10 min showing a maximum at 60-90 min whereby integral counts within 180 min induced by CSF-H (100 CFU/ml) were comparable to the effect of

phorbol-myristate-acetate (10 ng/ml). Activation lasted more than 3 h, thereafter PMN were completely deactivated to restimulation with the same mediator. The signal was significantly inhibited by superoxide dismutase (SOD) and D-mannitol, and only to a lower degree by catalase and DMTU suggesting the involvement of distinct ROS in generating the CL response. The effect on PMN was completely blocked by an antibody to CSF-H showing a specific effect of the mediator. Significant release of ROS was also demonstrated by measuring SOD-inhibitable cytochrome C reduction at concentrations of 10-1000 CFU/ml within 180 min. In contrast H_2O_2 release as measured by horseradish peroxidase-dependent reduction of phenol red was only minimal even at 10000 CFU/ml. Upon reversed phase HPLC granulocyte-stimulating activity overlapped with CSF activity. The results clearly demonstrate that CSF-H directly stimulates the release of ROS by human PMN. CSF-H may play a crucial role in activation of PMN turning them into active scavengers of parasites and microbial organisms. Future studies may elaborate the therapeutical use of this cytokine in treatment of skin disease.

170

HUMAN KERATINOCYTES RELEASE A LOW MOLECULAR WEIGHT DIFFFERENTIATION FACTOR WHICH INDUCES CLASS I (β_2 microglobulin) ANTIGEN EXPRESSION. M. Danner[1], A. Kock[1], W.Sterry[2], K.Kaltoft[3], T.A.Luger[1], [1]Dept. Derm.II and LBI for Dermven. Lab. for Cellbiol., Univ.of Vienna, Austria, [2]Dept.Derm.Kiel,FRG, [3]Institute of Hum.Gen., Univ.Aarhus,Denmark

Regulation of class I antigen expression within the skin is not yet clear. Class I antigens which have been shown to be closely associated with β_2 microglobulin (β_2m) expression and to be controlled by interferons (IFN) are important restriction elements recognized by cytotoxic T lymphocytes. Thus epidermal cells which are known to release a variety of cytokines were investigated for there capacity to release a β_2m-inducing factor different to IFN. For this purpose a surface marker lacking cell line (McCoy) derived from the synovial fluid of a patient suffering from degenerative arthritis was used. Class I antigen (β_2m) expression on this cell line was tested by indirect immune fluorescence and binding studies using ^{3}H-protein A and a monoclonal human β2m antibody (ATCC-HB28-clone). Supernatants of freshly isolated human epidermal cells as well as from different human epidermoid carcinoma cell lines (A 431, CCL-17, HTB 54, UBC, SCC) contained within 24 hr of incubation β_2m expression inducing factor. In contrast supernatants of a human fibroblast (CRL-1445) and from a human melanoma cell line (KRFM) as well as supernatants from a murine keratinocyte (Pam) and a murine monomyelocytic cell line (WEHI 3), both containing IL 3 activity, were unable to cause β_2m expression. In addition neither other cytokines (IL 1, IL 2, IFN , PGE_2, FTS, Thymosin α1, TP-5) nor mitogens (PHA, Con A, PMA) showed any capacity to induce class I antigen expression. Upon HPLC gel filtration (TSK-125 column) the β_2m inducing activity derived from A 431 and CCL 17 supernatants eluted within a mw of 2 kD. Pooled active fraction (mw 2 kD) also exhibited homogeneity on DEAE-ionexchange chromatography eluting as a single peak of activity. Using a human B lymphoma cell line (DAUDI) which lacks the gene for β_2m and is therefore negative for surface β_2m, the mediator was unable to induce β_2m expression excluding an unspecific cell membrane binding of a breakdown product cross reacting with the β_2m-antibody. These data indicate that human keratinocytes are able to release a low molecular weight differentiation factor which may participate in immunological skin reactions by regulation class I antigen expression.

171

DIFFERENTIATION OF KERATINOCYTES AND THE EXPRESSION OF RECEPTORS FOR EPIDERMAL GROWTH FACTOR AND LOW DENSITY LIPOPROTEIN. Marinus F.W. te Pas and Maria Ponec, Department of Dermatology, University Hospital Leiden, The Netherlands.

It has been reported earlier that the binding activity of the receptors for epidermal growth factor (EGF) and low density lipoprotein (LDL) is inversely related to the differentiation state of keratinocytes and squamous carcinoma cells (SCC) and to their differentiation capacity. These differentiation-induced changes in receptor binding activities may be either due to modulations on the level of receptor synthesis and/or to modulations of the physico-chemical properties of the plasma membrane. The present investigation has been undertaken to study the possible differentiation-related modulations on the level of receptor synthesis, namely on the DNA and RNA expression. For this purpose the following cells with decreasing receptor binding activities for EGF and LDL were used: A431 > SCC-4 > SCC-15 > SCC12F2 = SVK14 = normal keratinocytes. For differentiation capacity of these cells an opposite rank order has been observed.

For both EGF and LDL receptors the expression at both the DNA level (detected in Southern blot experiments) and at the RNA level (detected in dotblot experiments, with total cytoplasmic RNA) decreased in the same rank order as found earlier for EGF and LDL receptor binding activities. The increased DNA expression found in cells with reduced capability to differentiate is probably due to gene amplification of aneuploidy. Furthermore, for both receptors the RNA expression has been found to be higher in cells cultured under proliferating (low (Ca^{2+}) 0.07 mM) than under differentiating (normal (Ca^{2+}) 1.6 mM) conditions. This finding is consistent with previous reports on the EGF and LDL receptor binding activities.

It is concluded from the data presented that the binding activity of both the LDL and the EGF receptors is related to their expression at both the DNA and the RNA level on the following grounds i) the DNA expression as well as the binding receptor activity for EGF and LDL increases with decreasing capacity of cells to differentiate, ii) RNA expression for both the EGF and the LDL receptors is related to the DNA expression and iii) a decreased EGF and LDL receptor binding activity upon induction of keratinocyte differentiation is regulated on RNA level.