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001

Use of Dendritic Cells (DC) Genetically Modified by ALVAC Vectors Carrying MAGE Sequences to Induce Antimelanoma Immune Responses

M. Trakatelli, M. Toungouz, P. van der Bruggen, M. Lambermont, T. Velu, M. Heenen, and C. Bruyns

Free University, Brussels, Belgium

DC are highly efficient antigen presenting cells. Due to their capacity to induce primary immune responses, vaccination with DC genetically modified to express tumor-associated antigens holds the promise for successful cancer immunotherapy. DC gene delivery is most efficiently achieved by viral vectors. ALVAC viral vector was derived by Institut Mérieux (France) from a highly attenuated canarypox virus that does not replicate in mammalian cells. The safety and efficiency of ALVAC recombinant vectors to induce protective immune responses to their inserted foreign genes have been validated in humans within diverse infectious diseases trials or cancer trials. We generated DC from adherent blood monocytes from melanoma patients enrolled in ongoing clinical trials of vaccination (at Erasme Hospital) and infected them with a recombinant ALVAC virus encoding either a marker gene (EGFP) or the MAGE 1 and 3 minigenes. The aims of this study were to firstly evaluate the efficiency of DC transduction by ALVAC vectors, then to investigate if such infection induced or not their maturation and finally to control if MAGE-transduced DC were able to activate MAGE-specific CTLs clones. 5% to 60% (according to the patient-donor) of immature DC were successfully infected by ALVAC vectors as shown by EGFP expression. Flow cytometry analysis of surface markers expressed on DC after ALVAC infection did not reveal a mature phenotype when compared to control noninfected DC tested at the same day. But most importantly, our results demonstrated that DC from HLA-A1 patient-donors transduced with ALVAC vectors carrying MAGE 1/3 minigenes were capable of activating a MAGE 3/A1 CTL clone more efficiently than same DC loaded with MAGE 3/A1 peptide, as shown by increased IFN-γ secretion. These results could be the basis for the development of a new strategy in melanoma immunotherapy.

002

Elastin Peptides Up-Regulate proMMP-2 Activation and Melanoma Cells Migration Through Three-Dimensional Type I Collagen Matrix

C. Ntayi, W. Hornebeck,* and P. Bernard

*Department of Dermatology, CNRS FRE 2260; IFR 53-Biomolecules, Faculty of medicine, REIMS; *Department of Biochemistry, CNRS FRE 2260; IFR 53-Biomolecules, Faculty of medicine, REIMS*

Elastin, as a main constituent of elastin fibers, plays a prominent role in skin biology. Its enzymic degradation by gelatinases (MMP-2, MMP-9) leads to the liberation of elastin peptides (E.P.) which exhibit several biological functions. Particularly, interaction between E.P. and a truncated β galactosidase elastin/laminin receptor present at the plasma membrane of various cell types was shown to trigger MMP-expression (Brassart *et al*, *J Biol Chem* 2000).

We here evidenced that elastin peptides (50–200 μg per ml) from organo-alkaline or elastase hydrolyses of insoluble elastin could stimulate MMP-2, but not MMP-1, expression from highly tumorigenic melanoma cells, in a dose-dependant manner. When those cells were grown within type I collagen gels, in presence of EDPs, MMP-2 activation was strikingly exacerbated, an effect probably resulting from elastin-mediated MT1-MMP increased expression (Brassart *et al*, *Clin Exp Metastasis* 1998). Parallely, invasion of type I collagen matrix by melanoma cells was significantly increased.

This preliminary investigation, in keeping with our previous data (Capon *et al*, *Clin Exp Metastasis* 1999), indicate that the main fibrillar matrix macromolecules of human dermis i.e collagen and elastin actively contribute to tissue invasion, through modulation of MMPs expression and activation, by melanoma cells.

003

Identification by cDNA Microarray Technology of Genes Modulated by UV-B in Normal Human Melanocytes

C. Valery, J. J. Grob, and P. Verrando

LIMP, Université de la Méditérranée, Marseille, France

Although it is known that solar UV-B radiations play a major role in melanogenesis and melanocarcinogenesis, a list of function-related genes involved in such cutaneous stress response is lacking. By using the DNA microarray technology, this study characterized target genes of the UV-B stress response in normal human melanocytes, the pigment-synthesizing skin cells. Expression of more than 9000 genes was simultaneously checked 4 h after irradiation of the cells at 100 mJ per cm^2. 198 genes have a modulation factor $\geq$ 1.9 times. Among them, 159 corresponded to DNA known sequences, the encoded proteins being mostly in DNA- or RNA- binding/synthesis/modifications or ribosomal proteins. The others were transcription factors, receptors, tumors suppressors and (pro)oncogenes. Furthermore, 39 DNA sequences corresponded to unknown genes (''Expressed Sequence Tag'', EST) were also modulated. In order to identify new genes of UV-B stress, two of them were primarily selected. Their kinetics of expression following UV-B stress were characterised by RT-PCR confirming the DNA microarray data. Furthermore, mRNA of these ESTs were always found expressed in melanoma cell lines at differents levels. In return, they were not found in melanoma tumors at different stages. The 5′ end of one of this EST was amplified by primer extension (5′ RACE). The sequencing of this first amplification product showed an Alu element. This study, that established an expression profiles of melanocytic genes collectively modulated by UV-B stress allows to identify new proteins and genes that could represent new markers of UV-B induced melanocarcinogenesis.

004

The POH-1 Subunit of Proteasome is Involved in UVB DNA Damages Repair in Melanocytic Cells

V. Pellier, C. Valery, M. DeMéo, J. J. Grob, and P. Verrando

LIMP, Université de la Méditérranée, Marseille, France; Laboratoire de Biogénotoxicologie et Mutagénèse Environnementale EA 7030, Faculté de Pharmacie, Marseille, France

UVB radiations induce great amount of DNA damaged bases which are potentialy responsible for cutaneous carcinogenesis. Recent studies show that the proteasome is involved in DNA excision repair. The aim of this study was to evaluate implications of proteasome on DNA repair and cell resistance of normal and transformed human melanocytes exposed to an UVB stress. The phamacological inhibition of proteasic activity of the proteasome (ALLN and Lactacystin) causes deficient repair of cyclobutan-pyrimidin dimers (CPD) in cultured cells exposed to a 40 mJ per cm^2 UVB dose. In return, instrumental overexpression in melanoma cells of the POH-1 subunit of 19S proteasome leads to a better cells survival toward increased UV doses (0–250 mJ per cm^2). A speeding up to 25% of UVB-induced CPD repair is also observed. The presence of ALLN in cultured medium of cells overexpressing POH-1 maintains repair deficiency, suggesting that ALLN is able to reverse the effet of POH-1. An UVB irradiation of 100 mJ per cm^2 modifies POH-1 ARNm levels on a 24-h period of time. Taken together, the results show that a proteasic activity of proteasome and a POH-1 dependent activity are together involved in UVB-induced DNA damages repair in human melanocytes cells. These results open new phamacoloical perspectives in the field of the modulation of cutaneous melanocytes resistance to UVB stress and its possible alteration in melanocarcinogenesis.

005

GM3 Ganglioside of Human Melanoma Tumors Contains a Lactonized Sialic that is a Ligand of Interleukin-4

I. Popa, J. P. Zanetta,* J. Portoukalian, and L. Thomas

*Laboratory of Tumor Glycobiology, INSERM U.346, Faculty of Medicine Lyon-Sud, University Lyon-I, 69921 Oullins Cx, France; *Laboratory of Biological Chemistry, University of Sciences and Technologies of Lille, 56955 Villeneuve d'Ascq Cx, France*

The gangliosides of human melanoma tumors have structures different from those of gangliosides of normal melanocytes. Whereas the latter cells contain mostly GM3, the transformed melanocytes display a more complex profile with GM3, GM2, GD3 and GD2. These gangliosides, that are considered as markers of malignant melanoma, are shed into the extracellular medium during proliferation of malignant melanocytes. They can be the taken up by monocytes and disturb their biological activity. We have shown the presence of de-N-acetyl neuraminic acid in GD3 ganglioside, while this sialic acid is always N-acetylated in normal tissues. Moreover, we have shown that the sialic acid of GM3 ganglioside is mostly in the form of an internal lactone 1–7 which has just been reported to be a ligand of interleukin-4 (Cebo *et al*, *J Biol Chem* 2001, 276:5685–5691). The binding of interleukin-4 produced by activated leucocytes on GM3 that is present in high concentrations on malignant melanocytes is likely to disturb the activity of the immune system in the vicinity of the tumors.

006

Cutaneous T Cell Lymphoma Cells Express a Novel Allelic Form of the p140/Killer Cell Immunoglobulin-Like Receptor

M. Bagot, A. Moretta, S. Sivori, R. Biassoni, C. Cantoni, C. Bottino, L. Boumsell, and A. Bensussan

INSERM U448 and Service de Dermatologie de l'hopital Henri Mondor, Creteil, France; Dipartimento di Medicina Sperimentale, Università di Genova, Genova, and Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy

The tumor cells of patients with cutaneous T cell lymphomas (CTCL) have the cell surface phenotype of mature T helper lymphocytes and may be impossible to differentiate from non malignant lymphocytes, both in skin and blood. Until now, no specific cell membrane marker of CTCL has been reported. In the present study, we report for the first time that CTCL cells express the MHC class I binding p140/killer cell immunoglobulin-like receptor which has been described on a minor subset of NK lymphocyte and on a marginal circulating CD8+ T lymphocyte subset. Interestingly, the molecular characterization of this KIR expressed by CTCL allowed us to isolate a novel allelic form of p140/KIR3DL, resulting in four amino acid substitutions, three in the extracellular immunoglobulin-like domain of the protein and one in the cytoplasmic region. This finding is likely to be an important new issue, both for the pathophysiology and for the clinical management of CTCL patients (*Blood*, 1 March 2001, 97, in press).

007

Cutaneous T Cell Lymphoma Cells Express a Novel Early Activation Surface Membrane Receptor

M. Bagot, M. Nikolova, A. Tawab, A. Marie-Cardine, L. Boumsell, and A. Bensussan
INSERM 448 and Service de Dermatologie, Hôpital Henri Mondor, 94010 Créteil, France

Using a newly generated monoclonal antibody (mAb), we identified the 96 kDa transmembrane receptor SC5 expressed simultaneously on a human Sezary cell line and a minor T cell subset in normal individuals. SC5 antigen was detected mostly on CD45RO+ lymphocytes from both CD4+ and CD8+ subsets as well as on NK and B lineage cells. SC5 surface expression increased very early after polyclonal stimulation of CD3+ cells due to the transfer of intracellular SC5 molecules to the cell membrane. Engagement of SC5 receptor by its mAb inhibited the anti-CD3-induced proliferation and cytokine secretion of peripheral blood T cells and cell clones, while, SC5 mAb did not affect the cytotoxic activity of CD8+ T cell clones. Extensive phenotypic analysis revealed that the percentage of SC5+CD4+ circulating lymphocytes in SS patients was significantly increased in comparison with controls ($p < 0.001$) and correlated with the morphologically detected percentage of SS cells in peripheral blood ($p < 0.001$). In one patient we clearly demonstrated that the circulating malignant T cells coexpresses SC5 molecules. Importantly, ligation of SC5 receptor in a cutaneous T cell lymphoma (CTCL) cell line profoundly inhibited the anti-CD3 induced proliferation. Consequently, the expression of SC5 receptor in SS patients peripheral blood may serve not only to detect the presence of circulating malignant CD4+ cells but also a target for immunotherapy (*J Invest Dermatol*, 2001, in press).

008

Decreased Expression of Fas (Apo-1/Cd95) on Lesional CD4+ T Lymphocytes in Cutaneous T Cell Lymphomas: Correlations with Blood Data

O. Dereure, P. Portales, J. Clot, and J. J. Guilhou
CHRU Montpellier

Background: In a previous work, we demonstrated that the expression of apoptosis-mediating membrane receptor Fas/APO-1/CD95 was significantly decreased on CD4+ peripheral blood cells in CTCL compared with benign cutaneous lymphocytic infiltrates, which may favour the hypothesis of an initial accumulation of lymphocytes by a defective T-cell postactivation apoptosis. However, it is unknown whether skin-infiltrating lymphocytes displayed the same phenotype consistent with a defective Fas-dependent apoptosis.

Material and methods: Study by flow cytometry of fas expression on lesional CD4+ T cells extracted from cutaneous lesions of 12 patients with mycosis fungoides and 11 apparied patients with lymphocytic cutaneous benign inflammatory disorders; comparison with fas expression on CD4+ peripheral blood cells collected the same day.

Results: This study confirmed that fas expression on peripheral CD4+ lymphocytes was significantly lower in patients with CTCL and demonstrated that lesional lymphoid cells showed a similar pattern. Furthermore, correlation of Fas/CD95 expression between skin and blood lymphocytes appeared to be good.

Conclusion: These data support the hypothesis of an impaired Fas-mediated T cell apoptosis in CTCL physiopathology and establish additional correlations between blood and skin-based data in MF as to immunophenotyping profile of lymphocytes.

009

Inhibition of CGRP Expression in Alopecia Areata

D. Meyronet, K. Jaber,* A. Gentil-Perret, F. Cambazard, and L. Misery*
*Departments of Pathology and *Dermatology, University Hospital, Saint-Etienne, France*

The course of alopecia areata appears to be associated to stress. Denervation allows hair to grow again. Hence, the nervous system is involved in the pathophysiogeny of this disease.

Fifteen outpatients with alopecia areata have undergone biopsies of healthy scalp and lesions. We have searched for the expression of substance P and CGRP (calcitonin gene-related peptide), which are neurotransmitters coexpressed by sensitive nervous fibres. This immunohistochemical study has been performed on freezed slides. Monoclonal antibodies were revealed by immunoperoxidase.

Substance P was expressed in a same manner in healthy scalp and lesions. On the contrary, CGRP was strongly expressed on basal cells of epidermis and hairy folicles in healthy scalp whereas it was almost undetectable in lesions of alopecia areata.

This dramatic inhibition of CGRP expression could be due to a decrease of production or to a diminished expression of CGRP receptor. A recent study reveals a strong decrease of CGRP blood amounts. CGRP is a neurotransmitter with vasodilatator and immunosuppressive properties. We suggest the inhibition of CGRP expression in lesions favours vasoconstriction and the effects of lymphocytes, which are known to induce alopecia areata patches. Hence, CGRP agonists could be used to treat this disease. Works about other neurotransmitters are in process.

010

Drug Specific Cytotoxic T-Lymphocytes in the Skin Lesions of a Patient with Toxic Epidermal Necrolysis (TEN)

A. Nassif, A. Bensussan, G. Dorothée, F. Mami-Chouaib, N. Bachot, M. Bagot, L. Boumsell, and J.-C. Roujeau
Unité INSERM U 448 et Service de Dermatologie, Hôpital H. Mondor, Université Paris XII, Créteil, France

Objective: To characterize the T-lymphocytes present in the skin and suspect of being the effectors of the reaction in TEN, a drug reaction with widespread apoptosis of keratinocytes, considered to result from Fas–FasLigand interaction.

Methods: In a patient suffering from cotrimoxazole induced TEN, blister fluid cells were phenotyped by FACS and tested without any prior restimulation for cytotoxicity on autologous and allogeneic cells in the presence of the drug.

Results: Blister fluid cells were homogeneous for a phenotype of CD8+, DR+, CLA+, CD56+ T-lymphocytes and expressed preferentially two Vβ chains of the TCR repertoire. These lymphocytes were cytotoxic towards autologous EBV transformed lymphocytes and towards allogeneic cells sharing HLA-Cw4. Cytotoxicity occurred only in the presence of either cotrimoxazole or sulfamethoxazole but not with the hydroxylamine metabolite of sulfamethoxazole. The lysis was blocked by an anti-MHC Class I monoclonal antibody. It was abolished by EGTA, but neither by antifas nor by anti-TRAIL monoclonal antibodies, suggesting perforin/granzyme mediated cytotoxicity, without implication of fas at this stage.

Conclusions: This is the first direct evidence that T-lymphocytes present within the lesions of TEN may exhibit, without any re-stimulation, a drug-specific cytotoxicity against autologous cells. Harboring the markers of classical CTLs with MHC Class I restriction these lymphocytes reacted against the parent drug and not against its main reactive metabolite. These results challenge several current concepts and, if confirmed in further patients, could support new therapies.

011

Expression of High Affinity Immunoglobulin E Receptor (FceRI) on Mast Cells Increases the Intensity of the Inflammatory Response in a Model of Atopic Dermatitis (AD)

D. Staumont-Sallé,*† E. Delaporte,* M. Capron,† and D. Dombrowicz†
Department of Dermatology, CHRU, Lille, France; †INSERM U167, Institut Pasteur, Lille, France

The pathogenesis of AD still remains unclear, but FceRI is thought to be involved in the early and late stages of this disease. To confirm the potential role of FceRI, we performed epicutaneous sensitization with ovalbumin of wild-type mice (BALB/c), with FceRI expression restricted to mast cells and basophils, of FceRI-deficient mice (FceRIa−/−) and of transgenic mice, expressing a humanized FceRI (hFceRIa Tg) with a cell distribution similar to that in humans. The results obtained for BALB/c and FceRIa−/− mice suggest that FceRI expression on mast cells increases the intensity of the cutaneous and IgE responses, directly modulates mast cells recruitment to skin and lymph nodes and indirectly modulates cutaneous recruitment of eosinophils and antigen-presenting cells. On the other hand, the humanized cell distribution of FceRI does not enhance the immune response in this model. Furthermore, following a strictly cutaneous sensitization, we observed an inflammatory response in the lungs from the three groups of animals, correlating with the extent of skin inflammation. Works are in progress in order to study cytokines and chemokines released upon FceRI activation.

012

HIV Infection of a Reconstructed Mucosa Integrating Langerhans Cells

P. Sivard, O. Delézay, J. Kanitakis,* J.-F. Mosnier,† C. Genin, and L. Misery
*'Group Mucosal Immunity and Pathogen Agents', †Department of Pathology, University of Saint-Etienne; *Department of Dermatology, Edouard Herriot Hospital, Lyon, France*

We have performed a reconstructed *in vitro* mucosa integrating Langerhans cells (LC), and we have investigated the infection by HIV-1 of this model.

The epithelium consists of vaginal keratinocytes cultured on de-epidermized dermis in submerged medium for two weeks. LC precursors, obtained by differentiation of $CD34^+$ cord blood progenitor cells in presence of GM-CSF, TNF-α, TGF-β and Flt3L, are added in the reconstructed epithelium after 4–6 days of culture. Immuno-histochemical and ultrastructural studies showed that LC were detected in a physiological location in the well-stratified, differentiated epithelium.

HIV-1 strains (LAI and BaL) were added 1° on a epithelium of vaginal cells, 2° on isolated fresh LC, and 3° on the model of reconstructed mucosa. By nested PCR, we have shown the presence of proviral DNA in cultured LC and in LC integrated in reconstituted vaginal mucosa. In contrast, we could not detect any HIV-1 proviral DNA in epithelial cells. Then, we concluded that, in our experiments, only LC were infected by HIV isolates in this vaginal epithelial model. This one seems to be a useful tool for studying the mechanisms involved in heterosexual transmission of HIV.

013

In Freshly Isolated Human Langerhans Cells CD1a Molecules are Spontaneously Internalized, Gain Access to the Endosomal Pathway and Recycle to the Cell Surface

D. Lipsker, J. Salamero,* H. Bausinger,† A. M. Mommaas, D. Spehner, F. Proamer, J.-P. Cazenave,‡ B. Goud,* H. de la Salle, and D. Hanau

INSERM E 99–08 et ‡U 311, ETS, 67065 Strasbourg, France; **UMR 144, Institut Curie, 75248 Paris, France et* †*Departement de Dermatologie et Centre de Microscopie Electronique, LUMC, Leiden, Hollande*.

The cytoplasmic tail of CD1a molecules does not contain any of the targeting motifs known to direct membrane proteins into clathrin-coated pits and vesicles. In this study, we showed that in freshly isolated human Langerhans cells (LCs) CD1a molecules are not only present – at steady state – on the cell surface but also inside the cells, where they colocalized weakly with intracellular MHC class II molecules and with late endosomal/lysosomal markers. Immunoelectron microscopic analysis of ultrathin cryosections revealed the spontaneous presence of CD1a in clathrin-coated pits and vesicles as well as in Birbeck granules. Incubating freshly isolated LCs with an anti-CD1a FITC-labeled mAb confirmed the capacity of LCs (1) to internalize spontaneously CD1a molecules (2) the rapid localization of CD1a in early endosomes and (3) the recycling of CD1a from early endosomes to the cell surface. When endocytosis was blocked with latrunculin A or cytochalasin D, we observed (1) increased CD1a cell surface expression (2) the absence of CD1a from the $Rab11^{+}$ recycling endosomal compartments and (3) an enhanced capacity of LCs to stimulate a CD1a restricted T-cell clone.

014

Antagonist Effect of IL-4 and TGFβ1 on Langerhans Cell-Related Antigen Expression by Human Monocytes

G. Guironnet, C. Dezutter Dambuyant, C. Vincent, D. Schmitt, and J. Péguet-Navarro

INSERM U346, Hôpital E. Herriot, Pavillon R, Lyon, France

In this study we analyzed the specific effects of TGF-â1 and/or IL-4 on monocyte-derived cells. To this end, monocytes were cultured with either GM-CSF, GM-CSF/TGF-â1, GM-CSF/IL-4 or GM-CSF/IL-4/TGF-â1 before cell morphology, phenotype and function were assessed. As expected, IL-4 is mandatory for monocyte differentiation into potent allostimulatory dendritic cells. In its absence, monocyte-derived cells share many phenotypic and functional features with macrophages, such as CD14, CD16, CD64 and CD71 expression and lack of allostimulatory function. Interestingly, however, the cells express E-cadherin, independently of exogenous TGF-â1. Most importantly, a subset of monocytes cultured with GM-CSF/TGF-â1 expresses Langerin, as assessed by flow cytometry and confirmed by electron microscopy analysis. Langerin engagement with specific monoclonal antibody induces its internalization in coated pits and coated vesicles and the formation of typical Birbeck granules. Monocytes cultured in GM-CSF/IL-4 neither express E-cadherin nor Langerin. The simultaneous addition of TGF-â1 allows most of the cells to express E-cadherin but rarely Langerin, Taken together, the results add further evidence that LC can derive from monocytes and demonstrate an antagonistic effect of IL-4 and TGF-â1 on monocyte differentiation towards the LC pathway.

015

Germline and Somatic Mutations of the INK4a-ARF Gene in a XP-C Patient

N. Soufir, M. Rybojad,* T. Magnaldo,‡ O. Thibaudeau, L. Daya-Grosjean,‡ J. Rivet,† A. Sarasin,‡ and N. Basset-Seguin

U532, IRP **Service de Dermatologie,* †*Service d'Anatomie pathologique, Hôpital Saint-Louis, Paris;* ‡*UPR42,CNRS Vilejuif, France*

Xeroderma pigmentosum of group C (XP-C) is an inheritable autosomal recessive disease characterized by a defect in global genome repair, leading to the development of multiple skin tumours. A high proportion of these tumors harbor UV induced mutations (i.e. double CC > TT tandem transitions) of the p53 and/or the INK4a-ARF genes. Here, we report the clinical and molecular features of a 12-year-old XPC patient that in addition to severe cutaneous clinical symptoms also had 3 unusual tumors, a mediastinal lymphoblastic lymphoma, an atypical fibroxanthoma, and an epithelioid hemangioma. DNAs from eight skin tumors (4 actinic keratosis (AK), 2 microinvasive epidermoid carcinomas (ECs), 1 basal cell carcinoma (BCC) and one atypical fibroxanthoma) were examined by combining single strand conformation polymorphism and direct sequencing of the p53 and INK4a-ARF genes. Two different somatic mutations of $p16^{INK4A}$ were present in one tumour (a BCC) (12.5%), one of which affected also $p14^{ARF}$ reading frame. The same tumour also harbored a p53 tandem mutation. In addition, the patient carried also a germline variation affecting only the $p16^{INK4A}$ reading frame. This study confirms that simultaneous mutations of INK4a-ARF and p53 occur in some XP associated tumors. Furthermore, we show for the first time a germline variation of $p16^{INK4A}$, in association with the NER defect that could explain in part the patient's unusual phenotype.

016

Assessment of Ascorbic Acid and Iron Concentrations in Psoriatic Patient Dermis vs. Healthy Subjects

N. Leveque,* S. Robin,† P. Muret,*‡ S. Mary,* S. Makki,*§ J. P. Kantelip, A. Berthelot, and Ph. Humbert

**Laboratoire d'Ingénierie et de Biologie Cutanées,* †*Laboratoire de Physiologie* ‡*Laboratoire de Pharmacologie Clinique,* §*Laboratoire de pharmacie galénique, Faculté de Médecine et de Pharmacie, Place Saint Jacques, 25030 Besançon cedex-France*

Introduction: Reactive oxygen species (ROS) play an important role in skin inflammatory diseases such as psoriasis. Iron catalysed ros formation, while ascorbic acid (AA) scavenges ROS. The aim of this work was to assess AA and iron concentrations in involved and uninvolved psoriatic dermis and to compare them with AA and iron levels in healthy human dermis.

Materials and methods: 10 psoriatic patients and 8 healthy subjects were enrolled in this study. Two microdialysis probes (CMA/20) were inserted into the dermis with and without psoriatic lesions. A parallel study using microdialysis was performed in healthy subjects. AA and iron collected by microdialysis were assessed, respectively, by gas chromatography mass spectrometry and atomic absorption spectrometry.

Results: AA concentrations in psoriatic dermis with and without lesions were, respectively, 38.7 ± 1.6 µg per ml, 42.6 ± 1.2 µg per ml. Iron levels in involved and uninvolved psoriatic dermis were, respectively, 55.5 ± 1.9 µg per l et 47.0 ± 2.4 µg per l. AA and iron concentrations in healthy dermis were, respectively, 237.4 ± 8.7 µg per ml et 21.4 ± 2.3 µg per l.

Conclusion: These results demonstrate that AA concentrations are lower and iron levels higher in the dermis of psoriatic patients than in the skin of healthy subjects.

017

cDNA Cloning of Human Type I Peptidylarginine Deiminase and Expression Analysis

M. Guerrin,* A. Ishigami,† M. Sebbag,* T. Senshu,† M. Simon and G. Serre*

**Department of Biology and Pathology of the Cell, INSERM CJF 9602-IFR 30, Purpan School of Medicine, Toulouse, France;* †*Department of Cell Biology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan*

Deimination of cytokeratins and filaggrin occurs in the latest stages of epidermal differentiation and is thought to play a key role in the cornification process. Peptidylarginine deiminases (PADs) are a family of enzymes that convert arginine residues to citrulline residues. This post-translational modification changes the structure and possibly the function of the protein targets.

We cloned a full length cDNA for human epidermis type I PAD by a combination of RT-PCR using rat type I sequences, and RACE cloning. Human type I PAD displays 85% and 83% homology with mouse and rat type I PADs, respectively. Homology with other human PADs is more limited: 71%, 70%, 66% with type V (ortholog of mouse and rat type IV), type III, and type II, respectively. The expression of type I PAD was analysed by RT-PCR in a panel of 17 human tissues. In addition to epidermis, type I PAD was found to be mainly expressed in testis and placenta, and to a lesser extent in prostate and thymus. Very low or no expression was detected in heart, skeletal muscle, colon, small intestine, kidney, ovary, leukocytes, spleen, whole brain, lung, liver and pancreas. Recombinant type I PAD (*E. coli*) encoded by the cDNA exhibited enzymatic activity on bacterial extracts. Antisera elicited in rabbits are being used to determine the precise location of type I PAD in human epidermis.

018

Homophilic Interactions of Human Corneodesmosin

C. Caubet, S. Lawson, M. Guerrin, M. Simon, and G. Serre

Department of Biology and Pathology of the Cell, INSERM CJF 9602-IFR 30, Purpan School of Medicine, Toulouse.

Corneodesmosin (Cdsn) is a secreted glycoprotein located in the extracellular part of corneodesmosomes, the corneocyte-specific intercellular junctions. It is thought to play a major role in stratum corneum cohesion, and its degradation at the stratum corneum surface is necessary for desquamation to occur. Glycine- and serine-rich domains of Cdsn have been proposed to fold as structural motifs similar to glycine loops and to confer adhesive properties to the protein.

To test if Cdsn is really an adhesive protein, and to define the involved domains, full-length and truncated Cdsn forms, GST-tagged at their terminus, were produced in *E. coli* and tested *in vitro* for protein–protein interactions by membrane-overlay binding assays. Full-length Cdsn displayed homophilic binding properties but did not bind to GST alone, to BSA or to filaggrin. The interactions were dose-dependent, and incubation with calcium only induced a moderate increase in binding. Various forms of Cdsn deleted of the NH_2- or the COOH-terminal glycine loop domain, or of both domains, were also shown to display binding properties, but with a somewhat lower efficacy in the absence of the NH_2-domain. Interestingly, the full-length Cdsn and the various deletion mutants did bind another truncated form corresponding to the NH_2-terminal domain all alone. Therefore, this domain displays adhesive properties by itself.

The results provide the first experimental evidence for homophilic interactions of Cdsn, and confirm its involvement in intercorneocyte cohesion.

019

In Vitro Interleukin-15 and Interleukin-15 Receptor Expression by Normal Human Epidermal Keratinocytes

S. Leroy, J. Bernard, N. Chebassier,* A. C. Knol,* A. Plet, A. Godard, Y. Jacques, and B. Dréno*
*'Groupe de recherche sur les cytokines et leurs récepteurs', *Laboratoire d'Immuno-Dermatologie; INSERM U463, Nantes, France*

Interleukin (IL)-15 exerts similar biological activities to IL-2, notably by permitting lymphocyte activation.We have previously showed that IL-15 mRNA, but not IL-15 protein, could be detected *in vivo* in normal epidermis. The aim of this work was to study IL-15 and IL-15 receptor expression, and also their modulation by interferon a and ã, *in vitro* on monolayers of normal human keratinocytes.

IL-15, IL-15Rá, IL-2Râ and ãc mRNA expression was studied by RT-PCR, whereas IL-15 and IL-15Rá protein expression was examined using immunoperoxidase and Western blot.

By RT-PCR, we have observed that IL-15, IL-15Rá, IL-2Râ and ãc mRNA were expressed by normal human keratinocytes. IL-15 and IL-15Ra protein expression was also detected. Interestingly, IL-15Rá chain was detected in the nucleus of keratinocytes. This nuclear localization, which was confirmed using a Western blot technique, suggests that IL-15 may play a role at this level.

After stimulation by interferon á and ã, we observed an increase in IL-15 and IL-15, IL-15Rá and ãc mRNA expression, respectively. IL-15 and IL-15Rá protein expression were increased too.

Induction of IL-15 and IL-15 receptor production in normal human keratinocytes stimulated by IFNã suggests that this cytokine could play an important role in cutaneous T cell lymphoma development, a disease for which we previously have detected an elevated and specific IL-15 protein expression in keratinocytes.

020

DNA Damage and UV Sensitivity of Human Keratinocytes Harboring HPV 16

S. Mouret, M. T. Leccia, S. Sauvaigo,* A. Favier, and J. C. Beani
*Laboratoire de Biologie du Stress Oxydant, CHU Michallon, 38043 Grenoble cedex 03, France; *Laboratoire des Lésions des Acides Nucléiques, CEA, Grenoble, France*

Human papillomaviruses (HPV) in conjunction with ultraviolet (UV) radiation may play a role in the pathogenesis of nonmelanoma skin cancers from Epidermodysplasia verrucciformis individuals and some immunocompromised patients. E6 and E7 oncoproteins of high risk HPV may allow DNA damage persistence and modify UV-irradiated cells survival. The aim of our study was to determine the effects of UV irradiations on sensitivity and DNA damage of human keratinocytes harboring HPV 16. We used two keratinocyte lines, SKv-e and SKv-l, different in their viral genome integration, *in vitro* proliferative potential, autoregulation by TNFα, and *in vivo* tumorigenicity in mice. Firstly, we found significant different sensitivity between the two cell lines when exposed to a single UV irradiation. SKv-e cells with less proliferative and tumorigenicity potential, were most sensitive to UV irradiation than SKv-l cells, lethal dose 50 corresponding, respectively, to 0.6 and 1.5 J per cm^2. This difference between the two cell lines may be correlated with both E6 expression and p53–p21 pathway interactions. We showed secondly, using the comet assay, no significant difference between SKv-e and SKv-l cells in term of DNA strand-breaks and alkali-labile sites. But interestingly, we found an increased of oxidative DNA damage in SKv-e cells. In conclusion, these results suggest an interaction between viral oncoproteins and antioxidant status, which may play an important role in HPV-infected cell transformation.

021

Prevalence of Antidesmoglein Antibodies in the Tunisian Population

M. Kallel Sellami, M. Zitouni, M. Ben Ayed,* P. Martel,† D. Gilbert,† H. Masmoudi,* F. Tron,† P. Joly,† and S. Makni
*Hôpital La Rabta, Tunis. *Hôpital Habib Bourgiba, Sfax. †INSERM U519, Rouen*

In Tunisia, pemphigus has particular epidemiological characteristics, that mimic endemic pemphigus in some extend. Indeed, prevalence is increased and the superficial subtype (PF) prevales, involving mainly young females under 35. Since anti-Dsg1 antibodies have been found in 55% healthy Brazilian people living in endemic areas we tested the hypothesis that normal subjects living in Tunisia also have such antibodies.

Eighty four healthy blood donors from Tunis and Sfax were studied by immunoblot using an epidermal extract and were compared with 75 Tunisian patients with pemphigus (38 PF, 34 PV, 3 pemphigus herpetiformis).

Twenty-seven percent healthy controls (22/80) had autoantibodies that recognized polypeptides corresponding to pemphigus antigens: 15/22 reacted with a 160-kDa polypeptide (desmoglein 1), 5/22 with a 130 KD polypeptide (desmoglein 3), 8/22 with a 190-kDa polypeptide (periplakin) and 1/22 with a 250-kDa polypeptide (desmoplakin 1). IgG1 was the predominant subtype (77% anti-Dsg 1 antibodies, 60% anti-Dsg3 antibodies) and IgG4 subtype was never found. In contrat all patients with autoantibodies detected by immunoblot (65% PF, 44% PV) had IgG4, sometimes associated with other isotypes.

Anti-desmoglein antibodies and particularly anti-Dsg1 antibodies are prevalent in the Tunisian healthy population, which may be related to the high prevalence of PF in Tunisia. Furthermore, IgG4 are never found in healthy controls in accordance with the pathogenicity of this isotype in pemphigus. Factors that would explain the prevalence of antidesmoglein antibodies and the switch to pathogenic antibodies remain to be determined and probably involve both genetic background and environmental events.

022

Epistatic Interaction of DSG1 and HLA-DR4 in Pemphigus Foliaceus Susceptibility

P. Martel, D. Gilbert, M. Busson, L. Drouot, P. Joly, D. Charron, and F. Tron
INSERM U519, Rouen, France, Hôpital Saint Louis, Paris, France

We previously described a silent single nucleotide polymorphism (SNP) of the desmoglein 1 gene which consists of a T to C transition at position 809. To investigate the role of genetic background in pemphigus foliaceus and to ask whether PF, like other autoimmune diseases, is expressed as a complex trait, we simultaneously examined the role of major histocompatibility complex (MHC) class II polymorphism and SNP(809) in PF susceptibility.

Thirty-one Caucasian French patients and 84 healthy Caucasian French controls were studied by PCR-RFLP for SNP(809) genotyping and by PCR-SSO and PCR-SSP for DRB1 and DQB1 typing.

This analysis confirmed involvement of DRB1*04 ($p = 0.01$) and DRB1*14 ($p = 0.04$) generics in disease susceptibility and individualized DRB1*0102 ($p = 0.04$), DRB1*0402 ($p = 0.02$), DRB1*0406 (0.003), and DRB1*1404 ($p = 8.10^{-4}$) as susceptibility MHC class II alleles in French PF patients. Homozygous C/C(809) genotype was also found associated with the disease ($p = 0.03$). Furthermore, patients with both DRB1*04 and C/C(809) had a very significant risk to develope PF ($p = 1.10^{-4}$) and comparison, by logistic regression, of susceptibility given by both risk factors showed a significant interaction between DRB1*04 and C/C(809).

DRB1*04 and C/C(809) are significant risk factors to PF and interact to enhance disease susceptibility. PF therefore constitutes another demonstrative example of the role of epistatic interaction of individual genes in autoimmune diseases susceptibility.

023

The Reconstructed Epidermis with Sensory Neurons, a New Tool to Study Dermo-Cosmetic Products Properties

R. Steinschneider, C. Marteau,* E. Quelven, J. Franchi,* P. Andre,* and J.-C. Laurent
*B.E.T. Bio Expertise Technologies, 13011 Marseille; *Laboratoires R & D – LVMH Branche Parfums et Cosmétiques, 45804 Saint Jean de Braye*

Somatic sensory neurons transfer sensitive information from the skin to the Central Nervous System. In addition, the action potential is transfered anterogradely from dentritic fibers to the upper epidermis. This property allows sensory neurons to deliver neuropeptides to epidermal cells, particularly keratinocytes. The release of neuropeptides such as CGRP, substance P in skin is able to modulate both cutaneous cell proliferation and cell differentiation and is able to regulate secretion of both cytokines and growth factors. In order to reproduce a reconstructed sensory epidermis we have developed a culture chamber with two compartments. Rat sensory neurons from dorsal root ganglions were cultured on a biological matrix in the first compartment. On the other side of the matrix, in the second compartment a well-differentiated epidermis was constructed using normal human keratinocytes. Both microscopic and immuno-enzymatic analysis allowed us to demonstrate: *i.* keratinocytes have developed a pluristratified epidermis; *ii.* cell bodies of neurons are not directly in contact with keratinocytes; *iii.* Only neuritic fibers cross the biological matrix and establish a network among keratinocytes; *iv.* these cultured neurons are mature and functional, because they spontaneously release CGRP that is over expressed when neurons are stimulated *in vitro*. This novel model will be described and we will discuss potential applications.

024

Micrografts of Human Scalp Onto Nude Mice: Technical Improvement of a Human Hair Growth Model *In Vivo*

M. B. de Brouwer, T. Leroy, G. Shaker, and D. Van Neste
Skinterface, Tournai, Belgium

Several sources of evidence support the use of human scalp grafts onto nude mice as a model for the study of human hair growth *ex vivo*. Grafted hair follicles showed slowing down of the linear growth rate to 2/3 of the initial value and reduction of the expected life span of the hair follicles (up to 8 months) even though some follicles may engage into a second cycle of hair production. Testosterone conditioning of such mice bearing samples from affected scalp sites (androgenetic alopecia) has been proposed for assessing the efficacy of compounds having antiandrogen activity. At this stage however, information on the percentage of success of graft take (in terms of grafts and in terms of follicular units) is lacking.

In this study we performed micrografts (1–3 follicular units – unaffected androgen non sensitive donor site), aiming to document an eventual increase of the success rate usually obtained with the conventional punch grafts (10–15 follicles from affected scalp sites). These "micro" samples were implanted in mice and then monitored for hair growth during 7 months. Quantitative data were obtained from phototrichograms performed every month.

The analysis of the phototrichograms showed 58.33% productive micrografts compared to the 31.1% ratio obtained from punch grafts. The number of productive follicular units in micrografts reaches 47.22% of the initially active follicles while only 10.5% follicles remained active with the punch graft method.

Our results suggest that the improved grafting method inspired from cosmetic scalp surgery protocols must be further investigated as a clinically relevant experimental model.

025

Evaluation of the Convenience of the CD7 Marker in the Identification of Sezary Cells

A. C. Knol, N. Robillard, F. Accard, N. Chebassier, S. Leroy, M. C. Pandolfino, Y. Guilloux, F. Jotereau, and B. Dréno
INSERM U463 et CHU Hotel-Dieu, Nantes, France

Cutaneous T-cell lymphomas (CTCL) are characterized by the infiltration of the skin by T lymphocytes of the CD2+ CD3+ CD4+ CD45RO+ DR+ phenotype. CTCL represent a unique model in tumoral terms because both tumoral and reactive cells are T lymphocytes. Till now no specifical marker of tumoral cells has been described in the literature. Nevertheless, since the idiotype of the TCR represents a unique marker for a malignant clone of T lymphocyte, the clonal origin of tumoral cells allows us to think that the TCR-Vβ clone corresponds to the tumoral cell. Concurrently, loss of expression of CD7 is considered as a diagnostical criterium for CTCL because of its frequent publication and would be specific of Sezary cells. But this hypothesis remains unconfirmed. Our goal is to determine whether this CD7- population represents the Sezary cells. Our study turns on patients, cytologically confirmed and exhibiting more than 50% circulating Sezary cells. A sorting, by cell sorter, is realised with in order to harvest the CD4+ CD7+ and CD4+ CD7- populations, which are then submitted to a cytological evaluation on the basis of the cerebriform nucleus of Sezary cells. We found for our first patient that both CD4+ CD7+ and CD4+ CD7- populations contained Sezary cells, so this finding suggests that CD7 is not an appropriate marker to identify Sezary cells. Moreover we were able to note that that this cerebriform appearance was lost after a few days of *in vitro* culture with IL-2, IL-7 or IL-15, therefore this aspect would be directly related to the *in vivo* environment.

026

Expression of Retinoblastoma Gene in Melanoma Cell Line

A. Sparsa, C. Bédane, S. Boulinguez, H. Chable,* G. Barrière,* K. Faucher,* O. Berthier-Vergnes,* A. Varachaud,* M. Rigaud,* and J. M. Bonnetblanc
*Dermatology, *Molecular Biology, CHU Dupuytren, Limoges, France*

The high incidence of melanoma (4–14%) in retinoblastoma patients suggest that an oncosuppressor gene, the retinoblastoma gene could play a role in the progression of melanoma. Therefore we have studied the expression of retinoblastoma gene in three melanoma cell lines. Cell culture of 3 melanoma cell lines was performed in Mac Coy 5 A medium, 5% of CO_2. ARN extraction and amplification was done by RT-PCR. Western blot was performed with an antihuman pRb monoclonal antibody (G3-245). The level of protein was quantified by spectrophotometry. We confirm the expression of mRNA of retinoblastoma gene and of the protein Rb in 3 new melanoma cell lines. Despite a different tumorigenicity the level of expression of protein was identical in all cell lines and positive control MOLT-4. Thus our 3 cell lines express Rb protein at a pre and post transcriptional level. Quantitative RT PCR could assess wether there is or not a significant difference between the cell lines and positive control. We cannot exclude the possibility in some cell lines of a dysfunction of Rb protein secondary to mutations of other molecules or genes (CDKN2/p16…).

027

Transduction of Melanoma Cell Lines by AAV Type 2 Vectors

S. C. Cheong, L. Tenenbaum,* C. Bruyns,* T. Velu,* and A. Brandenburger
*IRIBHN, Université Libre de Bruxelles – Belgium, Campus Gosselies, *Campus Erasme.*

Recombinant adeno-associated virus type 2 (rAAV2) vectors are among the most promising viral vectors for gene therapy purposes and are already being tested in clinical trials. In cancer gene therapy the rAAV vector can be used to introduce genes that code for cytotoxic proteins into tumour cells. In one experimental approach we propose to transduce melanoma cell lines by rAAV vectors. However to date, the transduction efficiency by rAAV vectors in melanoma cells has not been well described.

The transducibility of three human melanoma cell lines Mel.Z2, 518.A2 and EB81 was studied by infection with rAAV-EGFP, a vector that carries the enhanced green fluorescent protein reporter gene. Infection was performed at multiplicities of infection (MOI) of 2.5, 5 and 10 transducing units (TU) per cell. Two days postinfection the expression of EGFP was measured by FACS analysis.

All three melanoma cell lines were found to be transducible by rAAV-EGFP. At an MOI of 2.5 TU/cell 10–25% of the cells were found to express EGFP. Increasing MOI resulted in over 50% EGFP positive cells.

These results show that melanoma cell lines are transducible by rAAV2 vectors *in vitro*. In contrast to most other viral vectors that require infection at high MOI, efficient transduction by rAAV can be obtained at relatively low MOI, suggesting small amounts of vector may also be sufficient for *in vivo* transduction.

028

Pattern of MIB-1 Distribution in Cutaneous Verrucous Carcinoma: Analysis of 5 Cases

J. C. Noel,* I. Fayt, G. De Dobbeleer, and T. Simonart
**Department of Pathology, Hôpital Erasme, Brussels, Belgium*

Introduction: Verrucous carcinoma (VC) is a low-grade squamous cell carcinoma (SCC). Its diagnosis requires evaluation of the clinical and microscopic appearance and biologic behaviour of the neoplasm. It is a slowly growing verrucous tumour that may ultimately penetrate deep into the tissue. Because of its high degree of histologic differentiation, it is often not recognised as a carcinoma for a long time. The goal of this study was to analyse the pattern of MIB-1 expression in VC and to compare it with SCC.

Material and methods: Routinely formalin-fixed and paraffin-embedded blocks were retrieved from the Erasme University Hospital, Brussels, Belgium. Five cases of VC and 10 cases of invasive well-differentiated SCC involving the skin were studied. Immunohistochemistry using MIB-1 (Immunotech, Marseille, France), a murine monoclonal antibody which reacts with the same epitope as KI67, was carried out on 4 µm paraffin-embedded sections using the antigen retrieval method.

Results: In VC the positive cells were principally located in basal and suprabasal layers at the periphery of the tubular epithelial strands. However, at the growing margin of the tumour, where areas of cellular anaplasia and high mitotic activity were the rule, MIB-1-positive nuclei were focally noted in intermediate layers. By contrast, in SCC, positive cells were randomly scattered throughout the lesion.

Conclusion: This peripheral pattern of MIB-1 distribution in VC may at least partially the differences in the clinical and morhological characteristics between VC and SCC.

029

Stromelysin 3 Expression is a Marker of Prognosis in Basal Cell Carcinomas

B. Cribier, G. Noacco, B. Peltre, and E. Grosshans
Laboratoire d'Histopathologie cutanée, Clinique Dermatologique des Hôpitaux universitaires, Strasbourg, France

Stromelysin 3 (ST3) is a member of the metalloproteinase family, which is expressed in various tissue remodeling processes. The prognosis of breast cancers and extracutaneous squamous cell carcinomas is correlated to the level of expression of ST3. The purpose of this work was to study the expression of ST3 in the 4 types of basal cell carcinomas (BCC).

We selected cases of primary BCC that were fully excised, without previous biopsy: 40 Pinkus tumors, 40 superficial BCC, 40 nodular BCC, 40 morpheiform BCC and 10 cases of BCC with deep subcutaneous or muscular invasion. Immunohistochemistry was carried out using the LSAB method, with monoclonal anti-ST3 Ab (MC Rio, IGBMC Strasbourg). A semiquantitative scale was used to evaluate the level of ST3 expression, from 0 to 3.

Positively stained cells were restricted to the periphery of the epithelial cells, and were concentrated around the most invasive strands. The global rate of expression was 27% of Pinkus tumors, 65% of superficial BCC, 72.5% of nodular BCC, 87% of morpheiform BCC and 100% of deeply invasive BCC. In addition, the rate of BCC with intense expression of ST3 (2 or 3) was, respectively, 7.5%, 20%, 45%, 63% and 100%.

This study confirms that ST3 is a marker of poor prognosis, because the rate of positively stained tumors was much higher in the aggressive BCC groups. Moreover, the majority of BCC which intensely expressed ST3 were morpheiform BCC and tumors with deep invasion, which are both of poor prognosis. These results are similar to those previously published in squamous cells carcinomas and keratoacanthomas (1). Altogether, the studies on cutaneous tumors are consistent with the theory of ST3 playing an active role in tumor progression.

(1) Asch PH *et al*, *Am J Dermatopathol* 21:146–150, 1999.

030

Cutaneous Human Dendritic Cells (DCs) Express CD1d

S. Ronger, G. Guironnet, M. J. Staquet, J. Peguet, D. Schmitt, A. Claudy, and D. Jullien
Unité Inserm 346, Hôpital Edouard Herriot, Lyon

The CD1 family of cell surface glycoproteins is a third lineage of antigen presenting molecules. Human CD1d tissular distribution is not well established. We previously reported CD1d expression on normal human keratinocytes (NHK). In the present report, we studied CD1d expression on cutaneous professional antigen presenting cell. Langerhans-like cells were generated *in vitro* from cord blood CD34+ stem cells (GM-CSF+ TNFá; 13 days) and from adherent PBMC (GM-CSF+ IL4+ TGFâ = GIT; 7 days). Dermal dendrocytes like cells were differentiated from PBMC (GM-CSF + IL4 = GI; 7 days). GI and GIT population were further matured by adding TNFá or cocultured with CD40L+ for 2 days more on the media. Immunochemistery was performed on normal skin specimens (n = 5, mAb NOR3.2). Double staining immunofluorescence suggested that dermal dendritic cells (Factor XIIIa+, CD83+) expressed CD1d and that to a lower level Langerhans cells (CD1a +) did too. Using specific primers, semiquantitative RT-PCR was used to assess CD1d mRNA expression. In Langerhans cells generated from cord blood, time course evaluation showed a progressive expression of CD1d mRNA. When TGFâ was added to the media, CD1d mRNA expression was progressively turned off, suggesting a regulatory role for TGFâ. CD1d mRNA was detected both in GI and GIT cells with a higher signal in GI population, which support the hypothesis of an inhibitory effect for TGFâ. Moreover, there was an increase of CD1d mRNA expression in these two populations during cells maturation with TNFá or CD40L. Cell surface expression was confirmed by flow cytometry on DCs developed *in vitro* (mAb CD1d 42.1). Our data suggest that CD1d is expressed on dermal dendrocytes and to a lower extent on Langerhans cells. TGFâ inhibits the expression of CD1d mRNA, while the expression is up-regulated when dendrocyte cells are further matured with CD40L or TNFá.

031

GM-CSF, TGFâ1 and TNFá Enable Peripheral Monocytes to Become Epidermal UV-Macrophages

N. Bechetoille, S. Dumont, V. André,* G. Guironnet, J. Péguet, S. Maréchal, D. Schmitt, E. Perrier,* and C. Dezutter-Dambuyant

*INSERM U346, Edouard Herriot Hosp., Lyon, France; *COLETICA, Gerland, Lyon, France*

After acute UVB irradiation of human skin, Langerhans cells (LC) leave the epidermis. However, the LC-depletion is transient and the epidermal compartment is rapidly colonized by $CD1a^-$ $DR^+CD11b^+CD36^+FcâRII^+$ monocytes/macrophages. This population was called UV-Macrophages (Meunier *et al, J Invest Dermatol* 1995, 105:782–788; Kremer *et al, Eur J Immunol* 1998, 28:2936–2946). To determine the origin of the UV-Macrophages which might either be issued from de-differentiation of residual epidermal LC or result of a colonization by migrating monocytes (Mo) from dermis, peripheral blood Mo were cultured in presence of 3 major keratinocyte-derived cytokines: GM-CSF, TGFâ1 and TNFá. After 6 days of culture with GM-CSF and TGFâ1, Mo differentiated into 2 distinct $CCR6^+$ subsets: (1) a low proportion (0.5–1%) of $CD1a^+$/$Langerin^{+high}$/$CCR6^+$ cells that strongly expressed the specific marker of LC, that is Langerin, and (2) a high number (40–50%) of $CD1a^+$/$Langerin^{+low}$/$CCR6^+$ cells. After addition of TNFáthe $Langerin^{+high}$ cells increased up to 2% and kept their phenotype while the phenotype of the $Langerin^{+weak}$ subset was modified. The $Langerin^{+weak}$ population progressively acquired the monocyte-macrophage profile, that is, $CD1a^{+weak}$/$Langerin^-$/$CCR6^{+weak}$ in presence of increasing amounts of TNFá (0–200 U/ml concentration). In presence of these 3 selected cytokines, the peripheral blood Mo may modulate their phenotype and become very similar to epidermal UV-Macrophages which were shown to originate from dermal monocytic cells entering epidermis after acute UV-irradiation. The capacity of these generated UV-Macrophages-like cells to produce IL12/IL10 and to stimulate allogeneic lymphocytes is under investigation.

032

The New BDCA Markers May Clarify the Controversy Between the Lymphoid and Myeloid Origins of the Langerhans Cells

S. Dumont, N. Bechetoille, S. Maréchal, D. Schmitt, and C. Dezutter-Dambuyant

**INSERM Unité 346, Hôpital Edouard Herriot, Lyon, France*

The lymphoïd vs. myeloïd origin of both cutaneous dendritic cells (DC), Langerhans cells (LC) and dermal DC is still a controversial issue. The identification of the new BDCA-1 to –4 markers could help us to solve this dilemma. We have studied the expression of these antigens on cells produced after 7 and 14 days of culture from CD34+ progenitors in presence of GM-CSF and TNFá as well as from CD14+ monocyte-derived in presence of GM-CSF, IL4 and TGFâ1 for 6 days and then, 2 days by adding TNFá. In both CD34+ and CD14+ systems, DC did not express BDCA2 even though BDCA4 was expressed by the majority of cells at the different culture times. In the CD34+ system, BDCA1 (CD1c) was expressed by 20–30% of cells, this percentage increased until day 14. In order to determine whether BDCA1 is a marker associated to an early LC differentiation stage, double cell surface labeling was perdormed with BDCA1 and, on day 7, E-Cadherin; on day 14, Langerine (Lang) (CD207, LC-specific type II lectine). On day 7, all cells were $BDCA1^+$/E-$Cadherin^+$ and on day 14, two subsets $BDCA1^+$/$Lang^+$ and $BDCA1^+$/$Lang^-$ were observed. In the CD14+ system, more than 60% of cells expressed BDCA1 on day 6 as well as on day 8, even though BDCA3 was present on more than half of the cells on day 6, BDCA3 expression was down-regulated after TNFá addition. In the CD14+ system, the strong BDCA1 expression on Lang+ cells seems to depend on the presence of TGFâ1 whereas in the CD34+ system, where TGFâ1 is absent, a BDCA1+ subset might not be able to acquire the LC marker. The presence of BDCA1 (described as a myeloïd DC-associated marker) on DC may be correlated to their ability to differentiate into LC. As BDCA3 expression in the CD14+ system was decreasing in parallel to Langerin appearance, after activation by TNFá, this BDCA3 marker (described as a myeloïd DC-associated marker) would be present on cells which would not have yet acquired the ability to differentiate into LC. Finally, BDCA4 (described as a plasmacytoïd DC-associated marker) may reveal all cells engaged in both LC and dermal DC pathways.

033

Keratinocyte Surface-Associated Proteolysis: Role in the "*In Vitro*" Shedding Process of BP180 (Collagen XVII)

A. L. Labrousse, W. Hornebeck,* and P. Bernard

*Department of Dermatology, CNRS FRE 2260, IFR 53, *Department of Biochemistry, CNRS FRE 2260, IFR 53, Faculty of medicine, REIMS*

Collagen XVII (BP180) is a hemidesmosomal transmembrane protein involved in adhesion of epithelial cells to the underlying basement membrane. It exists in two forms, including a 180-kDa full-length transmembrane protein and a 120-kDa polypeptide, corresponding to the collagenous ectodomain of the entire protein (Schäcke *et al, J Biol Chem*, 1998) and released as a soluble form in culture medium of normal human keratinocyte (NHK). The purpose of this study was the *in vitro* analysis of the keratinocyte surface-associated mechanisms of proteolysis that could lead to the shedded collagen XVII, and the matrix components influence on this shedding. Enhancement of active membrane gelatinase B (MMP-9), induced by exogenous treatment with neutral sphingomyelinase on NHK culture (Buisson *et al, J Invest Dermatol*, 2000), did not seem to increase the release of the 120 kDa soluble form in culture medium. However, concomittent treatment with sphingomyelinase and a peptide hydroxamate MMP inhibitor, batimastat, completely inhibited the shedding. Besides, treatment of NHK with a synthetic inhibitor of furin convertase, decanoyl-RVKR-chloromethylketone, markedly reduced the release of the 120 kDa soluble form. These data are not in favour of membrane MMP-9 involvement in the *in vitro* shedding process of collagen XVII and suggest the role of a furin-dependent proteolytic pathway. On the other hand, NHK culture on coated flasks, respectively, with laminin-5, laminin-1, collagen IV and collagen I, did not modify the amount of soluble form released in culture medium.

034

Early and Late-Stage Kaposi's Sarcoma-Derived Cells Can Invade De-epidermized Dermis

T. Simonart, C. Degraef,‡ R. Mosselmans,‡ P. Hermans,§ Y. Lunardi-Iskandar,¶ J.-C. Noel,† J.-P. Van Vooren,* D. Parent, M. Heenen, and P. Galand‡

*Department of Dermatology; *Department of Internal Medicine; †Department of Pathology; ‡Laboratory of Cytology and Experimental Cancerology, Erasme University Hospital, Brussels; §Department of Infectious Diseases, CHU St Pierre Brussels; ¶Institute of Human Virology, Baltimore, USA*

Background: Whether kaposi's sarcoma (KS) is a true neoplasm or a reactive endothelial cell outgrowth remains unclear.

Materials and methods: In this study, we investigated the differential invasive properties of activated endothelial cells and KS cells in a model of de-epidermized dermis, supplying the cells with matrix barriers similar to those found *in vivo*.

Results: Cells derived from early "patch-stage" and from late "nodular-stage" KS lesions exhibited similar invasive properties. Low proliferation index of the cells and expression of antiapoptotic proteins suggest that the progression of KS may be related to escape from cell death rather than to increased proliferation. The KS-Y1 cell line, which is tumorigenic in nude mice, also exhibited invasive properties. However, by contrast to the KS-derived spindle cells which were scattered between the collagen bundles, the KS-Y1 cell population displayed a multilayer arrangement. Inflammatory cytokines and KS cell supernatant could activate and stimulate the growth of human dermal microvascular endothelial cells but could not induce their invasion in this model.

Conclusion: These results confer to KS cells an invasive phenotype.

035

Antiviral Activity of Different Anti-Herpesvirus Compounds on Keratinocyte Organotypic Cultures Infected with Herpes Simplex Virus and Varicella-Zoster Virus

R. Snoeck, G. Andrei, E. De Clercq, and C. De Wolf-Peeters*

*Rega Institute for Medical Research, *Pathology Department, U.Z., K.U.Leuven, B-3000 Leuven, Belgium*

The three-dimensional organotypic "raft" culture system of keratinocytes recreates important features, both morphological and physiological, of epithelial differentiation *in vitro*. Human primary keratinocytes, isolated from neonatal foreskin, grown at the air–liquid interface, stratify and differentiate with the expression of specific keratins within 12–14 days. These cultures can be used as a model for skin tissue, the main viral replication site during both primary and recurrent herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections. We have shown that infection of cultures with HSV results in the production of cytopathic effect (CPE) independently of the stage of differentiation of the cultures. In contrast, infection with VZV after 8–10 days of differentiation resulted in reduced CPE compared to cultures infected after 2, 4 or 6 days of differentiation. In order to evaluate the effect of various antiherpesvirus drugs, organotypic cultures were infected with HSV-1 and HSV-2 after 8 days of differentiation and with VZV after 6 days of differentiation. At the moment of infection the cultures were incubated in the presence of medium containing different concentrations of the test compounds (acyclovir, brivudin, cidofovir and foscarnet). The cultures were incubated for 12 days, fixed and processed for histology. Morphological analysis of the organotypic cultures showed that treatment with cidofovir, acyclovir and brivudin at 40, 4 and 0.4 μg per ml and foscarnet at 200 μg per ml complete protected the epithelium against virus-induced CPE. As expected, only cidofovir and foscarnet showed activity against thymidine kinase-deficient viral strains. The evaluation of antiviral compounds in organotypic cultures may be of particular interest for VZV, given the fact that there is no *in vivo* model for VZV.

036

The â-Defensin hBD-2 is Expressed in Differentiated Human Reconstructed Epidermis, at mRNA and Peptide Levels, and is Up-Regulated in Response to Bacterial LPS

P. Chadebech, D. Goidin, C. Jacquet, J. Viac, D. Schmitt, and M. J. Staquet

INSERM U346, affiliée CNRS, Hôpital E. Herriot, Lyon, France

Defensins have been identified as key elements of innate immunity against microbial infections. Human β-defensins have been shown to be expressed in skin and airway epithelial tissues. In this study, human β-defensin-2 (hBD-2) mRNA and peptide expression were evaluated by RT-PCR and Western blotting in normal human keratinocytes, in function of their stage of differentiation. In proliferating, nondifferentiating keratinocytes generated in serum-free, low-calcium medium, a very low hBD-2 mRNA expression was found. A significant higher expression was detected in high-calcium cultivated keratinocytes grown either as monolayers or as multilayers under submerged conditions. In reconstructed epidermis using the dead de-epidermized dermis model, hBD-2 mRNA expression level was significantly higher than in the other conditions and displayed interindividual variability as observed in native epidermis. The protein was detected only in reconstructed epidermis. These results indicate that hBD-2 gene expression in normal human keratinocytes is dependent upon their stage of differentiation. Exposure of reconstructed epidermis to bacterial lipopolysaccharide resulted in a sustained stimulation of hBD-2 gene expression showing, for the first time, at mRNA and peptide levels, the functionality of hBD-2-coding gene in a human reconstructed skin model. These results also provide evidence that *in vitro* reconstructed epidermis represents a useful model for studying regulation of expression of a-defensins after skin challenge with pathogenic microorganisms in conditions as close as possible to the *in vivo* situation.

037

Cell and Collagen Concentration Effect on Retracted Collagen Lattices Thickness

C. Viennet, A. Degouy, J. Bride, and P. Humbert
Engineering and Cutaneous Biology Laboratory, School of Medicine and Pharmacy F-25030 Besançon

Introduction: *In vitro* dermal equivalent (also called collagen lattices) development leads to an original model of dermal tissue. The differentiation of fibroblasts cultured in 3 dimensions in a collagen matrix remains similar *in vitro* and *in vivo*. New technological performances in ultrasonic imaging allow precise measurements with a good resolution. The aim of this study was to assess, by ultrasonic imaging, the correlation between collagen lattices thickness and various collagen and cell concentrations.

Materials and methods: 3 different concentrations of human dermal fibroblasts ($F_1 = 8.10^5$ C per ml, $F_2 = 16.10^5$ C per ml, $F_3 = 32.10^5$ C per ml) and 3 concentrations of rat tail collagen were prepared ($C_1 = 2$ mg per ml, $C_2 = 3$ mg per ml, $C_3 = 4$ mg per ml). Collagen lattices were prepared as follow: F_2C_1, F_2C_2, F_2C_3, F_1C_1, F_2C_1, and F_3C_1 (n = 5 per case). Ultrasonic imaging was performed on day 0, 4, 6, 10, 12 and 14 using a Dermcup® 2020 scanner. Scans performed were numerized to measure collagen lattices thickness in their centre and periphery.

Results: Collagen lattices echogenicity was like dermis *in vivo*. For each assessment, we observed that collagen lattices thickness increases until day 12 and then stabilizes. When cellular concentration was more important, the lattice was thicker (at day 14: $F_1C_1 = 0.66$ MM, $F_2C_1 = 0.86$ MM, $F_3C_1 = 1.21$ mm). Collagen concentration does not influence lattices thickness significantly.

Conclusion: Collagen lattices thickness increases with retraction time and cellular concentration.

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Cultured Kaposi's Sarcoma Tumor Cells Exhibit a Chemokine Receptor Repertoire that Does Not Allow Infection by HIV-1

T. Simonart, C. Debussher, C. Liesnard,* M.-L. Delforge,* A. de Lavareille,† P. Hermans,‡ J.-P. Van Vooren,§ and P. Stordeur†
*Department of Dermatology, *Virology, †Immunology and §Internal Medicine, Erasme University Hospital, Brussels; and ‡Department of Infectious Diseases, Saint-Pierre University Hospital, Brussels, Belgium*

Background: HIV-1 is known to play a critical role in the pathogenesis of AIDS-associated KS. However, it remains controversial whether KS cells are target cells for HIV infection.

Material and methods: The expression of chemokine receptor mrna was studied by PCR in KS-derived spindle cell cultures and in an immortalized KS cell culture (KS-Y1). To determine whether these cells could be infected by HIV-1, we investigated the production of p24 antigen and HIV-1 RNA after infection of the cells with the HTLVIII-B T cell tropic strain and the JRFL macrophage tropic strain (1000 TCID50/ml).

Results: With the exception of CCR8 which is expressed by KS-derived spindle cell cultures but not by KS-Y1 cells, unstimulated KS cells express no significant levels of CD4, CCR3, CCR5 or CXCR4. HIV infectivity assays showed that KS cells were unpermissive to HTLVIIIB and JRFL strains, as evidenced by lack of p24 antigen and HIV RNA production as well as by absence of HIV-1 DNA integration. Although the expression of CXCR4 mRNA could be induced by interleukin (IL)-1β, stimulation of KS cells by this cytokine did not allow infection by HIV-1.

Conclusion: This shows that KS cells exhibit a chemokine receptor repertoire that does not allow infection by HIV-1.

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Chemosensitivity Modulation of Melanoma with an Anti-BRCA1 Ribozyme: Preliminary Results

C. Hesling,* M. D'Incan,*† C. D'Incan,† S. Lafarge, P. Souteyrand,† and Y. J. Bignon*
**Laboratoire d'Oncologie Moléculaire, Centre Jean Perrin; †Dermatologie, CHRU, Clermont Ferrand, France*

Background: Melanoma chemoresistance is in part due to genomic mechanisms (oncogene amplification, expression of dna repair genes, expression of detoxifying enzymes).

Aim: To study melanoma cell in chemosensitivity after transfection with a zibozyme directed against *brca1* which is a gene involved in dna repair processes, cell cycle control and apoptosis.

Material and methods: Three human melanoma cell lines were tranfected with two anti*brca1* ribozymes; two clones were generated in which more than 50% of *brca1* expression were down regulated. When cultivated with different drugs, cell growth (IC50) was compared between these clones and non transfected cells and cells transfected with the tranfection vector alone.

Results: An increase of chemoresistance against paclitaxel and platinium was evidenced but no effects with cystemustine was seen.

Comments: These preliminary results suggest that *brca1* could be involved in chemoresistance of melanoma on a way that is not yet known. Moreover, the consequence of its down regulation on chemosensitivity seems to depend on the genotoxic drug considered.

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Differential Secretion and Activation of MMPs in Two Reconstructed Skin Models using HaCaT or HaCaT-ras Cells

S. Holvoet, D. Nova, C. Le Griel, F. Duplan,* S. Charvat, E. Gentilhomme,† O. Damour,* D. Schmitt, and M. Serres
*Inserm U 346, HEH, Lyon, *Laboratoire des substituts cutanés, HEH, Lyon, †Département de Radiobiologie, C.R.S.S.A, 38702 La Tronche, France*

Matrix metalloproteinases (MMPs) play an important role in tissue regeneration, wound healing and tumor invasion. In monolayer cultures, our previous studies have shown a higher motility of HaCaT-*ras* cells compared with normal human keratinocytes (NHK) or HaCaT cells correlated with a higher secretion of MMP-2 (72 kDa) or MMP-9 (92 kDa), according to the medium used for cell cultures. Presently, the expression and activity of MMPs were investigated in two reconstructed skin models, using de-epidermized dermis (DED) or fibroblasts seeded in a collagen sponge matrix. In all experiments, MMP-9 is higher secreted in HaCaT-*ras* cells as compared with nontumoral keratinocytes, but its active form (86 kDa) is only detected in both reconstructed skin models according to keratinocyte differentiation. The MMP-2 which is highly secreted by HaCaT cells cultured in monolayer is not detected in reconstructed epidermis on DED. It is only secreted by living fibroblasts, cultured either in collagen lattices or in a dermal substitute. However, only in this last model, the modulation of MMP-2 secretion and activation occurred since HaCaT cells were cultured on the dermal substitute, but not with NHK. These results suggest a direct interaction of HaCaT with fibroblasts.