Guest lecture “Jean Thivolet”: Parvovirus and cancer. Pr J. Rommelaere, INSERM U375, Deutsches Krebsforschungszentrum, Heidelberg, Germany
Use of Dendritic Cells (DC) Genetically Modified by ALVAC Vectors Carrying MAGE Sequences to Induce Antimelanoma Immune Responses
For 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 antigens. The aim of this study was to further 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 T cells 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-specific CTL clone more efficiently than same DC loaded with MAGE-1/3 peptide, as shown by increased IFN-γ secretion. These results could be the basis for the development of a new strategy in melanoma immunotherapy.

Identification by cDNA Microarray Technology of Genes Modulated by UV-B in Normal Human Melanocytes
C. Valery, J. J. Grob, and P. Verrando
LMBP, Université de la Méditerranée, 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 cultures at 180 mJ/cm². This protocol was a modification of that used in previous reports. Among them, 159 corresponded to DNA known sequences, the encoded proteins being mostly in DNA- or RNA-binding/synthesis or cytoskeletal proteins. The others were transcription factors, receptors, tumor suppressors and (proto)cancer genes. 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 these were chosen. The kinetics of the expression of these genes following UV-B stress were characterized by RT-PCR confirming the DNA microarray data. Furthermore, mRNA of these ESTs were always found expressed in melanoma cell lines at different 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 expression profiles of melanocytes genes collectively modulated by UV-B stress allows to identify new proteins and genes that could represent new markers of UV-B induced melanogenesis.

Elastin Peptides Up-Regulate proMMP-2 Activation and Melanoma Cells Migration Through Three-Dimensional Type I Collagen Matrix
C. Nati, W. Hornebeck,* and P. Bernard
Department of Dermatology, CNRS FRE 2260, IRB, 53, Bismuthoud, Faculty of medicine, REIMS,*Department of Biochemistry, CNRS FRE 2260, IRB, 53, Bismuthoud, Faculty of medicine, REIMS
Elastin, as a main constituent of elastin fibers, plays a prominent role in skin biology. Its enzymatic degradation by gelatinases (MMP-2, MMP-9) leads to the liberation of elastin peptides (EP) which exhibit several biological functions. Particularly, interactions 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 (Braissant et al. J Biol Chem 2000). We here evidenced that elastin peptides (90–200 kDa 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-dependent manner. When those cells were grown within type I collagen gels, in presence of EPs, MMP-2 activation was strikingly exacerbated, an effect probably resulting from elastin-mediated MT1-MMP increased expression (Braissant 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.

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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 cultures at 180 mJ/cm². This protocol was a modification of that used in previous reports. Among them, 159 corresponded to DNA known sequences, the encoded proteins being mostly in DNA- or RNA-binding/synthesis or cytoskeletal proteins. The others were transcription factors, receptors, tumor suppressors and (proto)cancer genes. 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 these were chosen. The kinetics of the expression of these genes following UV-B stress were characterized by RT-PCR confirming the DNA microarray data. Furthermore, mRNA of these ESTs were always found expressed in melanoma cell lines at different 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 expression profiles of melanocytes genes collectively modulated by UV-B stress allows to identify new proteins and genes that could represent new markers of UV-B induced melanogenesis.

The POH-1 Subunit of Proteasome is Involved in UVB DNA Damages Repair in Melanocytic Cells
V. Pelletier, C. Valery, M. DeMèo, J. J. Grob, and P. Verrando
LMBP, Université de la Méditerranée, Marseille, France
UVB radiations induce great amount of DNA damaged bases which are potentially 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 pharmacological inhibition of proteasome activity of the proteasome (ALLN and Lactacystin) causes deficient repair of cyclobutan-pyrimidin dimers (CPD) in cultured cells exposed to a 40 mJ/cm² UVB dose. An UVB irradiation of 100 mJ per cm² modiﬁes POH-1 subunit of 19S proteasome leads to a better cells survival toward increased UV doses (0–250 nJ per cm²). 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 effect of POH-1. An UVB irradiation of 100 mJ per cm² modiﬁes POH-1 ARNm levels on a 24-h period of time. Taken together, the results show that a proteasome 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 pharmacological perspectives in the field of the modulation of cutaneous melanocytes resistance to UVB stress and its possible alteration in melanocarcinogenesis.

GM3 Ganglioside of Human Melanoma Tumors Contains a Lactonized Sialic acid that is a Ligand of Interleukin-4
I. Popa, J. P. Zanotta,* J. Portosukalan, and L. Thomas
Laboratory of Tumor Cytology, INSERM U 346, Faculty of Medicine Lyon-Sud, University Lyon-1, 69921 Oullins Ce, France, *Laboratory of Biological Chemistry, University of Sciences and Technologies of Lille, 59655 Villeneuve d’Ascq Ce, 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 melanomas display a more complex profile with GM3, GM2, GD3 and GD2. Those gangliosides, that are considered as markers of malignant melanoma, are shed into the extracellular medium during proliferation of malignant melanocytes. They can be 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 (Ceho et al. J Biol Chem 2001, 276:5655–5661). 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 tumor.

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. Biasioni, C. Cantonii, C. Bottino, L. Boumsell, and A. Bensussan
INSERM U 448 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. Bounaskell, and A. Bensussan
INSERM U 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 SCS expressed simultaneously on a human Sezary cell line and a tumor T cell subset in normal individuals. SCS antigen was detected mostly on CD35RO+ lymphocytes from both CD4+ and CD8+ subsets as well as on NK and B lineage cells. SCS surface expression increased very early after polyclonal stimulation of CD3+ cells due to the transfer of intracellular SCS molecules to the cell membrane. Engagement of SCS receptor by its mAb inhibited the anti-CD3-induced proliferation and cytokine secretion of peripheral blood T cells and cell clones, while SCS mAb did not affect the cytokytic activity of CD8+ T cell clones. Extensive phenotypic analysis revealed that the percentage of SCS+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 coexpressed SCS molecules. Importantly, ligation of SCS receptor in a cutaneous T cell lymphoma (CTCL) cell line profoundly inhibited the anti-CD3-induced proliferation. Consequently, the expression of SCS receptor in SS patients peripheral blood may serve not only to detect the presence of circulating malignant CD4+ cells but also as 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 Lymphoma: Correlations with Blood Data
O. Derreure, 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 homozygous 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 lesonal CD4+ T cells extracted from cutaneous lesions of 12 patients with mycosis fungoides and 11 appurated 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 Alopoeia Areata
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The course of alopoeia areata appears to be associated to stress. Derenervation allows hair to grow again. Hence, the nervous system is involved in the pathophysiology of this disease. Fifteen outpatients with alopoeia 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 nerve fibers. The immunohistochemical study has been performed on frozen 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 hairs follicles in healthy scalp whereas it was almost undetectable in lesions of alopoeia 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 vasoconstruction and the effects of lymphocytes, which are known to induce alopoeia areata patches. Hence, CGRP agonists could be used to treat this disease. Works about other neurotransmitters are in process.

010
Drug Specific Cytoxic T-Lymphocytes in the Skin Lesions of a Patient with Toxic Epidermal Necrolysis (TEN)
A. Naud, A. Bensussan, O. Dorendee, F. Mami-Chouab, N. Bachot, M. Bagot, L. Bounaskell, and J.-C. Roujean
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-Fad ligand interaction. Methods: In a patient suffering from cotrimoxazole induced TEN, bluster 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 CD3+, 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 monochomonal antibody. It was abolished by EGTA, but neither by antas nor by anti-TRAIL monochomonal antibodies, suggesting perforin/granzyme mediated cytotoxicity, without implication of fas at this stage. Conclusion: 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 react against the parent drug and not against its main reactive metabolite. These results challenge 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 Atoiic Dermatitis (AD)
D. Steunoumont-Sallé, *E. Delporte, *M. Capron, †D. Doubrowski²
*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 the epithelium consisting 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 the 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, submerged medium for two weeks. LC precursors, obtained by differentiation of CD34+ cord blood progenitor cells in the 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, submerged epithelium that increased 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. Delezay, J. Kanitakis, *J.-F. Mounier, C. Grun, and L. Misery
*Group Mucosal Immunity and Pathogen Agents’, ²Department 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 the 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 reconstructed 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
INSERM U 99-08 & U 311, ETS, 67606 Strasbourg, France, *UMR 144, Institut Canit, 75248 Paris, France and ‡Dipartement de Dermatologie et Centre de Microscopie Electronique, LIMHC, Lieuson, 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. Deutzer Damhuyten, C. Vincent, D. Schmitt, and J. Piguet-Navaro
INSERM U346, Hôpital E. Herriot, Pasteur R, Lyon, France.
In this study we analyzed the specific effects of TGIF-αI and/or IL-4 on monocyte-derived cells. To this end, monocytes were cultured with either GM-CSF, GM-CSF/TGF-αI, GM-CSF/IL-4 or GM-CSF/IL-4/TGF-αI 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 CD11b expression and lack of allostimulatory function. Interestingly, however, the cells express E-cadherin, independently of exogenous TGF-αI. Most importantly, a subset of monocytes cultured with GM-CSF/TGF-αI expresses Langensi, as assessed by flow cytometry and confirmed by electron microscopy analysis. Langern engagement with specific monoclonal antibody induces its internalization in coated pits and covered vesicles and the formation of typical Birbeck granules. Monocytes cultured in GM-CSF/IL-4 neither express E-cadherin nor Langensi. The simultaneous addition of TGF-αI allows most of the cells to express E-cadherin but rarely Langensi. Taken together, the results add further evidence that LC can derive from monocytes and demonstrate an antagonistic effect of IL-4 and TGF-αI on monocyte differentiation towards the LC pathway.

015
Germline and Somatic Mutations of the INK4a-ARF Gene in a XP-C Patient
M. Guerrin,* M. Ryborg,* T. Magdalou,* O. Toubiaudeau, L. Darya-Grenjean,* J. Rivet,* A. Sarasin,† and N. Beass-Seguer
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Xeroderma pigmentosum group of 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 tumours harbor UV induced mutations (i.e. double CC > TT (AK), 2 microinvasive epidermoid carcinomas (ECs), 1 basal cell carcinoma (BCC) and one atypical fibroxanthoma) were examined by combining single strand conformation polymorphism analysis and direct sequencing. A deletion of 200 bp at the 3′ end of INK4A characterized by a defect in global genome repair, leading to the development of multiple skin tumours. A high proportion of these tumours harbor UV induced mutations (i.e. double CC > TT (AK), 2 microinvasive epidermoid carcinomas (ECs), 1 basal cell carcinoma (BCC) and one atypical fibroxanthoma) were examined by combining single strand conformation polymorphism analysis and direct sequencing. A deletion of 200 bp at the 3′ end of INK4A characterized by a defect in global genome repair, leading to the development of multiple skin tumours. A high proportion of these tumours harbor UV induced mutations (i.e. double CC > TT (AK), 2 microinvasive epidermoid carcinomas (ECs), 1 basal cell carcinoma (BCC) and one atypical fibroxanthoma) were examined by combining single strand conformation polymorphism analysis and direct sequencing. A deletion of 200 bp at the 3′ end of INK4A characterized by a defect in global genome repair, leading to the development of multiple skin tumours. A high proportion of these tumours harbor UV induced mutations (i.e. double CC > TT (AK), 2 microinvasive epidermoid carcinomas (ECs), 1 basal cell carcinoma (BCC) and one atypical fibroxanthoma) were examined by combining single strand conformation polymorphism analysis and direct sequencing. A deletion of 200 bp at the 3′ end of INK4A.

016
Assessment of Ascorbic Acid and Iron Concentrations in Psoriatic Skin Dermis vs. Healthy Subjects
N. Leveque,* S. Robin,† P. Murer,* S. Mary,* S. Makki,*§ J. P. Kautelip, A. Berthelot, and Ph. Humbert
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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, 33.5 ± 1.9 μg per l and 47.0 ± 2.4 μg per l. AA and iron concentrations in healthy dermis were, respectively, 237.4 ± 8.7 μg per ml et 214.4 ± 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
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Degradation of collagen and fibrogenes occurs in the latter 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 (orthology 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. Antisa elicited in rabbits are being used to determine the precise location of type I PAD in human epidermis.

018
Homoplastic Interactions of Human Comedosmin
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Comedosmin (CduA) is a secreted glycoprotein located in the extracellular part of comedoform comedones, the comedo-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 CduA have been proposed to fold as structural motifs similar to glycine loops and to confer adhesive properties to the protein. To test if CduA was an adhesive protein, and to define the involved domains, full-length and truncated CduA forms, GST-tagged at their terminus, were produced in E. coli and tested in vitro for protein-protein interactions by membrane-overnight binding assays. Full-length CduA displayed homophilic binding properties but did not bind to GST alone, to BSA or to flaggrin. The interactions were dose-dependent, and incubation with calcium only induced a moderate increase in binding. Various forms of CduA deleted of the NH2- or 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 NH2-domain. Interestingly, the full-length CduA and the various deletion mutants did bind another truncated form corresponding to the NH2-terminal domain all alone. Therefore, this domain displays adhesive properties by itself. The results provide the first experimental evidence for homoplastic interactions of CduA, 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. Kiol,* A. Plé, A. Godard, Y. Jacques, and I. Deën* *Groupe de recherche sur les léshyres et leurs récepteurs; *Laboratoire d’Immuno-Dermatologie, INSERM U463, Nantes, France

Interleukin (IL)-15 exert 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 α and β, in vitro on monolayers of normal human keratinocytes.

IL-15, IL-15RA, IL-15Ra, and IL-15 mRNA expression was also detected by RT-PCR, whereas IL-15- and IL-15Ra protein expression was examined using immunoperoxidase and Western blot.

By RT-PCR, we have observed that IL-15, IL-15Ra, IL-15Ra, and IL-15 mRNA expression, respectively. IL-15 and IL-15Ra protein expression were increased as well.

Induction of IL-15 and IL-15 receptor production in normal human keratinocytes stimulated by IFNα 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.

021

Prevalence of Antidesmoglein Antibodies in the Tunisian Population

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In Tunisia, pemphigus has particular epidemiological characteristics, that mimic endemic pemphigus in some extend. Indeed, prevalence is increased and the superficial subtype (PF) prevails, 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 Tunisia and Sfax were studied by immunoblot using an epidermal extract and were compared with 35 Tunisian patients with pemphigus (30 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 kDa 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-Dsg1 antibodies, 60% anti-Dsg2 antibodies) and IgG4 subtype was never found. In control all patients with autoantibodies detected by immunoblot (65% PF, 44% PV) had autoantibodies corresponding to pemphigus antigens 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 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) genotypes in disease susceptibility and individualized DRB1*0402 (p = 0.04), DRB1*0402 (p = 0.02), DRB1*0406 (0.003) and DRB1*1404 (p = 8.10⁻⁶) 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 develop 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 Dermocosmetic Products Properties

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Somatic sensory neurons transfer sensitive information from the skin to the Central Nervous System. In addition, the action potential is transferred across the synapse to the target organ, particularly keratinocytes. The release of neurotransmitters such as CGRP, substance P in skin is able to modulate both cutaneous cell proliferation and differentiation and is able to regulate secretion of both cytokines and growth factors. In order to reproduce a reconstructed 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 allowed to develop. This allowed us to demonstrate: i) keratinocytes have developed a pluristratified epithelium; ii) cells 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 vivo. 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

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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 antianagen effect. 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
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Cutaneous T-cell lymphomas (CTCL) are characterized by the infiltration of the skin by T lymphocytes of the CD2+CD3+CD4+CD44+DR+ phenotype. CTCL represent a unique model in tumor terms because both tumoral and reactive cells act as T lymphocytes. Till now no specific marker of tumoral cells has been described in the literature. Nevertheless, since the idiootype of the TCR represents a unique marker for a malignant clone of T lymphocyte, the choral origin of tumoral cells allows us to think that the TCR-Vb clone corresponds to the tumoral cell. Concurrently, loss of expression of CD7 is considered as a diagnostic criterion 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 tumoral cells. A band, by cell sorter, is realised in order to have the CD4+CD7- and CD4+CD7+ populations, which are re-analyzed to a cytological evaluation on the basis of the cerebriform nucleus of Sezary cells. We found for our first patient that both CD7+ CD4+ and CD4− CD7− populations contained Sezary cells, so this finding suggests that CD7 is an appropriate marker to identify Sezary cells. Moreover we were able to note 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
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The high incidence of melanoma (4-14%) in retinoblastoma patients suggest that an onco-suppressor 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 S A medium, 5% of CO2, ARN extraction and amplification was done by RT-PCR. Western blot was performed with an anti-human 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 whether 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 dysregulation of Rb protein secondary to mutations of other molecules or genes (CDKN2/p16...).

027

Transduction of Melanoma Cell Lines by AAV Type 2 Vectors
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Recombinant adeno-associated virus type 2 (AAV2) 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 AAV 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, S18.2A and EB81 was studied by infection with AAV2-EGFP, a vector that carries the enhanced green fluorescent protein reporter gene. Infected was measured by multiplicity 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 AAV-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 rAAV 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 MB-1 Distribution in Cutaneous Verrucous Carcinoma: Analysis of 5 Cases
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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 MB-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 were included. Immunohistochemistry using MB-1 (Immunotech, Marseille, France), a murine monoclonal antibody which reacts with the same epitope as Ki67, was carried out on 4μm 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, areas of cellular anaplasia and high mitotic activity were the rule. MB-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 MB-1 distribution in VC may at least partially the differences in the clinical and morphological characteristics between VC and SCC.

029

Stromyulin 3 Expression is a Marker of Prognosis in Basal Cell Carcinomas
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Stromyulin 3 (ST3) is a member of the metalloproteases 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 mucosal invasion. Immunohistochemistry was carried out using the LSAB method, with monoclonal anti-ST3 Ab (MC Rov, 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 morphoform BCC and tumors with deep invasion, which are both of poor prognosis. These results are similar to those previously published in squamous cell carcinomas and keratoacanthomas [1]. Altogether, the studies on cutaneous tumors are consistent with the theory of ST3 playing an active role in tumor progression.


030

Cutaneous Human Dendritic Cells (DCs) Express CD1d

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The CD1 family of cell surface glycoproteins is a third lineage of antigen presenting molecules. Human CD1d cellular 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 + TNFα, 13 days) and from adherent PBMC (GM-CSF + IL4 + TG-Fα + GITF, 7 days). Dermal dendocytes like cells were differentiated from PBMC (GM-CSF + IL4 + GITF, 7 days). CD1d mRNA expression was progressively turned off, suggesting a regulatory role for TNFα. CD1d mRNA was detected in GI and GIT cells with a higher signal in GI population, which support the hypothesis of an inhibitory effect for TNFα. Moreover, there was an increase of CD1d mRNA expression in these two populations during cells maturation with TNFα or CD40L. Cell surface expression was confirmed by flow cytometry on DCs developed in vitro (mAb DCD1d). Our data suggest that CD1d is expressed on dermal dendocytes and to a lower extent on Langerhans cells. CD1d inhibits the expression of CD1d mRNA, while the expression is up-regulated when dendocyte cells are further matured with CD40L or TNFα.

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GM-CSF, TGFB1 and TNFα Enable Peripheral Monocytes to Become Epidermal UV-Macrophages


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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 CD14−/CD11c+CD36/FcγRII+ monocytes/macrophages. This population was called UV-Macrophages (Meunier et al, J Invest Dermatol 1995, 105:782–798; Kremer et al, Eur J Immunol 1998, 28:2536–2546). 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 monocytes (Mo) from dermis, peripheral blood Mo were cultured in presence of 3 major cytokines: GM-CSF, TGFB1 and TNFα. After 6 days of culture with GM-CSF and TGFB1, Mo differentiated into 2 distinct CD14+ CD16+ subsets: (1) a low proportion (0.5–1%) of CD14+/Langerin+/CCR6+/hBD-2+/hBD-1+/hBD-3+ cells that strongly expressed the specific marker of LC, that is Langerin, and (2) a high number (40–50%) of CD14+/Langerin+/low/CCR6−/hBD-2+/hBD-1+/hBD-3− cells. After addition of TNFα, Langerin+/high cells increased up to 2% and kept their phenotype while the phenotype of CD1a+/Langerin+high/CCR6+ cells that strongly expressed the specific marker of LC, that is Langerin or CD1a+/Langerin+/low/CCR6−/hBD-2+/hBD-1+/hBD-3− cells. After double cell surface labeling with BDCA1 and, on day 7, E-Cadherin; on day 14, Langerin (LgD) (CD207, LC-specific type II lectin). On day 7, all cells were BDCA1+/E-Cadherin+ and on day 14, two subsets BDCA1+/Langerin+/high and BDCA1+/Langerin−/low were observed. In the CD14+ system, more than 60% of cells expressed BDCA1 on day 6 as well as on day 8, even though BDCA1 was present on more than half of the cells on day 6, BDCA3 expression was down-regulated after TNFα addition. In the CD14+ system, the strong BDCA1 expression on Lg+ cells depends on the presence of TGFB1 whereas in the CD34+ system, where TGFα1 is absent, a BDCA1+ subset might not be able to acquire the LC marker. The presence of BDCA1 (described as a myeloid DC-associated marker) on DC may be correlated to their ability to differentiate into LC. A BDCA3 expression in the CD14+ system was decreasing in parallel to Langerin appearance, after activation by TNFα, this BDCA3 marker (described as a myeloid 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 plasmacytoid DC-associated marker) may reveal all cells engaged in both LC and dermal DC pathways.

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

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The lymphoid vs. myeloid origin of both cutaneous dendritic cells (DC), Langerhans cells (LC) and dermal DC is still a controversial issue. The identification of the new BDCA1–4 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 TNFα as well as from CD14+ monocyte-derived in presence of GM-CSF, IL4 and TGFB1 for 6 days and then, 2 days by adding TNFα. In both CD34+ and CD14+ systems, DC did not express BDCA2 even though BDCA1 was expressed by the majority of cells at the different culture times. In the CD34+ system, BDCA1 (CD1c) was expressed by 20–50% 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 performed with BDCA1 and, on day 7, E-Cadherin, on day 14, Langerin (LgD) (CD207, LC-specific type II lectin). On day 7, all cells were BDCA1+/E-Cadherin+ and on day 14, two subsets BDCA1+/Langerin+/high and BDCA1+/Langerin−/low were observed. In the CD14+ system, more than 60% of cells expressed BDCA1 on day 6 as well as on day 8, though BDCA1 was present on more than half of the cells on day 6, BDCA3 expression was down-regulated after TNFα addition. In the CD14+ system, the strong BDCA1 expression on Lg+ cells depends on the presence of TGFB1 whereas in the CD34+ system, where TGFα1 is absent, a BDCA1+ subset might not be able to acquire the LC marker. The presence of BDCA1 (described as a myeloid DC-associated marker) on DC may be correlated to their ability to differentiate into LC. A BDCA3 expression in the CD14+ system was decreasing in parallel to Langerin appearance, after activation by TNFα, this BDCA3 marker (described as a myeloid 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 plasmacytoid DC-associated marker) may reveal all cells engaged in both LC and dermal DC pathways.
Materials and methods: 3 different concentrations of human dermal fibroblasts (F1 = 8.105 C per ml, F2 = 16.105 C per ml, F3 = 32.105 C per ml) and 3 concentrations of rat tail collagen were prepared (C1 = 2 mg per ml, C2 = 3 mg per ml, C3 = 4 mg per ml). Collagen lattices were prepared as follow: F2C1, F2C2, F2C3, F1C1, F2C1, and F3C1 (n = 5 per case). Ultrasonic imaging was performed on day 0, 4, 6, 10, 12 and 14 using a Dersinc® 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: F1C1 = 0.66 MM, F2C1 = 0.86 MM, F3C1 = 1.21 mm). Collagen concentration does not influence lattices thickness significantly.

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

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

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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 vivo and in vitro. 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 (F1 = 8.105 C per ml, F2 = 16.105 C per ml, F3 = 32.105 C per ml) and 3 concentrations of rat tail collagen were prepared (C1 = 2 mg per ml, C2 = 3 mg per ml, C3 = 4 mg per ml). Collagen lattices were prepared as follow: F2C1, F2C2, F2C3, F1C1, F2C1, and F3C1 (n = 5 per case). Ultrasonic imaging was performed on day 0, 4, 6, 10, 12 and 14 using a Dersinc® 2020 scanner. Scans performed were numerized to measure collagen lattices thickness in their centre and periphery.

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

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

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Material and methods: Three human melanoma cell lines were transfected 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 modification of chemosensitivity against cystemustine was seen.

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