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Meeting held in honour of Professor Jean Thivolet

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Upholding the tradition of investigative interactions. To be inventive and creative, researchers need to communicate and discuss their findings. Nothing is more stimulating in this respect than the personal interactions during scientific meetings.

The founder of the French branch of dermatological research in the sixties and seventies, Professor Jean Thivolet, has understood this need and actively supported development of the freshly created Société de Recherche Dermatologique. Aware of the fact that the understanding of skin diseases increasingly depends on clinical and laboratory research, Jean Thivolet encouraged dermatologists to engage in such investigations. Under his incisive leadership, numerous present and future professors successfully developed their careers and several foreign fellows could appreciate the exceptional and stimulating atmosphere of his laboratory.

Proud of being his pupils, followers and friends, we were particularly happy to pay a tribute to this pioneer of dermatological research in his home city of Lyon.

Note: The abstracts of the communication N°8 and of the poster P8 were not submitted for publication. Posters N° 9, 10 and 29 were not presented and their abstracts are not published.

001

Placenta Growth Factor in Cutaneous Wound Healing

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Placenta growth factor (PlGF) is a dimeric glycoprotein, structurally and functionally related to the vascular endothelial growth factor (VEGF), a potent angiogenic/permeability factor known to play a role in the neoangiogenesis during wound healing. We decided to evaluate the expression of PlGF in human keratinocytes and to investigate its possible role during wound repair. Northern blot analysis on RNA from cultured keratinocytes revealed a transcript of 1.7 Kb and RT-PCR allowed the detection of two forms of PlGF (PlGF-1 and PlGF-2), generated by alternative mRNA splicing. PlGF homodimers as well as VEGF/PlGF heterodimers could be detected in keratinocyte culture medium. Increased levels of both PlGF mRNA and protein were observed upon treatment of keratinocytes with EGF, TGF-α, TGF-β and IL-6, all cytokines present at the wound site during the early phase of healing. By *in situ* hybridization on human full-thickness wounds at different times after injury, appreciable levels of PlGF mRNA were detected in the keratinocytes moving into the wound bed, starting from day 3. At day 5 a clear signal for PlGf mRNA was present on migrating keratinocytes, as well as endothelial cells lining the blood vessels close to the granulation tissue, while at day 7 PlGF mRNA was no more detectable. Immunohistochemical data confirmed that keratinocytes at the wound edge express PlGF starting from day 3 after wounding, with the staining gradually increasing up to day 7 and being completely abolished by day 13, when the wound is fully re-epithelialized and the granulation tissue regressing. These data indicate a role for PlGF in the neoangiogenesis process associated with cutaneous wound repair.

002

Zinc, Copper, and Manganese Enhanced Keratinocyte Migration Through a Functional Modulation of Keratinocyte Integrins

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The migration of keratinocytes over the wound bed plays an important role in the reepithelialization of cutaneous wounds. The trace elements zinc, copper and manganese are used *in vivo* for their healing properties and their mechanism of action is still only partially known. Thus, they have been shown both to promote keratinocyte proliferation and to modulate integrins expression on keratinocytes.

So, the aim of this study was to determine if trace elements induce an increase of the migration of keratinocytes and if this effect is related to the modulation of integrins by trace elements.

Two independent migration assays were used to study keratinocyte migration: the scratch assay using normal human keratinocyte and the modified boyden chamber using HaCaT cells. Inhibition studies using function-blocking antibodies directed to α3, α6, αV, and β1 subunits were performed to investigate the modulatory effect of trace elements on integrin function. In this way, zinc gluconate and copper gluconate increased α3, αV, and β1 function whereas manganese gluconate seems mainly able to modulate the function of α3 and β1. Moreover, the stimulating effect of these trace elements on keratinocyte migration does not appear to be related to α6 subunit.

Thus, zinc, copper, and manganese enhanced keratinocyte migration and one of the mechanism of this effect involved a modulation of integrin functions.

003

Identification of Two Secreted Phospholipases A_2 in Human Epidermis

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Phospholipases A_2 (PLA_2) are enzymes that catalyze the release of fatty acids from the *sn-2* position of phospholipids. Fatty acids have been suggested to play a key role in the barrier function of the epidermis. The aim of this study was to identify and characterize the type of secretory PLA_2 expressed in human epidermis. We report the molecular cloning of two secretory PLA_2 in the human epidermis. The first enzyme is identical to human pancreatic type IB PLA_2. Western blots revealed a 14-kDa protein localized in a soluble fraction. The second PLA_2 is identical to human synovial type IIA enzyme and is localized in the membrane fraction. By a new method using semiquantitative RT-PCR on horizontal sections of the epidermis, we found that the mRNAs of both PLA_2 were expressed mainly in the basal layers of the epidermis. Our data thus provide evidence for the expression of two secretory PLA_2 in human epidermis. The different localization of these two proteins strongly suggests a specific role of each enzyme in skin physiology and probably in the barrier function. Moreover, we developed an original technique which is quicker than *in situ* hybridization and can be used as a first approach to detect gene expression in different layers of the epidermis.

004

Epidermal Expression of Human Corneodesmosin in Transgenic Mice

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Corneodesmosin (Cdsn) is an extracellular component of corneodesmosomes, the corneocyte-specific intercellular junctions. It is expressed in the upper suprabasal layers of epidermis and its proteolysis at the stratum corneum surface is necessary for desquamation to occur normally. The terminal regions of Cdsn could be formed by glycine loop-related structural motifs that may confer adhesive properties to the protein. A genetic association between the Cdsn gene and susceptibility to psoriasis type 1 has recently been shown.

To test *in vivo* the function of Cdsn, the human Cdsn cDNA was introduced into a vector that drives an expression in suprabasal layers of squamous epithelia under the control of involucrin promoter (a gift of Dr J. Carroll). The resulting transgene was microinjected into fertilized mouse eggs (C57Bl/6 × CBA). Three transgenic mice were identified by polymerase chain reaction and Southern blot. Expression of human Cdsn in transgenic mice was tested by immunohistochemical staining of fixed tissue sections with a monoclonal antibody that reacts with human but not with mouse Cdsn. One mouse strongly expressed human Cdsn in cornified epithelia in a differentiation-appropriate fashion. This mouse was sterile but did not express Cdsn at a detectable level in testis and epididymis. The other mice were outbred to F1 animals to establish founder lines. They did not show detectable level of human Cdsn in skin, neither in newborns nor in adults. Homozygous F3 animals were then produced from one line. They showed a moderate expression of human Cdsn restricted to the inner root sheath of hair follicles. No gross phenotypical or histologic abnormalities were detected in the cornified epithelia or in hair follicles of all the mice expressing human Cdsn.

We concluded that overexpression of human Cdsn in the suprabasal layers of epidermis of transgenic mice is not sufficient to induce hyperkeratosis and to affect the normal process of desquamation.

005

A New Glycosylated Component of the Extracellular Part of Desmosome which is Different from Autoimmune Pemphigus Antigens

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Desmosomes are mechanical junctions composed of numerous intracellular and transmembrane proteins, some of which are known targets in autoimmune diseases. Detailed knowledge of desmosome composition should allow a better understanding of their implications in physiology and pathology.

Monoclonal antibody KM48 (IgM) recognises an extracellular antigen of desmosomes which shows, by immunofluorescence, a gradient of expression paralleling the degree of keratinocyte differentiation from the basal to granular layer. We report here preliminary characterisation of the antigen aimed mainly at excluding the possibility of its identity with already described cadherins. Mutual blocking experiments performed on normal human skin with KM48 and pemphigus vulgaris or pemphigus foliaceus sera indicated that each antibody recognised distinct epitopes. Additionally, tissue permeabilization revealed persistence of the antigen in the stratum corneum. Classical Western blot did not show reproducible data but "renaturated" proteins could be detected suggesting a high degree of the antigen glycosylation (approx. 130–300 kDa). Chemical deglycosylation on dot blot indicated that the antibody did not recognise a sugar epitope, what opens the possibility of molecular cloning. The pattern of expression of the KM48 antigen and its biochemical profile suggest that this protein may be a new component of desmosomes, potentially implicated in the process of epidermal differentiation and cohesion.

006

Interleukin-15 Expression by Normal Human Epidermal Keratinocytes

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Interleukin (IL)-15 is a 14–15 kDa cytokine of the four-alpha-helix-bundle family which shares many biologic activities with IL-2. Unlike IL-2, however, IL-15 mRNA have been identified in a variety of tissues and cell lines including epithelial cells. This ubiquitous expression of IL-15 mRNA does not correlate with a widespread production of IL-15 protein; indeed, IL-15 expression is regulated at multiple levels.

Production and secretion of IL-15 have been described in the adult T cell leukemia cell line HuT-102 and IL-15 is found at high concentrations in rheumatoid arthritis synovial fluid, suggesting that IL-15 secretion and production are induced by infections or chronic inflammatory stimuli.

The aim of our work was to study IL-15 expression by normal human epidermal keratinocytes stimulated by IFNα, IFNγ, TNFα, IL-1α or ciclosporine A. We examined IL-15 mRNA expression *in vitro* by RT-PCR and IL-15 protein production by immunocytochemistry. IL-15 mRNA levels were increased when keratinocytes were cultured in the presence of IFNγ and we detected IL-15 protein when cells were cultured in the presence of IFNα and IFNγ. No IL-15 induction was obtained, however, when cells were cultured in the presence of TNFα or IL-1α. Thus, IL-15 seems to be produced by keratinocytes only in inflammatory conditions associated with a IFNγ production, such as in activated T cell infiltrates in cutaneous lymphomas.

007

Jean Thivolet, a Pioneer of the French Dermatologic Research

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Born in Lyon, Jean Thivolet devoted his professionnal life to developing jointly both clinical practice and dermatologic investigation. ''Médecin des Hôpitaux'' in 1951, graduate in biology, ''Maître de Conférences'' in 1958, he became Head of the Department of Dermatology at the Antiquaille Hospital in 1962 and of the Department of Hygiene and Immunology in 1967. In 1972, he became Head of the Department of Dermatology at the Edouard-Herriot Hospital. His enthusiasm for clinical activity and biologic investigation is the source of numerous successes: the recognition of the field of dermatologic research at INSERM in 1977; the first INSERM group (FRA11) entitled ''Dermatological Research and Immunology'' created in Lyon in 1977, followed by the first INSERM Unit of Dermatological Research in 1981 (Unit 209, INSERM-CNRS). Jean Thivolet directed this unit from 1981 to 1992. In 1992, Unit 209 became Unit 346. He was at the first Society for Cutaneous Ultrastructure Research meeting, in Lyon in 1973, and at the first INSERM course on skin biology (COBIP), and was the author of the first book on the Langerhans cell (1988). Jean Thivolet was the initiator of the ''Société de Recherche Dermatologique'' (SRD) and the founder of the *European Journal of Dermatology*. Jean Thivolet was the representative of the modern french research in Dermatology in INSERM Commissions for several years. He was a teacher for numerous students, French and foreign, working in his department. Jean Thivolet is among the 20 authors nominated as the most cited dermatologists in the world. I propose the organization of a ''Jean Thivolet conference'' each year during the annual meetings (CARD) of the ''Société de Recherche Dermatologique'' (SRD).

008

Not submitted

009

Human Corneodesmosin/s Gene Polymorphism

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Psoriatic epidermis is characterised by a defective differentiation program leading to abnormal permeability barrier and impaired desquamation. Corneodesmosin (Cdsn) is a strong candidate in psoriasis susceptibility, due first to genomic position of its gene (''S'' gene, 160 kb telomeric to HLA-C) and recent genetic associations, and second to its location and function in epidermis. Cdsn is located in the desmosomes and corneodesmosomes where it is thought to have an adhesive function.

As Cdsn has been shown to be polymorphic, we analysed more precisely the genomic variability of different allele sequences. A 4.6-kb genomic fragment encompassing the first exon, the unique intron and the coding sequence of the second exon was amplified from HLA-Cw6-positive individuals: eight psoriatic patients whose DNA was provided by the ''National Psoriasis Tissue Bank'' in Dallas and six French healthy blood donors. Allelic discrimination was performed by RFLP for all patients and for three controls. After TA cloning, the entire coding sequence and the intron boundaries of 25 alleles were sequenced. Nine out of the 17 single nucleotide polymorphisms (SNPs) detected have not been previously reported. Among them, four accompany amino acid exchanges and two were detected in the intron boundaries. Moreover, one of the SNPs and one trinucleotide repeat were detected in the 3′UTR, close to the stop codon. These 17 polymorphic sites allowed the identification of 12 different alleles. Four alleles fulfil the criteria previously described by linkage studies as corresponding to a specific disease-related allele. Their identification will allow refined, additional family-based studies that could provide new genetic support for the S gene in psoriasis susceptibility. Moreover, Cdsn encoded by these alleles will be tested to understand SNP consequences on the protein function.

010

Correlation Between Mutations in Key Genes ($p16^{ink4a}$, $p14^{arf}$, and p53) in Skin Carcinomas of Xeroderma Pigmentosum Patients

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The INK4a-ARF locus encodes two tumor suppressor proteins, $p16^{INK4a}$ and $p14^{ARF}$ acting, respectively, through the Rb-CDK4 and p53 pathways. Mice knocked out for the entire locus, or specifically for ARF, develop skin carcinomas after UVB irradiation, and we previously reported 12% of INK4a-ARF mutations in human sporadic skin carcinomas. In this study, we examined the INK4a-ARF and p53 and gene mutational status in 28 skin carcinomas of patients affected by xeroderma pigmentosum (XP). Mutations were detected by PCR-SSCP and characterized by automated sequencing. Relevance of gene mutation association and comparison with sporadic tumors were evaluated by use of the two-tailed Fisher's exact test. Thirteen $p16^{INK4a}$ mutations (54% tandem CC:GG>TT:AA transitions) were detected in eight tumors (29%), a higher frequency compared with sporadic skin tumors (12%) ($p = 0.024$). Mutations affected in most cases a conserved codon and/or the functional domain of $p14^{ARF}$, and occurred at three particular hot spots codons. A strong positive association was found between mutations in p53 and INK4a-ARF ($p = 0.001$). Our data demonstrate for the first time the occurrence of INK4a-ARF UV-induced mutations in XP skin carcinomas, and enhances the role of this locus in skin carcinogenesis. The simultaneous inactivation of p53 and INK4a-ARF may represent an advantage for tumor progression, that may be linked to the XP genetic instability.

011

Role of p16INK4A in the Response of Human Keratinocytes to a Genotoxic Stress

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The p16INKA/ARF locus encodes by alternative transcription for two tumor suppressor genes implicated in cell cycle control. The first one, p16INK4A, acts through the Rb pathway, the second, p14ARF, regulates the cell cycle via the p53 protein. The implication of the p16INK4A gene in the cellular response to a genotoxic stress has been reported by various authors. Additionnally, UV-induced p16INK4/ARF mutations have been demonstrated in nonmelanoma skin cancer. Our objective was to precise the role and kinetics of P16INK4A/ARF in the response of human keratinocytes to a genotoxic stress and to compare it with that of p53. Normal human keratinocytes exponentially growing on NIH3T3 feeder layers (Green Method) were irradiated with sublethal UVB doses (30–100 mJ per cm^2, determined by a cytotoxic test). Expression of the locus was studied at various time intervals (2–48 h) by RT-PCR and Western blot. Our results showed that the p16INK4A protein was induced 2 h after irradiation, reached a maximum at 24 h and was maintained at 48 h. The absence of induction of its mRNA suggests that we observed a post-transcriptionnal stabilisation of p16INK4A. This induction was independant of the UVB dose conversely to what was observed for p53 and p53-related genes p21 and mdm2. Repeated UVB irradiation of keratinocytes still induced p16INK4A. On the other hand p14ARF did not seem to participate in the genotoxic stress response in these cells. The specific p16INK4A response to UVB could explain in part why inactivation of p16INK4A and UVB exposure participate in the development of skin tumors.

012

An Unusual Enhancer Confers Keratinocyte Expression of the Human LAMA3a Gene Through AP-1 Binding Sites

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We reported here the characterization, within the laminin α3A gene (*LAMA3*), of an enhancer fragment (FAP1) sufficient to confer strong keratinocyte-specific expression to heterologous promoters through the cooperative effect of three AP-1 binding sites. Each AP-1 site is equally bound by the members of the Jun/Fos families both in keratinocytes and in fibroblasts. Interestingly, the structural organization of the FAP1 fragment is essential since removal of the sequences located between the AP-1 sites did not alter the promoter activity in keratinocytes. Conversely, the distance between the AP-1 binding sites is crucial to maintain the promoter inactive in fibroblasts. EMSA experiments on the entire FAP1 enhancer fragment detected a slower migration of the DNA-proteins complexes with fibroblast nuclear extracts. The migration discrepancy of FAP1 probe observed with fibroblast extracts was lost as soon as the sequences located between the AP-1 binding sites were deleted but was restored when the deleted sequences were replaced by irrelevant spacer. The activity and migration of the different deleted or modified enhancer fragments, although modulated in fibroblasts, remained unchanged in keratinocytes These results demonstrate that the jun/fos heterodimers dictate a particular bending of the FAP1 enhancer fragment, leading to the anchorage of non-DNA-binding fibroblastic cofactor(s) to the AP-1/DNA complex to form an inhibitory ternary complex. Therefore, we characterized for the first time an unusual conformation-dependent enhancer within the *LAMA3* gene capable of strongly activating heterologous promoters in a cell specific manner.

013

Cooperation Without Direct Protein–Protein Interaction Between Smad and AP-1 in TGF-Activation of Human Type VIII Collagen Gene Expression

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Several reports suggest that the TGF-β specific Smad activation and the resulting transactivation of target genes is subject to crosstalks with other signaling pathways. In particular, it has been suggested that Smad proteins may interact with Fos and Jun family members via direct protein–protein interactions to regulate transcription of AP-1-driven gene promoters. In this report, using a genuine TGF-β response element found in the human type VII collagen promoter, known to bind Smad3 *in vivo* and containing a central consensus AP-1 binding site, we have investigated the functional interactions of the Smad and AP-1 signaling pathways in the context of COL7A1 gene transcription. We demonstrate that (1) both pathways activate transcription from the COL7A1 TbRE, (2) the AP-1 site is not required for binding of Smad complexes to the TbRE in response to TGF-β and for Smad-mediated transactivation, (3) the Smad site does not bind AP-1 members and does not allow AP-1-mediated transactivation, (4) the TGF-β-induced Smad complex does not contain any members of the Fos and Jun families, and (5) AP-1 and Smad complexes do not bind simultaneously to the TbRE. Together, our data indicate an absence of protein–protein interactions between Smad and AP-1 members in the context of TGF-β response, as Smads and AP-1 activate the COL7A1 promoter independently, through their cognate elements. It is therefore likely that the two transcription factors participate in COL7A1 promoter activation in a sequential manner, the AP-1 components being overexpressed as a result of the primary activation of Smads by TGF-β.

014

Differential Control by the Cytoskeleton of the Activation of MAP Kinases ERK and p38 During Mechanical Strain

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Physical forces play an important role in the regulation of cell function in many tissues, including skin. In order to mimic the *in vivo* situation, we choose to study the effects of controlled cyclic strain on human dermal fibroblasts.

Fibroblasts were seeded in monolayer on collagen type I-coated silicone rubber membranes. Confluent cultures were subjected to cyclic strain (1 Hz) during 1–12 min with a 20% maximal deformation. Cells were lysed immediately after and MAP kinases activation (ERK 1 & 2 and p38) was analysed by SDS PAGE from lysates followed by Western blotting with anti-phospho-ERK and anti-phospho-p38 antibodies. Mechanical strain clearly increased both ERK and p38 phosphorylation. Nevertheless kinetics of activation of these two protein famillies were different. ERK phosphorylation increased progressively from 1 to 12 min of stimulus whereas p38 phosphorylation was maximal after 1 min and then decreased during the additional time of traction. In addition, preincubation of fibroblasts with cytochalasine D or nocodazole (to disorganise the cytoskeleton networks of actin and microtubules, respectively) completely abolished the induction of ERK phosphorylation by mechanical strain, while it did not affect p38 response. Interestingly, fetal calf serum is necessary for p38 activation, suggesting that, in this case, mechanical strain effect is dependent on soluble factors.

In conclusion, depending on the structural organisation of the cytoskeleton, ERK and p38 will be differentially involved in mechanical strain response.

015

Human Cutaneous Dendritic Cells (DCs) Express CD1d

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The CD1 family of cell surface glycoproteins is a third lineage of antigen presenting molecules. Distribution of CD1d in human tissues is not well established. We previously reported CD1d expression on normal human keratinocytes (NHK). In this 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 d) and from adherent PBMC (GM-CSF + IL4 + TGFβ; 7 d). Dermal dendrocytes were differentiated from PBMC (GM-CSF + IL4; 7 d). Using specific primers, CD1d mRNA was detected by RT-PCR and cell surface expression by fow cytometry (MoAb CD1d 27.2 & CD1d 42.1, S Porcelli, Boston, MA). Immunochemistry was performed on normal skin specimens (n = 5, MoAb NOR3.2, Biosource). CD1d mRNA was present in all studied populations. Double staining immunofluorescence suggested that both Langerhans cells (CD1a+) and dermal dendritic cells (Factor XIIIa+) expressed CD1d. Cell surface expression was confirmed by flow cytometry on DCs developed *in vitro*; however, while CD1d27.2 MoAb gave a strong staining, no staining was observed using CD1d42.1 MoAb, which otherwise gave a normal staining on NHK and control transfectants. Our data suggest that CD1d is expressed on Langerhans cells and dermal dendrocytes. The pattern of epitopes expressed by CD1d molecules on NHK and DCs appeared to be not strictly overlapping.

016

Interactions of *In Vitro* Generated Langerhans-Like Dendritic Cells with Environmental Allergens

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The safety and efficacy of sublingual immunotherapy have been demonstrated in moderate allergic asthma and seasonal rhinitis. In order to define the precise mechanism of action of the allergen when it crosses the oral mucosa, we investigated the role of Langerhans cells in the capture, internalisation and presentation of allergens. We generated *in vitro* dendritic cells with the phenotypic characters of Langerhans cells (LLDC) from cord blood CD34+ progenitors cultured with GM-CSF and TNFα. We used two recombinant major allergens: Birch pollen allergen 1 (r-Bet v 1) and Phleum pratense allergen 1 (r-Phl p 1) labelled with FITC.

Internalisation of allergens was conducted by macropinocytosis. Part of the allergens accumulated in acidic vesicles, while the remaining part was observed in other cytoplasmic structures and left cells during vesicles recycling. The amount of allergens that use acidic vesicles pathway was maximum when LLDC were in an immature state (before day 12 of differentiation). Allergen internalisation was not followed by clear modifications of the phenotype whereas in three experiments out of seven LLDC were able to activate naïve T lymphocytes. These data suggest that *in vivo*, the low number of primary responses may be due either to a low frequency of specific T lymphocytes or to a lack of maturation and migration of LLDC.

017

CD100, the Immune Semaphorin, Inhibits the Migration of Monocyte-Derived Dendritic Cells

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The semaphorins, mostly expressed in the nervous system, exist as transmembrane and soluble molecules. Soluble semaphorins exert a repulsive effect on growth cone guidance. CD100 expressed on hematopoietic cells is still the unique immune member of this family. We had previously shown that transmembrane CD100 is involved in the regulation of the immune response through its association with CD45 and a serine-threonine kinase. A soluble form of CD100 (sCD100) can be generated from the surface of hematopoietic cells and inhibits the migration of B and monocytic cell lines. We studied the role of sCD100 in the migration of dendritic cells at various stages of their differentiation from purified monocytes cultured in the presence of GM-CSF and IL4. Monocytes have an important spontaneous migration of 40% in 6 h in a vertical transwell migration system. The spontaneous migration of dendritic cells at D3-D4 of *in vitro* differentiation is 30%. Dendritic cells at D6 of *in vitro* differentiation do not migrate spontaneously but only in response to chemokines such as MCP3 and MIP3β. We observed that sCD100 strongly inhibits (30%) the spontaneous migration of dendritic cells at D3-D4 of differentiation. SCD100 also inhibits the MIP3β-induced migration of dendritic cells at D6 of differentiation. These observations indicate that blood-derived dendritic cells have different migratory properties during their *in vitro* differentiation and that sCD100 ligand is expressed throughout their differentiation.

018

Monocyte-Derived Immature Human Dendritic Cells Internalise the Stress-Inducible Heat Shock Protein (HSP)70 Via a Specific Receptor

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HSPs are able to induce a specific, antigen presenting cell dependent, T cell response against tumor cells or viral-infected cells from which they are derived. The objective of this study was to assess the presence of a receptor for the stress-inducible cytosolic HSP70 on human dendritic cells (DCs). Monocyte-derived immature DCs were incubated for different time periods, at different temperatures, with either gold-labelled rhHSP70 (HSP-Au) or fluorochrome conjugated rhHSP70. FACS analysis revealed that immature DCs bind at 4°C rhHSP70. Confocal microscopy (CM) demonstrated the rapid internalisation of HSP70. Furthermore, CM showed that HSPs reached the MHC class II positive compartments. Electron microscopic studies demonstrated the spontaneous presence of HSP-Au in coated pits at 4°C. At 19.5°C for 20 min, abundant gold particles were found in coated pits and coated vesicles as well as in early endosomal structures. This staining disappeared almost completely in the presence of a 500-fold excess of unlabelled rhHSP70, while it was almost not affected by an excess of BSA. Finally, when DCs were incubated 15 min at 37°C in the presence of HSP70-Au, then washed extensively and chased for 30 min at 37°C, the gold particles accumulate in late endosomal structures, which were colabelled with an anti-HLA-DRα chain polyclonal antibody. Thus, monocyte-derived immature human DCs specifically and continuously internalise rhHSP70, thereby suggesting the presence of a receptor for HSP70 on the DC surface.

019

Effects of Tumor Products on the *In Vitro* Maturation of Human Epidermal Langerhans Cells

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Recent studies have demonstrated the capacity of tumor cell line supernatants to inhibit the differentiation of *in vitro* generated dendritic cells. In this study, we analysed the effects of VEGF, TGFβ and IL-10 on human Langerhans cell maturation. Langerhans cells were purified from normal human skin and incubated for 2–3 d in a serum free medium in the presence or not of the different cytokines, before analysing their phenotype and allostimulatory function.

Results showed that VEGF (3–20 ng per mL) and TGFβ (10 ng per mL) altered neither the increased expression of HLA-DR, CD40, CD54, CD80, CD86 and CD83 on cultured Langerhans cells, or their allostimulatory capacity. By contrast, IL-10 significantly decreased the cell membrane expression of all the antigens and led to an impaired allostimulatory function. Finally, we showed that supernatants from two human melanoma-derived clones did not affect Langerhans cell phenotype and function. These clones did not produce detectable amounts of IL-10 but, interestingly, they were quite able to inhibit the differentiation of dendritic cells from $CD34^+$ cord blood progenitors (O. Berthier *et al*, manuscript submitted).

020

Normal Human Fibroblasts can Maintain Dendritic Cells in an Immature State

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Inside a collagen I lattice retracted by fibroblasts, monocyte-derived dendritic cells (DC) are in an immature state (no expression of CD86, neither CD83) despite the presence of collagen I. In order to verify whether fibroblasts are implicated in this observation, we studied the effects of soluble factors secreted by fibroblasts on monocyte-derived DC, in contact or not with collagen I, using a Transwell. DC were cultured in the upper compartment, in contact or not with collagen I, while fibroblasts were seeded in the lower compartment in order to allow soluble factors to diffuse. As previously reported (Brand *et al*, *J Immunol*, 1998), DC matured when cultured with collagen I alone (HLA-DR was diminished, CD86 and CD83 were highly expressed, and allostimulatory function was strong). On the other hand, when fibroblasts were present in the system (DC + collagen I), they inhibited the DC maturation (CD86 and CD83 were poorly expressed and allostimulatory function was weak). So, fibroblasts secrete some soluble factors that can maintain dendritic cells in an immature state. Consistent with this, it is noteworthy that dermal DC have been described as immature DC.

021

CD34+ Cells Derived Dermal Dendritic Cells, Integrated in Collagen Lattices, are Potent Replicative Centers for HIV Type R5 Isolates and not for Type X4 Isolates

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Mucosal infection by HIV is mainly restricted to M-tropic strains of virus (type R5). We investigated the potency of three laboratory strains: BaL (type R5), Lai and HxB2 (type X4) to productively infect dermal dendritic cells integrated in collagen lattices.

CD34 + human myeloid progenitors can differentiate, in the presence of GM-CSF and TNFα, along two subsets of dendritic cell (DC) precursors identified by the exclusive expression of CD1a and CD14 at day 6 of culture. The CD1a+/CD14-precursors mature at day 12 of culture into Langerhans cells, and the CD1a-/CD14 + give rise to dermal DC. Cells were generated from a donor giving rise preferentially to the CD1a-/CD14 + population at day 6 of culture and were integrated into three-dimensional type I-collagen lattices with human fibroblasts. These integrated cells generated DC with the following phenotype: MHC class II++, CD86+, Factor XIIIa++, CD36+, CD11b+, CD80+, CD83+–. These DC-containing dermal equivalents were incubated with the HIV strains for 72 h. After extensive washing, these equivalents were cultures for eight extra days after infection. Culture supernatants were assayed every 2 d for p24 by ELISA test. Negative controls of infection were included: DC-depleted lattices, or DC-containing lattices treated with AZT. P24 analysis showed that a high replication of the BaL strain, but not of Lai and HxB2 strains in culture supernatants of DC-containing and nontreated by AZT. Next, we plan to explore, by PCR *in situ* amplification, the presence of provirus and to investigate whether nonproductive infection by type X4 strains may be confined to DC, putative reservoirs or latent vectors in the virus dissemination.

In transmucosal infection, this model of immunocompetent human dermis allows to study the capability of dermal effectors, such as interstitial DC, to be infected by, to replicate or not, and then to disseminate the HIV to other cell populations located in the superficial dermis.

022

Cutaneous T cell Lymphoma-Reactive $CD4^+$ CTL Clones Display a Th1 Cytokine Profile and use a Fas-Independent Pathway for Specific Tumor Cell Lysis

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We have previously described two CTL clones isolated from lymphocytes infiltrating a $CD4^+$ cutaneous T cell lymphoma (CTCL). These clones displayed a $CD4^+CD8dim^+$ (TC5) and $CD4^+CD8^-$ (TC7) phenotype and mediated a specific cytotoxic activity toward Cou-LB autologous tumor cell line. These studies were performed to precise the mechanism involved in T cell clone-mediated cytotoxicity and to determine the cytokine profile of both lymphoma cell line and specific CTL clones. Our results indicate that, despite surface expression of Fas receptor on Cou-LB and FasL induction on TC5 and TC7 cell membrane, the $CD4^+$ CTL clones do not use this cytotoxic mechanim to lyse their specific target. Blocking experiments of TC5 and TC7 cytotoxicity toward Cou-LB tumor cell line in the presence of EGTA, zVAD, TRAIL, and ZB4 indicated that ZB4 had no effect on the cytotoxic activity of both clones, in contrast EGTA significantly blocked TC7 activity while zVAD and anti-TRAIL MoAb blocked that of TC5. These results demonstrate that TC7 rather use a granzyme/perforin-dependent pathway while TC5 uses a TRAIL-dependent mechanism. Furthermore, quantitative analysis of cytokine mRNA expression in cell line and clones indicated that while the tumor cells display a Th3-type profile (IL-4, IL-6, IL-10, and TGF-β), the CTL clones express a Th1-type cytokines (IFNγ, GM-CSF, and IL-2). In addition, we showed by Elispot that preincubation of TIL clones with autologous tumor cells induced their activation and subsequent amplification of the Th1-type response. These results indicate a direct contribution of the malignant cells in the Th1/Th2 imbalance observed in CTCL patients and suggest their role in the inhibition of antitumor immunity.

023

Evolution of T Cell Repertoire in Treated Sezary Syndrome

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Sezary syndrome is a leukemic form of cutaneous T cell lymphoma related to the malignant proliferation of clonal CD4+ T cells. Extracorporeal photopheresis (ECP) or antineoplastic polychemotherapy may induce clinical improvement in some cases. In order to investigate the representation of the T cell clone in the peripheral blood of 10 patients receiving treatment with ECP (eight patients) or CHOP (two patients), we used a semiquantitative technique based on RT-PCR BV-BC and immunoscope determination of the CDR3 length. As previously reported, we did not find any BV preferential expansion. The percentage of Sezary cells was not correlated to the relative frequency of the clone. One of the eight patients treated with ECP showed a decrease of the relative frequency of the T cell clone from 15.6% to 0%, which was correlated to the complete clinical remission of the disease. In the seven remaining cases showing no clinical improvement, weak changes in the relative frequency of the dominant BV-BC rearrangement were observed. In one patient showing a partial remission following CHOP, a decrease of the relative frequency of the clone from 43% to 7% was observed. In the other chemotherapy-treated case, the relative frequency of the clone was stable in parallel to the absence of any clinical benefit of the treatment. In conclusion, immunoscope represents a semiquantitative technique that may be applied to the follow-up of the T cell clonal component in Sezary syndrome. In our study, the evolution of the relative frequency of the clone seemed to parallel the clinical course of the disease.

024

Arsenic Trioxide Induces Apoptosis of Sezary Cells *In Vitro*

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Arsenic trioxide (As2O3) has recently been shown to exhibit *in vitro* antiproliferative and apoptogenic properties against several myeloid and lymphoid malignant cell lines such as acute promyelocytic leukemia and acute lymphoid HTLV-I leukemia. *In vivo*, in APL patients, As2O3 induced complete remissions and was well tolerated.

In this study, we investigated the effects of As2O3 on the proliferation and the viability of the Sezary cell line HUT-78 and of peripheral blood mononuclear cells (PBMCs) derived from three patients with Sezary syndrome (SS), exhibiting a high proportion of tumoral cells in the peripheral blood. Viability/apoptosis were assessed by using flow cytometric analysis after propidium iodide and annexin-V staining, Terminal dUTP Nick End Labelling (TUNEL), and cell cycle analysis.

The results showed that a final concentration of 10 μM of As2O3 was needed to induce significant cell death of HUT-78. PBMCs derived from patients with SS exhibited a greater sensitivity to As2O3, in a time- and dose-dependent manner. Thus, 50% of cells were apoptotic after 72 h incubation with 2 μM As2O3. IL-2 + IL-7 did not alter the *in vitro* sensitivity of cells derived from the patients, and interferon-α did not potentiate this effect. Furthermore, the same range of *in vitro* kinetics and quantitative assesment of As2O3-induced apoptosis was found for PBMCs of healthy blood donors and SS patients. Using double staining with clonotypic anti-TCRBV antibodies and anti-CD3, we demonstrated that Sezary cells represented around 80% of T lymphocytes in each patient. Among living cells, this percentage remained stable during As2O3 treatment, suggesting that tumoral and normal lymphocytes in a given patient exhibited the same sensitivity to As2O3-induced apoptosis. The results from these preclinical studies are the first demonstration of the pro-apoptotic properties of As2O3 on Sezary cells *in vitro*, which were observed with clinically acceptable concentrations.

The therapeutic effect *in vivo* in Sezary patients remains to be assayed.

025

Pancreatitis Associated Protein-I (PAP-I) Alters Adhesion and Motility of Human Melanocytes and Melanoma Cells

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Pancreatitis Associated Protein-I (PAP-I) is a secretory stress protein first characterized in pancreas during pancreatitis, but also expressed in several tissues including hepatic, gastric, and colon cancer. Its concentration in serum can be significant. PAP-I relationship to skin cancers was investigated in normal melanocytes (NHM), melanoma tumors and in melanoma cell lines. None of them expressed PAP-I, as checked by indirect immunofluorescence and RT-PCR. In addition, melanocytic cell treatment by UV-B, cisplatin and hydrogen peroxide, three agents known to induce DNA damages and cell stress by different mechanisms, did not result in PAP-I expression; however, adenovirus-mediated PAP-I cDNA expression reduced cell adhesion to laminin-1 and fibronectin with a loss of integrin participation. Adenovrial PAP-I transfert stimulated haptotactic and directed migrations of some melanoma cells, but not of NHM. Importantly, directed migration of these melanoma cells was also activated in a dose responsive manner when 60–600 pM purified rat PAP-I were added to the culture medium of noninfected cells. This indicates that effects in PAP-I cDNA virus-transferred cells were elicited by PAP-I after its secretion. Exogenous PAP-I can therefore modify adhesion and motility of normal and transformed melanocytes, suggesting a potential role in invasion of melanoma.

026

Matrix Mediated Progelatinase a Activation Leads to Enhanced Invasive Property of Melanoma Cells

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Gelatinase A (Matrix Metalloproteinase 2: MMP-2) was implicated in matrix proteolysis during tissue invasion of melanoma cells (Brooks *et al*, *Cell*, 1996). M1Dor and M3Da melanoma cell lines were found to express in a constitutive manner only the pro form of MMP-2 (Capon *et al*, *Clin Exp Metast*). Co-culturing melanoma cells with dermal fibroblasts, in conditions where cell contacts were prevented, did not induce proMMP-2 activation; partial activation was noticed following contact between M3Da cell line and fibroblasts. On the contrary, complete proMMP-2 activation was noticed when melanoma cells were cultured in a three dimensionnal collagen lattice, in the presence or absence of dermal fibroblasts.

We further evidenced that proMMP-2 activation, in collagen lattice, was directly related to enhanced melanoma cell invasive property in a modified Transwell assay. Batimastat as well as a furin convertase inhibitor, inhibited enzyme activation and parallely empeded M3Da invasiveness. Cell migration was also modulated by an antibody blocking integrin $\alpha 2\beta 1$. These data highlight the crucial importance of proMMP-2 activation in the *in vitro* invasive property of melanoma cells.

027

Overexpression of the Proteasome Component POH-1 Stimulates Cell Resistance and DNA Repair in Human Melanoma Cells Exposed to UV-B Radiations

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Cell resistance to genotoxic agents may limit the effectiveness of anticancer therapies. In malignant melanoma, the mechanisms of genotoxic resistance remain largely unknown. Proteasome has been involved in some neoplastic processes and, recently, in nucleotide excision repair. To get insights on the possible roles of proteasome in melanoma cell resistance, proteasome relationships with UV-B stress were explored on melanoma cell viability and DNA repair. Following exposure of SK-MEL-5 and KAL melanoma cell lines to UV-B (40 mJ per cm^2), specific drug-inhibition of the proteasomal activity led to a slowing down of the removal of cyclobutane pyrimidine dimers (CPD) by DNA repair mechanisms. To analyse proteasome involvement, double-transfections of the cells were performed with plasmids bearing cDNAs coding for the human 26S proteasome component POH-1 and the rat CD2 surface membrane protein. Purified CD2/POH-1-positive cells were exposed to UV-B stress and subjected to direct and clonogenenic survival assays.

Results showed that overexpression of POH-1 conferred cell resistance to UV-B when compared to CD2-positive control cells bearing the empty POH-1 vector. Furthermore, kinetics of CPD removal following UV-B exposure were accelerated in SK-MEL-5 and KAL cells that overexpressed POH-1. Taken toghether, these preliminary data demonstrate that the proteasome is involved in melanoma cell resistance to UV-B genotoxic stress. Both proteasomal activity and POH-1-dependent activity seem involved in DNA repair mechanisms, thus opening perspectives for the pharmacologic modulation of melanoma cell resistance.

028

Not submitted

029

Autoantigen (Desmoglein 1) and HLA Class II Gene Polymorphisms are Involved in Susceptibility to Superficial Pemphigus (PF)

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We previously identified a polymorphic marker of the desmoglein 1 gene made of a silent substitution at position 809. In this study, we tested the hypothesis that autoantigen polymorphism and HLA class II molecules polymorphism are intricated in a multigenic model for PF susceptibility.

Distribution of mutation 809 in 26 caucasian patients and 85 caucasian controls was not significantly different as far as phenotypic frequency is concerned (84.6% *vs* 75.3%; $p = 0.3$) but barely reached significance when allelic frequency was examined (61.5% *vs* 48.2%, $p = 0.09$), which indicated a higher amount of homozygous in patients. HLA class II genotyping indicated a significant increase of DR4 generic in patients (58.3% *vs* 31.7%; $p = 0018$). Furthermore, of the 10 patients homozygous for mutation 809, nine were DR4+, whereas distribution of mutation 809 was homogenous between DR4+ and DR4-controls. Therefore, mutation 809 happened to be strongly associated with PF in DR4+ subjects ($p = 0008$), what is in accordance with a polygenic model of disease susceptibility. Preliminary results allow us to propose a hypothesis for susceptibility mecanism, involving an alternative splicing of the upstream exon to mutation 809, potentially dysregulated by the mutation itself. The resulting transcript would lead to a truncated and therefore secretory isoform of the autoantigen. This hypothesis is currently examined and will be discussed.

030

Leucocyte Elastase and Gelatinases and in Subepidermal Blister Formation during Bullous Pemphigoid

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In vivo (Backdahl, *J Clin Invest*, 1994; Liu, *J Exp Med*, 1998) and *in vitro* (Schäcke, *J Biol Cell*, 1998) recent studies have suggested metalloproteases (MMP_9, MMP_2) involvement in subepidermal blister formation during bullous pemphigoid (BP). We studied skin biopsies ($n = 17$) and blister fluid ($n = 6$) from patients with clinically typical BP lesions. All skin specimens showed immunoreactivity with MMP_9 antibodies localized in inflammatory cells and in keratinocytes. Zymograms confirmed the prescence of zymogenes forms of MMP_9 and MMP_2 in blister fluid. Quantification by ELISA revealed that the amount of TIMP1 was systematically higher than the amount of MMP_9 in blister fluid. Leucocyte elastase (LE) was also localized in polymorphonuclear cells in BP lesions and LE enzyme activity was found in BP blister fluid. Ectodomain (100 kDa) of recombinant PB 180 was partially cleaved by BP blister fluid and totaly cleaved by tested recombinant proteases with different sensitivity. Recombinant LE was more active than MMP9 for cleaving the antigenic protein. Our results confirm the presence of large amounts of MMP_9 and MMP_2 in BP lesions. Gelatinases are present only as inactive zymogenes and $TIMP_1$ concentration is higher than MMP_9 concentration. *In vitro*, LE is more active than MMP2 and MMP9 to cleave BP180 ectodomain. All these results suggest a prominent role of LE in subepidermal blister formation during BP. Nevertheless actived MMP9 could act locally at the membrane of the keratinocyte.

031

Pure Ocular Cictricial Pemphigoid: An Immunologic Study in Nine Patients

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Background. Cicatricial pemphigoid is a subepithelial autoimmune bullous dermatosis (AIBD) characterized by frequent and severe mucous involvement. In particular ocular lesions can lead to cecity. Ocular involvement can occur isolately, realizing pure ocular cicatricial pemphigoid (POCP). POCP is difficult to diagnose since clinical signs are not specific and immunologic criteria are ill-defined. This study was aimed to analyze the immunologic findings in nine patients with POCP.

Patients and methods. There were five women and four men, mean age was 75 y, all were presenting with chronic scarring conjunctivitis evocative of POCP. Direct immunofluorescence (DIF), indirect immunofluorescence (IIF) on salt-split skin, and immunoblot (IB) were performed in all patients.

Results. DIF showed linear deposits of igg, iga, or C3 along the basement membrane in seven of nine cases. IIF showed linear deposits of igg or iga in six of nine cases, located on the epidermal (three of nine cases), dermal (one of nine cases) or both (two of nine cases) sides. IB detected bands in five of nine patients with a molecular weight of 190 (one case), 180 (two cases), and 160 (one case) kDa.

Conclusion. We report for the first time in a series of patients with POCP that (1) in a majority of cases autoantibodies suggestive of AIBD can be detected and confirmed by different techniques, and (2) in some cases, it is possible to detect a 180 kDa antigen that may correspond to BPAG-2, the main antigen of CP.

032

Immunohistologic Study of the Skin of the First Human Allograft: Results at 16 Mo

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The first human hand allograft was performed in Lyon (23/9/98). The recipient (48-y-old man) and the donor (42-y-old man) were of the same blood group but showed six HLA mismatches. The patient was treated with prednisone, FK506, mycophenolate mofetil, antilymphocyte and anti-CD25 serum. This study was undertaken in order to assess the quality of the allografted skin and to detect possible signs of graft rejection. Skin biopsies were taken at various intervals postgraft and studied immunohistologically. Histologically, the skin showed a normal structure, and the main cell types expressed their characteristic differentiation antigens. CD1A+/Lag+ Epidermal Langerhans cells were present in normal amounts, and from D77 ≈ 10% of them were of recipient's origin (HLA-A24+). Epidermal Merkel cells and neurofilaments within dermal nerves were first observed on D342 and 472, respectively. On D57–77 a dense lymphocytic infiltrate of recipient's origin (HLA-A24+) was observed in the dermis; it had appeared concomitantly with erythematous macules that had developed following FK506 decrease. These lesions were considered as signs of acute graft rejection and regressed upon an increase of the immunosuppressive treatment. On D450 new erythematous lesions appeared on the allografted skin; histology showed an aspect identical with that of lichenoid GVH, and led to the diagnosis of chronic graft rejection. The increase of immunosuppressive treatment led to a slow regression of the lesions. Our results show that in this model of composite tissue allograft the skin maintains normal structure and trophicity; they also show that histologic examination of the skin can reveal acute or chronic graft rejection. The study of two hand allografts of a new patient is in progress in order to confirm these results.

033

Abnormal Melanosome Distribution with Normal Myosin-V Expression in the Melanocytes of two Patients with Griscelli Syndrome

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Griscelli syndrome (GS) is a rare autosomal recessive disease characterized by pigment dilution and cellular immunodeficiency. GS maps to chromosome 15q21 and was associated with mutations in the myosin-V gene; however, in one patient myosin-V was not mutated, suggesting that GS can be caused by a mutation in another gene. To understand the mechanisms of pigment dilution in GS, we established melanocyte cultures from two patients with GS. The shape of GS melanocytes was abnormal, with a round cell body, reflecting the accumulation of numerous melanosomes. On the other hand, GS melanocytes developed normal-looking dendrites, but the number of melanosomes in this region was markedly decreased. These melanosomes were normal in shape and maturation. Analysis of melanin content, TRP-1 and tyrosinase in GS melanocytes indicated that melanogenesis proceeded normally in these cells. Myosin-V expression and distribution was not significantly different in GS and normal melanocytes. These data confirm the possibility that another protein than myosin-V, may be essential for melanosome transport and involved in GS.

034

Pilomatrixomas Express the Hair Keratins α1, α2, and α5

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Pilomatrixoma (PM), a tumor of the hair matrix, expresses the basic hair keratin β1. The purpose of this work was to study the expression in PMs of the acidic hair keratins α1, α2, and α5, which have a specific expression pattern in the normal follicule.

We studied 30 PMs, 10 trichoblastomas, and 10 basal cell carcinomas using anti-α1, α2, and α5 monoclonal antibodies. We used a classic PAP immunohistochemistry method, after revelation by microwaves (20′), addition of trypsin and changes in pH.

The keratins α1, α2, and α5 were expressed in the 20 PMs showing transitional areas. None of the keratins were expressed in PM lacking basophilic cells, in trichoblastomas or in basal cell carcinomas. α1- and α5-antibodies stained large bands of cells, and α2 was only focally expressed. The cells positively stained with anti-α1 were closer to the basophilic cells, whereas those stained with anti-α5 were closer to the ghost cells.

This study shows a strong expression of α1, α2, and α5 in transitional areas of PMs, confirming the complex hair matrix differentiation of this tumor, whereas pilar tumors without matrix differentiation (trichoblastoma, basal cell c.) do not express those keratins. The pattern of expression of α1, α2, and α5 is similar in PM and in normal follicules, showing a high degree of differentiation in PM. Keratin α1 is expressed in the same areas as keratin β1 (*in situ* hybridization results, Cribier *et al*, *Br J Dermatol* 140:600–604, 1999), confirming the validity of these results, as α1 and β1 form pairs in the intermediate filaments.

035

***In Vitro* Reconstitution of Cutaneous Microvascularisation** Method**ologic Approach**

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If dermal equivalents represent valuable *in vitro* models of human dermis, cutaneous vascularisation plays a key role in many physiologic and pathologic events. We propose here a methodologic approach to reproduce a cutaneous microvascularization in a dermal equivalent. A three dimensional coculture of microvascular cells and fibroblasts, both from human dermal origin, has been realized in a collagen gel. At day 21, capillary like structures can be noted in this dermal equivalent. Organization of cells forming tubular structures is observed at the histologic level. Formation of vascular lumen from a cellular cluster is clearly seen at the ultrastructural level. Numerous intercellular junctions are present around this internal lumen. Intracytoplasmic Weibel-Palade bodies and extracellular collagen deposits along the plasmic membrane, outline of basal membrane, sign the vascular origin of these cells. This vascular origin is confirmed by a strong positive labelling, at the histologic and ultrastructural levels, by CD31.

This model of endothelialized dermal equivalent will enable us to study *in vitro* the roles of cellular interactions and growth factors in various cutaneous events, particularly during wound healing.

P1

Evaluation of the Avene Spring Water Effect on Oxygen Radicals Generation

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During cellular metabolism, cutaneous inflammatory process or pathogenesis of human diseases, oxygen radicals can be generated. The release of reactive oxygen species (ROS) is indicative of cell stress and their overproduction induces an important cellular alteration especially of the plasmic membrane components, of the proteins and of the genomic material. Avene spring water (ASW) has been widely used in the treatment of various inflammatory skin diseases (atopic dermatitis and psoriasis). In order to evaluate the ASW effect (from 0 to 100% in millipore water) on oxygen radicals generation, we have used different *in vivo* and *in vitro* models. We have first analysed the ASW effect on ROS production by phorbol ester (TPA)-stimulated mouse peritoneal macrophages, using a chemiluminescence assay. The effect of ASW on macrophage oxidative metabolism was investigated by measuring the peak of luminol-enhanced chemiluminescence response. The chemiluminescence inhibition (ROS production inhibition) following ASW treatment was significant with 50% and 75% of ASW. We have secondly analysed the *in vitro* ASW potential protective role against alteration of the main cellular targets (membrane lipids, ADN and proteins) induced by ROS generated during ultraviolet (UVA) irradiation. So, we used UVA-irradiated human skin cells in which we measured the lipid peroxidation level by assaying the TBARS (ThioBarbituric Acid Reactive Substances), the DNA damages using the comet assay, and the oxidized proteins by immunodetection of carbonyl group. The membrane lipid oxidation assay (TBARS) clearly showed the protective effect of ASW which is maximal for 50% and 75% ASW. The qualitative and quantitative evaluation of DNA strand breaks showed the genomic protection efficiency of ASW with a maximal effect at 50% of ASW. Moreover, the ASW also decreased the protein oxidation in a dose-dependent manner. These studies clearly show a significant antioxidant activity of the ASW. In conclusion, by its antiradical properties, Avene spring water preserves the cutaneous tissue from dramatical effect of free radicals during inflammation process of the skin.

P2

PAI1 Expression is Induced in Human Skin Keratinocytes After Repeated UVB Irradiation

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Sunlight, and particularly ultraviolet (UV) light, is an important environmental insult to skin. UV radiation plays a major causative role in skin cancer and can activate the expression of a wide variety of genes.

By using the Atlas Human Cancer cDNA Expression Array (Clontech), we investigated the modulation of gene expression in primary human skin keratinocytes following UVB injury. In order to get closer to a model of chronic UV irradiation, human keratinocytes cultured in monolayer were irradiated daily with a sublethal dose of UVB for 5 d. Forty-eight hours after the last irradiation, RNA was extracted from human cells and the expression of 588 genes was studied. We observed the repression of numerous genes (including for example CDK6 and the gene coding for versican core protein) and a large induction (40 times) of the Plaminogen Activating Inhibitor I (PAI1) gene. The latest modulation was confirmed using RT-PCR and Northern blot, and with different strains of primary human skin keratinocytes. Moreover we showed that PAI1 gene induction was observed in human epidermis following UVB irradiation. Interestingly, an acute UVB irradiation on keratinocytes in monolayer lead to the repression of the PAI1 gene, demonstrating that repeated or acute irradiation can lead to different gene modulation.

To our knowledge this is the first demonstration of UV modulation of PAI1 gene. As this gene has been shown to be implicated in carcinogenesis we wonder if this induction of PAI1 expression in keratinocytes after repeated UVB irradiation could not participate to the photocarcinogenesis process.

P3

Comparative Effects of UVB/A1 Radiations and Prostaglandin E2 on the Production of Vascular Permeability Factor (VEGF) by Cultured Human Fibroblasts and Keratinocytes

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We previously reported a differential effect of UVB and UVA1 radiation on the release of vascular permeability factor (VEGF) by human keratinocytes (Longuet-Pernet *et al, Br J Dermatol* 138:221–224, 1998). This study was aimed at determining the effects of UVB/A1 and prostaglandin E2 (PGE2) on the production of VEGF by cultured human fibroblasts (HF) in comparison with keratinocytes (HK). The skin cells derived from foreskin were cultured in defined medium after treatment by either UVB (312 nm, 100 and 200 J per m^2), UVA1 (365 nm, 2 and 4 J per cm^2), or PGE2 (10^{-7}–10^{-5} M). The expression of VEGF was evaluated at the mRNA (RT-PCR) and protein levels (ELISA). The results showed major differences between the type of skin cells. The basal level of VEGF was lower in HF than in HK. UVB radiation induced a greater increase of VEGF in HK than in HF. UVA1 radiation that did not change VEGF secretion in HK, strongly upregulated VEGF mRNA and protein in HF. Whereas UVA1, when associated with UVB radiation, inhibited the UVB-induced stimulation of VEGF secretion in HK, they exhibited an additive effect on VEGF secretion in HF. PGE2 whatever its concentration, did not modify the secretion of VEGF in HK but moderately increased in a dose-dependent manner the expression of VEGF in HF. These results indicate different signalling pathways according to the cell type and the wavelength of UV radiation used and suggest a role for VEGF in the UV-induced erythema, likely independent of PGE2.

P4

Persistance of Unscheduled DNA Synthesis in Human Skin Irradiated with a Solar Simulator and Protected with a Sunscreen

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The use of high sun protection factor (SPF) sunscreens is proposed to reduce skin cancer incidence; however, persistence of DNA damage has been observed after irradiation by suberythematous dose of UV. Six volunteers were exposed to UV on one forearm, unprotected, at 3 MED and on the other one at 12 MED after application of a sunscreen with a SPF of 12.

Biopsies were performed 15 min and 24 h after irradiation for autoradiographic determination of UDS and immunohistochemical p53 detection. In the skin protected with a 12 SPF sunscreen, UVB-induced erythema was completely inhibited. There was, however, a significant amount of DNA damage.

DNA damage was still detectable 24 h after irradiation in sunscreen protected skin and the normally observed induction of p53 was significantly reduced.

In conclusion, in spite of an efficacious protection against UV-induced erythema, sunscreens are unable to avoid induction of DNA damage.

P5

#245#245iEα-Tocopherol does not Prevent UVB-Induced Epidermal Vitamin A Depletion in Hairless Mice, but Accelerates its Reconstitution

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We previously showed that a single UVB exposure induces the depletion of epidermal vitamin A (retinol) and its esters, the storage form of vitamin A, in hairless mice. Since UVB induces lipid peroxidation, and this process involves the formation of free radicals and reactive oxygen species, we assessed the rate of lipid peroxidation induced by UVB in hairless mice, and applied topical α-tocopherol (vitamin E) 0.25%, an endogenous free radical scavenger, in order to prevent UVB-induced epidermal hypovitaminosis A. Lipid peroxidation, as measured by the formation of organic lipid peroxides, was not increased by UVB irradiation, in the conditions giving rise to epidermal vitamin A depletion. Moreover, topical α-tocopherol did not prevent retinol and retinyl ester depletion following UVB exposure, although a 60-fold increase in epidermal α-tocopherol. However, a pretreatment with topical α-tocopherol accelerated retinol and retinyl ester reconstitution following UVB exposure. When applying a topical mixture associating 0.5% ascorbic acid and 0.25% melatonin to 0.25% α-tocopherol, epidermal retinol, but not its esters, was protected from UVB-induced degradation. In conclusion, UVB-induced epidermal vitamin A depletion is probably not correleted to free radical and lipid peroxide production, and topical agents with other properties than only free radical scavenging and lipid peroxide reduction, in association with α-tocopherol, would have some benefits in preventing epidermal hypovitaminosis A following UVB exposure.

P6

Photoimmunosuppression in the Skin: *In Vitro/Ex Vivo* Evaluation of the Immunoprotective Effect of an Organo-Mineral Sunblock

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Exposure of the human skin to UV radiation causes an imbalance of its homeostasis and a reduced cutaneous immunity. Langerhans cells and keratinocytes play a fundamental role in the immune response. The immunosuppression brought about by UVB is assumed to result from the direct effects of UVB on the antigen-presenting function of the Langerhans cells and from indirect effects mediated by immunosuppressor mediators derived from the keratinocytes such as Transforming Growth Factor-β1 (TGFβ1) and interleukin 10 (IL10). The aim of this study was to determine, in the context described, the immunoprotector effect of the organo-mineral sunblock by means of two models: (1) a model of TGFβ1 expression by human HaCaT keratinocytes exposed to UVB (0.01–0.02 J per cm^2) and directly in contact, or not, with the organo-mineral sunblock in the culture medium. The UVB induce the production of TGFβ1: an increase of +87% in TGFβ1 production at the dose of 0.015 J per cm^2. This production was significantly decreased when the keratinocytes were irradiated in the presence of the organo-mineral sunblock in the medium: 30% inhibition of TGFβ1 production caused by 0.015 J per cm^2. (2) A human skin explant model protected, or not, by the application of a topical preparation containing the organo-mineral sunblock (2 mg per cm^2) and irradiated with increasing levels of UVB (312 nm; 0.5–1 J per cm^2); after irradiation the total pool of epidermal cells was isolated and the allostimulating capacity of the Langerhans cells was tested in mixed lympho-epidermal culture (MLEC). UVB inhibited the lymphocyte proliferating response which was evaluated in the presence of ^{3}H thymidine (5 d of coculture): 0.75 J per cm^2 caused a 43% inhibition of the response. The response was totally restored when the explant was protected by the organo-mineral sunblock. UV radiation plays a critical role in the development of a state of cutaneous immunosuppression. Our results demonstrate the immunoprotecting effect of the organo-mineral sunblock and its ability to limit cutaneous photoimmunosuppression particularly by maintaining the quantities of cytokine TGFβ1, which is immunosuppressive, at levels similar to cutaneous homeostasis but also by protecting the function of the Langerhans cells.

P7

UVAI Immunosuppression and Photoprotection Afforded by a Broad-Spectrum Sunscreen

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Ultraviolet AI (UVAI) (340–400 nm) irradiation of human skin results in Langerhans cell depletion and reduction of epidermal antigen presenting cell (APC) activity. We assessed the protection afforded by a broadspectrum sunscreen against immunosuppression induced by a high UVAI exposure (60 J per cm^2). For each volunteer ($n = 10$), epidermal cell (EC) suspensions were obtained from irradiated skin after sunscreen (SS-UV-EC) and vehicle (Veh-UV-EC) treatment, and from nonirradiated and vehicle-treated skin (Veh-EC). T cell proliferation was assessed by incubation of EC with allogeneic $CD4^+$ purified T lymphocytes. The APC activity of Veh-UV-EC (25001 ± 5095 cpm) was reduced from 45% compared to that of Veh-EC (41548 ± 6849 cpm) ($p = 0.005$). The sunscreen axerted a partial protection since the APC activity of SS-UV-EC was 33890 ± 6184 cpm. The percentage protection against immunosuppression (PPIS) was 57 ± 9% ($n = 10$). The protection factor against UVAI (UVAI-PF) was 3 ± 0.2 ($n = 9$) and the coefficient correlation between PPIS and UVAI was 0.79.

Conclusion The downregulation of epidermal APC activity induced by high UVAI exposure is partially prevented by prior application of a sunscreen with a low UVAI-PF value. These results indicate that increasing the absorption of UV filters for long UVA wavelengths may lead to an improved immune protection.

P8

Not submitted

P9

Not submitted

P10

Not submitted

P11

Evaluation of the Avene Spring Water Effect on Generation of Oxygen Radicals

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During cellular metabolism, cutaneous inflammatory process or pathogenesis of human diseases, oxygen radicals can be generated. The release of reactive oxygen species (ROS) is indicative of cell stress and their overproduction induces an important cellular alteration especially of the plasmic membrane components, of the proteins and of the genomic material. Avene spring water (ASW) has been widely used in the treatment of various inflammatory skin diseases (atopic dermatitis and psoriasis). In order to evaluate the ASW effect (from 0 to 100% in millipore water) on oxygen radicals generation, we have used different *in vivo* and *in vitro* models. We have first analysed the ASW effect on ROS production by phorbol ester (TPA)-stimulated mouse peritoneal macrophages, using a chemiluminescence assay. The effect of ASW on macrophage oxidative metabolism was investigated by measuring the peak of luminol-enhanced chemiluminescence response. The chemiluminescence inhibition (ROS production inhibition) following ASW treatment was significant with 50% and 75% of ASW. We have secondly analysed the *in vitro* ASW potential protective role against alteration of the main cellular targets (membrane lipids, ADN and proteins) induced by ROS generated during ultraviolet (UVA) irradiation. So, we used UVA-irradiated human skin cells in which we measured the lipid peroxidation level by assaying the TBARS (ThioBarbituric Acid Reactive Substances), the DNA damages using the comet assay, and the oxidized proteins by immunodetection of carbonyl group. The membrane lipid oxidation assay (TBARS) clearly showed the protective effect of ASW which is maximal for 50% and 75% ASW. The qualitative and quantitative evaluation of DNA strand breaks showed the genomic protection efficiency of ASW with a maximal effect at 50% of ASW. Moreover, the ASW also decreased the protein oxidation in a dose-dependent manner. These studies clearly show a significant antioxidant activity of the ASW. In conclusion, by its antiradical properties, ASW preserves the cutaneous tissue from dramatical effect of free radicals during inflammation process of the skin.

P12

Substance P Effects on CCR5 and CXCR4 Receptors on Langerhans Cells and their Progenitors

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Substance P (SP), a neuropeptide, has been shown to enhance Human Immunodeficiency Virus type1 (HIV-1) proliferation in macrophages. Langerhans cells, main targets of this virus in mucosae, allow only a weak viral replication. Thus we studied the effects of SP on HIV-coreceptors CXCR4 and CCR5 expression on Langerhans cells.

We cultured cord blood CD34+ cells with GM-CSF, TNFα and TGFβ1 for 12 d in order to obtain dendritic cells/Langerhans cells (DC/LC). We studied several surface antigens on Langerhans cells cultured with different SP concentrations (10^{-9} M, 10^{-7} M, and 10^{-5} M).

SP had no effect on CD34 and CD1a expression on dendritic cells/Langerhans cells, suggesting that dendritic cells/Langerhans cells maturation is not affected by this neurotransmitter. Without SP during culture, CXCR4 and CCR5 expression on dendritic cells/Langerhans cells increased until the fifth day of culture (29% of CCR5-positive cells, 48% of CXCR4+ cells), decreased at the eighth day of culture (11% of CCR5+ cells, 12% of CXCR4+ cells) and increased again at the 12th day (37% of CCR5+ cells, 40% CXCR4+ cells). After 1 d of culture with SP at a concentration of 10^{-7}M, we observed that CCR5 is overexpressed on dendritic cells/Langerhans cells (35% of positive cells). After 8 d of culture, SP induced a decrease of CCR5 expression (6% of positive cells). Yet, SP did not to affect CXCR4 expression.

Thus SP seems to increase infectivity of dendritic cells/Langerhans cells progenitors but to decrease infectivity of matured cells. Infection studies are in progress in order to confirm these conclusions.

P13

Expression of CXCR4 and CCR5 Coreceptors on the Plasmic Membrane of ME180 and SiHa Cells

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HIV infection occurs mainly during sexual intercourse and involves Langerhans cells in the vaginal mucosa; however, it seems that exocervical epithelial cells could be directly infected by the virus during HIV exposure. In order to clarify this point, we have analyzed the expression of the two main coreceptors for HIV-1 (CXCR4 and CCR5) on two cell lines derived from human exocervix (ME180 and SiHa cells).

With the aim to develop an *in vitro* epithelial model close to the *in vivo* mucosa, the cells were cultured on different supports: plastic, de-epidermized dermis (DED) or Matrigel. Optical microscopy observations of the immunohistochemical sections revealed that the cells grown on biologic supports (DED or Matrigel) could form a multilayered epithelium, as described for the malpighian epithelium. The study of CXCR4 and CCR5 coreceptors on our different models was performed by immunolabellings and analyzed by FACS after a gentle cell dissociation. Cells cultured on plastic expressed the two coreceptors, but this expression was exclusively intracellular. In contrast, cells grown on DED or Matrigel showed a membraneous signal for the coreceptors that was evaluated to 15–30% of living cells.

Then, it seems that the presence of HIV coreceptors on the plasmic membrane is dependent on the epithelium organisation and more particularly is regulated by cell differentiation. This functional expression in a pluristratified epithelium close to the normal exocervix suggests that HIV transmission could occur via normal epithelial cells. Current studies in our laboratory could permit us to confirm this hypothesis, and the importance of HIV coreceptors in the genital epithelium during the HIV transmission process.

P14

Expression of CD1d Molecule in Human Skin: Histologic Study and Correlation with Keratinocyte Differentiation

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Introduction CD1 molecules are a family of nonpeptidic antigen presenting molecules. We report CD1d expression in normal human skin and demonstrate that its expression is correlated with keratinocyte differentiation.

Material and methods Immunohistochemical study was performed on normal human skin specimens (n = 15) with anti-CD1d antibody (clone NOR3.2). Normal human keratinocytes were grown in medium supplemented with calcium (0.03, 0.15, and 1.2 mm) and CD1d surface expression was analyzed by flow cytometry, using anti-CD1d 42.1 (S. Porcelli, Boston).

Results CD1d was widely expressed on epidermal keratinocytes, except in the stratum corneum, giving a chicken-wire aspect. The staining was increasing from the basal to the outermost layers suggesting that CD1d expression paralleled keratinocyte differentiation. A similar pattern could be observed on the inner and outer root sheath epithelium. CD1d was also expressed on epithelial cells in sebaceous glands and eccrine duct epithelium. Flow cytometry showed that calcium-switch induced an increase in CD1d surface expression, correlated/associated with keratinocyte differentiation.

Conclusion CD1d is widely expressed by epithelial cells in normal human skin. Cell surface expression on keratinocytes is correlated with differentiation.

P15

Differentiation of Embryonic Stem Cells into Keratinocytes

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Embryonic stem (ES) cells are pluripotent cells that can differentiate into various cell types, including squelettal and cardiac muscle, endothelial, neuronal or adipocytes cells as well as from the hematopoietic system. After 20 d in suspension culture, a small population of ES cells differentiate spontaneously to basal keratinocytes, as judged by their positive staining against keratin 14 (K14) antibody; however, the stimuli that would force ES cells to differentiate into keratinocytes and the specific genes turned on/off remain totally unknown. The overall goal of our study is to search for culture conditions needed to transform undifferentiated ES cells into keratinocytes. This will then allow us to identify the genes which, activated in differentiated cells, will induce the commitment of primitive ES cells into keratinocytes. To detect keratinocytes in the ES population, we constructed a plasmid carrying the green fluorescence gene (GFP) under the control of keratinocyte-specific promoter/enhancer sequences. Analysis of the regulatory sequences of laminin α3 gene delineated a strong keratinocyte-specific enhancer fragment. This enhancer was cloned in tandem upstream the K5 promoter and GFP reporter gene. As expected, transcriptional activity of this construct (p2AK-GFP) was strong in keratinocyte cell lines but absent in fibroblasts and undifferentiated ES cells. Therefore, ES neo^R clones were selected after electroporation with p2AK-GFP. Most of the 40 individual clones isolated displayed some fluorescent cells after spontaneous differentiation. We are currently testing whether these positive cells coexpressed basal keratinocyte-specific genes. These stable cell lines will then allow us to identify, through testing different culture conditions and various stimuli, the appropriate way to force ES cells to become keratinocytes.

P16

Epidermal Cell Differentiation is Linked to Notch Signalling

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Epidermal homeostasis involves the monitoring of continuous proliferative and differentiative processes, as keratinocytes migrate from the basal layer to the skin surface. The Notch pathway is characterized by cell–cell interactions between transmembrane proteins and was first implicated in cell binary choices and patterning. It may also play a crucial role in regulating cell proliferation and differentiation. The spatio-temporal sequence of expression of Notch signalling genes during embryonic stratification of mouse (Thélu *et al*, *J Invest Dermatol* 1998) epidermis has been described previously, but the site of action and functions of this pathway have not yet been investigated in human skin. To begin to address these questions, the expression of Notch1–3 receptors, their Delta1, Jagged1 ligands and their Fringe modulators expression were studied by *in situ* hybridization in normal and pathologic adult human skin. The results show that these genes are transcribed in the epidermis of nonlesional skin, particularly in the basal and lower suprabasal cell layers. Conversely, when keratinocytes are hyperproliferating, as in basal cell carcinoma, psoriasis, and the first stage of wound healing, expression is only faint or nonexistent. Furthermore, normal levels of transcription were rescued in psoriatic plaques when treated by phototherapy, as well as in newly regenerated stratified epidermis following wound healing.

The Notch pathway thus appears to be involved in the differentiation program of normal adult human epidermis. It remains to be established if the absence of this pathway is a cause or a consequence of the hyperproliferative epidermal process.

P17

Localization of Members of the Notch System and the Differentiation of Vibrissa Hair Follicles: Receptors, Ligands and Fringe Modulators

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Hair vibrissa follicle morphogenesis involves several cell segregation phases, in the dermis as well as in the epidermis. The expression of *Notch*-related genes, which are well established mediators of multiple cell segregation events in Drosophila development, was studied by *in situ* hybridization during embryonic mouse vibrissa follicle morphogenesis and the first adult hair cycle. The results show that two receptors, *Notch1* and *-2*, three ligands, *Delta1*, *Serrate1*, and *-2*, and the three *Fringe* regulators *Lunatic*, *Manic*, and *Radical*, are expressed in different locations and morphogenetic stages. First, the appearance of hair vibrissa primordia involves the expression of complementary patterns of *Notch2*, *Delta1*, and *Lunatic Fringe* in the dermis and of *Notch1*, *Serrate2*, and *Lunatic Fringe* in the epidermis. Second, this expression pattern is no longer found after stage 3 in the dermis. Meanwhile in the epidermis, the expression of *Notch1*, *Serrate2*, and *Lunatic Fringe* before the formation of the placode may be involved in determining two populations of epidermal cells in the developing follicle. Third, complementary expression patterns for *Notch1*, *Manic*, and *Lunatic Fringe*, as well as *Serrate1* and *-2* as previously shown (Powell *et al*, 1998), are progressively established from stage 4 of embryonic development both in the outer root sheath and in the hair matrix. These patterns are consistent with the one found in the adult anagen phase. During the hair vibrissa cycle, *Notch1* and *Manic Fringe* display temporal and spatial changes of expression, suggesting that they may intervene as modulators of trichocyte activities.

P18

Promotion of Human Keratinocyte Differentiation and Improvement of Dry Skin by Ethyl α-D-Glucoside, Identified in Japanese Traditional Rice Wine (Sake)

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Sake, traditional rice wine, is familiarized in Japan as well as wine in Europe. For a long time, Japanese people have used it not only as a beverage, but also as a lotion for skin treatment. Sake contains as a main component a specific glycoside, ethyl α-D-glucoside (α-EG), which gives it its characteristic taste. In this study, activities of α-EG were studied on cultured human keratinocytes and human subjects. Addition of α-EG to the culture significantly promoted differentiation of keratinocytes, but had a weak effect on proliferation, whereas ethyl β-D-glucoside, an isomer of α-EG, had no effect on differentiation. Topical application of a solution containing α-EG on human skin improved dry skin after daily treatment for more than 2 wk, as well as daily application of sake. In dry skin, an imbalance between proliferation and differentiation has been reported, in which proliferation is higher than differentiation. These results indicate that α-EG contained in sake might show its improvement effect on dry skin via correction of this imbalance.

P19

Low Frequency Sonophoresis of Insulin: An *In Vivo* Study

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Low frequency ultrasound has been shown to increase percutaneous absorption of drugs, a phenomenon referred to as sonophoresis. In this work, we reported the results of the sonophoresis of insulin performed *in vivo* on hairless rats. Ultrasound (20 kHz) was applied in pulsed mode during 1 h in a plastic cylinder glued on the animal abdomen and filled with 3 mL of insulin (100 UI per mL). Blood samples (0.5 mL) were taken during and after sonication in order to monitor the animal glycemia and its insulin concentration. This treatment was repeated for three intensities. At the end of each experiment, skin samples were taken for histologic study.

When used at 0.25 W per cm^2, no decrease in the animal glycemia was observed. On the other hand, 0.5 W per cm^2 and 1 W per cm^2 intensities provoked a decrease in the animal glycemia and the amount of insulin was found to be eight times higher than control. With 1 W per cm^2, however, clinical and histologic skin lesions were observed. This was not the case with 0.5 W per cm^2. So, our results show that there seems to be a window in intensity around 0.5 W per cm^2 where effective percutaneous administration of insulin is possible without involving observable skin lesions.

P20

Role of H-ras Oncogene in HaCaT Migration and MMP Secretion. Comparison Between Cells in Monolayers and Reconstructed Skin Models Using Fibroblasts or Dead Dermis

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Matrix metalloproteinases (MMPs) play an important role in tissue regeneration, wound healing, and tumor invasion. Our previous studies have shown a higher motility of HaCaT-*ras* cells compared with normal human keratinocytes or HaCaT. According to cell culture medium, a higher secretion of MMP-2 (72 kDa) or MMP-9 (92 kDa) was detected in *ras*-transfected cells.

In this study, the invasive capacity of HaCaT-*ras* clones is investigated in reconstructed skin models, using de-epidermized dermis (DED) or fibroblasts seeded in a collagen sponge matrix. As MMPs are directly implicated in cell motility, their expression and activity are determined in each model to evaluate the role of fibroblasts cultured either in collagen lattices or in a dermis substrate. In opposite to cell monolayers, MMP-2 is faintly detected in reconstructed epidermis model on DED and unchanged during all the culture. In all models, MMP-9 is higher secreted in HaCaT-*ras* cells but its active form (86 kDa) is only detected in reconstructed epidermis, suggesting an important role of extracellular matrix. Currently, the specific role of living fibroblasts in keratinocyte invasion and MMP secretion are under investigation.

P21

Heterogeneity and Quantitative Differences of Steroid Metabolizing Enzymes in Human Cultured Hair Follicle Cells

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Various steroidal enzymes control the level of intracellular active estrogens and androgens in the hair follicle. Besides the reductive pathway of testosterone (e.g., 5α-reductase), there is an oxidative pathway in the hair follicle that is mediated by 17β-hydroxysteroid dehydrogenase (17β-HSD), which catalyses interconversions between testosterone and androst-4-enedione and between DHT and 5α-androstane-3,17dione. The 3β-hydroxysteroid dehydrogenase enzymes are responsible for the oxidation and isomerization of 5-ene-3β-hydroxysteroid precursors into 4-ene-ketosteroids.

The aim of this study on testosterone metabolism in hair follicle is to identify type I 17β-HSD, type II 17β-HSD and 3β-HSD mRNA in various cultured hair follicle cells: dermal papilla cells, root sheath keratinocytes, and dermal fibroblasts. This study is performed by RT-PCR analysis.

The type 1 17β-HSD is expressed by all the cell types. The expression is higher in dermal papilla cells and dermal fibroblasts than in keratinocytes.

As for the type II 17β-HSD, only the outer root sheath keratinocytes express this isoenzyme. The 3β-HSD is present similarly in dermal papilla cells and dermal fibroblasts; however, the expression of this enzyme is not observed in the outer root sheath keratinocytes culture.

These results showed a different expression of these enzymes according to the hair follicle cell type, suggesting a different control of steroid biosynthesis in hair follicle cells.

P22

Determination of Ascorbic Acid in Human Dermis (Microdialysis in Skin Exploration)

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Ascorbic acid (AA) plays a significant role in preventing photobiologic damage in human skin. Until now, invasive methods (biopsies) were used to determine AA concentrations. In this work, microdialysis, a noninvasive pharmacologic method, was employed in order to quantify AA in cutaneous interstitial fluid *in vitro* and to compare our results with those published in the literature.

Results AA was sampled from the dermis after insertion of probes (CMA/20) and determined by gas chromatography-mass spectrometry. Seven fragments of human abdominal skin obtained from plastic surgery were used just after their excision to determine AA levels. AA concentrations were 825 ± 66 nmol per mL (759–891 nmol per mL).

Discussion Results obtained were comparable with AA concentrations reported in the literature (403–1043 nmol per mL) and obtained in human skin biopsies. This work demonstrates the ability of microdialysis to determine biologic molecules in dermis noninvasively. This technique could also be employed *in vivo*; determination of AA *in vivo* in human dermis is in progres.

P23

An *Ex Vivo* Microdialysis Model to Study Skin Histamine Release

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The aim of this study was to focus on a new *ex vivo* model which combines histamine release in fresh skin fragments with microdialysis. Atracurium (A) (muscle relaxant able to induce histamine release in skin) and substance P (SP) were selected to validate the model.

Abdominal skin fragments (n = 8) were immediately used after surgical intervention. Placebo, A or SP were injected into dermis and induced histamine released was collected using a microdialysis technique. The histamine was assessed by an E.I.A.

Method (Immunotech). Dose–response and kinetic studies were performed and the release of histamine was plotted over a 2 h period. The time-dependent survival of the skin explants was also evaluated in terms of response towards stimulants.

In our model, the optimal concentrations of A and of SP giving the maximum histamine release were of 10^{-3} M and of 10^{-5} M, respectively. With these concentrations of drugs, the histamine levels peaked at 1972.86 ± 590.98 nmol per L and at 1369.43 ± 672.30 nmol per L (mean ± SEM), respectively. The peaks were observed 10 min after stimulation. Besides, the two substances showed different profiles in their kinetic of histamine release activity. In addition, the use of skin explants in a long-term fashion was possible until the sixth hour after the beginning of the experiments.

In conclusion, this new *ex vivo* model of microdialysis on human cutaneous explants appears to be particularly adequate for pharmacologic studies on human skin allergic-like reactions and could be used as a reproducible screening method for testing putative inducers or inhibitors of histamine release.

P24

Spatial Organization of Tense Collagen Lattices Visualized with Confocal Microscopy

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The purpose of this work was to observe the spatial organization of disc-shaped tense collagen lattices using a confocal microscope. To visualize fibroblasts, we used α SM actin revelation with FITC.

During the first days of culture, stellate cells with no preferred orientation became spindle-shaped and orientated whereas the lattice became very taut. These lattices were thin and could be mounted directly on glass slides. They were examined with a confocal microscope as a series of horizontal optical sections. After 5 d of culture, the lower layer of the lattice consisted of well-labelled spindle-shaped myofibroblasts orientated parallely to their long axis. This picture was similar to confluent monolayer cultivated fibroblasts. Six days later, the upper layer became orientated too, perpendicularly to the cells beneath in the same way of overlapping hyperconfluent cells. The cells in the middle part of the lattices remained less-labelled and randomly arranged.

Confocal microscopy studies showed that behavior and differentiation of fibroblasts in disc-shaped tense collagen lattices exhibited great similarities with monolayer fibroblasts. The two external layers orientated orthogonally made this tense collagen lattice a tough tissue which could be particularly resistant to mechanical stress and completely separate from surrounding medium.

P25

Contraction of Collagen Lattices by Striae Distensae Fibroblasts: Drug-Induced Changes

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Striae distensae are characterized by linear, smooth bands of atrophic-appearing skin. Excessive steroid activity, genetic factors and inherited defects of connective tissues are the most frequent causes of this disease. Fibroblasts derived from women presenting striae distensae lesions were investigated for the ability to retract a reconstituted collagen matrix. Two drugs were incorporated into the lattice to assess possible effects on the rate of lattice contraction: the corticoid "Triamcinolone acetonide" and a mixture of three terpenes extracted from a tropical plant "Titrated Extract from Centella asiatica".

To measure the retracted lattice diameter, the Petri dish was placed on a transparent metric scale. We have used an isometric force system to study quantitatively contraction of cells in lattice.

Striae distensae fibroblasts contracted collagen gels slower than normal human fibroblasts but the final contraction was similar. They produced a greater isometric contractile force which was associated with enhanced $\alpha 2\beta 1$ integrin expression. The capacity for contracting the gel was inhibited in a dose-dependent fashion by Triamcinolone acetonide. This effect was abolished by the addition of Titrated Extract from Centella asiatica 1%.

Striae distensae fibroblasts display a mechanical activity in culture which can be modulated by the action of drugs of dermatologic interest.

P26

***In Vitro* Decrease of Advanced Glycation End Products and the Induced Skin Coloration by the Hypotaurine**

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The amino groups of proteins react with reducing sugars to form advanced glycation end products (AGEs), which are insoluble brown products. Glycation is an oxygen dependent reaction generating reactive oxygen species as U.V. A radiations. We investigated the role of the hypotaurine, an antioxidant molecule, on the formation of AGEs and the induced skin brown/yellow products. The *in vitro* AGEs formation, obtained with type IV collagen or bovine serum albumin incubated 10 d at 37 °C with ribose, was inhibited by 44% and 81%, respectively, when the hypotaurine (10 mM) was added to the incubation medium. 31% fewer cells adhered to glycated collagen than to collagen (Anova, Newman-Keuls). These cells were rounded and poorly plated. The hypotaurine increased by 26% the cellular adhesion on glycated collagen and restored a normal cellular morphology. The glycation of a cutaneous explant that was maintained in survey for 10 d on a microporous filter induced a brown coloration of the skin. The addition of the hypotaurine to the incubation medium reduced the brown/yellow skin explant coloration.

In conclusion, the hypotaurine is able to inhibit the *in vitro* protein glycation reaction, process leading to skin darkening.

P27

An Approach of Chronologic Aging of Human Skin by Glycation of the Collagen in a Skin Equivalent Model

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Glycation is a chemical reaction between sugars like glucose or ribose and free amine residues in proteins. *In vivo* this slow nonenzymatic reaction may create new residues or formation of cross-links (AGE Products) in slowly renewing macromolecules like collagen or other extracellular matrix of the dermis. Chronologic aging is a complex phenomenon taking place in all organs and tissues of the body which affects their biologic properties. In skin formation of bridges between molecules could be responsible for loss of elasticity or modification of other properties of the dermis observed during aging. Glycation may therefore play an important role in chronologic aging of the skin. In order to examine this hypothesis we have developed a reconstructed skin model made of a modified dermal compartment which is a fibroblast-contracted collagen lattice prepared with preglycated collagen. The presence and the distribution of AGE-products in skin equivalents was studied using specific antibodies against pentosidine, carboxymethyllysine, or total AGE-products. Moreover, immunostaining experiments were performed using antibodies directed against vimentin, procollagen I, collagen IV, $\beta 1$ and $\alpha 6$ integrins to study the effect of glycation on various markers of skin. We observed that the shape and the orientation of the fibroblasts could be modified by the presence of glycated collagen. We also found that extracellular matrix molecules of the dermis and basement membrane seemed to be enhanced in the presence of glycated collagen. In addition, stainings of integrins like $\beta 1$ and $\alpha 6$ were also increased in epidermal cells. By comparing to young and old skin these *in vitro* findings could at least in part be related to aging *in vivo*.

P28

Expression of Fas and Fas Ligand by Human Dermal Endothelial Cells: Involvement in the Control of Inflammatory Processes

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Human dermal endothelial cells (HDMEC) contribute to homeostasis by preventing extravasation of circulating cells toward tissue. These cells constitutively express Fas (a type I membrane protein belonging to the TNFα receptor family) in human normal skin (*Arch Dermatol Res* 286:396, 1994). Although in many cellular types, Fas initiates an apoptotic signal when bound to its ligand FasL, our results demonstrate that cultured HDMEC *in vitro*, in which 50–75% express Fas at their surface, were resistant to apoptosis after Fas ligation with a specific anti-Fas (clone 7C11: 0.1–5 ng per mL) or with recombinant soluble FasL (absence of apoptosis shown by Annexin-FITC or TUNEL). Moreover, FasL transcripts have been depicted in HDMEC by RT-PCR and protein detection has been obtained by western blot as well as by flow cytometry (20–40% positive cells with clone H11). The function of FasL was demonstrated by coculturing HDMEC and Fas-positive cells such as Jurkat cells or isolated neutrophils, leading to apoptosis of the latter. However, activation of HDMEC by TNFα (100 U per mL, 48 h) downregulated transcripts and FasL expression at their surface (no staining after 48 h of cell activation). Furthermore, the passive transmigration of nonapoptotic polymorphonuclear cells through a monolayer of endothelial cells was higher if these latter have been previously activated by TNFα during 48 h.

Taken together, our results suggest a potent role of FasL in the limitation of cellular infiltration in human normal skin, this function being alterable under the effect of soluble mediators released during inflammatory reaction, allowing transmigration of viable circulating cells within cutaneous tissue.

P30

Heterogeneity and Quantitative Differences of Steroid Metabolizing Enzymes in Human Cultured Hair Follicle Cells

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Various steroidal enzymes control the level of intracellular active estrogens and androgens in the hair follicle. Besides the reductive pathway of testosterone (e.g., 5α-reductase), there is an oxidative pathway in the hair follicle that is mediated by 17β-hydroxysteroid dehydrogenase (17β-HSD) which catalyses interconversions between testosterone and androst-4-enedione and between DHT and 5α-androstane-3,17dione. The 3β-hydroxysteroid dehydrogenase enzymes are responsible for the oxidation and isomerization of 5-ene-3β-hydroxysteroid precursors into 4-ene-ketosteroids.

The aim of this study on testosterone metabolism in hair follicle is to identify type I 17β-HSD, type II 17β-HSD and 3β-HSD mRNA in various cultured hair follicle cells: dermal papilla cells, root sheath keratinocytes, and dermal fibroblasts. This study is performed by RT-PCR analysis.

The type 1 17β-HSD is expressed by all the cell types. The expression is higher in dermal papilla cells and dermal fibroblasts than in keratinocytes.

As for the type II 17β-HSD, only the outer root sheath keratinocytes express this isoenzyme. The 3β-HSD is present similarly in dermal papilla cells and dermal fibroblasts; however, the expression of this enzyme is not observed in the outer root sheath keratinocyte culture.

These results show a different expression of these enzymes according to the hair follicle cell type suggesting a different control of steroid biosynthesis in hair follicle cells.

P31

Effect of White Lupin Peptides on the Regulation of Matrix Metalloproteinases (MMPs)

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Repeated exposure to solar radiation damages human skin leading to photoaging (PA). There exists evidence that suggests that PA results largely from UV induction of matrix metalloproteinases (MMPs) which degrade extracellular matrix components within the dermis. Thus, the development of new potent MMPs inhibitors is of interest with regard to the prevention of PA. The purpose of this work is to delineate the precise role of lupin peptides on the regulation of MMPs pathway.

Low molecular weight purified White lupin peptides are obtained from lipid free seeds using a biotechnologic process which eliminates the polysaccharides potentially involved in the secondary reactions of glycation. Using the Enz/Chek Gelatinase/Collagenase kit from Prolabo, we demonstrate that these pure peptides have a significant inhibitory effect on MMP activity. This effect is both time and dose dependent. We also check MMP secretion in UVA-irradiated human skin fibroblasts. Forty-eight hours postirradiation, lupin peptides almost completely inhibit UVA-induced-MMP secretion (both MMP-3 an –9), as measured in cell culture medium. MMP are zinc dependent enzymes. Thus, we verify if lupin peptides are zinc chelators. As measured *in vitro*, they are able to chelate zinc.

In conclusion, these data suggest interesting applications of White lupin peptides in the development of a new generation of topical products intended for the prevention of PA.

P32

Iron Chelators and Kaposi's Sarcoma

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Iron is suspected to be involved in the induction and/or progression of various human tumors. More particularly, iron may be involved in the pathogenesis of Kaposi's sarcoma (KS), a human herpesvirus 8-associated vascular tumor. This hypothesis may partly explain the high prevalence of KS in geographic areas with iron oxide-rich volcanic clays, such as Sicily, Iceland, or the East African Rift System (1,2). It may also provide a nonhormonal explanation for the lower prevalence of KS among women, as they are known to have lower iron reserves than men. This prompted us to investigate the effects of iron chelators on KS cells.

Desferrioxamine and deferiprone, two chemically unrelated iron chelators, induced a time- and dose-dependent inhibition of KS cell growth. The inhibition of cell growth was paralleled by a decrease in Ki-67 and in both stable and total PCNA expression, suggesting that iron chelation blocked these cells in the G0/G1 phase of the cell cycle. TUNEL assay, flow cytometry with propidium iodide and morphologic analysis indicated that iron chelation also induced a time- and dose-dependent apoptosis. This suggests that iron chelators may represent an experimental therapeutic approach for the treatment of KS.

1. Ziegler JL: Endemic KS in Africa and local volcanic soils. *Lancet* 342:1348–1351, 1993.
2. Simonart T, Noel JC, Andrei G, *et al*: Iron as a potential cofactor in the pathogenesis of KS. *Int J Cancer* 78:720–726, 1998.

P33

A Precursor Common to the Myeloid and NK Cell Lineages with Skin Homing Property

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Mechanisms of skin involvement observed in some tumor proliferations are not well understood. We reported a case of *leukemia cutis* without circulating leukemic cells which may be related to a special phenotype of tumor cells.

A 56-y-old man presented with disseminated cutaneous nodules and his clinical examination was normal except for lymphadenopathy in the parotid region. Skin histology demonstrated a dermal infiltrate of large blast cells without cytoplasmic granules; these cells expressed no T cell antigens (CD2, CD3, CD5, CD7, TCR $\alpha\beta$ and $\gamma\delta$), no B cell antigens (CD19, CD20), nor cytotoxicity markers (CD8, granzyme B) but some markers of the myelomonocytic (Mm) (CD4, CD68, MPO) and the NK cell lineages (CD56, TIA1) were positive. Hemogram was normal. Bone marrow and lymph node were infiltrated by blast cells which expressed Mm markers (CD36, CD38, DR) and only one NK cell marker (CD56+ in 36% of cells). Culture of these blast cells during 24 h with GM-CSF or IL2 and IL7 induced appearance of azurophilic granularity and expression of CD33. PCR genomic studies failed to detect any T cell or B cell clonal rearrangement in skin, blood, bone marrow, and lymph node samples.

This clinical and biologic picture was very special because of: (1) the unusual phenotype of blast cells expressing Mm and NK cell markers at the same time and demonstrating Mm maturation in culture, this result was in favor of a common origin to both lineages and a normal counterpart cell has been identified in healthy individuals; (2) a very high level of CD56 expression on the tumor cells in the skin (90%) and a low level in the bone marrow. CD56 is an isoform of neural cell adhesion molecule (N-CAM) involved in the cell homing mechanisms and its high level of expression on the blast cells may thus explain the predominant skin involvement of this disease.

P34

Frequency and Prognostic Value of Clonal T Cells PCR Detection in Skin, Peripheral Blood Lymphocytes and Bone Marrow in Cutaneous T Cell lymphomas

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We have studied the frequency of T cell clone PCR detection in skin, peripheral blood lymphocytes (PBL) and bone marrow in a series of 90 patients with cutaneous T cell lymphomas-CTCL-(57 mycosis fungoides-MF–, six Sézary syndromes × SS- and 27 nonepidermotropic CTCL: 11 with CD30+ large cells and 16 with CD30-pleomorphic cells), according to their initial stage and to their outcome, in order to determine the prognostic value of this detection.

PCR detection frequency of a cutaneous T cell clone varied according to the stage of MF: from 52.5% in early stages (Ia-IIa) to 96% in the late stages (IIb-IV,SS). A T cell clone was detected in PBL in 40% of the early MF and in 74% of the late MF but in early stages, the T cell clone in PBL was more frequently different from the cutaneous one than in late stages (11 of 16 *vs* four of 17) suggesting a reactive clonal population. In nonepidermotropic CTCL, a T cell clone was detected in the skin in 80% of the cases and in PBL in 44%. Similar results were observed in bone marrow, but the detection of a T cell clone identical to the skin in PBL or bone marrow was not predictive for a medullar histologic involvement that was extremely rare (two of 65).

In MF, SS and nonepidermotropic CTCL, the detection of an identical T cell clone in skin and PBL was associated with a 17% remission rate (complete or partial), while this rate was 76% when the T cell clone was observed only in the skin, and was 87% when a polyclonal profile was observed in skin. Indeed, these results strongly suggested the prognostic interest of PCR study in skin and PBL in such patients.

P35

Compared Immunogenicity in Mice of GD3 Ganglioside Purified from Human Malignant Melanoma *Versus* Glyco-Replica Peptides Mimicking GD3

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Glyco-replica peptides mimicking GD3, that is the major ganglioside of human malignant melanoma, were isolated with a mouse monoclonal antibody (MoAb) specific for GD3 ganglioside, from a phage-displayed peptide library. The peptides were sequenced and analyzed, then synthetized. The immunogenicity of peptide R4, the most reactive peptide with MoAb anti-GD3, was studied following immunization of mice with the peptide R4 coupled to keyhole limpet hemocyanin (KLH), and compared with that of ganglioside GD3. All immune sera reacted as IgG and IgM antibodies with both GD3 and R4 peptide, as seen by ELISA and immunostaining on PVDF membranes; however, the IgG subtypes were quite diffrent with respect to the antigen tested. Mice immunized with R4 had a high titer of specific IgG3 when tested on GD3, whereas the titer of IgG3 bound to R4 was weak. Mice immunized with GD3 had a high titer of IgG2a and IgG1 specific for both GD3 and R4. Thus, peptide R4 induced an IgG3 response displaying antibodies that had a higher affinity for GD3 than for the peptide itself. A study by flow cytometry on murine cells expressing GD3 showed that, although the binding of immune sera is stronger for mice immunized with GD3, the sera of mice immunized with R4 also react with these cells. These results suggest that glyco-replica peptides mimicking gangliosides may be useful to trigger an immune response against tumor cells such as human malignant melanocytes expressing those gangliosides.

P36

Expression of Ceramide Species in Sphingolipids of Human Melanoma Tumors

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Ceramides are key molecules in apoptosis, cell growth and differentiation phenomenons where they have important signal transduction functions. Moreover, ceramides are an important component of the skin and disregulation of their metabolism occurs in some pathologies such as psoriatic disease and cancer. Few studies have focused on ceramide species and metabolism in melanoma, which is one of the major emerging pathology of skin cancers. The expression of free ceramide species in fresh human melanoma tumors was studied with special reference to the sphingoid backbone. Ceramide isolated from more complex sphingolipids such as sphingomyelin and glycophingolipids were also investigated. This was done after purification and fractionation of sphingolipid classes by solid phase extraction onto aminopropyl columns. Long chain bases (LCB) were identified by different chemical treatments and according to different chromatographic criteria by HPTLC, by gas chromatography of their aldehyde derivatives after periodate treatment, and by GC-MS of their heptafluorobutyric acid derivatives. The results indicate that in the different sphingolipid classes the major long chain base is d18:1 sphingosine. d18:0 dihydro-sphingosine was found in high proportion in free ceramides, sphingomyelin, and glyco-sphingolipids (40% of LCB in free ceramides). Trace amount of t18:0 phytosphingosine was also detected in neutral glycosphingolipids as well as O-methyl and 3-O ethyl derivatives of LCB. The presence of high amounts of dihydrosphingosine is unusual since this LCB is found only in trace amounts in human tissues. As dihydroceramides cannot induce apoptosis, concentration of such compounds in melanoma tumors could be relevant and have some important consequences in ceramide apoptosis pathway. This possibility is under investigation.

P37

Regression of Melanoma Metastases (Stage III) after Vaccination with Dendritic Cells

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Human melanoma cells express a number of antigens that can be recognized by T cells and can provide potential targets for immunotherapy. Dendritic cells (DC) are the most potent antigen presenting cells of the immune system capable of stimulating naïve T cells.

In stage III and IV melanomas, we initiated a vaccination with autologous DC pulsed by MAGE-1, –3 peptides in HLA-AL, -A2 and/or -B44 patients, with or without help (KLH: Keyhole Limpet Haemocyanin).

We present the case of a 65-y-old patient suffering from a nail melanoma with epitrochlear and axillary node metastases. After surgical resection, some residual tumor detectable by PET scan using labelled fluro-D-glucose (FDG) required a MAGE3-HLA2 vaccination with KLH.

As early as the second injection, cytometry analysis of blood sample disclosed a transient and specific increase of T lymphocytes secreting IFN-γ in response to MAGE3 antigenic peptide as shown by ELISPOT and FACS analysis. After three series of injections we observed a progressive regression of the PET scan hypercaption.

P38

Melanoma Cell Uptake of Iododobenzamides

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Iodobenzamides are a class of radiopharmaceuticals developed as melanoma-seeking agents for the diagnosis of ocular form and the detection of metastasis. Undergoing clinical trial with N-(2-diethyl aminoethyl)-2-iodobenzamide (^{123}I-BZA2) shows promising results with a good specificity.

The aim of our *in vitro* work, using various experimental models, was to evidence the cellular uptake mechanism of ^{125}I-BZA2 to melanoma. The cellular fixation of ^{125}I-BZA2 depends on the cell lines. ^{125}I-BZA2 uptake on pigmented melanoma cells was higher than those obtained on other cell lines (fibroblast, mammary carcinoma, unpigmented melanoma). For various melanin contents, the fixation of ^{125}I-BZA2 in B16 cells (murine melanoma) appeared proportional. Moreover, our results showed a good affinity of ^{125}I-BZA2 to synthetic melanin. Finally, we have studied the potential implication of ^{125}I-BZA2 binding to sigma receptors, by the competition of ^{125}I-BZA2 and haloperidol on a melanoma pigmented cell line, a hypothesis often suggested. Our results are in accordance with a predominant melanin binding of ^{125}I-BZA2 to explain the good scintigraphic clinical results.

P39

Epidermal Fatty Acid-Binding Protein (E-FABP) and Proteins S100A7, S100A8, and S100A9 in Patients with Cutaneous Melanoma

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It has been shown that S100 proteins and recently E-FABP play important roles in melanoma. The presence of S100A7, S100A8, S100A9, and E-FABP was analyzed in the urine of patients with cutaneous melanoma at various stages (according to the AJCC/UICC staging system) to know if these proteins could be used as early markers.

A multicentric study was conducted in 31 patients with cutaneous melanoma and 17 healthy volunteers. Sex ratio was about one in each group. The first voided morning urine samples were concentrated and submitted to SDS-PAGE immunoblotting.

All these proteins were detected. We observed that frequencies of urinary detection in melanoma patients were higher for S100A7, lower for S100A9, and identical for S100A8 than in control group, without a modification in function of the staging: 77% (24 of 31) *vs* 41% (seven of 17) ($P < 0.02$), 16% (five of 31) *vs* 65% (11 of 17) ($p < 0.001$), and 61% (19 of 31) *vs* 71% (12 of 17) (NS), respectively. In addition, we found an inverse relationship between E-FABP detection and the extent of melanoma (I/II: seven of 10, III: five of 10, IV: none of 11 and control: five of 17, $p < 0.001$). No difference was detected for all these proteins between urine sample collected before and 48 h after tumorectomy ± adenectomy in patients with stage I/II ($n = 5$) or III ($n = 5$).

Our results suggest that these proteins are not melanoma tumor markers, but the host response to the melanoma tumor process might be reflected in this differential urinary detection. Further studies are needed to determine which cells release these proteins.

P40

Overproduction of Active Matrix Metalloproteinase-9 is Associated with Invasion of Human Melanoma Cells Through the Dermal–Epidermal Junction

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We recently reported that human melanoma cells from a metastatic clone (T1C3), but not from a nonmetastatic clone (IC8), penetrated through a preserved dermal–epidermal basement membrane of human reconstructed skin. This local invasion coincided with the dissolution of native basement membrane collagens types IV and VII. The purpose of this study was to determine whether the gelatinases MMP-2, MMP-9, the tissue inhibitor TIMP-1 are involved in this process. To this end, the time-course of MMP-2, MMP-9, and TIMP-1 produced in the culture fluids harvested from the skin reconstructed with invasive or noninvasive melanoma cells was compared with those collected from the skin reconstructed with keratinocytes alone, over a 3-wk culture period. Gelatin zymograms, Western blots, and ELISA analysis demonstrated that MMP-9 was the predominant secreted gelatinase, remaining latent only in skin reconstructed in the absent of melanoma cells. When melanoma cells proliferated into the epidermis, a processed form of 82 kDa, corresponding to its active form, was present and became overexpressed only in the presence of the invasive melanoma cells. This overproduction of active MMP-9 was concomitant with a low amount of TIMP-1, as assassed by ELISA and Western blots. Collectively, our data demonstrate that ratio of active MMP-9/TIMP-1 is crucial in the penetration of human melanoma cells through the dermal–epidermal basement membrane. The activation process of MMP-9 may thus represent a pivotal event in the earliest invasive steps of human cutaneous melanoma.

P41

Study of Congenital Nævus's Formation in a Model of Reconstructed Epidermis

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Congenital nævi are common lesions constituted by pigment cells bearing common features with melanocytes but showing altered growth and localization characteristics. In a reconstructed epidermis containing nævocytes, we studied the formation of nests of nævocytes and the dermal passage of those cells in correlation with the secretion of matrix metalloproteinases (MMP2 and 9). The same study was done with normal melanocytes and melanoma cells. On reconstructed epidermis, a positive correlation was established between the increasing percentage of seeded nævocytes and the pigmentation of reconstructs as well as the clustering of cells in jonctionnal nests. The presence of nævocytes in the dermis of reconstructs was never detected. We noted a differential expression of MMP9 in neonatal nævi and a probably constitutive expression of MMP2 by congenital nævus cells. Previous studies had shown that melanocytes produced comparable amounts of both MMP2 and MMP9, and that Bowes melanoma cells secreted a marginal level of MMP2. So, nævus cells would correspond to an intermediate status of differentiation between normal melanocytes and melanoma cells. Activation of MMPs by a cofactor or the activation of another pathway seem necessary to provoke the dermal passage of nævus cells.

P42

Massive Skin Necrosis Associated to the Prothrombin Gene Pt20210a Mutation

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Background Skin necrosis is an infrequent feature related with multiple causes, including an evolving spectrum of abnormalities of the haemostasis system.

Case report A 43-y-old white woman presented with a 1 y history of painful, progressive cutaneous lesions. She referred compulsive intake up to 20 tables by day of pyrithyldione associated with diphenhydramine. Symmetrical erythemato-purpuric plaques with serohaematic bullae and necrotic eschars were observed on 30% of total body surface. Biopsy specimens disclosed massive thrombosis in small and middle-size dermal and subcutaneous vessels, without signs of vasculitis. Laboratory tests revealed raised titres of antinuclear antibodies (1/2560) and a positive rheumatoid factor. A complete study of other thrombosis risk factors was normal, except for slightly raised plasma levels of IgM antiphosphatidylcholine, IgM antiphosphatidylserine, factor VIII and factor II. Analysis of the 3′-untranslated region of the prothrombin (PT) gene 20210 variant showed that the patient was heterozygous carrier of this mutation.

Comment. The PT 20210 A mutation has been recently described as a clear risk factor for venous thrombosis (*Blood* 88:3698, 1996; *N Engl J Med* 338:1793, 1998) and only one case of skin necrosis related to this mutation and induced by warfarin has been reported (*N Engl J Med* 340:735, 1999). Abusive intake of pyrithyldione associated with diphenhydramine could contribute to the development of thrombosis (*Dermatology* 193:50, 1996).

P43

T Cell Response Patterns in T Cell Reactive Leprous and Sarcoid Granulomas

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A variety of mycobacterial antigens including peptides, lipids, and glycolipids is responsable for the diverse nature of the cutaneous immune response in the T cell reactive forms of leprosy with stimulation of α/β T cells, γ/δ T cells, and NKT cells.

In order to study the role of NKT cells in the formation of cutaneous granuloma of infectious and idiopathic origin and to study the nature of antigenic stimulation of α/β T cells in these conditions, we took biopsies from six patients with cutaneous sarcoidosis and compared them with five biopsies of patients with tuberculoid leprosy, three patients with lepromatous leprosy, two patients with the reversal form, and one normal skin biopsy by analysing the invaded T cell populations using immunohistochemistry, RT-PCR, the Immunoscope technique, and sequence analysis. This approach showed restricted Vα usage in sarcoidosis as well as in leprosy granulomas. In sarcoidosis an antigen-driven-like pattern could be found with different V α bearing T cells on expansion. Sequence analysis of the CDR3 region of these peaks showed an individual dominant sequence in the majority of patients which was not shared between patients. In tuberculoid and reversal leprosy patients besides the expected finding of Vα 24 positive cells a strong bias towards Vα 6 and Vα 14 with a polyclonal expansion pattern could be detected. CDR3 sequence of these expansions revealed oligoclonal expansions with repetitive sequences which were not shared between the patients. We could identify in all leprosy patients with active cellular immune response the canonical Vα24-Jα18 rearrangement of the α/β TCR which is typical for human NKT cells. Moreover six of seven patients scored positive for Vα 24 positive T cells in the immunohistochemistry. Sarcoidosis patients, however, were found negative for the presence of NKT cells by both techniques. In summary we could identify NKT cells in the investigated infectious leprous granuloma lesions but not in the sarcoidosis patients. Analysis of the TCR α chain in sarcoidosis suggests an antigen driven process with individual clones on expansion. In contrast the investigated leprosy patients showed biased TCR Vα chain usage with oligoclonal expansions mainly for Vα 6 and Vα 14.

P44

Thymidine Phosphorylase and Skin Cancers: Lack of Expression in Basal Cell Carcinoma

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Thymidine phosphorylase (TP) or platelet-derived endothelial cell growth factor (PD-ECGF) is implicated in tumoral growth and has been found to be a major determinant of the toxicity of 5-fluorouracil (5FU) and its prodrugs, which are extensively studied as anticancer agents. The aim of our study was to assess TP expression in melanoma, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC). TP was detected on paraffin-embedded sections by using a specific monoconal antibody directed against human TP (*n*°654–1, Roche Laboratory, Japon). TP was strongly expressed in normal epidermis with a nuclear and cytoplasmic distribution. TP expression in SCC (n = 10) was homogeneous and either strong (n = 5) or moderate (n = 5). TP reactivity was heterogeneous in superficial spreading (n = 5) and nodular melanoma (n = 5) beeing absent or weak in all tumors. As opposed to SCC, there was no TP expression in BCC (n = 10). This lack of expression may be related to the origin and/or differenciation of these tumors. Although TP has angiogenic activity *in vivo*, the upregulation of TP activity in tumors that weakly express this enzyme may be helpful to potentiate 5FU cytotoxicity.

P45

Immunosuppressed and Nonimmunosuppressed Patients Suffering from Squamous Cell Carcinoma: Modulation of Expression of MMP-2, -9 and TIMP-1

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Matrix metalloproteinases (MMPs), enzymes containing a zinc atom in their catalytic domain, are involved in physiologic processes like wound healing and also in the development of pathologic processes like cutaneous carcinomas. A few studies demonstrated that MMPs could play a role in the progression of squamous cell carcinoma (SCC). Observations revealed a clinical evolution of SCC more agressive for immunosuppressed (IS) patients. The aim of this work was to study *in vivo* the expression of MMP-2, –9 and an inhibitor, Tissue Inhibitor of Metalloproteinases (TIMP)-1, in SCCs among 24 immunosuppressed and 35 nonimmunosuppressed (NIS) patients.

Cutaneous carcinomas were divided into three states following histology: *in situ*, differentiated, and undifferentiated. Modulation of expression was analysed by immunohistochemistry using the immunoperoxidase technique. Expression of MMPs and TIMP-1 were evaluated in tumor cells and surrounding epidermis according to: 0, none; +, weak; ++, moderate; +++, strong.

MMPs and TIMP-1 are weakly expressed in the tumor and surrounding epidermis in 20 of 35 NIS patients (all SCC stages); however, eight of 13 IS patients with an *in situ* SCC, present a moderate to strong expression of MMPs and TIMP-1 in the overlying epidermis. No difference of expression in the tumor itself was observed between IS and NIS. It seems that MMPs and TIMP-1 are highly expressed by immunosuppressed patients suffering from SCC *in situ*.

P46

Comparison of Keratoacanthomas and Squamous Cell Carcinoma, the Point of View of Microsatellites

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Keratoacanthomas (KA) and squamous cell carcinoma (SCC) are two types of tumors difficult to distinguish by clinical and histologic means. The aim of this work was to compare the frequencies of loss of heterozygosity (LOH) in 10 KA and 27 SCC tissues. Eight microsatellites markers were used to study abnormalities within three regions, 17p13.1/9p21/9q22.3, encoding p53/p16/PTCH, respectively. Amplified PCR products were analysed by automatical sequencer.

Among the 27 SCC, 77% tumors displayed LOH in the 17p13.1 region and 70% in the 9q22.3 region. The results confirm the role of p53 and PTCH in the development of SCC. Moreover, 59% of SCC and 60% of KA showed LOH within the 9p21 region. Thus, as it was demonstrated for melanoma, p16 located in this region may play an equally important role in the development of KA and SCC.

Finally, LOH was found in the 17p13.1 and 9q22.3 regions, in 40% and 30% of KA lesions, respectively. Therefore LOH in the 9q22.3 and 17p13.1 regions appears to be significantly less frequent in KA than in SCC (70% *vs* 30%/77% *vs* 40%; $p < 0.05$, chi2 test).

In conclusion, this study implies that in addition to p53 and PTCH also the mutation in p16 locus may be responsible for SCC phenotype, and that SCC and KA may be distinguished by the underlying genetic lesions.

P47

Loss of Heterozygoty (LOH) in Malignant Nerve Sheath Tumors (MNSTS) Associated with Neurofibromatosis 1 (NF1)

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NF1 is associated with a high risk of MNSTs. P53 gene mutations have been identified in MNSTs. Our aim was to study the implication of other genes in the pathogenesis of these tumors.

Patients and methods Six NF1 patients with MNSTs were studied. For each patients, lymphocytes and MNST were disposable. A LOH research was performed at NF1 and INH4 loci studying microsatellites. Seven markers were studied at NF1 locus, four intragenic and three extragenic, both telomeric (D17S250) and centromeric (D17S925 and D17S783). At INK4 locus, three markers recovering a large part of the locus including p14, p15, and p16 genes were studied (D9S1870, D9S974, and D9S975).

Results Four tumors presented with a LOH at NF1 locus, the fifth tumor was not informative. Three tumors presented with a LH at INK4 locus, one was not informative and the last seemed to be not deleted.

Discussion This study highlights the role of tumor-suppressor genes in the malignant tumor transformation and progression in NF1 patients. These results should be confirmed with a genic dosage targeted to NF1, p14, p15, and p16.

P48

Role of the 1Q21 Locus in the Genetics of Psoriasis: Study of Three Microsatellite Sequences

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Psoriasis is a frequent disease involving 2% of the general population. It is characterized by an increase of the keratinocytes turnover associated with abnormalities in epidermal differentiation and inflammation. Data on the genetics of psoriasis give clues to a polygenic transmission. Genomic studies by microsatellites discovered several loci with binding desequilibrium: 20p, 17q, 6p (HLA), 4q, 1q. The 1q21 area, named Epidermal Differentiation Complex, is occupied by many duplicated genes, which code for calcium binding proteins and for neutrophil chemotactic factors. Some of those proteins are overexpressed on psoriasis skin biopsy. Thus, this area is a well-designed candidate, and Novelli demonstrated on a study of 22 Italian family its links with psoriasis.

We studied 88 unrelated patients with psoriasis followed in the dermatology and rheumatology departments of our hospital. We compared the allelic repartition of three microsatellites of the 1q21 area (D1S 514, 498, 305) in our patients and in a control population of 196 patients. DNA was extracted from lymphocytes. Amplification by PCR was performed, and the PCR products were then analyzed by capillary electrophoresis. Statistical comparison (chi2 test) of allelic repartition was unable to demonstrate any difference. In our opinion this area is not involved in psoriasis.

P49

Autoreactive T Cell Response Against a Polymorphous Domain of Desmoglein 1, in Patients with Pemphigus Foliaceus

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Pemphigus foliaceus (PF) is an autoimmune blistering skin disease that is characterized by the production of autoantibodies directed against desmoglein1 (Dsg1). An autoreactive T cell response against Dsg1 has also been demonstrated. We have previously evidenced a genetic polymorphism of the EC4-EC5 domains of Dsg1. In this study, we studied the T lymphocyte proliferative response against wild type and mutated EC4-EC5 domains of Dsg1 in five patients with PF, in order to determine the functional role of these mutations.

We first produced wild type and mutated EC4-EC5 domains of Dsg1 in *E. Coli*. We then studied the proliferative response of peripheral blood mononuclear cells (PBMC) against two recombinant proteins and synthetic overlapping peptides covering these domains, in five PF patients and six healthy individuals.

A proliferative response to synthetic peptides was observed in two wild type patients with an active disease. Interestingly, the proliferative response of PBMC could be measured in one patient at different stages of the disease and was correlated with the disease activity. No proliferative response could be observed in the three mutated patients, but their disease was in remission under treatment at the time of the study. This study confirms the presence of T cell epitopes in the EC4-EC5 domains of Dsg1. Further studies in mutated patients with an active disease are necessary to confirm the functional role of these mutations.

P50

HLA Class II Alleles Influence Anti-BPAG 1 and Anti-BPAG 2 Autoantibody Response in Bullous Pemphigoid in French Caucasians

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Bullous pemphigoid is an autoimmune bullous disorder of the elderly characterized by autoantibody binding to the dermal–epidermal junction. Two major antigens are targeted by autoantibodies BPAG1 and BPAG2. The aim of this study was to determine wether specific immunization against those antigenic determinants was influenced by HLA class II alleles or not. Specific HLA -DRB1, -DQB1, and -DQA1 typing was performed in 49 BP patients and 106 healthy controls by high resolution. BPAG1 and BPAG2 autoantibodies were determined by immunoblotting on epidermal extracts at the time of diagnosis. In BP compared with controls, an increased frequency of HLA-DRB1*11 (21.4% *vs* 11.3%, p = 0,02, pc = NS) and of HLA DQB1*0302 (13.3% *vs* 5.2%, p = 0.01, pc = NS) was observed. Generic DQB1*02 frequency was decreased (10.2% *vs* 25%, pc = 0.02), suggesting a protective effect of this allele in BP. Anti-BPAG and anti-BPAG2 were detected in, respectively, 47.7% and 50% of BP sera. Target antigens recognized by anti-BMz antibodies were dependant on HLA DRB1*1101. In the subgroup of DRB1*1101 positive patients the frequency of anti-BPAG1 response was increased (76.9% *vs* 38.7%, p = 0.03), whereas in the DRB1*1101 negative patients the occurence of anti-BPAG 2 antibodies was decreased (14.3% *vs* 58.1%, p = 0,03).

These results suggest that the presence of HLA DRB1*1101 allele influences the auto antibody response to BPAG1 in BP.

P51

Malignant Langerhans Cell Tumor: Study of Blood Dendritic Cells

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Among abnormal proliferations of Langerhans cells, the single malignant Langerhans cell tumor is different from histiocytosis X and is the most rare (about 10 cases). We have studied blood dendritic cells in one patient.

Some blood samples have been taken in 2 y. Mononuclear cells have been studied by flow cytometry. CD34+ precursors have been isolated and cultured 12 d with granulocyte/monocyte-colony stimulating factor (GM-CSF) and tumor necrosis factor alpha.

At day 0, 1.02–4.35% of mononuclear cells were CD1a+ cells whereas 1 ± 0.05% were CD34+. No abnormality of proliferation of CD34+ cultured cells was noted in 12 d. In this patient, CD34+ or CD1a+ cells were more numerous than in healthy subjects and even than in histiocytosis X (< 1%) but less numerous than in widespread burns (> 20%). Like in histiocytosis X, the high number of CD34+ and CD1a+ cells suggest that Langerhans cell transformation occurs at the blood or bone marrow precursors but not in the skin and does not seem to be a factor of prognosis. Long-term culture of blood dendritic cells of this patient could lead to obtention of a Langerhans cell line.