BIODERMA
CONGRESS **REPORTS**

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INTRODUCTION

Because the dermatology of tomorrow is already being developed in laboratories today, we attended the Annual Congress of Dermatological Research in Brest, France.

At the crossroads of basic sciences and clinical applications, this congress provided us with a real overview of the research topics that are of interest to French-speaking laboratories.

As an eminent forum for exchanging, sharing and learning, the congress not only met our expectations but also gave us all hope for future innovations. Working hand in hand, doctors and scientists debated, reflected and built the future of dermatology, while enjoying *crêpes bretonnes* and salty oysters.

The summaries below will give you a brief, non-exhaustive overview of the topics covered.

The congress began with a review of the skin's primary function, i.e. its barrier function, given by Joachim Fluhr, a Berliner from Brest, during the first plenary session entitled "Epidermal barrier and inflammation". In five points, the essential roles of the skin were addressed.

SKIN BARRIER: INTERFACE AND REGULATOR

Speaker: Prof. Joachim Fluhr (Berlin)

Prof. Joachim Fluhr described the skin barrier function in terms of its composition and structure, but also as a regulatory organ through its interactions with the intestinal and pulmonary microbiota and the nervous system.

He discussed the link between inflammation and the central nervous system (CNS), based on recent articles highlighting the role of impaired skin barrier function in autism spectrum disorders and the reduction in total epidermal ceramides in schizophrenia.

He then talked about the many strategies aimed at restoring the epidermal barrier in hand eczema. He also discussed the superiority of multi-lamellar preparations compared with conventional emulsions in improving epidermal barrier function.

Lastly, he presented work on exposure to a standardised environment enriched with ozone (linked to pollution) and pollen in atopic and non-atopic patients, showing a more pronounced decrease in barrier function in atopic individuals.

CHARACTERISING THE COMPOSITION AND REGULATION OF THE SECRETOME OF SENESCENT KERATINOCYTES

Speaker: Florence Debacq-Chainiaux (Namur)

Cellular senescence is age-related but increases with UV exposure. Senescent cells are morphologically and metabolically different, giving rise to a secretome (the set of proteins secreted by cells) called senescence-associated secretory phenotype (SASP), composed of pro-inflammatory factors. This secretome has a pro-tumour effect and induces cell dysfunction through a paracrine effect which, if better understood, could help to identify new therapeutic targets in skin ageing.

Inès Bouriez et al. (Namur) studied the influence of SASP on adjacent keratinocytes and attempted to understand the pro-tumour mechanism of action.

To do this, they exposed primary keratinocytes to UV rays and observed an increase in galactosidase activity, DNA damage, and suppressed proliferation, which persisted even seven days after the end of exposure to UV rays.

They then studied the impact of the secretome on cancer cells using Boyden chambers (a tool for studying cell migration and invasion), in which keratinocytes were exposed to the secretome of senescent keratinocytes. A pro-migratory effect was observed after UV exposure; this was shown to be of protein origin as it was inhibited by proteinase K.

Two SASP proteins were identified by mass spectrometry: calpain-1 (known for its role in melanoma progression) and the S100A4 protein (known for its pro-tumour effect).

To continue this research project, the research team plans to study the molecular pathways involved in senescence and develop physiological models to study interactions between senescent keratinocytes and the skin.

ROLE OF SUBTILISINS IN THE ADHERENCE OF DERMATOPHYTES TO THE HOST EPIDERMIS

Speaker: Emilie Faway (Namur, Liège, Lausanne, Tokyo)

Dermatophytosis is the most common fungal infection in the world, with an estimated prevalence of 20%, and the emergence of resistant strains is prompting research into the virulence mechanisms/factors of dermatophytes in order to identify potential therapeutic targets.

In the skin, the 25 most over-expressed genes include three subtilisins (SUBs), a type of enzyme with keratinase activity. The over-expression of SUBs 6, 8 and 10 has been confirmed in reconstructed human epidermis, but not in simple keratin-enriched media, which are too far removed from human physiology.

The kinetics of SUB expression were assessed by Emilie Faway and her team (Namur), who found over-expression at early stages.

The next step involved inactivating SUBs in *Trichophyton benhamiae* strains, resulting in a lower level of colony development, confirmed by use on reconstructed human epidermis (RHE) which showed, for the strain deleted for SUB6, a decrease in adherence at early stages, compensated for after two hours, suggesting compensatory mechanisms following the loss of SUBs. Moreover, RHE infected with the SUB6 strain showed greater and earlier barrier function impairment. Subtilisins are therefore virulence factors involved in the adhesion mechanisms of dermatophytes and are paving the way for new therapeutic strategies.

IDENTIFICATION OF A PROTEASE INVOLVED IN FILAGGRIN METABOLISM AND CORNIFICATION: THE PREP PROLYL ENDOPEPTIDASE

Speaker: Julie Briot (Toulouse)

Marie-Claire Méchin *et al.* (Infinity Toulouse) worked to identify the proteases involved in filaggrin proteolysis and the formation of natural moisturising factors.

Following an analysis of omics data, the PREP protease was selected.

It was expressed in the stratum granulosum/stratum corneum, colocalised with filaggrin, and *in vitro* enzymatic activity tests confirmed its ability to cleave a filaggrin-derived peptide. Interestingly, substrate deimination promoted its activity.

In addition, PREP knockdown (using shRNA) in reconstructed epidermis resulted in a decrease in trans-epidermal resistance, as well as a decrease in the expression of other proteases involved in the metabolism of filaggrin (capase-14, bleomycin hydrolase) or epidermal differentiation proteins. PREP could therefore be a potential therapeutic target.

DEVELOPMENT OF AN IN VITRO MODEL OF INNERVATED HUMAN SKIN USING THE MICROFLUIDICS TECHNIQUE

Speaker: Thomas Bessy (Lyon)

In vitro skin models are becoming increasingly complex and are tending to reproduce interactions found *in vivo* with increasing accuracy. Organs-on-chips are providing 3D models recreating the innervation and vascularisation of the skin. In order to reproduce the innervation of the skin, co-cultures of primary keratinocytes and sensory neurons are produced. Each population of cells is separated by microchannels in which only the dendrites of neurons can fit to reach the keratinocytes. This artificially recreates the distance between the neuronal bodies and the keratinocytes. Electrodes can be placed on either side to induce stimulation or take electrophysiological measurements, for example. In addition, since the two populations of cells are separated by a fluid-filled space, the compounds released into the medium on either side of the microchannels can be studied independently.

Furthermore, the skin is also an irrigated system, as well as being innervated. Another model also enables this vascularisation to be recreated.

The aim is to create a tripartite system with irrigated and innervated skin.

FIBROBLAST CHARACTERISATION FOR THE DEVELOPMENT OF AN INNOVATIVE SKIN SUBSTITUTE

Speaker: Lucile Guillot (Lyon)

The treatment of acute (major burns) or chronic (diabetes, vascular wounds, etc.) wounds is a major public health challenge. One of the most effective treatments is autologous skin grafting. However, it is sometimes difficult to implement this technique (lack of donor areas, for example). The aim of this study was to characterise potential skin substitutes that could be used as ready-to-use grafts formed from fibroblasts (referred to here as X fibroblasts). The ability of these fibroblasts to synthesise, organise and regulate the extracellular matrix was studied in comparison with adult and foetal fibroblasts. Various techniques, including immunofluorescence staining and transmission microscopy, have shown that X fibroblasts produce a more abundant and differently organised collagen network, with finer, denser collagen fibrils. These properties could be of interest in wound healing processes.

IN VITRO PIGMENTED SKIN MODELS

Speaker: Nicolas Berthelemy (Lyon)

Hyperpigmentation problems are common in darker phototypes, and they increase with age and inflammatory diseases.

To study hyperpigmentation in acne and during ageing, *in vitro* models combining keratinocytes derived from induced pluripotent stem cells and melanocytes were studied.

The depigmenting effect of a botanical extract applied to the models was evaluated.

For the ageing model, a decrease in melanin content and a dose-dependent decrease in tyrosinase activity were observed in the presence of the botanical extract compared with the control.

To mimic post-inflammatory hyperpigmentation in acne, interleukin-1 (IL-1) was added to the model. In the presence of the botanical extract, a decrease in melanin content and IL-1 release was observed.

An *in vivo* study in humans then showed favourable results with the botanical extract for acne-related hyperpigmentation.

INVOLVEMENT OF SKIN-MICROBIOTA COMMUNICATION IN NEUROGENIC INFLAMMATION

Speaker: Marc Feuilloley (Evreux/Rouen)

The skin is the largest neuroendocrine organ in the human body.

Cutaneous neurogenic inflammation is a complex mechanism based on the local release of neuromediators, including calcitonin gene-related peptide (CGRP), independent of extrinsic factors.

The cutaneous microbiota is exposed to these neurohormones via diffusion in the intercellular space and in sweat, which can influence the capacity for biofilm formation or bacterial adhesion.

Prof Feuilloley et al. presented the various impacts of cutaneous neurohormones on bacteria.

Molecules such as substance P can increase the potential cytotoxicity and virulence of bacteria such as *Bacillus cereus* and *Staphylococcus aureus* and can increase the production of *S. aureus* biofilms. However, the response varies depending on the strain.

The bacterial receptor (gram+ and gram-) for substance P is elongation factor thermo unstable (EfTu), which shows no homology with the substance P receptor in eukaryotes. It is a "moonlight receptor", i.e. it combines two functions: elongation factor and environmental sensor.

Prof Feuilloley also presented his work on the influence of CGRP and natriuretic peptides on bacteria and their respective receptors.

He also presented the effect of catecholamines on *Cutibacterium acnes*, known for its role in acne. Exposure to catecholamines increases the bacterium's effect on lipogenesis in sebocytes, making *C. acnes* a potential relay between stress mediators and acne.

Cutaneous bacterial endocrinology is a factor that should be taken into account in the skin cell dialogue.

CAN A PERSONALISED APPROACH BE TAKEN TO THE TREATMENT OF ECZEMA?

Speaker: Audrey Nosbaum (Lyon)

Eczema is a heterogeneous disease in terms of age of onset, distribution of the lesions, and co-morbidities. A distinction is made between atopic dermatitis, allergic eczema, and irritant eczema. These multiple phenotypes need to be identified as accurately as possible to be able to offer the right treatment, at the right time, for the right patient. One possible approach would be to analyse the molecular profile (endotype) of patients in order to determine their inflammatory profile. To achieve this, nothing could be better than looking directly at the skin. After a simple routine biopsy, we can analyse the inflammatory genes expressed (using the RT-qPCR method), the pJAK/pSTAT signalling pathway (using immunohistochemistry), and also the histological profiles of the samples. These techniques will enable atopic dermatitis to be distinguished from allergic contact eczema or irritant eczema when the clinical picture is misleading.

We will have to wait a bit longer before we see this method used in routine practice. These techniques first need to be optimised and their favourable economic impact validated.

ATOPIC DERMATITIS: A MODEL DISEASE FOR STUDYING THE THERAPEUTIC POTENTIAL OF MULTI-LAMELLAR LIPOSOMES

Speaker: Antoine Bernasqué (Bordeaux)

As we have already seen, lipids play a central role in the skin's barrier function. A key theme in dermatological research is the formulation of compounds capable of restoring this lipid layer when it is damaged.

The speaker presented the effectiveness of multi-lamellar liposomes as vectors for active ingredients in the skin. Liposomes are small spheres with a wall made up of lipids. With multi-lamellar liposomes, several layers are superimposed. These compounds can transport a large number of hydrophobic and hydrophilic active ingredients. By varying several physical and chemical factors, such as water content, we can control the size and elasticity of the liposome, which will have an effect on the target of these liposomes. The smaller and more rigid the liposome, the deeper it will go into the skin, whereas a more elastic liposome will tend to stay on the surface.

Using a skin reconstruction model combining dermis and epidermis and recreating the characteristics of AD skin, multi-lamellar liposomes were used as corticosteroid vectors. Using this technique, it has been shown that corticosteroids are restricted to the stratum corneum, thus limiting systemic uptake.

In addition, the epidermal atrophy that can be observed with the conventional use of topical corticosteroids is reduced.

Even without corticosteroids, multi-lamellar liposomes play a beneficial role in barrier function and are therefore relevant candidates for the treatment of a large number of skin diseases.

CHARACTERISATION OF THE TRANSCRIPTOMIC PROFILE OF PSORIATIC SKIN IN RESPONSE TO AN ANTI-TNF A THERAPY

Speaker: Ewa Hainaut (Poitiers)

It had previously been shown that an inflammatory skin signature persisted in patients with severe cutaneous psoriasis who were non-responders (NR) to adalimumab (ADA) treatment. The aims of the study were firstly to analyse the modification of the cytokine network and the transcriptomic signature at M4 of treatment in psoriasis patients treated with anti-TNF α therapy, and secondly to identify predictive markers of response to treatment.

It was a multi-centre study carried out in mainland France.

Of the 65 patients included, 49 responders (R) were identified. The initial characteristics of responders compared with non-responders showed that patients in the responder group were significantly taller, but unlike in other, larger-scale studies, male gender did not appear to be significant.

At M4, IL-23p19, IL-22 and IL-36 mRNA expression was significantly different between R and NR patients.

Serum ADA levels were similar in all patients and no anti-ADA antibodies were detected.

A univariate analysis found several skin markers predictive of response to treatment: CXCL10, IL-12p40, and IL-22. It should be noted that the skin markers were not detected in serum. These response markers could therefore be of interest in clinical practice, subject to confirmation of the results in a larger population.

DERMAL FIBROBLASTS ARE SKIN CELLS HIGHLY SENSITIVE TO IL-1 RELEASED BY KERATINOCYTES DURING STERILE INFLAMMATION INDUCED BY EPIDERMAL INJURY

Speaker: Sevda Cordier-Dirikoc (Poitiers, Orléans)

This study aimed to understand the effects of IL-1 (a pro-inflammatory mediator) released by keratinocytes during “sterile inflammation” (not induced by infectious agents) on the various cells that make up the skin (melanocytes, keratinocytes, fibroblasts, and endothelial cells).

Keratinocytes are major reservoirs of IL-1, which is released following a breach of the skin barrier and, among other things, leads to the recruitment of numerous immune cells. In this study, keratinocyte lysates (containing IL-1) were brought into contact with melanocytes, fibroblasts, keratinocytes, and endothelial cells. The expression levels of the IL-1 receptor and the production of pro-inflammatory cytokines in response to IL-1

were measured in these four cell populations. Fibroblasts were found to be the best responders to IL-1, even at very low IL-1 concentrations.

It was then shown that fibroblasts activated in this way played a role in the recruitment of neutrophils. Lastly, *in vivo*, mice genetically modified to not express IL-1 showed less inflammation and a lower level of neutrophil recruitment.

Fibroblasts therefore appear to be the most important cells in relaying the inflammatory message mediated by IL-1 released from keratinocytes during sterile inflammation.

STUDY OF TRPV1 IN PSORIASIS

Speaker: Emilie Marie-Joseph (Brest, Poitiers, Lyon)

TRPV1 is a cation channel activated when the temperature exceeds 43°C.

It is expressed by neurons and to a lesser extent by keratinocytes and endothelial cells. TRPV1 is involved in chronic inflammatory dermatoses. The work of Emilie Marie-Joseph (Brest) consists in investigating the role of TRPV1 in regulating inflammation in psoriasis.

Firstly, TRPV1 expression was found to be reduced in the lesional skin of patients compared with non-lesional skin and the skin of healthy patients.

Then, exposure of a two-dimensional (2D) culture of keratinocytes to a standardised cocktail of inflammatory cytokines ("M5") led to a reduction in the TRPV1 transcript in keratinocytes exposed to M5 compared with unexposed keratinocytes, confirmed by immunohistochemistry. The same was true for endothelial cells.

The impact of TRPV1 on IL-8 secretion by keratinocytes was then studied by assessing the impact of adding a TRPV1 inhibitor to a 2D culture and "M5".

No change in IL-8 transcription was found, but in the supernatant, TRPV1 inhibition was associated with a decrease in IL-8. The same was true for the endothelial cell response to TRPV1 inhibition.

Conclusion: The TRPV1 transcript in keratinocytes and endothelial cells.

A next step could be to study TRPV1 expression in psoriatic patients with and without pruritus.

DECIPHERING THE CUTANEOUS NEURO-IMMUNE RESPONSE: FROM BASIC SCIENCE TO THE DEVELOPMENT OF NEW THERAPIES

Speaker: Nicolas Gaudenzio (Toulouse)

N. Gaudenzio et al. (INSERM) are specifically studying cutaneous mast cells, which are fixed immune cells located close to blood vessels. The MGPRB2 and MGPRX2 receptors, expressed respectively by murine and human mast cells, have several ligands, both endogenous (including substance P) and exogenous (such as drugs), which are responsible for triggering pseudo-allergic reactions.

The team has identified the neuro-immune sensory units, consisting of a TRPV1+ neuron in contact with mast cells, which play a fundamental role in the development of a Th2-type immune response upon contact with an allergen.

One of the projects carried out by the laboratory was the mapping of mast cells in each mouse organ. It was shown that these resident cutaneous mast cells originate from "primitive" haematopoiesis and are all found in contact with neurons. They are divided into seven sub-types.

Another of the laboratory's projects involved studying atopic dermatitis in infants (affecting 1 in 5 infants), which often resolves within a few months. The biological hypothesis was that this form of AD is pre-conditioned by an event occurring *in utero*. Using healthy and MRGPRX2-deficient mouse models, it was shown that this phenotype could be caused by the *in utero* degranulation of cutaneous mast cells.

N. Gaudenzio then presented a final study on the prediction of local cutaneous injection site reactions with cetrotide (an LH-RH antagonist used in infertility and known to induce local injection site reactions) using human skin explants. The transcriptomic profile of the explants following cetrotide injection showed a Th2 profile, with activation of pathways linked to endothelial cells and neurons, suggesting that neuro-immune sensory units may play a role in triggering these reactions.

APPEARANCES ARE OFTEN DECEIVING... THE EXAMPLE OF CLINICALLY HEALTHY SKIN IN VITILIGO PATIENTS

Speaker: Laure Migayron (Bordeaux, Brive-la Gaillarde, Rennes, Paris)

In vitiligo, it is known that the peri-lesional skin is made up of an infiltrate of tissue-resident memory T cells (Trm cells).

The pathophysiology of vitiligo is linked to the secretion of Th1-type cytokines and chemokines leading to the recruitment of immune cells which secrete metalloproteinases and cleave E-cadherin, causing melanocytes to adhere to the basal membrane.

But are Trm cells also found in non-lesional skin?

An analysis of TCR clones present in lesional skin (LS) and non-lesional skin (NLS) showed similar distributions in the two areas.

Single-cell RNA-seq transcriptomic analysis of the dermal and epidermal infiltrate showed over-representation of T-cells, with similar profiles in LS and NLS.

Analysis of CD8+ clusters did not find any difference in cytokine expression, and Trm cells responded similarly after activation in lesional and non-lesional skin.

Future research will aim to understand the factors that explain the absence of depigmentation if the same populations, transcriptomic profiles and activation capacities are found in LS and NLS. The over-expression of PD1 in NLS is one avenue to be explored.

From a practical point of view, it is worth considering the benefits of proposing a local treatment rather than a systemic treatment for vitiligo.

MODULATION OF NK CELL ACTIVITY BY CALCIUM IONS RELEASED BY AN ALGINATE DRESSING: CONTROL OF INFECTION AND WOUND DEBRIDEMENT

Speaker: Yara Adib (Paris)

Alginate dressings are the devices of choice in the treatment of chronic wounds, especially exudative wounds. Thanks to a transfer of calcium ions from the dressing and sodium ions from the wound, a gel can be formed.

Moreover, it is known that NK cells are immune cells that are rapidly mobilised during the healing process, enabling lysis of infected cells and good biological wound debridement.

The aim of this study was to investigate the effect of alginate dressings on NK cells. Two types of dressings were studied – a pure alginate dressing (Algosteril) and an alginate dressing with carboxymethyl cellulose (CMC) fibres (Biatin Alginate) – in comparison with a control medium. The Algosteril dressing significantly increased NK cell cytotoxicity associated with increased secretion of inflammatory molecules compared to the control and the Biatin Alginate dressing, which had no effect on cell cytotoxicity. This effect was induced by the entry of intracellular calcium into the NK cells and was lost when the calcium ions were neutralised.

It was therefore via calcium exchanges that the pure alginate dressing activated NK cells.

PRODUCTION OF FUNCTIONAL PLASMACYTOID DENDRITIC CAR-T CELLS FROM PATIENTS FOR THE TREATMENT OF INFLAMMATORY DISEASES

Speaker: Blandine Cael (Besançon)

CAR-T cells constitute a new therapeutic option for the treatment of certain blood disorders and autoimmune diseases. The technique is based on the ability to generate T cells from the patient's own immune cells capable of recognising certain patterns on the surface of the cells to be eliminated.

The aim of this study was to generate CAR-T cells that recognise plasmacytoid dendritic cells, which are known to be involved in auto-inflammatory and/or autoimmune diseases (such as lupus and scleroderma). Using blood mononuclear cells from 23 patients with scleroderma, psoriasis, Verneuil's disease or dermatomyositis, it was possible to produce CAR-T cells capable of lysing the plasmacytoid dendritic cells of these patients *in vitro*. This capacity was also observed in an *in vivo* humanised mouse model. This technique therefore appears promising for the treatment of diseases in which plasmacytoid cells play a harmful role.

MELANOMA IMMUNOTHERAPY BY ADOPTIVE TRANSFER OF T CELLS WITH OPTIMISED FUNCTIONS

Speaker: Nathalie Labarriere (Nantes)

Melanoma is the most aggressive type of cancer and has the highest mutational load, promoting the emergence of neoepitopes recognised as "non-self" by the immune system, making immunotherapy all the more effective. However, almost 50% of patients do not respond to immunotherapy, which is why alternative treatments are needed.

Adoptive transfer of T cells (TILs) with optimised functions can be carried out using effector T cells from the tumour or the patient's blood (phase 3 study published in NEJM at the end of 2022).

The responder patients in the first studies were those whose TILs had specificity for melan-A and MELOE.

MELOE, in particular MELOE-1, is highly specific to melanoma because it is produced by a lncRNA that is only translated in melanoma and not in healthy melanocytes.

Lymphocyte populations specific to MELOE-1 were sorted and amplified; their high immunogenicity was confirmed and persisted for up to seven days post-injection.

However, PD-1 and TIGIT were found to be activated, paradoxically inhibiting lymphocyte activation. TILs invalidated for immune checkpoints (TIGIT and PD-1) were produced. TIGIT^{KO} showed high anti-tumour reactivity, maintenance of proliferation capacity because they had no effect on the cell cycle, and inhibition of PD-1 expression.

In conclusion, to optimise TILs, it is necessary to address the immunosuppressive tumour microenvironment, the mechanisms of tumour escape, and the maintenance of the transferred T cells' capacity over time.

ANTI-TUMOUR CYTOTOXIC CD4 TH1 CELLS ARE INVOLVED IN THE LONG-TERM RESPONSE TO PD-1 INHIBITORS IN CD4 TH1 MELANOMA PATIENTS

Speaker: Jessica Mathiot (Besançon)

In metastatic melanoma treated with PD-1 checkpoint inhibitors, the tumour response observed in 50% of patients is associated with a high mutational load, an interferon-gamma signature, and a high number of tumour-infiltrating lymphocytes (TILs). The presence of an anti-*hTERT* CD4 Th1 response prior to immunotherapy is associated with a better response, and is mainly observed in patients diagnosed at an early stage with a Breslow thickness <1 mm.

The aim of this study was to characterise the anti-tumour Th1 phenotype of responder patients.

With the interferon-γ ELISPOT assay, 64% of long responders had an anti-tumour response against *hTERT* that was often multi-specific (two or three antigens).

Among these cells, there was a predominance of effector memory CD4 cells and overexpression of checkpoint proteins.

It was also shown that the Th1 anti-tumour response was inversely correlated with the level of *TIE2 MDSCs* (myeloid-derived suppressor cells expressing the TIE2 receptor).

The next steps following this study will be to characterise the intra-tumour response, to be correlated with the circulating response, and characterise the Th1 response after re-challenge with PD-1 inhibitors in initial responders presenting a recurrence.

FUNCTIONAL ANALYSIS OF GENOMIC ABNORMALITIES IN NON-UV-INDUCED CUTANEOUS SQUAMOUS CELL CARCINOMA

Speaker: Carmen Al Youssef (Paris)

Cutaneous squamous cell carcinoma (cSCC) is a malignant tumour derived from keratinocytes; most cases are UV-induced. A fraction of these tumours occur in non-photoexposed areas with chronic inflammation (ulcers, burns, etc.).

The aim of this study was to understand genetic abnormalities in non-UV-induced cSCC.

To do so, NGS sequencing of 500 genes involved in skin tumours was carried out with DNA from formalin-fixed, paraffin-embedded tissue samples.

The same genes were found as in UV-induced tumours, but they were mutated at lower frequencies: 26% versus 60% for UV-induced cSCC.

A new gene family, *KMT2B*, was mutated in 35% of samples, at a significantly higher frequency than for UV-induced carcinoma. Seventy percent of mutations had a negative effect on the protein, which plays a role in regulating cell cycle transcription and development. Inhibition of *KMT2B* by shRNA showed an increase in migration, with no effect on proliferation, suggesting a tumour suppressor gene function.

TSLP CYTOKINES PRODUCED BY KERATINOCYTES INDUCE TUMOUR GROWTH AND METASTASIS BY REGULATING THE TUMOUR MICROENVIRONMENT

Speaker: Mei LI (Strasbourg)

The TSLP receptor (TSLPR) is a heterodimer expressed by keratinocytes.

Expression of TSLP (thymic stromal lymphopoietin, an IL-7-related cytokine), induced by skin barrier impairment, acts on dendritic cells via TSLPR, which stimulates T cells expressing GATA3. TSLPR stimulates Th2 and Treg cells, inhibiting CD8 and the interferon-γ response.

The result is a Th2 and Tfh response that plays a major role in allergen sensitisation.

The aim of this study was to determine the role of TSLP in the formation of the melanoma microenvironment.

TSLPR is induced in melanoma. In TSLP knockout mice, tumour growth is slowed down, whereas growth is accelerated when TSLP is overexpressed. Treg cells are thought to play a major role in these pro- and anti-tumour mechanisms.

TSLP stimulates the expression of markers involved in inhibiting CD8+ T cells and producing interferon-γ in Treg cells expressing GATA3.

TSLP inhibits the proliferation of cytotoxic CD8 T cells and the production of interferon-gamma.

In conclusion, dendritic cells expressing TSLPR appear to be involved in inhibiting the anti-tumour immune response.

XERODERMA PIGMENTOSUM: FROM MODELLING THE CONDITION USING CRISPR-CAS9 TO UNDERSTANDING THE PATHOPHYSIOLOGY OF SKIN CANCER

Speaker: Ali Nasrallah (Grenoble)

Xeroderma pigmentosum (XP) is a genodermatosis in which mutations occur in the *XPC* gene involved in DNA repair, resulting in early UV-induced carcinoma and melanoma. No treatments are available to date, and there are no human models.

The aim of this study was to produce an XP model from human skin cells.

To this end, the CRISPR-Cas9 tool directed against the *XPC* gene was introduced as a ribonucleoprotein complex into a human cell line, and cells with an *XPC* knockout (XPC^{KO}) were isolated and cloned.

In a two-dimensional culture, 24 hours after irradiation, mutations were accumulated in the XPC^{KO} cells and viability was reduced, with a time- and dose-dependent effect.

Overexpression of JAK/STAT proteins was observed in all KO cells, especially in irradiated cells.

In a three-dimensional culture on irradiated fibroblasts, SPRR and involucrin proteins were overexpressed. Morphologically, reconstructed epidermis derived from KO cells showed abnormal differentiation compared with healthy epidermis, and this difference was more pronounced in irradiated cells.

In conclusion, JAK/STAT deregulation could constitute a therapeutic strategy for XP patients.

MELANOMA: RESISTANCE AND THERAPY

Speaker: Marie-Dominique Galibert (Rennes)

The prognosis for melanoma has changed radically in recent decades with the development of targeted therapies (MEK and BRAF inhibitors) and checkpoint inhibitors. However, tumour cells have the formidable ability to mutate at high speeds, making them resistant to our therapies. It is therefore essential to clarify the mechanisms of genetic mutation leading to resistance to treatment. This study showed that activation of a transcription factor called AHR (involved in epidermal differentiation and cell detoxification) induces a resistance programme in melanoma cells that were initially susceptible to treatment.

Conversely, inactivation of AHR in resistant cells leads to loss of expression of resistance genes.

In a mouse model, the combination of a BRAF inhibitor and AHR reduced tumour proliferation and increased the lifespan of the mice. The underlying mechanisms that explain how AHR activation leads to the formation of resistant cells are not yet fully understood. AHR directly or indirectly regulates around 50 genes. But it has been shown that cells activated by AHR acquire an increased invasion capacity, leading to the emergence of resistant phenotypes.

This is a major discovery in that the use of an AHR inhibitor could limit therapeutic escape.

PATHOPHYSIOLOGY OF KELOID SCARS: IDENTIFICATION OF INTER- AND INTRA-KELOID HETEROGENEITY BY ANALYSING THE TRANSCRIPTOMIC AND FUNCTIONAL PROFILES OF KELOID FIBROBLASTS

Speaker: Kevin Serro (Paris)

Keloid scars are the result of an abnormal healing process leading to the formation of unsightly hypertrophic scars that can sometimes be painful. Keloid scars vary greatly in terms of morphology and topography, making them complicated to treat. There are two different profiles: nodular keloid scars and extensive keloid scars.

Considering that fibroblasts play a major role in keloid scar formation, the aim here was to characterise the different profiles of these fibroblasts between the different types of keloid scars and also within the same keloid scar.

Initially, it was shown that fibroblasts found in keloid scars expressed more genes involved in the cytoskeleton and cell adhesion than fibroblasts from normal skin. Within a nodular keloid scar, fibroblasts located in the centre of the scar had a greater capacity for contraction and produced more extracellular matrix. Fibroblasts in the periphery had reduced migratory capacity. In addition, fibroblasts in the papillary dermis had a greater capacity for proliferation than reticular fibroblasts, which produced more growth factors. These factors explain the nodular structure of certain keloid scars.

For extensive scars, the proliferation and contraction capacities of the fibroblasts in the centre of the scar were reduced. Conversely, fibroblasts in the periphery produced more growth factors and had a greater capacity for migration and contraction, especially when the fibroblasts were located in the papillary dermis. Fibroblasts in the reticular dermis were more involved in the production of the extracellular matrix.

Two different models have therefore been identified to explain the expansion of nodular and extensive keloid scars, enabling more targeted treatments to be proposed.

ROLE OF THE PCPE-2 PROTEIN IN SKIN HOMOEOSTASIS AND WOUND HEALING

Speaker: Manon Napoli (Lyon)

It is crucial to clarify the enzymatic pathways involved in skin homoeostasis and wound healing. This study therefore focused on a specific protease called PCPE-2 (procollagen C-proteinase enhancer) belonging to the large BTP (BMP-1/tolloid-like) family, which plays a role in collagen fibre assembly, angiogenesis, and lipid metabolism.

Unlike its cousin PCPE-1, PCPE-2 does not induce collagen cleavage, enabling collagen fibres to form. Experiments showed that PCPE-2 was produced by keratinocytes and was deposited in the extracellular matrix. PCPE-2-deficient female mice showed a reduction in vascular density and had smaller adipocytes; this was only observed in females.

During the healing process, the deficient mice showed a delay in wound closure and granulation tissue formation. Therefore, PCPE-2 appears to influence neoangiogenesis in a sex-dependent manner, suggesting the potential role of sex hormones such as oestrogen.

A COLD ATMOSPHERIC PLASMA-ACTIVATED MEDIUM PROMOTES WOUND HEALING BY STIMULATING THE MIGRATION OF HUMAN KERATINOCYTES

Speaker: Aurélie Marches (Toulouse)

Cold plasma is a special physical state; it is a partially ionised gas capable of interacting with liquid media and producing reactive oxygen species. And yet reactive oxygen species are known to play a role in the healing process.

The aim here was to study the effect of applying a liquid medium activated by helium plasma to cultured keratinocytes. The medium was activated by the plasma for a period ranging from 10 to 120 seconds. Keratinocytes treated with a medium activated by cold plasma for more than 60 seconds proved to be cytotoxic and had a reduced migratory capacity with an increase in oxidative stress.

On the other hand, for a medium treated with cold plasma for less than 60 seconds, there was no cytotoxicity or oxidative stress. There was also no increased proliferation or differentiation. However, the migratory capacity of the keratinocytes was improved and confirmed by accelerated re-epithelialisation in 3D skin models.

Therefore, a medium activated by cold plasma for less than 60 seconds can accelerate the re-epidermalisation of a wound (without inducing cytotoxicity or oxidative stress, as is the case if the medium is treated for more than 60 seconds).

ROLE OF SEROTONIN SIGNALLING IN SKIN HOMOEOSTASIS AND WOUND HEALING

Speaker: Stéphanie Matar (Paris)

Serotonin is a neurotransmitter that may play a role in skin homoeostasis. Ninety percent of serotonin is stored in platelets, which act during the first phase of wound healing. Sequencing data have shown that certain serotonin receptors are ubiquitously expressed in skin cells.

In addition, serotonin-deficient mice show a longer healing time than wild mice, reinforcing the assumption that serotonin is involved in skin homoeostasis. The idea was therefore to use topical serotonin reuptake inhibitors to promote healing.

The X molecule tested here, i.e. a partial serotonin receptor agonist applied topically to mouse wounds, significantly reduced the size of the wounds by promoting keratinocyte proliferation and stimulating neoangiogenesis. Topical serotonin reuptake inhibitors could therefore be a treatment of choice for chronic wounds.

C-TACTILE FIBRES

Speaker: Emmanuel Bourinet (Montpellier)

The somatosensory neurons involved in pain pathways include low-threshold mechanoreceptors (LTMRs). In these neurons, a CAV3 sodium channel is involved in amplifying the action potential. In mice, CAV3 inhibition results in analgesia.

The expression pattern of CAV3 is mainly in cLTMR sensory neurons, and to a lesser extent in Aδ and Aβ fibres.

Patch-clamp and nerve-skin studies have characterised CAV3 as an amplifier of mechanosensing.

In *in vivo* mouse models with a specific deletion of CAV3 in cLTMR fibres, a change in sensations of cold and the sense of touch was identified, with no alteration in sensations of painful heat.

A partnership between Montpellier University Hospital and Brest led to the creation of a programme to collect dorsal root ganglia from the spinal cords of deceased human donors. The electrophysiological characteristics and expression pattern of CAV3 were the same in humans and mice.

In mouse models with a CAV3 deletion, there was a decrease in social behaviour, and when CAV3 was overexpressed, an increase in social behaviour and a decrease in anti-social behaviour were observed.

Another project focused on autism spectrum disorders (ASDs) and the alteration of sensory pathways.

In humans, the SHANK3 mutation is involved in ASDs. Mice with a SHANK3 deletion in sensory fibres have impaired nociception.

Germline deletion of SHANK3 in mice induces self-harming behaviour and hypersensitivity to chemical pruritus. The nerve-skin technique showed that only cLTMRs are altered in SHANK3-deficient mice.

STUDY ON THE ROLE OF MERKEL CELLS IN THE INITIATION OF MECHANICAL PRURITUS

Speaker: Adeline Bataille (Brest)

Mechanically induced pruritus is clinically equivalent to alloknesis.

The detection of light touch in hairy and hairless skin is made possible by Merkel complexes (containing keratinocytes, Merkel cells, and Aβ, Aδ and C fibres).

As Merkel cells (MCs) are involved in psoriasis and prurigo, this study aimed to understand the role of MCs in the initiation of mechanical pruritus.

Immunohistochemistry on human and rat skin identified synaptic contacts between MCs and Aδ and C fibres. MCs may stimulate activating interneurons and cause mechanically induced pruritus.

Contact functionality was demonstrated using a co-culture model of rat nerve cells and MCs, and immunohistochemistry revealed synaptic vesicles opposite the point of contact between the fibre and the MC.

As a result of this work, which found synaptic contacts between MCs and Aδ and C fibres for the first time, it is assumed that the MC communicates with the synapse to initiate mechanically induced pruritus at the inhibitory interneurons.

ROLE OF TRANSIENT RECEPTOR POTENTIAL CATION CHANNEL SUBFAMILY V MEMBER 1 (TRPV1) IN LACTIC ACID-INDUCED SUBSTANCE P RELEASE FROM KERATINOCYTES AND NEURONS IN VITRO

Speaker: Raphael Leschiera (Brest)

Lactic acid is used in clinical practice to help diagnose sensitive skin. In these patients, contact with lactic acid rapidly causes an unpleasant sensation. But what are the mechanisms underlying the effects of lactic acid at cellular level? This study focused on the role of keratinocytes and substance P.

After determining the cell culture conditions required for cell viability in the presence of lactic acid, it was observed that substance P was released after adding lactic acid, even at very low doses.

The effects of lactic acid on substance P and TRPV1 activity were verified with neurons derived from IPS cells.

These results suggest that TRPV1 is involved in the release of neuropeptides when the environment is acidified.

MOUSE CHEEK TESTING TO STUDY SENSORY DISTURBANCES IN CIGUATERA: EXPECTED AND UNEXPECTED BEHAVIOURS

Speaker: Raphaële Le Garrec (Brest, Davis)

Ciguatera is a foodborne illness endemic in tropical countries, and sensory disturbances are the most frequent symptom. The pathophysiology is poorly understood, and the predominance of certain sensory disturbances suggests that ciguatoxins (CTX) may be involved.

To distinguish between pain responses and pruritus induced by CTX, mouse cheek testing was carried out.

Localised pruritus was triggered at doses as low as 0.1 nM, whereas pain induced by CTX was dose-dependent and triggered from 10 nM. At higher doses (> 1 nM), diffuse pruritus and central neurological signs (ataxia) were also observed in mast cell-deficient mice, ruling out anaphylaxis.

NEUROPROTECTION OF THE EPIDERMIS DURING INFLAMMATORY STRESS

Speaker: Numa Deydier (Brest)

It is now known that neurons play a major role in dermatological diseases, especially pruritus, but not exclusively. It has been shown *in vitro* that keratinocytes demonstrate improved differentiation with an increase in filaggrin and loricrin upon co-culture with neurons. This study showed that keratinocytes activated by an inflammatory cocktail mimicking the inflammation found in atopic dermatitis had reduced inflammatory stress when cultured in the presence of neurons. In the absence of neurons, keratinocytes on their own activated by this inflammatory cocktail showed a reduced level of filaggrin expression. The addition of neurons partially restored the level of filaggrin expression.

The neuroprotective mechanisms at play here still need to be clarified. It seems that brain-derived neurotrophic factor (BDNF) may play a role.

HYPERION AND SKIN APPLICATIONS

Speaker: Nadège Marec and Patrice Hémon (Brest)

The Hyperion (HYPE Research in Immunology and ONcology) platform was created in 2019 with the acquisition of an imaging mass cytometer. Conventional flow cytometry has several limitations, including overlapping spectra, which limits the simultaneous analysis of multiple markers. The development of imaging mass cytometry has overcome this problem, enabling 40 markers to be analysed simultaneously.

Technically, antibodies are coupled to metallic isotopes, and after the slides have been prepared and stained, a plasma torch ionises the metallic isotopes and the time of flight of each isotope is measured. The data are analysed to represent the intensity and spatial distribution of the various isotopes.

This technology has been used to identify prognostic biomarkers and study the tumour microenvironment in recurring cutaneous squamous cell carcinoma.

DEVELOPMENT OF A TESTING AND DIAGNOSTIC PLATFORM DEDICATED TO RARE GENETIC VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS) IDENTIFIED IN PATIENTS WITH CONGENITAL ICHTHYOSIS

Speaker: Nuria Pell Vidal (Toulouse)

Congenital ichthyoses are rare monogenic disorders, more than 50% of which are caused by pathogenic mutations that deregulate ceramide metabolism. In 5 to 10% of patients, variants that have been little or never described, known as "variants of uncertain significance (VUS)", are found in genes involved in congenital ichthyoses, making it impossible to make a formal diagnosis and include them in clinical studies. The aim of this study was to develop a methodology for classifying VUS as pathogenic or benign.

Two approaches were used. The first was a direct functional analysis, which involved transducing keratinocyte lines with a lentiviral vector containing the mutation and analysing the functionality of the enzyme after 48 hours.

An indirect functional analysis was then performed; it involved transducing immortalised keratinocyte lines (N/TERT keratinocytes) previously inactivated for a key ceramide metabolism gene (*PNPLA1*) with a lentiviral vector containing the mutation.

The cells were then multiplied and cultured in reconstructed epidermis and analysed.

These two techniques enabled three VUS to be reclassified as pathogenic.

MECHANICAL SIGNATURE OF EPIDERMAL STEM CELL NICHES BY ATOMIC FORCE MICROSCOPY

Speaker: Sarah Miny (Lyon)

The skin is renewed by epidermal stem cells that are organised into niches, creating a specific environment that is conducive to maintaining the stem cell pool. These niches can be located above the dermal papillae or above the epidermal ridges.

This study sought to further characterise these stem cell niches by using atomic force microscopy, which studies the mechanical forces to which a medium is subjected. The results showed that stem cells in niches in the dermal papillae were more rigid than other basal cells in the epidermal ridges.

Comparisons between young skin and the skin of patients over the age of 60 showed that ageing was accompanied by a decrease in the height of the dermal papillae and that the difference in rigidity observed in young skin between the stem cell niches of the dermal papillae and the other basal cells tended to diminish.

Homogenisation of tissue rigidity is therefore observed with age, with an overall increase in the rigidity of all basal cells. This technique is therefore very useful for distinguishing between the various sub-populations of cells in the basal layer of the epidermis.

INVESTIGATION OF CUTANEOUS MITOCHONDRIAL METABOLISM AT CELLULAR AND TISSUE LEVEL

Speaker: Justine Dugrain (Lyon)

Cutaneous homoeostasis depends in part on skin vascularisation. The aim of this study was to understand the metabolic function and in particular the parameters of mitochondrial respiration in endothelial cells. Two types of endothelial cells were analysed: microvascular (HDMEC) and macrovascular (HUVEC) cells.

A technique for measuring oxygen consumption (called Seahorse) was used to establish the respiratory profile of the cells. This technique showed that macrovascular cells had a greater capacity to respond to stress via glycolytic metabolism. They also had a greater capacity for a metabolic switch between mitochondrial respiration and glycolysis.

The development of this technique has also made it possible to characterise the metabolism of cells in pathological conditions, as in the case of type 2 diabetes, where a reduction in the mitochondrial metabolism of endothelial cells has been demonstrated, contributing to the delayed healing observed in these patients.

RECONSTRUCTION OF HUMAN EPIDERMIS IN AN ANIMAL-FREE MEDIUM

Speaker: Julia Bajsert (Namur)

The skin cannot be studied without reproducible and robust *in vitro* epithelial models. The development of keratinocyte culture media dates back to the 1970s. Keratinocytes are isolated from children's foreskins or abdominoplasty samples and immortal keratinocytes can be created. Initially, keratinocytes were cultured on a nourishing bed of fibroblasts to which serum enriched with growth factors was added. Some of these compounds are derived from animals, whose availability can vary.

A culture technique was presented here that does not use animal-derived compounds. One of the reasons why this technique was developed was the COVID-19 pandemic, which had led to numerous supply shortages. Thanks to open-source data and the cooperation of numerous researchers, it was possible to create reconstructed epidermis independent of any animal product.

But the adventure is not over, as the keratinocytes cultured in this way show an unexpected inflammatory profile. It will therefore be necessary to continue working to find the best "recipe" for a medium for culturing keratinocytes as close as possible to *in vivo* conditions, while maintaining the open-source approach that enabled this technique to be developed.

CONCLUSION

It was with this magnificent example of knowledge-sharing that this superb congress came to an end.

The cable car took us one last time over the Penfeld river, leaving the Capucins workshop behind us. We are already looking forward to the next edition of the congress, which will take place in Lyon on 27 and 28 June 2024 – the video presentation promises an event packed with new discoveries!