EPITHELIAL CELLS: CONDUCTORS OF THE INFLAMMATORY ORCHESTRA

One Day GREMI Meeting

MARCH 14, 2025 | PASTEUR INSTITUTE PARIS | DUCLAUX AMPHITHEATER

Yasmine Belkaid (President of the Institut Pasteur)
Plenary Lecture "Immune control of lactation"

SESSION : Epithelial cell sensing and interaction with the inflammatory and infectious environment

Mélanie Hamon
Institut Pasteur, Paris, France

Bacterial targeting of host chromatin, a lasting impression

SESSION : Transdisciplinary and new approaches for epithelial cell studies

Jean-François Berret
Université Paris Cité, France

Organ-on-a-chip models for lung functions: Applications in therapy and environmental toxicology

SESSION : Epithelial cells as cornerstones of inflammatory diseases

Yves Poumay
University of Namur, Belgium

Epidermal keratinocytes as actors and sensors of inflammatory conditions

SESSION : Diversity and unconventional functions of epithelial cells in inflammatory context

Malin Johansson
University of Gothenburg, Sweden

Epithelial protection by different goblet cells

Posters and 10 oral communications will be selected from the submitted abstracts

- Abstract submission dead line and registration : february 24, 2025 **gremi.asso.fr**
- Registration fees 150€,
  Reduced price (post-docs, technicians) 70€
- Reduced price (students) 50€
  The price include coffee breaks and lunch

Organizing Committee

Jean-Claude Lecron, Isabelle Couillin, Céline Deraison, Jessica Quintin, Nathalie Vergnolle, Jean-Paul Motta, Vincent Lagente, Xavier Norel, Mustapha Si-Tahar

GREMI
Groupe de Recherche et d'Etude des Médiateurs de l'Inflammation

One day GREMI meeting

Epithelial Cells: Conductors of the Inflammatory Orchestra

Mars 14th 2025

Pasteur Institute

Organizing Committee -

Isabelle Couillin, Céline Deraison, Jean-Claude Lecron, Jean Paul Motta, Xavier Norel, Vincent Lagente, Jessica Quintin, Mustapha Si Tahar, Nathalie Vergnolle

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One-Day GREMI Meeting

"Epithelial Cells: Conductors of the Inflammatory Orchestra"

March 14, 2025 - Pasteur Institute Duclaux Lecture Theater

8:30-9:00 Registration of the participants in the Hall of the Duclaux Building

9:05-9:10 Opening and welcome remarks (Xavier Norel)

Session 1: **Epithelial Cell Sensing and Interaction with the Inflammatory and infectious Environment**

(chairpersons: Céline Deraison & Mustapha Si-tahar)

9:10 **Mélanie HAMON, Institut Pasteur, Paris, France**
"Bacterial targeting of host chromatin, a lasting impression"

Selected talks from the submitted abstracts with 3 min for discussion

- 9:40-**David Ribet**, Inserm, Université de Rouen, France

"Gut bacteria modulate intestinal inflammation by targeting epithelial SUMOylation"

- 9:55-**Joan Bestard-Escalas**, University Hospital, Palma, Spain

"The endocannabinoid-derived prostaglandin glycerol esters and prostaglandin ethanolamides modulate intestinal epithelial hallmarks of colitis"

- 10:10- **Adeline Cezard**, Inserm, Université de Tours, France

"A host-derived metabolite with dual antiviral and anti-inflammatory properties protects the lung mucosa against *Influenza* virus infection"

10:25-10:55 Coffee Break, Poster session and Exhibition stand visit

Session 2: **Epithelial cells as cornerstones of inflammatory diseases**

(chairpersons: Isabelle Couillin & Jean-Claude Lecron)

11:00 **Yves POUMAY, University of Namur, Belgium.**
"Epidermal keratinocytes as actors and sensors of inflammatory conditions"

Selected talks from the submitted abstracts with 3 min for discussion

- 11:30- **Emma Fraillon**, CNRS, Université C Bernard, Lyon, France
"Exploring the role of the TRPV3 calcium channel in psoriasis"
- 11:45- **Zhipeng Li**, Inserm, Bichat, Paris
"Impaired treprostinil-induced relaxation in COPD bronchi and effects of metformin and AICAR on bronchial tone"

12:00 - 14:05 Lunch, Poster session and Exhibition stand visit

14:05 **GREMI PRIZE - Michel CHIGNARD**, laureate -**Mélanie DOUTÉ**

Session 3: **Diversity and unconventional functions of epithelial cells in inflammatory context**

(chairpersons: Nathalie Vergnolles & Jean-Paul Motta)

14:20 **Malin JOHANSSON, University of Gothenburg, Sweden**
"Epithelial protection by different goblet cells"

Selected talks from the submitted abstracts with 3 min for discussion

- 14 :50- **Florian Hermans**, Haselt University, Belgium,
"Tooth organoids and single celltranscriptomics position epithelial cell rests of Malassez as periodontal signaling hubs"
- 15 :05- **Hugo Main**, Université de Poitiers, France
"Expression of Heparanase 1 in Psoriatic Skin Inflammation"

15:20-15:55 Coffee Break, Poster session and Exhibition visit

16:00 **Keynote Speaker, Yasmine BELKAID, President of the Institut Pasteur,**
(Chairperson: Jessica Quintin)

“Immune control of lactation”

Session 4: **Transdisciplinary and new approaches for epithelial cell studies**

(chairpersons: Valérie Urbach & Moez Rhimi)

16:30 **Jean-François BERRET, Université Paris Cité, France**
“Organ-on-a-chip models for lung functions: Applications in therapy and environmental toxicology”

Selected talks from the submitted abstracts with 3 min for discussion

- 17:00- **Lisa Morichon**, CNRS, Université de Montpellier, France

“Healthy and COPD iPSC derived lung organoids response to SARS-CoV-2 infection”

- 17:15- **Khadija Oukacha**, Institut Pasteur, Paris, France

“Impact of microbial metabolites on intestinal barrier function during inflammation using gut-on-chip models”

- 17:30- **Sarahdja Cornelie**, CNRS, Université de Montpellier, France

“A Ca2+ antagonist limits inflammatory damage by reducing epithelial calcium activity in a zebrafish model of cystic fibrosis”

17:45 Closing remarks (Xavier Norel)

GREMI PRIZE – Michel Chignard

Laureate Mélodie Douté

Exploring the interplay between chronic thrombo-inflammation and megakaryopoiesis, in the development of microvascular lesions during vasculitis-associated glomerulonephritis

Thesis highlights presented by the co-director of her thesis, MARC CLEMENT,

Platelets, traditionally known for their role in hemostasis, have recently emerged as key players in inflammation, particularly in fostering microvascular thrombo-inflammation. A critical consequence of chronic thrombo-inflammation is pathological tissue remodeling, or fibrosis, often driven by TGF-ß, a factor enriched in platelets. Microvascular inflammation is a hallmark of autoimmune inflammatory diseases, especially vasculitis-associated glomerulonephritis, which affects the kidneys and leads to organ dysfunction. In these diseases, chronic inflammation is initiated by immune complexes (ICs) that activate complement and coagulation cascades, promoting platelet and leukocyte recruitment. ICs also stimulate leukocytes to release pro-inflammatory cytokines and proteases, which damage vesselsand trigger thrombosis. This interplay of thrombosis and inflammation, consumes platelets and leukocytes. Hematopoietic growth factors (HGFs) regulate hematopoiesis to compensate for this excessive consumption, and prevent thrombopenia and leukopenia. Interestingly, the kidney is a known source of HGFs under normal conditions. I hypothesized that in chronic thrombo-inflammatory injuries, the kidney's sustained release of HGFs could exacerbate inflammation, fibrosis, and organ dysfunction by promoting the overproduction of platelets and leukocytes.

During my thesis, I investigated this hypothesis using an experimental model of antibody-mediated chronic kidney disease (AMCKD), which mimics vasculitis-associated glomerulonephritis. I discovered that chronic thrombo-inflammation in AMCKD induces the kidney to produce HGFs, particularly Thrombopoietin (TPO). This promotes excessive megakaryopoiesis and platelet production. Notably, platelets generated under these conditions are dysfunctional and contribute to glomerular fibrosis via TGF-ß. Importantly, neutralizing TPO in this model prevented excessive platelet production, reduced glomerular fibrosis, and preserved kidney function. These findings highlight a previously unrecognized role of the kidney in driving thrombo-inflammation and fibrosis through HGF production. Targeting TPO could represent a promising therapeutic strategy to mitigate platelet-driven fibrosis and organ dysfunction in autoimmune inflammatory diseases.

Invited Speakers' Abstracts

MELANIE HANNON,
Chromatin and Infection laboratory, Institut Pasteur
Paris – France

Bacterial targeting of host chromatin, a lasting impression

Epithelial cells are the first point of contact for bacteria entering the respiratory tract. *Streptococcus pneumoniae* is an obligate human pathobiont of the nasal mucosa, carried asymptomatically but also the cause of severe pneumonia. The role of the epithelium in maintaining homeostatic interactions or mounting an inflammatory response to invasive *S. pneumoniae* is currently poorly understood. However, studies have shown that chromatin modifications, at the histone level, induced by bacterial pathogens interfere with the host transcriptional program and promote infection. In my presentation, I will present evidence that *S. pneumoniae* induces histone modifications during infection and during asymptomatic colonization. Furthermore, *S. pneumoniae* leaves a lasting histone mark, which remain after bacterial clearance and are transmitted through cell division. In fact, infection establishes a unique epigenetic program affecting the transcriptional response of epithelial cells, rendering them more permissive upon secondary infection. Thus, *S. pneumoniae* infection leaves a memory in epithelial cells after bacterial clearance, which alters cellular responses for subsequent infection.

YVES POUMMAY
University of Namur, Namur Research, Institute for Life Science
Namur - Belgium

Epidermal keratinocytes as actors and sensors of inflammatory conditions

In the epidermis, keratinocytes constantly proliferate and differentiate to establish, maintain, and eventually repair the crucial skin barrier that separates the organism from its environment. It happens in the most superficial cornified layers of the epidermis where cells become keratinized and intercellular spaces are normally closed in order to prevent the entry of foreign substances or micro-organisms, while preventing any loss of body fluid. In addition to the physical barrier, the epidermis represents an immune barrier, hosting innate dendritic antigen-presenting Langerhans cells, but also being able to detect abnormal substances and organisms through the innate immune involvement of keratinocytes. To more deeply investigate properties of keratinocytes, *in vitro* reconstructed human epidermis has become on one hand a valuable tool for cutaneous toxicology, but also on the other hand for analysis and understanding of roles played by keratinocytes in inflammatory conditions, such as those encountered in atopic dermatitis lesions where the epidermal barrier is weakened by Th2-related cytokines (e.g. interleukins (IL) 4 and 13), or still encountered when epidermal fungal infection occurs and concomitantly alters the barrier formed by keratinocytes. In each condition, epidermal keratinocytes detect anomalies resulting from cytokines and growth factors released in their environment. They indeed express a vast number of various receptors including those for IL-4 and IL-13. They also modulate expression of several receptors and can then transmit information and alerts to neighboring epidermal and immune cells by their own release of peptides, growth factors and cytokines. In summary, as a first line of defense, keratinocytes are normally devoted to finally dying silently while they form the most superficial cornified layer of the epidermis. However, living keratinocytes remain highly reactive up to the granular layer in order to quickly perceive tissue intruders and react to initiate the protective tissue response.

MALIN E.V.JOHANSSON
University of Gothenburg, Inst Biomedicine, Dept. Medical Biochemistry and Cell Biology
Gothenburg -Sweden

Epithelial protection of different goblet cells

The intestine experiences several hazards including chemicals, mechanical stress, and microbes. To maintain a good environment at the epithelium mucus is secreted forming a barrier which can filter, modulate diffusion, provide binding sites, serve as a microbial niche, and reduce mechanical force. Mucus is produced by and secreted from goblet cells, which have a specific biosynthesis machinery. Differentiation results in a variety of goblet cells wit different expression profiles. The goblet cells and the mucus they produce vary depending on site and requirement coupled to functions as protection and absorption. Both the mucus and the goblet cells are affected in inflammatory conditions which could be part of the pathogenesis. In conclusion mucus is a very dynamic part of our defence of the intestinal epithelium.

JEAN-FRANÇOIS BERRET
Laboratoire Matière et Systèmes Complexes, Université Paris Cité / CNRS UMR 7057
Paris-France

Organ-on-a-chip model for lung functions - Applications in Therapy and Environmental Toxicology

The transport and fate of inhaled nanoparticles in the lungs, as well as their interactions with pulmonary fluids, remain poorly understood despite their critical implications for drug delivery and toxicology. In this study, we develop microfluidic models that replicate alveolar expansion and contraction during respiration, as well as mucociliary clearance, to evaluate the penetration and distribution of inhaled particles under physiologically relevant conditions. In particular, we focus on the role of pulmonary fluids—mucus and pulmonary surfactant—by investigating their structure and physicochemical properties. To model native pulmonary mucus, we have developed a bioinspired mucus surrogate based on crosslinked snail slime, which exhibits rheological and structural similarities to human lung mucus.This system should allow us to assess how mucus properties influence the transport of inhaled corticosteroid nanoparticles, commonly used in the treatment of inflammatory lung diseases. By integrating alveolar biomechanics and mucociliary clearance dynamics, our approach provides a powerful platform for studying drug-mucus interactions and nanoparticle behavior in the lungs. These models offer a promising tool for screening new aerosol formulations, ultimately improving drug delivery strategies for respiratory diseases

Selected abstracts for oral communication

DAVID RIBET
U1073, Université de Rouen
ROUEN- Frnace

Gut bacteria modulate intestinal inflammation by targeting epithelial SUMOylation

Loison Léa, Ezzine Chaima, Huré Marion, Montbrion Nadine, Lefranc Benjamin, Leprince Jérôme, Bôle-Feysot Christine, Coëffier Moïse, RIBET DAVID
1- Univ Rouen Normandie, INSERM, Normandie Univ, ADEN, UMR 1073 "Nutrition, Inflammation and Microbiota-Gut-Brain axis", F-76000 Rouen, France

SUMOylation is a ubiquitin-like post-translation modification playing essential roles in intestinal physiology. Several pathogens were shown to interfere with host intestinal SUMOylation in order to promote infection. In contrast to pathogenic bacteria, the effect of commensal bacteria from the gut microbiota on intestinal SUMOylation remains poorly characterized. We recently showed that branched chain fatty acids (BCFAs), produced by the gut microbiota, increase protein SUMOylation in intestinal epithelial cells (1). We demonstrated that these metabolites inactivate intestinal deSUMOylases and promote the hyperSUMOylation of specific epithelial proteins. This hyperSUMOylation inhibits the NF- B pathway, decreases pro-inflammatory cytokine expression, and promotes intestinal epithelial integrity. In contrast to BCFA-producing bacteria, we also showed that some bacteria from the gut microbiota dampen SUMOylation. We identified in particular that the bacterium *Staphylococcus warneri,* a natural member of the human gut microbiota, secretes a toxin named Warnericin RK which impairs SUMOylation (2). This toxin induces the degradation of key components of the host SUMOylation enzymatic machinery in both intestinal epithelial and immune cells. We demonstrate that this loss of SUMO-conjugation promotes inflammation and affects the integrity of the intestinal epithelium. Taken together, our results show that bacteria from the gut microbiota use different mechanisms to manipulate host SUMOylation at the level of the intestinal epithelium. They also suggest that a shift in the balance between bacterial effectors promoting or dampening SUMOylation, for example in the context of a dysbiosis, may have a significant impact on gut inflammation.

1. Ezzine C, Loison L, Montbrion N, Bôle-Feysot C, Déchelotte P, Coëffier M, Ribet D. Fatty acids produced by the gut microbiota dampen host inflammatory responses by modulating intestinal SUMOylation. *Gut Microbes* 14(1):2108280. (2022)
2. Loison L, Huré M, Lefranc B, Leprince J, Bôle-Feysot C, Coëffier M, Ribet D. *Staphylococcus warneri* dampens SUMOylation and promotes intestinal inflammation. *Gut Microbes* 17, 2446392. (2025)

JOAN BESTARD-ESCALAS
Functional Genomic Research Group,
Palma - Spain

The endocannabinoid-derived prostaglandin glycerol esters and prostaglandin ethanolamides modulate intestinal epithelial hallmarks of colitis

JOAN BESTARD-ESCALAS, Olivia Sasportes, Hafsa Ameraoui, Mar González-Nicolau, Mireille Alhouayek, Giulio G Muccioli
1- Functional Genomic Research Group Son Espases Universitary Hospital.Module G. Floor -1.Ctra. Valldemossa, 79. 07120 Palma

Inflammatory bowel diseases (IBD) are chronic relapsing-remitting inflammatory disorders of the gastrointestinal tract. IBD has a high impact on patients' quality of life and their prevalence and incidence is increasing. Therapeutic options control the symptoms of IBD but induce significant side effects and lose efficacy over time. The impact of IBD and the lack of effective treatments makes it necessary to explore new therapeutic approaches for IBD. The epithelial cells of the gastrointestinal tract are traditionally considered to function as a selective barrier that separates the lumen from the connective tissue. In fact, the disruption of theepithelial layer is one hallmark of IBD. However, epithelial cells play also an active role during inflammation. Indeed, these cells participate in the coordination with the immune system through the production of cytokines and the presentation of antigens to immune cells. In addition, the recovery of the lost epithelial cells during the inflammatory insult is essential to return to homeostasis. 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamine (AEA) are bioactive lipids capable of modulating inflammation. When metabolized by cyclooxygenase (COX)-2, 2-AG and AEA are transformed into glycerol ester (PG-G) and ethanolamide (PG-EA) derivatives of the prostaglandins (PG). PG-G and PG-EA are understudied besides their significant anti-inflammatory properties. Indeed, PGD2-G exerts anti-inflammatory effects in the context of colitis in a PGD2-independent way, as we proved previously. Thus, we used Caco-2 spheroids and primary colon organoids to evaluate how PG, PG-G, and PG-EA modulate three epithelial hallmarks of colitis: the epithelial barrier integrity, the production of cytokines, and the wound healing process.PGD2-G decreased the secretion of TNFa and MCP-1 in inflamed Caco-2 spheroids. On the other hand, PGE2-G modulated the production of TNFa, MIP2a, and KC in colon organoids and improved their survival in a DSS-platting efficiency assay. Interestingly, the increased plating efficiency was not associated with proliferation of stem cells. Thus, our results suggest that PGE2-G could be a promising therapeutic option to treat the epithelial component of colitis without increasing the risk of colorectal cancer.

ADELINE CEZARD
INSERM, Centre d'Etude des Pathologies Respiratoires (CEPR), UMR 1100, Tours -France

A Host-Derived Metabolite with Dual Antiviral and Anti-Inflammatory Properties Protects the Lung Mucosa Against Influenza Virus Infection

ADELINE CEZARD[1,2], Déborah Brea-Diakite[1,2], Virginie Vasseur[1,2], Alan Wacquiez[1,2], Loic Gonzalez[1,2], Ronan Le Goffic[3], Bruno Da Costa[3], Delphine Fouquenet[1,2], Severine Heumel[4], Arnaud Machelart[4], Eik Hoffmann4, Priscille Brodin[4], François Trottein[4], Cyrille Mathieu[5], Lola Canus[5], Florentine Jacolin5, Pierre-Olivier Vidalain[5], Laure Perrin-Cocon[5], Vincent Lotteau[5], Julien Burlaud-Gaillard[6], Dominique Tertigas[7], Michael G. Surette[8], Antoine Legras[2,9], Damien Sizaret[10], Thomas Baranek[1,2], Christophe Paget[1,2], Antoine Guillon[1,2,11] and Mustapha Si-Tahar[1,2]
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2. Université de Tours, Tours, France
3. Université Paris-Saclay, INRAE, UVSQ, UMR892 VIM, Jouy-en-Josas, France
4. Université de Lille, CNRS, INSERM, CHU Lille, Institut Pasteur de Lille, U1019 – UMR 9017 - CIIL - Center for Infection and Immunity of Lille, F-59000 Lille, France,
5. Centre International de Recherche en Infectiologie (CIRI), Université de Lyon, Inserm, U1111, CNRS, UMR5308, Université Claude Bernard Lyon 1, Ecole Normale Supérieure de Lyon, 69007 Lyon, France
6. Plate-Forme IBiSA des Microscopies, PPFASB, Université de Tours and CHRU de Tours, France.
7. Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada
8. Department of Medicine, McMaster University, Hamilton, Ontario, Canada
9. Service de Chirurgie Thoracique, CHRU de Tours, France.
10. Service de Pathologie, CHRU de Tours, France.
11. Service de Médecine Intensive -Réanimation, CHRU de Tours, France.

Influenza remains a major public health concern, with current therapies offering limited efficacy. As the primary site of infection, lung epithelial cells play a central role in both viral replication and the initiation of inflammatory responses. Influenza pathogenesis results from direct cytopathic effects on epithelial cells and excessive inflammation, highlighting the need for therapies targeting both mechanisms. We investigated the potential of host-derived metabolites to modulate influenza infection in human lung epithelial cells. Cis-aconitate, a Krebs cycle derived-metabolite, demonstrated potent dual activity. Cis-aconitate significantly inhibited viral mRNA and protein expression, effectively blocking replication of both influenza A and B virus strains in epithelial cell cultures. Beyond its antiviral effects, cis-aconitate reduced the hyperinflammatory responses of epithelial cells triggered by influenza infection and others inflammatory stimuli, such as TNF-a. In 3D culture model of primary human airway epithelial cells grown at an air-liquid interface, cis- aconitate decreased influenza A virus-induced cell death, indicating its protective effect on epithelial integrity. In a mouse model of influenza pneumonia, cis-aconitate treatment lowered viral loads, reduced leukocyte infiltration, decreased inflammatory mediator release, and limited lung tissue damage. Strikingly, mice treated with cis-aconitate at day 2 post-infection showed an 80% survival rate compared to 0% in untreated controls, following a lethal viral dose. In conclusion, cis-aconitate is a host-derived metabolite that directly modulates epithelial cell responses, exerting both antiviral and anti-inflammatory effects to protect against influenza pneumonia. These findings highlight the pivotal role of epithelial cells in influenza pathogenesis and position cis-aconitate as a promising therapeutic candidate.

EMMA FRAILLON
UMR CNRS 5305
Lyon, France

Exploring the role of the TRPV3 calcium channel in psoriasis

EMMA FRAILLON[1,2] Ph.D student., Sylvie Ducreux Ph.D[3]., Fabien P. Chevalier Ph.D[1,2]., Bérengère Fromy Ph.D[1,2].
[1]Laboratoire De Biologie Tissulaire Et Ingénierie Thérapeutique - UMR CNRS 5305 – Lyon, France
[2]Université Claude Bernard Lyon 1 – Lyon, France
[3]Laboratoire Cardiovasculaire, Métabolisme, Diabète et Nutrition - INSERM U1060 – Lyon, France

Psoriasis is a chronic inflammatory dermatosis with a worldwide prevalence ranging from 0.09 to 11,3%. Characterized by erythematous-squamous plaques, psoriasis results from an alteration in the signals exchanged between epidermal keratinocytes and immune cells, resulting in an aberrant and constant Th-17 inflammatory response in the skin of patients. While significant progress has been made in understanding the role of immune cells in psoriasis, the involvement of keratinocytes in the psoriasis-associated inflammation remains largely unexplored. Keratinocytes express various sensory receptors, such as members of the TRPV *(Transient Receptor Potential Vanilloid)* family, including TRPV3. TRPV3 is a permeable non- selective cation channel with a high affinity for calcium which is abundantly expressed in the epidermis. Its activation is known to lead to the overexpression of pro-inflammatory cytokines like IL-6 and IL-8, supporting its role in inflammatory processes. Furthermore, TRPV3 is overexpressed and hyperactive in some chronic inflammatory skin disorders like atopic dermatitis and the Olmsted syndrome, which share numerous histological features with psoriasis. Despite these findings, the contribution of TRPV3 to psoriasis has been poorly studied. We have demonstrated by *TRPV3* mRNA *in situ* hybridization that *TRPV3* was overexpressed in the human psoriatic epidermis compared to the healthy epidermis. Likewise, we also find TRPV3 overexpression in human primary keratinocytes cultured under psoriasis- like inflammatory conditions. In addition to TRPV3 overexpression, we have also demonstrated by FURA2-AM calcium imaging that the TRPV3-dependent calcium influx is increased in keratinocytes cultured under psoriasis-like pro-inflammatory environment compared to untreated keratinocytes. On another hand, we showed that TRPV3 increases psoriasis severity in the Imiquimod-induced psoriasis like mouse model as mice deficient for TRPV3 (TRPV3- KO mice) present less severe psoriasis features apparition compared to Wild-Type mice. Finally, we highlighted the involvement of TRPV3 in the regulation of the secretion of some psoriasis-related pro-inflammatory cytokines such as IL-17A, IL-8 and TNF-α in a 3D model of Human Reconstructed Epidermis (RHE) using ELISA. The investigation of the TRPV3- dependant inflammatory pathways, especially the TRPV3-EGFR-NF-kB pathway, will enable us to further advance our mechanistic understanding of how TRPV3 regulates the expression and the secretion of these typical psoriasis-related pro-inflammatory cytokines.

LI ZHIPENG
Université Paris Cité and Université Sorbonne Paris Nord
Paris – France

Impaired treprostinil-induced relaxation in COPD bronchi and effects of metformin and AICAR on bronchial tone

*ZHIPENG LI[1], Badji Hichem[1], Gaelle Merheb[1], Dan Longrois[1,2], Xavier Norel[1]**
1/ Université Paris Cité and Université Sorbonne Paris Nord, INSERM, LVTS, F-75018 Paris, France;
2/ Hôpital Bichat-Claude Bernard, Assistance Publique-Hôpitaux de Paris, Université Paris Cité Paris, France.

Chronic obstructive pulmonary disease (COPD) is a multifactorial disease characterized by a decline in lung function, chronic airway inflammation, and airflow obstruction. Studies have shown that metformin and AICAR can inhibit pulmonary inflammation, reduce airway remodeling, and mitigate cellular senescence in COPD(1,2). However, their effects on bronchial tone remain unexplored. This study aims to investigate if metformin and AICAR regulate the human bronchial tone in both control and COPD subjects. Since the epithelium plays a critical role in bronchial tone regulation, with epithelium is known to reduce airway contraction (3), we further investigated whether metformin and AICAR exert their effects via epithelium.
Method: Human bronchial rings with or without epithelium were incubated in the presence or absence of metformin (3mM, 2h) or AICAR (3mM, 30min), then mounted in organ baths system. Dose-response curves were generated: contractions were induced by histamine, followed by relaxations using treprostinil a PGI2 analogue.
Result: Histamine-induced bronchial contraction showed no difference between control and COPD groups. However, treprostinil-induced relaxation was significantly reduced in COPD (n=5) compared to controls (n=8). Although epithelial removal showed a similar trend, the difference was no longer significant. Metformin (n=7) significantly decreased histamine-induced contraction by 26% compared to controls without treatment (n=9), with a consistent effect after epithelial removal, but had no effect on COPD bronchi. AICAR (n=8) significantly reduced treprostinil-induced relaxation by 30% compared to controls without treatment (n=9), independent of
epithelium, but had no effect in COPD subjects.
Discussion and Conclusion: Our findings reveal key differences in bronchial relaxation responses to Treprostinil between control and COPD groups, as well as distinct effects of metformin and AICAR on bronchial tone
regulation. Treprostinil-induced relaxation was significantly reduced in COPD bronchi compared to controls, since treprostinil has affinity not only for IP receptor but also for EP2 and DP1 receptors, this result suggests impaired function of IP/EP2/DP1 receptors in COPD airways. Notably, this difference was no longer significant
after epithelial removal, suggesting impaired epithelial prostanoid receptor function in COPD. Additionally, metformin and AICAR modulated bronchial tone in controls and this effect remained persisting after epithelial removal, suggesting a direct action on airway smooth muscle cells. However, they had no effect in COPD subjects, indicating a potential loss of responsiveness in diseased airways. Future studies should explore the molecular mechanisms underlying these differences.

[1] Polverino F. Am J Respir Crit Care Med. 2021 Sep 15;204(6):651-666. PMID: 34033525.
[2] Cheng XY. Oncotarget. 2017 Apr 4;8(14):22513-22523. PMID: 28186975.
[3] Vanhoutte PM. Am J Physiol Cell Physiol. 2013 May 1;304(9):C813-20. PMID: 23325407.

FLORIAN HERMANS
Biomedisch Onderzoeksinstituut (BIOMED), Agoralaan - Gebouw C
Diepenbeek - Belgium

Tooth organoids and single cell transcriptomics position epithelial cell rests of Malassez as periodontal signaling hubs

FLORIAN HERMANS (1,), Steffie Hasevoets (1), Mai Hoang Ngoc Phuong (1), Reindert Jehoul (2,3), Werend Boesmans (2,3), Annelies Bronckaers (1), Ivo Lambrichts (1)*
(1) Department of Cardiology and Organ Systems (COS), Biomedical Research Institute (BIOMED), Faculty of Medicine and Life Sciences, Hasselt University, Diepenbeek, Belgium
(2) Department of Neuroscience, Biomedical Research Institute (BIOMED), Faculty of Medicine and Life Sciences, Hasselt University, Diepenbeek, Belgium
(3) Department of Pathology, GROW-Research Institute for Oncology and Reproduction, Maastricht University Medical Center, Maastricht, The Netherlands *Corresponding author

Epithelial cell rests of Malassez (ERM) are enigmatic stem cells dispersed throughout developing dental follicle (DF) and mature periodontal ligament (PDL). These presumed quiescent cells derive from the ameloblast-forming dental epithelial stem cells following disintegration of Hertwig's epithelial root sheath during root formation. ERM are localized in clusters or networks throughout the periodontium, are innervated and frequently associated with blood vessels and antigen-presenting cells. ERM are attributed important roles regulating periodontal homeostasis, response to tissue damage, as well as (orthodontic) tooth movement. Previous studies suggest ERM can directly contribute to cementum and PDL following epithelial-to-mesenchymal transition, and can acquire ameloblast-like features. In addition, we hypothesize that ERM act as paracrine signaling hubs, regulating these diverse functions. In order to shed light on the paracrine signaling factors employed by ERM, we take advantage of previously established single cell transcriptomic periodontal atlases, together with our recently established, novel 3D in vitro models of ERM from human tooth designated as epithelial tooth organoids (TO). Human TO mirror in vivo ERM and are capable of differentiation toward ameloblast-like cells. We aim to explore the secretory profiles of human TO as avatars for in vivo ERM by mapping the presence of niche-modulating proteins in their secretome. Antibody array screening revealed TO express pro-angiogenic (e.g. IL-6, VEGFA, uPAR), neurogenic (e.g. beta-NGF) and osteoregulatory (e.g. CCL2) factors, validated by enzyme-linked immunosorbent assay (ELISA). Single-cell transcriptomic analysis and in situ RNA hybridization confirmed these findings in native human ERM. Conditioned medium from ERM-derived TO promotes endothelial tube formation in vitro, functionally supporting the pro-angiogenic ERM secretome. Next, we aim to address the ERM secretome's ability to recruit monocytes and promote osteoclastogenesis via CCL2, using human peripheral blood-derived monocytes. Better understanding of these puzzling cells not only holds potential to unravel fundamental aspects of tooth development, tooth movement and periodontal biology, but also in advancing tissue engineering of artificial enamel organ and periodontal tissues – bringing us one step closer to biological tooth repair and regeneration.

HUGO MAIN
Litec
Poitiers - France

Expression of Heparanase 1 in Psoriatic Skin Inflammation

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Psoriasis is a chronic inflammatory disease affecting 3% of the world's population. Psoriatic lesions are characterized by a disruption in epidermal differentiation, hyperproliferation of keratinocytes, and immune cell infiltration. Resident tissue cells and immune cells contribute to the organization of an inflammatory microenvironment based on a complex network of pro-inflammatory cytokines, as well as the production of numerous enzymes. Heparanase 1 (Hpa1) is an endo-β-D-glucuronidase that selectively degrades heparan sulfate chains, participates in extracellular matrix remodeling, and releases inflammatory mediators, thus facilitating the recruitment of immune cells. Well-described in a tumor context, Hpa1 is also involved in chronic inflammatory diseases such as Crohn's disease and rheumatoid arthritis. Our objective is to study the involvement of Hpa1 in psoriasis. We have demonstrated 1) ex vivo, an increased expression of Hpa1 by keratinocytes in the skin lesions of patients with psoriasis, as well as normalization of its expression after anti-TNFα immunotherapy; 2) in vitro, an overexpression of Hpa1 in primary keratinocytes stimulated by the synergistic action of IL- 17A and TNFα; 3) in vivo, an increased expression of Hpa1 in the skin lesions of a murine model of imiquimod-induced psoriasiform inflammation. To understand the role of Hpa1 in psoriasiform inflammation, we also treated mice with intraperitoneal injections of a commercial Hpa1 inhibitor, OGT2115, at a dose of 10 mg/kg for 6 days. Under these experimental conditions, we observed, among inflammatory mediators, a decrease in the expression of chemokines CXCL2/3, involved in immune cell recruitment, as well as a reduction in neutrophil infiltrate in psoriasiform lesions. These results suggest that the production of Hpa1 by inflammatory keratinocytes may be involved in the establishment and/or maintenance of psoriatic lesions, particularly in neutrophil recruitment. The invalidation of the Hpa1-coding gene, in vitro and in vivo, should allow us to analyze in more detail the role played by Hpa1 in skin inflammation.

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Healthy and COPD iPSC derived lung organoids response to SARS-CoV-2 infection

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Respiratory infections are a global health priority, highlighted by the COVID-19 pandemic. To this end, preclinical in vitro study models have been developed to mimic the multiciliated airway epithelium, including the induced pluripotent stem cell (iPSC)-derived air- liquid interface culture (iALI)*. Using iPSCs dedifferentiated from cells from patients with chronic obstructive pulmonary disease (COPD), we found that we could produce complete, functional bronchial epithelium with the hallmarks of COPD: goblet cell metaplasia, basal cell hyperplasia and tissue inflammation. SARS-CoV-2 infection of healthy and COPD iALI was validated for virus replication by RTqPCR, for infection by plaque assays and analysed for virus localisation by immunofluorescence coupled to 3D high resolution confocal microscopy. In both samples, the virus infects and replicates for several weeks with a peak at 2-3 days post infection. Cilia destruction and increased mucus secretion were observed in infected iALI. In addition, the innate immune response of infected and uninfected iALI was measured by RTqPCR and immunoassays. Our results indicate differential expression of interferon-stimulated genes (ISGs) and pro-inflammatory cytokines following SARS-CoV-2 infection**. Infected COPD iALI have higher tissue response with increased numbers of basal cells, goblet cells and innate immune response compared to healthy iALI. In conclusion, our results show that the iALI lung model is a tool of choice to study respiratory viral infections and lung tissue responses, including those of patients with COPD pathology.

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Impact of microbial metabolites on intestinal barrier function during inflammation using gut-on-chip models

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In patients with chronic inflammatory bowel disease (IBD), the intestinal barrier is altered, with loss of epithelial integrity, excessive activation of the immune system and changes in the microbiota composition. Our previous works revealed that 3-oxo-C12:2-HSL, a bacterial quorum sensing molecule of the N-acyl-homoserine-lactone family, is present in significant quantities in the gut ecosystem of healthy subjects, but decreases in IBD patients. We have previously demonstrated the protective effects of 3-oxo-C12:2-HSL on tight junctions of epithelial cells under inflammatory conditions, as well as its immunoregulatory action on immune cells. To better understand the effects of this molecule, we used “organ-on-chip” (OOC) technology. OOC technology recreates the characteristics of the intestinal epithelial interface, integrating mechanical forces (shear induced by continuous fluid flow, and stretch caused by peristaltic motion) and 3-dimensional (3D) cellular organization. Our aim was to set up an inflammation-on-a-chip (IOC) model of intestinal inflammation to assess the effects of 3-oxo- C12:2-HSL on intestinal barrier function, as well as its impact on immune cell recruitment. Our OOC model consists of human intestinal epithelial cells in the upper channel of the chip, and in the lower channel, human endothelial cells and immune cells isolated from the blood of healthy donors (Peripheral Blood Mononuclear Cells (PBMCs) or neutrophils). 3-oxo-C12:2- HSL was supplied to the upper channel, and inflammation was induced by adding pro- inflammatory cytokines or lipopolysaccharide (LPS). Under these experimental conditions, we measured paracellular permeability, the secretion of several pro-inflammatory cytokines and chemokines, and the recruitment of circulating immune cells (PBMC and neutrophils) to the epithelial compartment. Our results show that 3-oxo-C12:2-HSL reduces paracellular hyperpermeability induced by both inflammatory stimuli as well as the secretion of several pro-inflammatory cytokines. Moreover, this quorum sensing molecule limits the recruitment of immune cells (PBMCs or neutrophils) into the epithelial cell monolayer under inflammatory conditions. In conclusion, we demonstrate the protective effects of 3-oxo-C12:2-HSL during inflammation using our IOC model integrating the different cellular compartments involved in intestinal barrier function.

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A Ca2+ antagonist limits inflammatory damage by reducing epithelial calcium activity in a zebrafish model of cystic fibrosis

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In people with cystic fibrosis (CF), a disorder resulting from mutations in the CFTR, overactive neutrophil (PNN)-dominated inflammation is responsible for pulmonary damage and premature death. Today, therapies targeting of inflammation in CF have been hampered by a lack of understanding the mechanisms involved in the inflammatory pathogenesis of CF. The deleterious role of epithelial calcium (Ca2+) has been proposed to explain the onset of inflammation in CF. However, how the defective CFTR/Ca2+ axis contributes to CF-related
inflammation remains unclear. Herein, using Cftr-depleted zebrafish as an innovative model of CF, we sought to i) elucidate how Cftr dysfunction affects Ca2+ signaling and thereby understand how alterations of Ca2+ fluxes are involved in CF-related inflammation, and ii) test the efficacy of Ca2+ antagonists aimed at preventing inflammatory damage in CF. To do so, expression of cftr was knocked-down using morpholino and inflammation was induced by tail fin injury. To examine the characteristic patterns of epithelial Ca2+ during inflammation, we generated a transgenic line expressing GCaMP6 specifically in epithelial cells. Using this tool, combined with dynamic imaging, we showed that CF larvae exhibited an abnormal elevation of Ca2+ fluxes at wounds compared to WT animals. Importantly, genetic and pharmacological inhibition of voltage-gated Ca2+ channels reduced inflammation in CF larvae both by reducing excessive ROS production and hyper PNN mobilization toward wound. Remarkably, a Ca2+ antagonist used for cardiac diseases, efficiently reduced PNN inflammation by reducing calcium and ROS over-production. Consequently, this drug also reduced tissue damage in CF larvae. Our findings develop evidence for the role of Ca2+ signaling in CF-related inflammation, a promising therapeutic target to prevent inflammatory damage in CF individuals, thereby improving their quality of life and longevity.

Selected abstracts for poster presentation

Poster #1

Oncostatin M in chronic rhinosinusitis with nasal polyps

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Chronic rhinosinusitis with nasal polyps (CRSwNP) is an inflammatory condition of the sinonasal mucosa that leads to the formation of polyps. Histologically, polyps are characterized by fibrosis and epithelial barrier disruption. The presence of polyps causes nasal blockage and loss of smell in patients. The management of CRSwNP has recently evolved with the introduction of biologics: targeted antibodies against molecules in the inflammatory cascade. However, some patients are resistant to these biologics, making it necessary to identify other potential targets for targeted therapies. Oncostatin M (OSM) is a pro-inflammatory cytokine from the interleukin (IL)-6 family that targets fibroblasts and epithelial cells in several skin or lung models. Here, we investigate the effects of OSM on fibrosis and the epithelial barrier in CRSwNP. OSM counteracts the pro-fibrotic effect of IL-4 and TGF-β1 by inhibiting the nuclear translocation of Smad3. Regarding epithelial barrier function, OSM alters epithelial permeability through the degradation of tight junction proteins.
After injury, OSM increases the rate of epithelial repair but impairs ciliary function. Through its action on fibrosis and epithelial permeability, OSM could be an interesting target for innovative new treatments in patients resistant to biotherapies in severe CRSwNP.

Poster #2

Involvement of epithelial CD146 in severe asthma

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Asthma is a chronic inflammatory lung disease affecting 300 million people worldwide, 2-10% of whom suffer from severe asthma. In asthma, the bronchial epithelium is dysregulated, contributing to the pathophysiology of the disease. CD146 is an adhesion molecule that is highly expressed on all the cells from the vascular system, including endothelial cells. It is also expressed by epithelial cells, but its functions in these cells are poorly understood, as is its role in severe asthma. The aim of our study is to elucidate the role of CD146 in the bronchia epithelium and its implication in severe asthma.

Methods: Bronchial epithelia of healthy controls and severe asthma patients were reconstituted in vitro using air-liquid interface culture. Measurements of CD146 expression were made on differentiated epithelium (n = 20 control and 20 severe asthma patients). Then, undifferentiated and differentiated bronchial epithelial cells were sorted based on the expression level of CD146. The sorted undifferentiated bronchial epithelial cells were cultured and monitored during the differentiation process until obtention of a fully differentiated epithelium (n= 3). The sorted differentiated bronchial epithelial cells were used to perform RNA-Seq experiments (n= 4).

Results: We showed that CD146 is highly expressed in severe asthma patients compared to healthy controls both at mRNA and protein levels in differentiated epithelium. Then, we have shown that CD146 expression is not ubiquitous in the differentiated bronchial epithelium. Cell sorting and RNA-seq experiments demonstrated that CD146 is mostly expressed by the basal epithelial cells in the differentiated epithelium. Finally, morphological analysis revealed that CD146 seems to play a role in the bronchial epithelium differentiation process. Indeed, fully differentiated epithelium obtained from CD146+ and CD146- sorted undifferentiated bronchial epithelial cells were different in morphology and cell composition.

Conclusion: Our results suggest that CD146 is only expressed by basal cells of the bronchial epithelium and supports an important role in the differentiation of this epithelium. Moreover, we demonstrated that CD146 is overexpressed in severe asthma, suggesting a possible role of this molecule in this disease notably during repair epithelial process.

Poster #3

Ex vivo modelling to study the role of airway epithelial cells in defective lung defense during sepsis

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Sepsis-induced immunosuppression confers increased susceptibility to secondary pneumonia. Studies in septic hosts have revealed several dysfunctions in immune cells. However, little is known about alterations of the respiratory epithelium, despite its role of first-line mucosal defense. We hypothesized that epithelial cells could be impaired in their functions of maestro of lung immune defense during sepsis and could contribute to susceptibility towards secondary pneumonia. We generated consistent data demonstrating airway epithelial cells impairment in their barrier and immune functions following a remote polymicrobial sepsis.

The main goal of our study is to decipher the role of airway epithelium in defective post-septic lung defense with a specific objective to dissect epithelial responses towards a double-infectious hit.

Methods: We established a primary 3D ex-vivo model derived from mouse tracheobronchial epithelial cells (mTEC) cultured in air-liquid interface. Epithelial cells were subjected to sequential infectious challenges (hit 1: TLR agonists at day0 and hit 2: Heat-killed P. aeruginosa (PAO) at day 6).

Results: No significant modifications were observed in Transepithelial Electrical Resistance (TEER) before and after epithelial stimulation.

Interestingly, histological analysis revealed that epithelial cells thinned drastically within 24 hours of PAO either without no prior stimulation or when pre-stimulated with a single TLR agonist; suggesting a physiological process of epithelial repair post-injury. In contrast, when pre- stimulated with a combination of 3 TLR agonists; we observed an hyperplasia of epithelial cells post-PAO secondary stimulation; suggesting a default of epithelial repair in response to PAO when epithelial cells are subjected to a prior “large enough” first inflammatory/infectious hit.

Expression of inflammatory mediators (e.g., KC) were measured via Luminex in culture supernatants; data are currently being analysed.

Conclusion: Overall, our preliminary data reveal differential epithelial responses to bacterial stimulation depending on an initial inflammatory stimulus via TLR pathways, suggesting the concept of trained immunity in epithelial cells. This 3D ex vivo model will allow us to elucidate the cellular and molecular mechanisms involved in such modulation of epithelial responses to infection. Ultimately, this will help in the development of epithelium-targeted therapies to restore lung immune function during sepsis.

Poster #4

Contribution of the respiratory epithelium to shape the response to inhaled particles

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Depending on material characteristics like fiber shape, inhaled nanomaterials (NMs) are known to induce acute or persistent pulmonary inflammation. To better understand the response of the epithelial niche to carbon-based NMs, we conducted a longitudinal analysis of cellular perturbations in intratracheally exposed mice to carbon black (CNP), double-walled carbon nanotubes (DWCNT), and multi-walled carbon nanotubes (MWCNT) using single-cell RNA sequencing (scRNAseq). Subsequently, murine alveolar epithelial cell cultures and lung organoids were investigated towards their potential to reflect signatures of epithelial injury and inflammatory activation in vitro.

At the chosen dose, all NMs induced comparable levels of neutrophilia in the airspace at 12h, sustained until d6 for DWCNT and until d28 for MWCNT, accompanied by eosinophilia for the latter. Only CNTs caused injury to alveolar epithelial cells and particularly MWCNTs also to alveolar macrophages (AM), evident by AT1 cell death (TUNEL), alveolar barrier disruption (BAL protein) and alarmin (IL33, IL1α) release into the airspace till d6. The epithelial niche signature was further characterized elevated DAMP gene expression with a transient accumulation of inflammatory AT2 cells (Lcn2, Il33 and CXCL5) for all NM exposures. scRNAseq, however identified material-specific proinflammatory epithelial cell circuits resulting in either, acute but resolving (CNP), versus persistent responses with chronic damage (CNTs). For MWCNT the iron-regulated cell death pathway ferroptosis was identified to kill epithelial cells as validated by ACSL4 immunohistochemistry. MWCNT triggered epithelial damage further induced the emergence of Krt8+ alveolar differentiation intermediates (ADI), which were recently described for their importance in balancing regeneration or fibrotic development. Concurrently, recruited inflammatory macrophages (SPP1), together with from dying AMs (IL1a) and epithelial cells (IL33) released DAMPs produced a Th2-environment in MWCNT exposed lungs which apparently generated the profibrotic fibroblast activation (Eln, Timp1, Mmp2, IL6) known to precede fibrosis. The Krt8+ ADI signature and inflammatory activation (Lcn2, Cfs2 and Lamp3 immunohistochemistry) could be reproduced in mouse lung organoids treated with IL1β or supernatants released from MWCNT treated AM.

In summary our data supports a strong implication of the epithelial barrier in the fate of the inflammatory responses to inhaled carbon NMs, and our transcriptomic signatures suggest that cell circuits between different niches underly the profibrotic responses to fiber shaped NMs.

Poster #5

Human organotypic model of the gastrointestinal epithelium to study inflammatory mechanisms and efficacy of anti-inflammatory treatments.

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Organotypic models using primary human cells are commonly used for in vitro studies of inflammatory mechanisms in different human tissues. Regarding gastrointestinal tract, Mattek's EpiIntestinal model reproduces the morphological and functional characteristics of the small intestine. Histological and fluorescent microscopic imaging of the EpiIintestinal model showed in vivo-like tissue architecture consisting of an epithelial layer with columnar organization, villi structures and brush borders. Majority of intestine cell type are present with enterocytes, Paneth cells, Tuft cells, and Goblet cells. The model is also characterized for barrier function, drug transporter and first passage metabolizing machinery Exposure of the EpiIntestinal model to proinflammatory cytokines (TNF-α and IFN-γ) induced upregulation of cytokines/chemokines such as IL-6, IL-8, and GRO-α. In another study, it has been shown that exposure to nanoparticles used as food additives induced different profile of inflammatory response with IL8 secretion and induction of oxidative stress mechanisms. In an EpiIntestinal model incorporating fibroblasts and macrophages, the exposition to ligands for TLR 4 (lipopolysaccharide lipopolysaccharide; LPS), NOD 1(C 12 -iE -DAP) and NOD 2(L 18 -MDP, showed a higher number of upregulated genes (4400) compared to the model without macrophages (1400). In this system, synergetic effects of TRL4, NOD 1 and NOD 2 activation on proinflammatory cytokine secretion have been demonstrated by quantification of IL-8, IL-6 and TNF-a secretion. In a recent study, EpiIntestinal model was used to assess the efficacy of 5-HT2A receptor ligands (psilocybin, 4-AcO-DMT, ketanserin) and TRP channel ligands (eugenol, curcumin, capsaicin) on TNF-α- and IFN-γ-induced inflammation. The effect of the treatments on different inflammatory markers such as TNF-α, IFN-γ, IL-6, IL-8, MCP-1 and GM-CSF showed that psylocibin and Eugenol could be promising candidates for further research into anti-inflammatory therapies, especially for conditions like inflammatory bowel disease (IBD).
Overall, these results illustrate the value of this model in the study of inflammatory mechanisms,
and its use in the early evaluation of the efficacy of treatments or treatment combinations. These innovative approaches are not limited to the gastrointestinal system, and are also applicable to other tissues/organs with the human organotypic models of the respiratory, cutaneous, hepatic and renal systems developed by Mattek

Poster #6

Mechanisms involved in the protective effects of a bacterial quorum sensing molecule on the intestinal epithelial barrier under inflammatory conditions.

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Inflammatory bowel disease (IBD) is characterized by an altered intestinal barrier and an excessive immune response to the microbiota. Certain bacterial metabolites have been implicated in the maintenance of the intestinal barrier. Our team discovered several quorum sensing molecules of the N-acyl-homoserine-lactone (AHL) family in the human intestinal ecosystem and showed that the most abundant, 3-oxo-C12:2-HSL, is lost in IBD patients, suggesting a beneficial role. 3-oxo-C12:2-HSL exerts anti-inflammatory properties on immune cells, notably via a bitter taste receptor (TAS2R). Its protective role in the integrity of tight junctions of intestinal epithelial cells under inflammation has been highlighted, but the mechanism remains to be determined. Our aim is to evaluate the involvement of TAS2R in the protective effects of 3-oxo-C12:2-HSL in epithelial cells and to determine the molecular targets of this AHL. We use Caco-2/TC7 cells, a human intestinal epithelial cell line, cultured on semi-permeable filters with inflammatory condition induced by addition of pro-inflammatory cytokines Interferon-γ (IFNγ) and Tumor Necrosis Factor-α (TNFα). Our results show that, in addition of maintaining tight junction integrity in Caco-2/TC7, 3-oxo- C12:2-HSL attenuates MCP-1 chemokine secretion induced by IFNγ/TNFα. Among TAS2Rs that can be activated by 3-oxo-C12:2-HSL, we show that TAS2R13 is expressed in Caco-2/TC7 cells and human intestinal tissues. Our first results using siRNA approach show that TAS2R13 appears to be involved in the effect of AHL on the maintenance of tight junctions under inflammatory conditions. We generated Caco-2/TC7 cells invalidated for TAS2R13 by CRISPR-Cas9 to confirm this result. In parallel, we are investigating the protein targets of AHL in intestinal epithelial cells using proteomic analysis. Preliminary results suggest that 3-oxo-C12:2-HSL may attenuate pro- inflammatory cytokine-induced responses by modulating the levels of certain proteins in the NF- kB and JAK/STAT pathways. This work will provide essential data to understand the protective role of an intestinal microbiota molecule on host cells. As the level of 3-oxo-C12:2-HSL is decreased in IBD patients, knowing the mechanisms underlying the protective effects of this AHL on host cells may open the way to new therapeutic approaches in IBD

Poster #7

Study of the role of IL-20 related cytokines in Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD), encompassing Crohn's disease and ulcerative colitis, is a group of inflammatory pathologies affecting the gut whose incidence has greatly increased in Europe in the last decades. By regulating the inflammatory response, cytokines are important actors of the development and maintenance of IBD. Recently, studies have shown that the expression of IL-20-related cytokines (IL-19, IL-20, IL-22 and IL-24) is upregulated in the biopsies and sera of IBD patients. Moreover, certain polymorphisms in their genes have been associated with an increased risk of developing the disease. Our team and others have already demonstrated that IL-22 has a protective effect in IBD. We are now investigating the role of the other cytokines of this family in this disease. To this end, we first investigated the effect of the IL-20-related cytokines on the murine gastrointestinal tract and on colorectal cell lines. While IL-22, our positive control, induced phosphorylation of STAT3 both in cell lines and in the digestive tract of healthy mice, IL-19, IL-20 and IL-24 did not. Furthermore, we have used two mouse models of colitis – the Citrobacter rodentium model and the Dextran Sulfate Sodium model – on mice deficient for Il24 or the Il20rb receptor chain required for IL-19, IL-20 and IL-24 signaling, and found no difference in terms of colitis severity between the WT and KO groups. This suggest that IL-19, IL-20 and IL-24 do not play an important role in the murine colon, both in healthy and inflamed conditions. However, mice and human biology often differ, especially in the field of immunology. In the future, we plan to investigate the effect of these cytokines on human organoïds derived from patient biopsies. We hope this research will enable us to better understand the role of these cytokines in IBD and how they could potentially be targeted in patients.

Poster #8

Microbial serine proteases and IBD: new actors under the spotlight

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Background: Proteases are key players in the gastrointestinal physiology. However, the disruption of proteolytic homeostasis has emerged as a significant contributor to Inflammatory Bowel Diseases (IBD). Most studies have focused on host proteases in gut inflammation, overlooking the potential contribution of gut microbiota. Our project aims to functionally characterize microbial serine proteases that are overabundant in IBD patients compared to healthy individuals. This study reports the functional analysis of SP-1, a new microbial serine protease identified from the human gut microbiota.
Materials & Methods: The gene encoding SP-1 from the human gut microbiota was cloned and expressed in E. coli. To further investigate its role, we purified the recombinant SP-1. Additionally, to better understand the physiological role of this protease, we conducted its biochemical characterization, analysing its kinetic parameters, stability across different pH values and temperatures, and the effects of metal ions. We also provided the in vivo demonstration of the proinflammatory role of the SP-1 microbial protease.
Results: We succeeded in the production and purification of active SP-1 with high recovery yields for functional analysis. This protease showed subtilisin-like activities with a strong preference for Arg and Lys in the P1 position. Using a mouse model of colitis, we show that oral administration of recombinant bacteria secreting SP-1 compromises intestinal barrier function and exacerbates colitis. Moreover, these effects occurred with an alteration of the mouse gut microbiota composition.
Conclusion: A growing body of literature has shown the association between the alteration of the gut microbiota composition and functions and IBD pathogenesis. However, the gut microbiota's role in the proteolytic dysregulation observed in IBD remains understudied. Here, we report for the first time the ability of SP-1, a serine protease from the commensal microbiota, to exert potential pro-inflammatory responses during colitis and exacerbate inflammation and shed light on the relevance of gut protease activities and the mechanisms involved in IBD pathogenesis.

Poster #9

From GWAS to function in IBD: A focus on ORMDL3, GSDMA and GSDMB

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Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract driven by a complex interplay between genetic susceptibility, immune dysregulation, and epithelial barrier dysfunction. Genome-wide association studies have identified several genetic loci associated with IBD, including ORMDL3, GSDMA, and GSDMB, which are located within the 17q21 locus. Evidence suggests that these genes may be co-regulated, but their precise role in the pathogenesis of Crohnâ€™s disease and ulcerative colitis remains unclear. To elucidate their contribution to IBD pathogenesis, we use knock-in and knock-out models in both intestinal epithelial cells and T cells to assess their individual and combined effects In the context of intestinal epithelial cells, we evaluate the impact of these three genes on barrier integrity using the CACO-2 monolayer model, assessing parameters such as transepithelial electrical resistance (TEER), FITC-Dextran permeability assays, and the expression of differentiation markers.

We also explore their role in T cells, using genetically modified Jurkat cells. We analyze proliferation, signal transduction following T-cell receptor activation, and cytokine production to determine the impact of these specific genes on T-cell activation and modulation of immune response.

By integrating these approaches, our study aims to define the individual and synergistic roles of ORMDL3, GSDMA, and GSDMB in maintaining epithelial integrity and T-cell function, providing new insights into their contribution to IBD development.

Poster #10

Understanding the early response to urinary tract infections in female and male mice

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Urinary tract infections (UTI) are one of the most common bacterial infections, affecting 150 million people every year, worldwide. The incidence of infection greatly varies as 50% of woman and only 5% of men will experience at least one UTI in their lifetime. Women tend to experience recurrent UTI, whereas men develop chronic UTI. We can recapitulate human infection outcomes using a mouse model of UTI in which uropathogenic Escherichia coli (UPEC) is inoculated transurethrally into the bladder. Female mice clear their infection spontaneously within a week, whereas male mice remain infected at least one-month post-infection. Although bacterial burden peaks at 24 hours post-infection in both sexes, at this timepoint, female mice exhibit more robust innate immune cell infiltration and cytokine expression compared to male mice. At only one-hour post-infection, female mice had more bacteria in their bladder, and more specifically in their urothelial cells compared to male mice. We also found that the transcription factor NF-kB in urothelial cells is activated before infection and peaks earlier in infection in female mice. This suggests the first hours of infection are essential for initiating a strong immune response and promoting resolution. To more closely understand the early response, I performed RNA sequencing of peeled bladder urothelium to detect immune gene activation. Preliminarily, I found there was higher gene expression in female mice compared to male mice at one-hour post- infection. With functional analysis, I detected upstream regulators that may control downstream activation of the immune response to UTI. I will test downstream effects of these activators using genetic models and inhibitory molecules in our infection models. Identification of essential regulators of the immune response between the sexes will drive the development of ‘tailor-made’ treatment for women and man in the clinic.

Poster #11

Abnormal biosynthesis of specialized pro-resolving lipid mediators by airway epithelial cells and its impact on epithelial integrity in cystic fibrosis

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Abstract: Specialized pro-resolving mediators (SPMs) such as lipoxins (LX), resolvins (Rv), protectins (PD) and maresins, are bioactive lipids promoting the resolution of inflammation and return to tissue homeostasis. We and others reported an altered biosynthesis of LXA4 in the airways of cystic fibrosis (CF) patients. With this study we investigated the role of CF airway epithelial cells in SPM biosynthesis and the impact of SPMs on epithelial barrier function We used primary cultures of airway epithelial cells (hNEC) obtained from CF and non-CF subjects and cell line cultured in air-liquid interface. The epithelial secretions were collected in the basal compartment of the culture inserts for the quantification of 21 SPMs and related metabolites by mass spectrometry. The expression of the main enzymes involved in SPMs biosynthesis was assessed by western blotting of protein extracts. Epithelial differentiation was evaluated by the transepithelial electrical resistance (TEER) measurements. Epithelia repair was studied using an epithelial wound assay under video microscopy (Incucyte). We detected a broad range of SPMs in the secretion medium of hNEC. The LXA4, LXB4, RvD2, RvD5, PD1 and RvE3 levels were significantly lower in samples derived from CF patients compared with non-CF subjects. Within CF samples, the RvD3, RvD4 and PD1 were significantly lower in samples derived from females. While the mean expression of the enzymes involved in SPMs biosynthesis (15-LO, 5-LO and 12-LO) did not significantly differ either between CF and non-CF or between female and male samples, the SPM concentrations correlated with their expression levels. Furthermore, we found that SPMs enhanced airway epithelial differentiation and repair after a mechanical lesion.
Our results provided evidence of a role for airway epithelial cells in mediating the resolution phase of inflammation through the biosynthesis of bioactive lipids. More specifically, we found lower abilities of airway epithelial cells derived from CF patients and more markedly, females to produce SPMs. We also showed a role for SPMs in airway epithelial integrity. These data are consistent with the contribution of CF airway epithelium in the abnormal SPMs biosynthesis which participates to enhanced epithelial vulnerability.

Poster #12

Placental immune response during cytomegalovirus infection

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The maternal-fetal interface, consisting of the maternal decidua and the fetal chorion, plays an essential role in fetal development. It forms a physical barrier against infection. Transmission of cytomegalovirus (CMV) during pregnancy is a major public health problem and the main cause of non-genetic congenital malformations. This study aims to assess the adaptive immune response in CMV-infected placentas and to evaluate the impact of valacyclovir treatment on this response. We analyzed 68 placentas divided into three groups: 26 from medical terminations of pregnancy (TOP) (between 21 and 26 weeks of gestation), categorized by severity of fetal brain lesions; 16 term placentas (≥32 weeks of gestation), grouped by CMV transmission to the neonate; and 14 pairs of infected placentas, matched for gestational age and ultrasound findings, comparing treated (valacyclovir) vs. untreated placentas. Immunohistochemistry was performed using anti- CD3, anti-CD20, and anti-MUM1 antibodies to identify T cells, B cells, and plasma cells, respectively. Quantitation was performed using QuPath, and statistical analysis was performed using R and GraphPad (Mann-Whitney test). T cells and plasma cells were significantly more abundant in infected placentas, particularly in cases with severe fetal brain lesions. No significant difference was observed for B cells. Immune cell density was higher in the second half of the second trimester (21-26 weeks of gestation) compared to the third trimester (≥32 weeks of gestation). There were no significant differences in immune cell density between treated and untreated placentas, regardless of lesion severity or gestational age.
These findings suggest that the placenta mounts an adaptive immune response to CMV infection. The response seems higher in severe cases, possibly due to higher viral load. The response appears to peak in the second trimester. Although valacyclovir treatment did not significantly alter immune cell density, further studies are needed to evaluate its effects.

Poster #13

Cystic Fibrosis Airway Epithelium Secretome Profiling: new insights into CFTR- related dysregulations

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The airway epithelium contributes to host defense through a variety of mechanisms, including the secretion of a wide range of defense proteins and peptides, antioxidants, anti- proteases, growth factors, chemokines and cytokines. In cystic fibrosis (CF), a genetic disease caused by mutations in the CFTR gene encoding the CFTR chloride channel, there is a vicious circle of respiratory infection, exaggerated neutrophil-predominant lung inflammation, epithelial remodeling and lesions favoring mucus stasis and respiratory infection. Lung lesions appear to be largely the consequence of a failure of airway epithelial defense. It has also been suggested that airway inflammation is due to epithelial dysfunction secondary to CFTR mutations, resulting in impaired innate host defense systems. However, the link between altered innate host defense proteins in CF surface liquid and CFTR defects has not been clearly demonstrated. Our aim was to assess the impact of the CFTR defect on the secretome of CF bronchial epithelium, without contamination by inflammatory cells and bacterial secretions, while retaining the influence of inter-patient variability and directional epithelial secretion associated with polarization and differentiation.

Bronchial epithelial cells from CF patients (F508del/F508del; n=8) and healthy subjects (lung donors; n=) were cultured at the air-liquid interface for 35 days until complete epithelial differentiation. The 24-hour secretions from these epithelia were collected and analyzed by quantitative liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Proteome profiling identified and quantified 1629 proteins, of which 118 were found differentially abundant between non-CF and CF secretions (log2 fold change≥0.58; p<0.05), with 29 proteins upregulated and 89 proteins downregulated in CF secretions. Ninety-four differentially expressed proteins (80%) were classified as secreted according to the SecretomP and SignalP databases and the Human Secretome Atlas initiative. Seventy-three biological processes (Gene Ontology) were enriched. According to the Reactome database, 18 signaling pathways were enriched in differentially identified proteins, among which immune system pathways are the most enriched and represented, together with protease/antiprotease activity.

Our results are indicative of a CF airway epithelium with a constitutive altered innate immunity, suggesting that the downstream consequences of CFTR mutation set the stage for chronic inflammation and infection in the cystic fibrosis airways.

Poster #14

Comparative study of the effects of LPS and TGFβ stimuli on lung alveolar organoids.

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Abstract: Organoids are attracting new interest in the study of inflammatory and chronic lung diseases, notably idiopathic lung disease (ILD). ILD is mainly linked to an abnormal response of alveolar epithelial cells to repeated aggression. This response results in excessive activation of fibroblasts and myofibroblasts, leading to exaggerated deposition of extracellular matrix. In some patients, acute exacerbations represent a major clinical turning point, often triggered by bacterial infections, notably by Pseudomonas aeruginosa. The release of inflammatory mediators and free radicals exacerbates the fibrosis process. The complexity of this disease is widely recognized, but cellular research remains very difficult due to the lack of appropriate experimental models that reproduce the interaction between cells. Alveolar organoids could enable us to assess the involvement of several cell types exposed to different stimuli, such as TGFβ, a major mediator driving the progression of fibrosis, or Lipopolysaccharide (LPS) from Pseudomonas aeruginosa inducing pulmonary inflammation. The aim of this study is to evaluate the reactivity of the 3D alveolar model in vitro from mouse lungs, after exposure to TGFβ or LPS. We used progenitor cells isolated by filtration from pieces of mouse lung and cultured in Matrigel for fourteen days. We then exposed the cultures to LPS for two hours, or to different doses of TGFβ for twenty-four hours. We then assessed the expression of several inflammation and tissue remodeling genes by RT-qPCR. We observed that inflammatory genes expression, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor α (TNF-α) and chemokine-2 ligand CCL2, was strongly increased in alveolar organoids after LPS exposure.
Regarding tissue remodeling genes expression, notably matrix metalloproteinases 2 and 9 (MMP2/9) and tissue inhibitor metalloproteinase-1 (TIMP-1), we can observe a dose-dependent increase in the expression of these genes after exposure to TGFβ. However, exposure to TGFβ induced a decrease in tissue inhibitor metalloproteinase-3 (TIMP-3) gene expression in alveolar organoids.
Our results show that alveolar organoids derived from mouse lungs are able to respond to exposure to LPS or TGFβ. These organoids can therefore be used as multicellular experimental models to study the pathophysiology of fibrosis or inflammation processes in chronic lung disease.

Poster #15

NLRP6 promotes pulmonary inflammation and fibrosis through activation of caspase-3/gasdermin E pyroptotic pathway in lung epithelial cells

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Idiopathic Pulmonary Fibrosis (IPF) is the most common chronic and progressive interstitial lung diseases with fatal ending. Immunological processes involved remain poorly understood. Innate immune sensors such as nucleotide-oligomerization domain-like receptors (NLRs) are able to form cytosolic complexes named inflammasomes that mediate inflammatory cytokines production and immunological cell death. We and others showed that the NLRP3 inflammasome is involved in the establishment of pulmonary inflammation and fibrosis. However, the role of the NLRP6 inflammasome in IPF remains unknown. Using the experimental model of IPF through oropharyngeal administration of bleomycin (BLM), we explored NLRP6 contribution in the context of programmed cell death induced after lung injury and its consequence on lung inflammation and fibrosis. Flow cytometry analyses showed that Nlrp6 deficiency dampens airway inflammation with reduced neutrophils, eosinophils, dendritic cells, T and B lymphocyte influx in the bronchoalveolar space. In addition, we observed attenuated inflammation, collagen deposition and fibrosis-related gene expression in lung tissue of Nlrp6 deficient mice in comparison to WT mice.
Since lung fibrosis is due to repeated injury of lung epithelium that leads to alveolar epithelial cell (AEC) death, we analyzed the mechanisms by which BLM induces AEC programmed cell death and in particular the role of gasdermin (GSDM) E, known to be involved in non-conventional pyroptosis. Indeed, GSDME cleavage by activated caspase-3 was reported to be induced by anti- tumor drugs promoting pyroptotic cell death of cancer cells and activation of immune responses in favor of anti-tumor therapy. However, GSDME-mediated pyroptosis may also be responsible for pathological inflammation in chronic diseases. Our results show that BLM administration promotes caspase-3 and GSDME cleavage in AEC, which is reduced in Nlrp6 deficient mice. These results suggest that NLRP6 inflammasome contributes to pulmonary inflammation and fibrosis through GSDME-dependent pyroptosis. Together, our data indicate that NLRP6 is a key player in BLM- induced pulmonary inflammation and fibrosis in mice, potentially through induction of programmed cell death in lung epithelial cells.

Poster #16

ATP released from tracheal tuft cells as a mediator for protective immune responses in pneumonia

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Tracheal epithelial tuft cells detect bacterial metabolites and subsequently initiate protective respiratory reflexes and innate immune responses, such as cough and neurogenic inflammation. These cells display chemosensory traits as they express components of the canonical bitter taste signalling cascade, notably its member the Trpm5 (transient receptor potential melastatin 5) ion channel. To date, tuft cell-dependent acute immune responses have been attributed to the release of acetylcholine, interleukin-25 and cysteinyl leukotrienes. However, it remains unclear whether tuft cells also impact the adaptive immune system. Using whole-cell current recordings in primary tuft cells combined with membrane fluorescence imaging in ATP sensor cells, we found that tuft cells release ATP via pannexin 1 channels. This was depended on activation of Trpm5. The tuft cell-derived ATP led to an increased dendritic cell migration, activation and phagocytosis of bacteria. In line with this, three days after in vivo tuft cell stimulation by inhalation of denatonium or in an experimental model of *Pseudomonas aeruginosa* infection, dendritic cells were activated and recruited to the trachea, lungs and the draining lymph nodes. Moreover, three days after in vivo tracheal tuft cell stimulation with denatonium, we observed activation of the adaptive immune system, characterized by an increased TH17 cell count in the lungs, accompanied by elevated IL-17A plasma levels. Importantly, mice deficient for pannexin 1 or Trpm5, which do not release ATP from tuft cells, showed decreased survival during *P. aeruginosa* infection. Conclusively our results show a crucial role for tuft cell-released ATP in the regulation of protective innate as well as adaptive immune responses in the airways to fight bacterial infections.

Poster #17

Involvement of IL-24 in skin carcinogenesis

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Interleukin-24 (IL-24) belongs to the IL-20 family, which acts on non-immune cells. Its two heterodimeric receptors are highly expressed on keratinocytes in which IL-24 promotes proliferation and induces the production of chemokines and antimicrobial peptides. Its role in the pathogenesis of several inflammatory skin diseases such as psoriasis or contact dermatitis has also been demonstrated. Beyond its role in the skin, IL-24 has been of particular interest in cancer research. Indeed, the intracellular overexpression of IL-24 in cancer cells, using adenovirus, inhibits their growth. This effect appears to be mediated by the induction of apoptosis in tumor cells but also by the inhibition of angiogenesis and the development of metastases. However, the tumor suppressor activity of IL-24 has never been demonstrated in the context of physiological expression.

In this study, we analyzed the development of skin papillomas in IL-24 deficient mice. We used the two-stage skin carcinogenesis model induced by dermal application of dimethylbenz(a)anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA). While DMBA induced mutations in epithelial cells, repeated application of TPA induced a chronic inflammatory response leading to cell proliferation and papillomas development. Contrary to what was expected, IL-24-deficient mice had fewer papillomas than WT mice and their tumors were also smaller. These results were confirmed in mice deficient in IL-20Rb, the common chain of the two IL-24 receptors, which are also protected against the development of papillomas. These results demonstrate that the role of IL-24 in tumor development remains unclear. The tumor context, the expression level of IL-24 and its mode of action (intracellular or via its extracellular receptor) may play a role in this apparent contradiction. We are currently investigating the mechanisms that lead IL-24 to have pro-tumor properties in the DMBA/TPA model.

Poster #18

Investigating *C. albicans* colonisation and the shaping of gastrointestinal epithelial responses to unrelated infections

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The fungal pathobiont Candida albicans colonises up to 95% of all humans, with intestinal carriage detected in nearly 80% of human samples. Our understanding of how C. albicans interacts with the host and governs local and global immunity is not understood, as in vivo modelling has traditionally used the reference strain SC5314, which requires antibiotics to establish colonisation. In this project, we establish a commensal C. albicans murine model. We are also investigating whether commensal C. albicans changes intestinal epithelial cell composition and immunity, and if these changes impact the control and pathogenesis of systemic candidiasis or unrelated helminth infections, such as the whipworm Trichuris spp., which has a global incidence of over 513 million, and harbours the same niche as C. albicans.

Poster #19

Role of Transglutaminase 2 in mucus plugging in asthma

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In asthma, airway obstruction by mucus plugs impairs breathing and can lead to patient death. Mucin post-translational modifications have been involved in airway mucus plugging. We hypothesized that the crosslinking enzyme Transglutaminase (TG)2 could reticulate airway mucins and participate in mucus tethering to the airways. Methods: Colocalization of mucins and of TG2 and its crosslinking product (isopeptide) was explored in bronchial biopsies using immunofluorescence and Proximity-Ligation Assay (PLA). Air liquid interface-regenerated bronchial epitheliums were obtained from severe asthmatics and controls. Mucus tethering was modeled by IL-13 exposure followed by mucin immunodetection after one apical wash. Mucins were purified from apical secretions and incorporation of biotinylated amine by TG2 was evaluated by western-blot. TG2 involvement was assessed using the pharmacological inhibitor ZDON.
Results: In bronchial biopsies from severe asthmatics, TG2 and isopeptide were detected at the epithelium surface in association with tethered mucus. PLA assay showed interactions between MUC5AC and both secreted and membrane-anchored MUC1. Moreover, isopeptide was detected on MUC5AC, MUC5B and MUC1. In regenerated epitheliums, TG2 mRNA was upregulated in a sub-group of asthmatics characterized by higher serum IgE, and in control epitheliums exposed to IL-13. Secreted mucins were used as substrates for TG2 in a ZDON-sensitive manner. Finally, ZDON facilitated removal of tethered MUC5AC from the surface of hypersecretory epitheliums.
Conclusion: TG2 crosslinking activity may participate in mucus tethering to the bronchial epithelium in asthma and its pharmacological targeting may reverse mucus plugging in the airways.

Poster #20

Highlighting the roles of ACKR3 in skin homeostasis and response to UV light-induced damage in full-thickness 3D-epithelial cell cultures

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CXCL12 and its receptor CXCR4 are recognized as critical regulators of the intricate processes that ensure skin homeostasis. The CXCL12/CXCR4 pair regulates the biology of and communication between epidermal keratinocytes and fibroblasts. It organizes immune cell migration in steady state and recruitment during inflammation, its contribution being essential in wound healing. The CXCL12/CXCR4 couple also participates in age-dependent fibrotic and regenerative responses and serves as the gatekeeper of human papillomavirus (HPV, the most prevalent eukaryotic viruses in the epidermal virome) infection and pathogenesis. However, the possible role of ACKR3, the second atypical receptor of CXCL12, in regulating these processes remains unknown, as does its expression pattern and regulation in this tissue. To provide insight into these questions, we have leveraged optimized full-thickness organotypic 3D-epithelial cell cultures (3Deps) that recapitulate human skin keratinocyte–fibroblast communication. By characterizing the expression pattern of ACKR3 in the 3Deps, we have confirmed the presence of this receptor in native conditions with similar distribution as in the human skin, as well as the production of CXCL12 by dermal fibroblasts. This prompted us to use 3Deps to investigate the contribution of ACKR3 to skin homeostasis and response to ultraviolet (UV) light-induced stress. To do this, we used innovative compounds developed within the ONCORNET 2.0 consortium to modulate endogenous ACKR3. First, we showed that ACKR3 activation promotes epithelial development at the steady state. Then, using a setting in which UV exposure compromises fibroblast survival and epithelium development, we demonstrated that ACKR3 agonism rescued fibroblasts in a CXCL12-dependent manner and promoted keratinocyte proliferation through a cell-intrinsic mechanism associated with maintained p53 levels. Finally, we established that HPV replication in 3Deps protected these tissues from UV light-induced damage without rescuing fibroblasts and abolished ACKR3 agonism-induced protection. Together, our findings unravel the overlooked role of ACKR3 in skin homeostasis and response to UV damage, with cell-selective effects, and identify this atypical receptor as a valuable target for a more comprehensive understanding of processes where CXCR4 alone may not provide a complete picture.