Abstracts of poster communications

1- Weight loss consecutive to obesity: consequences on epididymal adipose tissue

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Introduction: Obesity is characterized by an excessive accumulation of white adipose tissue (WAT) associated with pathological remodeling leading to fibrosis. The healthy WAT displays critical homeostatic functions: energy storage and mobilization through lipolysis activation and adipokines secretion. Obesity-induced WAT pathological remodeling results in altered adipocyte function and favors metabolic alterations leading to co-morbidities such as type 2 diabetes.
Obesity management relies on fat mass loss in order to improve metabolic disorders.
In this context, we aimed to characterize the WAT remodeling after weight loss and assessed whether weight loss improves adipocytes functions.
Methods: We set up a mouse model of weight loss: high fat diet (HFD)-fed mice lose weight following changing HFD by a low-caloric diet allowing us to compare features of epididymal WAT (eWAT) among 3 groups of mice: lean, obese and formerly obese mice (Fob).
Results: In FOb animals, with body composition, adipocyte size and glycemic control normalized to lean mice, eWAT study revealed higher fibrosis deposition and a loss of perilipin-1 expression. Lipolysis investigation showed an increase in basal lipolysis and a loss of sensitivity to β-3-receptors stimulation in obese and FOb mice compared to lean mice.
We then examine the mechanism underlying the increase in fibrosis deposition and analyzed the mRNA expression of fibrosis markers. Surprisingly, while fibrosis increased in FOb eWAT sections, the expression of messengers encoding for fibrosis markers decreased to the level measured in lean mice, indicating an uncoupling between fibrosis production and deposition.
Conclusion: Even if weight loss is metabolically favorable, eWAT keep a scar of obesity. The lack of fibrosis resolution could be instrumental in the loss of adipocyte functions following weight loss. The uncoupling between mRNAs coding for fibrosis markers expression and fibrosis deposition remains to be elucidated, but our first results suggest an alteration in the collagen degradation processes.

Keywords: Obesity, weight loss, fibrosis, collagen

2- Critical role of adipocyte ABCG1 expression in adipose tissue remodeling and progression of insulin resistance during diet-induced obesity

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Aim (background & objectives). Our previous work brought to light the key role of the membrane ATP-Binding Cassette G1 (ABCG1) transporter in triglycerides storage in adipocytes and fat mass formation in obesity. Such a role involves the control of the bioavailability of lipoprotein lipase through the remodeling of plasma lipid membranes. However, the consequences of this mechanism on the appearance of metabolic disorders during diet-induced obesity are not yet determined. The objective of this study is to analyze the impact of adipocyte Abcg1 deficiency on the development of diet-induced obesity and associated metabolic disorders.
Method. To achieve this goal, adipocyte Abcg1 deficient mice (Abcg1ADIPOKO, Abcg1flox/flox x Adipoq-CreERT2) were generated and fed a high-fat diet (HFD) for up to 30 weeks.
Results. A significant reduction of fat mass and body weight was observed in Abcg1ADIPOKO mice as compared to control animals on HFD. Whereas an alteration of insulin sensitivity and glucose tolerance was detected in Abcg1ADIPOKO mice after a 12-week of HFD, those parameters were improved when HFD was prolonged up to 30 weeks. Analysis of fat depots revealed that the Abcg1 deficiency improved the metabolic health of adipose tissue and could impact thermogenesis. Such a deleterious role of Abcg1 was also observed when Abcg1 deficiency in adipocytes is induced in obese mice. Finally, adipocyte Abcg1 deficiency in Abcg1ADIPOKO mice was accompanied by a reduction of plasma cholesterol concentrations in comparison to control mice.
Conclusions. Taken together, our findings indicate that the expression of Abcg1 in adipocyte plays an important role in adipose tissue remodeling and contributes to the development of metabolic disorders in diet-induced obesity.

Keywords: Obesity, Adipocyte, Metabolism, ABCG1

3- Gut microbiota metabolites and the enteroendocrine sweet taste signaling pathway in obesity

Moret Dounia

Sorbonne Université / INSERM UMRS 1269 Nutriomics – Nutrition and obesities: systemic approaches (Pr. K. Clément) - Site Pitié Salpêtrière, Paris, France

Background: Gut microbiota produces metabolites such as short chain fatty acids (SCFA) that modulate enteroendocrine cells (EEC) and hormone secretion. In EEC two pathways may lead to GLP-1 secretion in response to carbohydrates and sweeteners: the glucose transport pathway and the sweet taste signaling pathway (STSP). Gut microbiota dysbiosis related to metabolic diseases may result in altered STSP in EEC thus contributing to impaired GLP-1 secretion (1). My project aims to investigate whether prebiotics such as fructooligosaccharides (FOS) and SCFA are beneficial to GLP-1 secretion in obesity. The effect of FOS supplementation was evaluated during the onset of obesity in mice and the GLP-1 secretion was studied in response to a sweetener and SCFA in a murine EEC line.

Material and methods: Mice were supplemented with FOS during the onset of obesity with a high-fat diet (HFD) and genes encoding factors of the STSP were studied in the intestine. In the murine EEC line STC-1 STSP gene expression was performed, and GLP-1 secretion was evaluated in response to sucralose a specific activator of the STSP and to SCFA.

Results: During the onset of obesity, FOS supplementation appears to have a beneficial preventive role against HFD deleterious effects on mouse metabolic parameters but does not improve the STSP gene expression. The STSP seems to be responsible for 50% of GLP-1 secretion in STC-1 cells and as expected, glucose, sucralose and SCFA (butyrate, acetate) stimulate this secretion.

Conclusion: As expected, HFD induced obesity and insulin resistance while FOS supplementation dampened these alterations without affecting the intestinal STSP gene expression. GLP-1 secretion in EEC appears to be equally dependent on the glucose transport pathway and the STSP. The interaction between SCFA and the STSP to modulate GLP-1 secretion in EEC remains to be demonstrated. I will pursue this question using a murine enteroid model.

(1)- Le Gléau & al, AJP Endocrinol & Metab, 2021.

Keywords: enteroendocrine cells, sweet taste, gut microbiota metabolites, obesity, enteroids

4- PCOS metabolomic profile according to BMI

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Introduction: PCOS is frequently associated with metabolic disorders such as obesity and/or insulin resistance. A metabolic assessment is recommended by 2023 ESHRE PCOS guidelines regardless of BMI. However, literature on PCOS normal-weight patients is less prolific and increased risk of diabetes is contested in this population. The aim of this study was to characterize normal-weight PCOS metabolomic profiles.
Materials and methods: We conducted a retrospective study in the Pitié-Salpêtrière endocrinology department between January 2019 and October 2022, collecting clinical and biological data from the check-up performed during day hospitalization. To identify metabolomic profile according to BMI, we used a combined mass spectrometry and machine learning approach.
Results: We included 397 patients, 152 normal body weight patients, 96 overweight patients and 149 obese patients. No difference was found for age with a median age of 26 years. Obesity was predominantly associated with insulin resistance (median HOMA-IR: 3,8) in contrast to normal-weight patients (median HOMA-IR: 1,2, p<0,0001). Obesity was also associated with metabolic abnormalities (dyslipidemia and liver function tests abnormalities, and higher blood pressure). Due to a lower SHBG, bioavailable testosterone was significantly higher in obese patients, but no difference was found in total testosterone levels. A negative correlation was found for AMH and antral follicle count as a function of BMI. In normal-weight patients, we found an interesting increase in the delta5 pathway steroids. Using a machine-learning approach, metabolic parameters were discriminative in obese patients, whereas the steroid profile was discriminative in normal-weight patients.
Conclusion: Obesity in PCOS was associated with metabolic disorders, particularly insulin resistance, whereas an increase in steroids, precisely the delta5 pathway, was found in normal-weight patients. The next step is to compare our cohort with a control cohort and to develop a machine-learning model to discriminate PCOS and control patients.

Keywords: PCOS, metabolomic, mass spectrometry, machine learning

5- Deciphering the precise c-Fos connectome of ocular pain in mice

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Background:
Chronic ocular pain dramatically affects the quality of life of patients and the therapies are still unsatisfactory. To characterize the central corneal pain network, we used the "c-Fos connectome" based on c-Fos neuronal staining, in a preclinical mouse model of chronic ocular pain developed in our team.
Material and methods: Adult male mice received either a topical instillation of 0.2% benzalkonium chloride (BAC) (10µl, twice a day, 7 days; n=5) or no instillation (naive animals; n=5). The spontaneous and evoked ocular pain was evaluated using eye closure ratio and von Frey test.
The brain stem was cut in coronal sections and the rest of the brain in sagittal sections and c-Fos immunostaining was performed. All the sections were imaged with NanoZoomer and 1/5 sections (n=22) per brain was used to build the c-Fos connectome. These sections were aligned with the Allen Brain Atlas using ABBA plug-in in Image J software. Then, regional c-Fos quantification was performed using QuPath software and the c-Fos connectome was built using Cytoscape software.
Results:Topical 0.2% BAC induced corneal damage, ocular discomfort and corneal hypersensitivity. In the brainstem, the projection site of the corneal neurons, a significantly higher number of c-Fos positive cells was observed in BAC mice compared to naive mice. Regional quantification (820 regions per brain) uncovered increased c-Fos positive cells in several brain regions associated with the pain pathways such as the somatosensory cortex, anterior cingulate area and the thalamus.
Conclusions: We develop a new and efficient pipeline of analysis of c-Fos staining in the entire brain that allows us to build a c-Fos connectome to characterize precisely the ocular pain pathways. This study is encouraging to provide valuable insights into the therapy for chronic corneal pain.

Keywords: Oucular pain, Benzalkonium chloride, c-Fos connectome, Brain network

6- Eating behavior and associated quality of life in adults with obesity and neurodevelopmental disorders

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Introduction : Individuals with neurodevelopmental disorders (NDD) are at greater risk of developing overweight or obesity. The development of obesity may be polygenic, but may also be associated with genes/syndromes causing alterations in the neuronal networks involved in the control of hunger and/or satiety, leading to eating disorders (ED). Prader-Willi syndrome (PWS) is characterized by hyperphagia and other severe eating-related behaviors, but phenotypes in other NDD are still poorly described.
Method : 142 adults with NDD from the ObeRar cohort divided into four groups, including the most frequent syndromic forms of obesity including (1) PWS (n=100), (2) Bardet-Biedl syndrome (BBS) (n=8) but also (3) other forms of genetic obesity classified as "certain" or "probable" (n=10) and (4) other NDD not/rarely associated with obesity (n=24) were included. Clinical data and questionnaires completed by parents/caregivers were collected.
Results : Adults with NDD had a high hyperphagic score that was comparable between groups. Patients with BBS were associated with lower foraging perseverance than PWS and other NDD (7(3) vs. 11(5), p=0.002 vs. 10(4), p=0.031). Food selectivity was lower in PWS. Finally, the age of increased interest in food was earlier in BBS (2 years) and PWS (6 years) than in other NDD (11 years). The quality of life associated with these ED was comparably and significantly degraded for the caregivers of adults with NDD (Moderately: 24%; A lot: 51%, p=NS).
Conclusion : Adults suffering from obesity with NDD, whatever the aetiology, present an increase in food intake and a significant hyperphagia. In syndromic forms, this hyperphagia develops early in childhood, and later in other forms, until adulthood. It is therefore essential to detect these ED as early as possible, using specific questionnaires, in order to manage them early, preventing onset of obesity and improve the quality of life of adults/caregivers with NDD.

Keywords: Neurodevelopmental disorder, obesity, eating behaviour, quality of life

7- Obesity as a model of premature immune aging

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In one hand, ageing is defined by a decline in immune competence, coined immunosenescence, and is associated with chronic, low-grade inflammation termed “inflammageing”. During lifetime, the accumulation of metabolism alterations and cellular damages caused by oxidative stress result in increased comorbidity. Altogether, this leads to the development of an immune risk phenotype related to poor vaccine efficacy and increased morbi-mortality. In the other hand, it has been postulated that the central mechanism underlying obesity, one of the leading causes of death worldwide, comes from a persistent low-grade chronic inflammation. Moreover, obese individuals experience a higher prevalence and severity of persistent viral infections with lower immune responses to infections and vaccines. Thus, given the commonalities between ageing and obesity, it has been speculated that overweight could lead to premature immunosenescence.
The project aims to compare the expression of immunosenescence markers between obese and healthy individuals, while investigating the impact of dietary intervention on immune capacity. We have access to samples from 90 obese adults (before and after a 6-week dietary intervention) as well as 50 sex-and age matched healthy individuals. Thus, the immune phenotype and functional responses will be studied by spectral cytometry while the degree of inflammation and crucial markers of obesity will be analyzed by multiplex. Finally, metabolic studies will be performed, and telomeres length will be measured by qPCR in order to assess the immune risk profile of obese volunteers.
Understanding the relation between nutritional factors and immune responses in obese individuals is crucial to design innovative food solutions able to improve immune function in vulnerable individuals.

Keywords: Ageing, Obesity, Immunosenescence, Diet, Vaccines

8- A high-fat diet and prebiotics impact the microbiota throughout the digestive tract

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NutriOmics

The gut microbiota (GM) has been identified as a major player involved in the pathophysiology of metabolic diseases. Obesity and type 2 diabetes are known to be associated with a decrease of microbial diversity and altered GM composition and functions. Modification of the GM by introducing prebiotic supplements may partially restore this imbalance and thus improve the metabolic profile.
Most studies aiming to determine the relationships between GM composition and metabolic health focus on the bacteria present in the stool. Although fecal microbiota is sometimes considered to be a proxy for the GM, variation in environmental conditions in each segment of the digestive tract (DT) result in regional differences in microbial ecology that merit exploration. In addition, prebiotic compounds may also affect the microbial composition of the DT segments differently.
Consequently, we aim to characterize and compare for the first time the bacterial communities specific to the different segments of the TD in a model of non-obese mice versus mice made obese by the high-fat diet and treated or not with a prebiotic (fructo-oligosaccharide) during 15 weeks. In the different experimental arms, we extracted bacterial DNA from ileal, jejunal, caecal and fecal contents of mice and characterized the bacterial communities by Nanopore sequencing.
Our results demonstrate that the composition of the GM differs according to its location in the DT, according to the diet/prebiotics administered and, by extension, according to the metabolic health of the animals. Furthermore, we found strong and significant correlations between metabolic variables in mice and key phyla in both the fecal and small intestine. Finally, we characterized the profile of a bacterial family particularly associated with metabolic health
Our data underscore the relevance of studying the proximal GM to further understand the relationships between the GM and metabolic health.

Keywords: Microbiota, digestive tract, prebiotics, obesity, diabetes

9- Characterization of the anti-Aspergillus fumigatus activity of the antimicrobial peptide ETD151.

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Bronchial colonization with Aspergillus fumigatus (Af) is the starting point for severe infectious complications in patients with chronic pulmonary diseases. The antifungal armamentarium remains very limited and the emergence of azole-resistant-Af became recently a threat. Antimicrobial peptides (AMPs) may exhibit both antimicrobial activity and immunomodulatory effects being thus a tantalizing alternative to conventional antifungals. ETD151, an AMP derived from a lepidopteran, exhibits a strong activity against a phytopathogenic filamentous fungus, Botrytis cinerea. Our objectives are to assess the antimicrobial activity of ETD151 against Af and its effect on bronchial epithelial cells (BEC).
The activity of ETD151 was evaluated on azole-sensitive and -resistant Af clinical isolates. The impact of ETD151 on Af was assessed by analysing the microscopic morphology, by quantifying the hyphal growth, using the galactomannan assay (ELISA) and measuring the Af metabolic activity using a resazurin test. The fungicidal activity was visualized by dual staining of Af with Calcofluor and Sytox-green. The fungal target of ETD151 was explored by competition of EDT151 with glucosylceramides. Finally, the antifungal activity, cytotoxicity (LDH assay) and immunomodulatory activity (IL-8 and 10 synthesis) of EDT151 were measured in a model of infection of BEC (Beas-2B cell line) with Af.
Whether Af isolates were susceptible or resistant to azoles, ETD151 induced morphologic alterations of Af, presenting with short, highly-branched hyphae, inhibited growth and reduced the metabolic activity of Af. The use of Sytox-green confirmed the partial lytic effect of EDT151. Competition studies with glucosylceramides support the role of these glycolipids in ETD151 activity. ETD151 did not increase IL-8 or IL-10 release by BEC and did not induce any cell toxicity detectable.
Finally, ongoing transcriptome analysis should provide a better understanding of the impact of EDT151 on Af. Acquisition of resistance will be also a pivotal step in the assessment of the activity of this AMP.

Keywords: Aspergillus, antimicrobial peptide, antifungal, infaction

10- sMadCAM is decreased after alloHCT, along with gut microbiota dysbiosis, and is associated with hematopoietic recovery

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Background: The use of high-dose chemotherapy conditioning and broad-spectrum antibiotics during allogeneic hematopoietic cell transplantation (allo-HCT) leads to gut microbiota dysbiosis, which negatively affects patient outcomes. Microbiota composition may influence hematopoiesis, and fecal microbiota transplantation after allo-HCT is associated with higher neutrophil levels. However, no study has evaluated the impact of microbiota dysbiosis on hematopoietic recovery. Recent findings have shown the implication of MAdCAM-1–α4β7 axis in the migration of hematopoietic cells outside the bone marrow. Notably, low sMAdCAM-1 levels are a hallmark of gut microbiota dysbiosis. Hence, we aimed to assess whether sMAdCAM-1 levels correlate with hematopoietic recovery after allo-HCT.
Material and Methods: We analyzed sMadCAM-1 levels in a cohort of 279 patients that underwent allo-HCT in Saint-Antoine Hospital, Paris, between 2012 and 2018. sMadCAM-1 levels were assessed by ELISA on serum samples collected before the start of the conditioning regimen, at day 0 and at day 20, around hematopoietic recovery. Antibiotic exposure data were collected from day -30 to day 30 after allo-HCT, with a particular focus on antibiotics with a broad anti-anaerobic spectrum.
Results: We found a significant decrease in sMadCAM-1 level between the preconditioning sample and day 0 (median 8815 pg/mL, versus 4234 pg/mL, $p<0.0001$) and a further decrease in sMadCAM-1 between day 0 and day 20 (4234 versus 3355, $p<0.0001$). In a subgroup of 60 patients with stool and serum sampling at the same time point (day 0), we found a correlation between Shannon diversity index and sMadCAM-1 (spearman r, 0.21, $p=0.056$), and the median sMadCAM-1 concentration was significantly lower in patients with a low versus high Shannon diversity index (4685 versus 6240, $p=0.04$). We then assessed the impact of antibiotic exposure on sMadCA-1 level and found that early use of antibiotics (before day 0), was associated with a significantly lower sMadCAM-1 level at day 0 (3980 versus 4891, $p=0.01$). We were not able to assess the impact of antibiotics on sMadCAM-1 level at day 20, given that only 20 patients did not receive antibiotics during alloHCT. We then assessed the impact of sMadCAM-1 level at day 20 on engraftment. Median absolute neutrophil count (ANC >0.5 G/L) and platelet recovery (>20 G/L) were significantly lower in patients with higher sMadCAM-1 levels at day 20, being 15 days and 10 days, respectively, versus 16 days ($p<0.0001$) and 12 days ($p=0.004$), respectively, in patients

with lower sMadCAM-1 levels. The day 28 cumulative incidence of ANC >0.5 G/L and platelets >20 G/L was significantly higher in patients with a higher sMadCAM-1 level, being 99.0% and 91.6% versus 94.8% (p=0.002) and 83.3% (p=0.02), respectively, in patients with lower levels.
Conclusions: We found that alloHCT is associated with significant decrease in sMadCAM-1 levels, which correlate with gut microbiota diversity, particularly in patients receiving broad-spectrum antibiotics. Furthermore, we found that sMadCAM-1, known to be involved in hematopoietic cell output from the bone marrow, was associated with neutrophil and platelet recovery after alloHCT, suggesting that microbiota dysbiosis mediates a detrimental effect on hematopoiesis through modulation of the MAdCAM-1–a4b7 axis.

Keywords: sMadCAM-1,allo-HCT,antibiotics, microbiota, hematopoietic recovery

11- Etiopathogenesis of Alzheimer's disease : role of Herpes Simplex Virus Type 1 (HSV-1)

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Neurotropic herpesviruses, and in particular Herpes Simplex Type 1 (HSV-1), are among the infectious agents that focus many of the attention regarding the hypothesis of a microbial actor in Alzheimer's disease (AD). HSV-1 primo-infection leads to a life-long presence of the virus in the body, the trigeminal ganglia (TG) being the main latent viral reservoirs from which the virus can periodically reactivate. Each TG is directly connected to the brainstem making the virus able to neuroinvade the brain.
HSV-1 is highly prevalent in the aged population and genomic-proteomic studies underscore an HSV-1 enrichment in AD brains. Epidemiological data have confirmed the link between Herpes Viruses infection and risk of developing AD. Moreover, a limited number of experimental studies indicate that HSV-1 could trigger Aß production-deposition as well as increased phosphorylation of Tau reminiscent of AD brain lesions.
Using a multidisciplinary approach based on histological (immunohistochemistry and in situ hybridization) and biochemical analysis, we study the virus-induced initial development of AD-like proteinopathies in the cotton rat model to confirm that HSV-1 is able to induce Tau and Aß pathologies, especially at the level of the brainstem where early AD lesions have been described in human tissues. Our results show that HSV-1, following peripheral infection, is able to invade the brain of cotton rats (brainstem and cerebellum) and that this viral neuroinvasion is associated with increased inflammation and tissue damage. We also showed that Aß peptide is abnormally deposited in the brains of infected rats and that hyperphosphorylated Tau protein staining is higher in infected rats compared to uninfected rats. We also initiated epidemiological studies in patients with Alzheimer's disease vs aged non-demented subjects to evaluate the associations, in humans, between herpes virus infection (serological status and titers) and AD biomarkers.

Keywords: Alzheimer's disease / Herpes Simplex Virus Type 1 / Neuroinvasion / Histology

12- Altered septin functions in intracellular infection of bronchial epithelial cells by Pseudomonas aeruginosa in cystic fibrosis

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Lung disease is the main cause of mortality in cystic fibrosis (CF). Infection of the lungs by pathogens and subsequent inflammation alters bronchial tissue, leading to a decline in lung function. Pseudomonas aeruginosa (P. aeruginosa), a bacterium present in almost 50% of adult patients, is highly resistant to antibiotics and requires new treatments. We investigated the involvement of the septin cytoskeleton (SEPT) in the intracellular infection process of P. aeruginosa. SEPTs have been implicated in the control of bacterial infection. We studied the role of SEPT in the control of P. aeruginosa infection in CF bronchial epithelial cells.
We used CF and non-CF primary bronchial epithelial cells, two bronchial epithelial cell lines, 16HBE (wild-type and F508del/F508del) and BEAS-2B. P. aeruginosa PAK-GFP (Green Fluorescent Protein) strains were used. Bacterial internalization experiments were performed with tobramycin to eliminate extracellular bacteria.
The mRNA expression levels of the 13 SEPTs are similar in CF and non-CF cells. Our work then focused on the three SEPT most highly expressed in bronchial cells: SEPT2, 7 and 9. Whether infected or not, protein expression of these SEPTs showed no difference between CF and non-CF cells. We observed that SEPT7 was able to form cage-like structures around intracellular P. aeruginosa in all our models. We also found that inhibition of each of the three SEPTs led to an increase in the number of intracellular bacteria in non-CF cells. In contrast, this effect was not observed in CF cells. We also found that the differences observed in non-CF cells did not occur at the start of infection, suggesting that SEPT do not play a role during the bacterial internalization phase, but later, when the bacteria are intracellular.
Our study shows that SEPT2, 7 and 9 are essential for controlling P. aeruginosa infection of bronchial epithelial cells. We have also shown that, in the context of cystic fibrosis, they are not altered quantitatively but functionally, inducing greater infection of bronchial epithelial cells. The next step will be to characterize this process in primary bronchial epithelial cells differentiated at the air-liquid interface and then study persistence.

Keywords: Cystic fibrosis, septin, Pseudomonas aeruginosa

13- Divergent ecological trajectories of fecal microbiota transplantation in IgA deficient mice versus wildtype mice

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Objective: The absence of secretory IgA (sIgA) is associated with an altered gut microbiota ecosystem in selective IgA-deficient patients (Fadlallah et al. 2018;PMID:29720448). Mucosal secretion of sIgA into the gut lumen is a major contributor to immune-regulation of the gut microbiota. Here we propose to study the ecology of the intestinal microbiota in IgA deficient and wildtype mice colonized by fecal microbiota transplantation (FMT). We aim to demonstrate that the ecological impact of IgA-deficiency observed in humans is indeed causally linked to the absence of IgA.
Methods: Mice were rendered axenic with a high-dose broad-spectrum antibiotic treatment and gavaged with homogenized human feces. Gut microbiota composition was monitored by 16S rRNA gene analysis over 3 weeks. The proportion and identify of IgA-bound gut commensals was analyzed by flow cytometry, cell sorting and sequencing (IgAseq).
Results: Gut microbiota colonization post-FMT was successful since we could demonstrate temporal changes in microbiota composition both in IgA-deficient (PERMANOVA=0.002) and wildtype (PERMANOVA=0.004) mice. Indeed, 3 weeks post-FMT, two new ecological steady-states were installed in IgA-deficient and wildtype mice, respectively (PERMANOVA=0.034). We observed several differentially abundant microbial species between IgA-deficient and WT mice, which are known to be frequently IgA-bound (e.g. Staphylococcus and Akkermansia genus).
Conclusions: Here we demonstrate a causal link between the absence of secretory IgA and gut microbiota colonization. This is well in line with previous associative studies in humans. Our research paves the way for our understanding of how host immunity regulates gut microbiota ecology.

Keywords: Microbiota ecosystem, FMT, IgA deficiency

14- Impact of prenatal prebiotic intervention on humoral immunity to maternal and infant gut microbiota during pregnancy and early life

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Objective: We showed that alterations of microbiota composition at birth and proportion of bacteria bound to immunoglobulins precede later development of allergy in children (Villette et al. in revision). We want to assess if prebiotic supplementation during pregnancy impacts early-life colonization and allergic manifestations. Indeed, prenatal prebiotic supplementation reduces the risk of developing allergic disease in mice (Selle et al. 2022 PMID:35069524). Here we aim to evaluate the impact of prenatal prebiotic intervention on IgA-bound gut microbiota and colonization during the first year of life in atopic children.
Methods: We analyzed longitudinal samples from a randomized, double-blinded cohort with prenatal prebiotic intervention including 115 allergic mothers and their children. Stool samples were analyzed at 8 time points: pregnancy, birth, two months and one year after birth. Proportions of Immunoglobulin-bound microbiota were analyzed by cytometry. Bacteria bound and unbound to IgA were separated and analyzed by 16S rRNA gene sequencing (IgASeq).
Results: Children from supplemented mothers present a higher microbial load (microbes per gram of stool) during the first week (FW) of life ($p=0.014$). The proportion of bacteria bound to IgA increases during pregnancy and the first year of life. Breastfed children present a higher proportion of bacteria bound to IgA than formula fed children. Prenatal prebiotic tends to modify the IgA-bound microbiota profiles at FW.
Conclusions: Prenatal prebiotic increases the quantity of bacteria in the gut. Age and breastfeeding influence immunoglobulin profiles during pregnancy and the first year of life. We will identify IgA-bound microbes and their association with allergic manifestations.

Keywords: Microbiota, IgA, Early life, Pregnancy, allergy

15- Characterization of adaptive NK cells during paradoxical reactions in Tuberculosis

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Tuberculosis (TB) remains a major public health problem, with about 10 million new cases each year. TB is a curable disease but its treatment takes time, with sometimes serious side effects. The paradoxical reaction (PR) usually occurs within the first few months of treatment and may occur in up to 30% of cases in immunocompromised patients (IRIS). PR is characterised by excessive and disseminated inflammation that warrants the use of anti-TNF therapies. Natural Killer (NK) cells are among those that produce the most TNF-α in TB patients. These innate immune lymphocytes also produce IFN-γ and have direct cytotoxic functions on infected cells. NK cells were found to be more numerous and more active in HIV-TB patients with IRIS compared to those without IRIS, suggesting a deleterious role of NK cells in the worsening of the disease.
The objective of our study is to determine the role of these NK cells in TB and in particular in PR. We performed phenotypic and functional (degranulation, cytokine production) analyses of NK cells by flow cytometry from PBMC in 28 patients with disseminated tuberculosis with or without paradoxical reactions from the national ParaTB cohort (PHRC-N09-01-25, NCT012529). Initial results show the presence of a subpopulation of NK cells co-expressing the NKG2C activator receptor and CD57 differentiation marker, characteristic of adaptive NK cells, in TB patients and more preferably in patients with PR (Fisher test, p=0.04). We have also shown that these adaptive NK cells produce more TNF-α than "conventional" NK cells.
Our results suggest that adaptive NK cells may play a deleterious role by producing TNF-α during tuberculosis disease, particularly in patients who develop PR.

Keywords: NK cells, Tuberculosis, Inflammation, Innate immunity

16- Overcoming Treg cell instability for cell therapy

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T cells are the major players in the specific immune response. The T cell population includes CD4 T cells, which are not a homogeneous population. However, after leaving the thymus, two populations of CD4 T lymphocytes can be distinguished: conventional CD4 T lymphocytes, which differentiate into helper T lymphocytes after encountering a foreign antigen, and natural CD4 regulatory T lymphocytes (tTregs), which play a central role in controlling autoimmune diseases. Another population of Tregs called induced peripheral Tregs (iTregs) are derived from naive CD4 T lymphocytes.
These iTregs have a CD25+FOXP3+++ phenotype and share the same immunosuppressive properties as natural regulatory T cells, but have a broader TCR repertoire directed towards foreign antigens. The high number of naive CD4 cells present in peripheral blood could be an interesting alternative based on the conversion of naive CD4 cells into iTregs with immunosuppressive properties.
Although their therapeutic potential is undeniable, the use of regulatory T lymphocytes in cell therapy raises some difficulties. The maintenance of the suppressive function of the natural human and induced Treg population (iTreg) appears to depend essentially on high and stable FOXP3 expression. The expression of FOXP3 is unstable and could lead to the conversion of Treg cells into effector cells producing cytokines with effector pathogenic potential such as IL-2, IFNγ and IL-17. A better understanding of iTreg cell signaling pathways and the identification of new transcriptomic signatures of these cells will not only provide new information on iTreg cell biology but also allow the optimization of culture conditions to obtain large numbers of stable and functional cells for injection into patients.

Keywords: Tregs, FOXP3, Naive CD4 cells, Thymus,TCR

17- Tertiary Lymphoid Structures role in rejection after uterus transplantation

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Centre d'Immunologie et des Maladies Infectieuses; Equipe 9 - NK and T cell immunity, Infection & Cancer

Background: Women with absolute uterine infertility (1 in 500) faced bleak prospects for conception until the successful clinical demonstration of uterus transplantation (UTx) in 2014 by Prof. Brännström's Swedish team, resulting in the birth of a healthy baby. With over a hundred UTx procedures performed globally and more than 30 live births recorded, this emerging technology is transitioning from experimental to clinical practice. However, UTx presents unique immunological challenges as a composite vascularized tissue, encompassing issues of the transplanted organ, pregnancy, and the semi-allogeneic fetus. Despite advancements, graft rejection remains a significant concern in 60% of UTx patients, predominantly within the first three months, impacting pregnancy progression and delivery. Conversely, the decidua provides an immunosuppressive milieu regulating alloimmune responses. Additionally, UTx patients may experience miscarriages, implantation failures, pre-eclampsia, and pregnancy-related infections.

Materials and Methods: Drawing from preliminary insights, the primary aim of the project is to conduct a comprehensive analysis of tertiary lymphoid structures (TLS) in cervical biopsies to elucidate the immune mechanisms underlying rejection in UTx. Scientific objectives encompass characterizing TLS, evaluating their impact on graft outcomes, the materno-fetal interface, and pregnancy complications, identifying TLS-associated biomarkers in non-invasive samples (cervical smears and peripheral blood), and enhancing understanding of UTx rejection and pregnancy pathophysiology for improved diagnostic and therapeutic strategies.

Results: Initial findings from cervical biopsy samples of five UTx patients in Sweden revealed graft rejection-associated gene expression changes, notably skin and immune system markers, including T and B cell and macrophage activation and differentiation. Mass cytometry imaging confirmed TLS presence in three samples.

Conclusion: The research plan comprises four work packages focusing on biological sample collection and analysis, bioinformatic and statistical analysis, and correlation with clinical and histological data. The project aims to unravel the role of TLS in UTx rejection and pregnancy outcomes while identifying potential biomarkers for rejection diagnosis and treatment.

Keywords: Uterus transplantation, tertiary lymphoid structures, cervical biopsies, infertility

18- Machine learning to accelate characterization of single plasma cells

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Quantification of antibody secretion and antibody affinity for their antigen is essential to understand the immune response to different stimuli. DropMap is a droplet microfluidic technique coupled to time-lapse microscopy for studying antibody-secreting cells with single-cell resolution. Antibody secretion by droplet-encapsulated cells and the affinity for their antigen is kinetically measured using a fluorescent immunoassay, which require large scale image analyses. DropMap is currently limited in its throughput and robustness by requiring manual verification to select only droplets containing a single cell.
We have developed a machine learning model able to classify droplets as empty, single-cell, or multiple-cell based on the convolutional neural network ResNet-50. Importantly, our algorithm can classify droplets also containing non-cellular structures, unlike previously described models, and achieve accuracies above 90%. This model makes the analysis of DropMap experiments faster by 100-fold, more reproducible, and less biased. Our method may be applied to other functional droplet-based bioassays, provided that the kernel used during image preprocessing is adapted to efficiently remove the non-cellular structures inside the droplet.
Taken together, DropMap assays and automated droplet classification enables fast plasma cell characterization. This combined approach will be used to study the development of autoimmune diseases and the immune response to infection and vaccination.

Keywords: Microfluidics, machine learning, image classification

19- Characterization of immune cell dynamics during low-dose interleukin-2 treatment in healthy volunteers

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Interleukin-2 (IL-2) plays a central role in the regulation of the immune system. Low-dose administration of IL-2 (IL-2LD) has been shown to be effective in activating regulatory T cells (Treg) without affecting effector T cells (Teff). Thus, IL-2LD has been proposed for treating autoimmune diseases with promising results in various indications (PMID: 25882245). Understanding the direct and indirect effects of IL-2LD on a healthy immune system is crucial.
To understand these effects, we have samples from the HEALTHIL-2 clinical trial (NCT03837093). In this trial, 26 healthy volunteers received different doses of IL-2 (0.33, 1.0, 1.5 or 3.0 MUI per day) or placebo. The administration schedule consisted of a 5-days induction phase (daily injections) followed by a maintenance phase (weekly injections over 2 months). During this trial, blood samples were taken at different times from baseline to 2 months and processed using different omics techniques: cytometry, immunoproteomic and transcriptomic.
The results show that IL-2LD significant modulates the Treg/Teff ratio in favour of Tregs in a dose-dependent manner. We identified a continuum of Treg differentiation following treatment. Surprisingly, the Treg response was associated with a dose-dependent increase in IL-2 and its soluble receptor in serum. In parallel, we identified a transcriptomic signature common or specific to the different doses. We observed an increase in CD56++ NK cells and eosinophils in a dose-dependent manner. Monocytes also responded to treatment, with an increase in transient monocytes and a decrease in conventional monocytes. Finally, a reduction in the proportion of B cells was observed, with IL-10 production by these cells.
This project will provide an in-depth understanding of the mechanisms of response to IL-2LD. It will improve treatments by defining an optimal dose that would increase the efficacy of treatment while limiting the risks, leading to more personalized medicine.

Keywords: Low-dose interleukin-2, autoimmune diseases, systems immunology, personalized medicine

20- Interactions between intestinal microorganisms: mechanisms, roles and therapeutic potential in inflammatory bowel diseases

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The human gut harbors a large microbial community where diverse species interact with each other. This large community is shaped by positive and negative interactions ranging from competition (ex: bacteriocins) to cooperation (ex: cross-feeding), which contribute together to eubiosis in the gut. In comparison to the gut microbiome in healthy population, dysbiosis in microbiota of inflammatory bowel disease (IBD) patients is often highlighted. We hypothesize that the interplay between microorganisms (competition and cooperation) in the gut is perturbed in IBD patients with impact on host metabolism and immune response and therefore disease development and chronicity. My PhD project aims at (i) exploring the interplay between the intestinal microorganisms and identifying the molecular mechanisms (metabolites, genes, signaling pathways…) underlying these interactions, (ii) analyzing the biological effects of these interactions on host and (iii) assessing the relevance of this interplay in humans. As interplays rely mostly on secreted molecules, we screened the effects of culture supernatant between IBD-associated microbes and we observed surprisingly that supernatants from Fusobacterium nucleatum and Veillonella parvula that belong to pro-inflammatory species affect positively the fitness of two anti-inflammatory species Agathobacter rectalis and Blautia hansenii, respectively. Coculture and membrane-separation model results indicated that the interactions in these two pairs are unidirectional and contact-independent. We also found that the molecules responsible for the effects in the supernatants of F. nucleatum and V. parvula are heat-resistant, enzymes-resistant and less than three kilodaltons. Identification of the molecules of interest will now be achieved by metabolomics and genetic screens. The impact of the interactions on the microbiota and on the host will further be studied, using SHIME® and in vivo models respectively. Understanding the ecological relations between gut microbiota is an important aspect of treatment of disease and can help us to develop a new therapeutic in future.

Keywords: Inflammatory bowel disease, dysbiosis, micobial interactions

21- Characterization of the immune-mutanoma and discovery of new immunomolecular biomarkers for non-small cell lung cancers in people living with HIV: the IDeATIon program

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CIMI-Paris Equipe NK and T cell Vincent Vieillard

Background : Non-small cell lung cancer (NSCLC) is the leading cause of cancer-death worldwide, and especially in people living with HIV (PLWHIV), who face poor prognosis. However, the specific molecular and immunological traits of NSCLC in PLWHIV remain largely unexplored. We hypothesize that the HIV infection influences these parameters by default of immune selection pressure. This study aims to analyze the impact of HIV infection on NSCLC molecular (Tumor Mutational Burden (TMB), oncogenic pathways, and molecular signatures) and immunological (neoepitope burden, immunogenicity, and tumor microenvironment (TME)) profiles.
Materials : NSCLC patients have been prospectively recruited: PLWHIV (cases) and immunocompetent (IC)(controls). Whole exome sequencing (WES) and bulk RNA sequencing (RNAseq) were performed on tumor samples. Tumor neoepitopes were predicted through local bioinformatics, HLA typing, and WES, augmented by RNAseq when available. Selected immunogenic neoepitopes were synthesized for ex-vivo assessment of systemic antitumor response using ELISPOT IFN-γ and flow cytometry.
Results: Analysis of 27 patients (15 PLWHIV, 12 IC) revealed comparable clinical profiles between groups. Predominant molecular alterations manifested as single nucleotide variations (SNVs), primarily C>A and C>T transitions. Molecular profiles exhibited similarity between the two groups concerning TMB, molecular signatures, and affected oncogenic pathways. Predicted neoepitopes (50,432) were predominantly HLA2-predicted (84%) and derived from missense SNVs (91%). Functional validation entailed testing 655 neoepitopes (median 30/patient) with IFN-γ ELISPOT uncovering systemic responses against neoepitopes in 8/20 patients (3/10 PLWHIV, 5/10 IC), notably absent in PLWHIV with CD4 nadir <200/mm3 (0/4). High DNA variant allele frequency correlated with neoepitope immunogenicity. Ongoing analysis includes evaluating the immunogenicity of common neoepitopes from shared mutational hotspots and further TME exploration.
Conclusion: In conclusion, HIV infection, particularly in the context of low CD4 nadir, modulates NSCLC immunogenicity despite similar molecular profiles. HIV-induced immunosuppression within the TME may attenuate lymphocytic antitumor responses, potentially exacerbating the prognosis for NSCLC patients.

Keywords: Non-small cell lung cancer / HIV / Molecular profile / Immunological profil

22- Exploration of the role of the ICOS/ICOSLG interaction in glioblastoma in a humanized mouse model

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Immunotherapies targeting immune checkpoint inhibitors represent a significant advancement in cancer treatment by unleashing the body's immune system against tumors. However, while these therapies have shown remarkable success in various cancers, glioblastoma (GBM), the most aggressive brain cancer, has proven resistant due in part to its immunosuppressive microenvironment partialy driven by regulatory T lymphocytes (Treg). Then, strategies aimed at neutralizing or modulating Treg activity within GBM tumors hold promise for enhancing current GBM treatments.

ICOS is a co-stimulatory molecule expressed on T lymphocytes following activation of the T cell receptor. Its sole ligand, ICOSLG, is expressed on the surface of antigen-presenting cells as well as cancer cells, with elevated expression levels observed in GBM cells compared to healthy tissue. Moreover, heightened ICOSLG expression correlates with reduced survival rates and disease-free intervals in GBM patients. Given that ICOS is predominantly expressed by Treg, we hypothesize that ICOSLG plays a pivotal role in GBM pathogenesis by influencing Treg development.

Conventional GBM mouse models are constrained by inherent interspecies disparities between mice and humans. To overcome this limitation, we have transplanted patient-derived GBM cell lines orthotopically into humanized mice, allowing for studying human anti-tumor immune responses in a more physiologically relevant setting. Our findings demonstrate robust tumor growth in both humanized and non-humanized mice, indicating a lack of immune rejection. Additionally, utilizing flow cytometry, we have identified various populations of human leukocytes (myeloid cells and lymphocytes) within the brains, underscoring the feasibility of our approach.

Our ongoing investigations will focus on unraveling the mechanisms by which ICOSLG expressed by GBM cells influences the immune system and Treg dynamics. Anticipated outcomes of this study promise to enrich our understanding of the functional implications of ICOSLG expression within tumors, potentially paving the way for more effective therapeutic interventions against GBM.

Keywords: Glioblastoma, immunotherapy, regulatory T cells

23- Exploration of the dialogue between T helper and B lymphocytes in multiple sclerosis: contribution of a microfluidic 3D culture model

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Multiple sclerosis (MS) is a chronic autoimmune disease affecting the central nervous system (CNS). Demyelination, the damage to the protective myelin sheath around nerve fibers, is the result of an inflammatory autoimmune process, in which the Epstein-Barr virus (EBV) may play a critical role.
EBV is a common herpesvirus that infects over 90% of adults worldwide. It establishes a lifelong persistent infection in memory B cells, with periodic reactivations. Recent research suggests elevated immune responses against EBV can distinguish individuals who will develop MS after primary EBV infection (Bjornevik et al., 2022). These heightened EBV-specific immune responses persist during disease progression and correlate with disease activity (Farrell et al., 2009).
The elevated EBV-specific immune responses may drive EBV-specific CD8+ T cells into the CNS (Gottlieb et al., 2024; Schneider-Hohendorf et al., 2022). This could generate cross-reactive T and B cell populations recognizing myelin autoantigens (Lanz et al., 2022; Tengvall et al., 2019; Thomas et al., 2023; Wang et al., 2020) and compromise immune control of autoreactive B and T cell responses (Vietzen et al., 2023a).
To further investigate these mechanisms, the project will use a set of complementary approaches. First, the frequency and differentiation status of EBV-specific T cells will be compared between MS patients and healthy controls using intracellular cytokine staining (ICS) and an activation-induced marker (AIM) assay. Second, memory B cells specific for EBV antigens will be amplified using the microfluidic chip system, frequency of plasmablast induction and CD4+ T cell/B cell cluster formation will be measured upon antigenic restimulation. Cross-reactive antibodies capable of binding both MS and EBV antigens will also be detected in the chip effluent. Third, additional specific CD4+ help will be provided to increase the sensitivity of cross-reactive memory B cell detection in the chip.

Keywords: Immunology, Multiple sclerosis, EBV, Organ-on-chip

24- Investigating the metabolism of T cells in physiological contexts

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The regulation of cellular metabolism is a central element governing the stability and function of T cells. Current experimental procedures assessing metabolism are performed in-vitro in artificial culture media and do not accurately recapitulate physiological conditions. In addition, most techniques have a limited capacity of evaluating the metabolism of rare immune populations such as tissue T cells. A newly innovative method was developed, derived from the SCENITH technique, allowing to investigate cellular metabolism in-vivo. The metabolic profile of regulatory T cells, conventional CD4+ T cells and CD8+ T cells in vivo are different from the ones obtained when performing SCENITH in-vitro. In-vivo, the metabolism of T cells appears to be tissue specific. Splenic T cells have a metabolism dependent on glycolysis. In the lungs, T cells preferentially use oxidative phosphorylation to generate energy. The maintenance of Foxp3 expression of lung regulatory T cells appears to be affected by oxidative phosphorylation. These results highlight the importance of studying immunometabolism in-vivo, potentially of improving the successful translation of pharmaceutical agents targeting metabolic pathways.

Keywords: Immunology, metabolism

25- The Immunoregulation of Aorta Inflammation in Large-Vessel Vasculitis

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Background: Large-vessel vasculitis (LVV) are rare systemic vasculitis affecting the aorta, represented by Takayasu's arteritis (TAK) and Giant cell arteritis (GCA), which share pathogenic, clinical, therapeutic and prognostic features. Distinct infiltrating immune cells triggers inflammation across all vascular layers, with plasticity across blood and target tissues according to the inflammatory milieu. The phenotype, molecular mechanisms and plasticity of circulating and tissue-infiltrating immune cells in LVV are not fully known. We have previously demonstrated a great enrichment in several inflammatory pathways across aortas and circulating T cells following transcriptomic analyses of bulk aortic tissue and T cells from GCA aortitis patients. We now proceed to a more holistic approach regarding LVV.

Material and methods: Blood from active TAK and GCA patients and healthy donors are analyzed. Multiplex cytokines assays will be performed in patients' sera. Flow cytometry deep immunophenotyping characterize several immune cells subpopulations. Microarray blood transcriptomics on MACS-sorted lymphocytes will unravel dysregulated genes. FFPE inflammatory aortic samples from TAK and GCA patients undergoing surgical repair have been retrieved, as well as non-inflammatory and disease controls (i.e., clinically isolated aortitis). Single-cell (Chromium®) and spatial (Visium®) transcriptomic aortic analyses are ongoing. Single cells from adventitia and media-intima aortic layers are analyzed separately thanks to laser microdissection (Leica®) of the external elastic membrane. Eight-plex immunofluorescence experiments (Opal®) will be used to confirm specific cells' co-localization in inflamed aortas.

Results: Preliminary analyses of deep immunophenotyping from TAK patients reveal an increase in several lymphocyte subpopulations (eg, Th17.1, Tfh, Tfr, TLM B-cell, CD8+TEMRA, etc.) as compared to controls. Single-cell and spatial transcriptomic analyses have been performed for 6 and 8 aortic samples, respectively, and allows the differentiation of the transcriptional profile and dysregulated pathways of various cell subpopulations between vascular layers and between LVV.

Conclusions: State-of-the-art techniques will allow for unprecedented pathogenic insights in LVV.

Keywords: Large-vessel vasculitis, Aortitis, Takayasu's Arteritis, Giant Cell Arteritis, Clinically Isolated Aortitis

26- Association of immune checkpoint molecules on recombinant Virus-Like Particle-based tolerogenic vaccines for oral administration

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UMRS 938, CRSA, Système immunitaire et neuroinflammation

Our team has designed tolerogenic vaccines based on antigen recombinant virus-like particles (tVLPs) displaying Immune Checkpoint (IC) at their surface to induce antigen-specific immune tolerance. The proof of concept with the CTLA-4 IC has shown efficacy in a mouse food allergy model. We currently investigate the possibility to use alternative IC and/or combine different IC to improve their therapeutic efficacy. We also propose to optimize tVLPs for oral route administration.
We have selected different IC, known to negatively modulate the activity of dendritic cells (DC), effector T cells (Teff), conventional T cells (Tconv) or improve regulatory T cells (Tregs). We evaluated the effect of OVA-recombinant tVLPs displaying single or combo IC on DC and DO11.10 TCR-transgenic T cells (OVA-specific Tconv and Treg cells) in vitro or in vivo. We also evaluated the impact of adding parasite-derived VSP proteins conferring protection from the conditions of the digestive tract.
tVLPs carrying IC stimulate tolerogenic responses inducing either a tolerogenic phenotype of DCs, proliferation and/or activation of Tregs, suppression of Teff proliferation and/or an exhaustion-like phenotype of Tconv. Each tVLP acts differently according to the carried IC and we observed a synergic effects of combined IC. We also observed that adding VSP on tVLPs protects the vaccine without affecting IC activity. According to our first results, specific IC can be selected to induce tolerogenic response and combined onto tVLPs to optimize their effectiveness. Adding VSP opens the possibility of using tVLPs orally to beneficiate of the intestinal pro-tolerogenic environment. We plan now to evaluate their therapeutic efficacy in allergy.

Keywords: Tolerogenic vaccine, immune checkpoint, allergy

27- Development and optimization of Interleukin-2 (IL-2) self-sufficient Tregs as cell therapy for the treatment of cGvHD

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i3 lab, team of David KLATZMANN

Regulatory T cells (Tregs) are crucial in preventing autoimmune diseases, making them a promising avenue for treatment. One approach to harness their therapeutic potential is their activation and expansion by in vivo administration of interleukin-2 (IL-2), i.e. the cytokine supporting the differentiation, survival and fitness of Tregs. However, this may not be sufficient for highly inflammatory diseases. To address this, the lab generated IL-2 self-sufficient enhanced-Tregs (e.Tregs) which express a mutated form of IL-2 (IL-2m) tailored specifically for Tregs. Yet, the optimal IL-2m production level remains elusive. Insufficient IL-2m may fail to maintain e.Tregs' long-term survival and expansion, while excessive production could lead to eTregs' exhaustion or bystander activation of effector T cells and Natural Killers. Therefore, our primary objective was to engineer a vector with an optimal promoter for controlling IL-2m production by eTregs.
To achieve this, we evaluated promoters of different strengths: strong, medium and weak. These vectors express IL-2m together with a Thy1.2 reporter gene. The best promoter will be selected based on its capacity to provide optimal e.Tregs survival measured by their long-term persistence; and its bystander activation of endogenous Tregs measured by their expansion and expression of activation markers. Finally, therapeutic efficacy will be assessed in a GvHD preclinical model.
We effectively transduced murine Tregs with the different vectors. In vitro, the Mean Fluorescence Intensity (MFI) of Thy1.2 (reflecting IL-2m production) corresponded to the promoter strengths, with the strong promoter yielding the highest MFI and the weak promoter the lowest. This pattern persisted in vivo when eTregs were injected in both immunodeficient and immunocompetent mice. Furthermore, eTregs survival was dependent on the promoter strength, as eTregs harboring the strong promoter displayed long-term survival. In conclusion, IL-2m production appears critical for eTregs' survival and expansion, and it correlates with the strength of the promoter employed.

Keywords: Tregs, IL-2, cell therapy, autoimmunity, GvHD

28- Preclinical development of a 3rd generation interleukin 2 targeted to inflammatory sites

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Immunology - Immunopathology - Immunotherapy (I3) David Klatzmann

Stimulation of regulatory T cells (Tregs) holds significant promise for the treatment of autoimmune diseases. Our laboratory has pioneered the use of low-doses IL-2 (IL-2LD) in clinic to preferentially stimulate and expand Tregs in vivo, resulting in beneficial effects in autoimmunity. However, off target immune cell activation and short serum half-life limit the clinical potential of IL-2 treatment. Another concern is that while Tregs efficiently control low-grade inflammation, they can lose their efficacy in highly inflammatory environments. In this context, we have developed a 3rd generation of targeted IL-2 that will target IL-2 to inflammatory sites where this cytokine is much needed.
Our targeted IL-2 (IL-2IT) consists in an IL-2 moiety (native or mutated) and a scFv for the targeting (from a prototypic anti-OSE Ab) both linked to a fragment of C4 binding protein beta (C4BPβ) allowing dimerization of the IL-2IT. We have evaluated the PK and PD profiles of the proteins, as well as their in vitro and in vivo targeting capacities to OSE. Furthermore, we have examined the therapeutic properties of these molecules in two different models of auto-inflammatory diseases (DSS-induced colitis and psoriasis).
In this study, we demonstrate that by incorporating the scFv and dimerizing the construct, we substantially enhance the pharmacokinetics and half-life of IL-2. IL-2IT effectively expands Tregs in vivo while maintaining better control over effector cells under steady-state conditions. Additionally, IL-2IT exhibits specific binding to OSE in vitro and in vivo in a mouse model of psoriasis. Furthermore, compared to native IL-2, IL-2IT demonstrates enhanced therapeutic efficacy in mouse models of both colitis and psoriasis. These findings offer guidance for the engineering of cytokines with enhanced therapeutic efficacy, paving the way for their potential clinical application in treating autoimmune diseases.

Keywords: Autoimmunity, Tregs, IL-2 mutein, Immunotherapy

29- Exploring the role of tumor 4-1BBL on anti-tumor response in kidney cancer in humanized mice

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CIMI U1135

Kidney cancer is a major issue in oncology, with an increasing incidence related to obesity and aging. Current immunotherapy treatments only benefit a minority of patients highlighting the necessity to explore other mechanisms to prevent disease progression. Among the many co-stimulation molecules associated with poor clinical prognosis, the molecule 4-1BBL has captured our attention. While the stimulating effect of 4-1BBL expressed by antigen-presenting cells on the effector response is well-documented, the impact of 4-1BBL expressed on the surface of renal cancer cells on the immune response remains unclear. The project aims to address this question using genetically modified human kidney cancer cell lines that no longer express 4-1BBL. With these tools, we will determine the role of 4-1BBL in controlling tumor growth in an orthotopic xenograft model in humanized mice. Furthermore, we will explore the possibility that a toxic "reverse signaling" for the tumor cell could be generated in vivo using proprietary antibodies directed against 4-1BBL. The feasibility of the project is demonstrated by numerous preliminary results, from the establishment of the orthotopic xenograft model to the phenotypic and functional analysis of the immune system in humanized mice with a thirty five-color spectrum flow cytometry panel.

Keywords: Oncology - 4-1BBL - kidney cancer - immune system

30- Phenotypic and functional characterization of mutations in the biliary transporter ABCB4: towards development of targeted pharmacotherapy

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In the liver, a series of ATP-Binding Cassette (ABC) transporters catalyze the generation of bile and their dysfunctions lead to rare, evolutionary diseases for which there is often no therapy. My project is focused on ABCB4, which is a specific hepatocellular canalicular transporter involved in biliary phosphatidylcholine (PC) transport. PC is crucial to ensure the solubilization of cholesterol into mixed micelles and to prevent bile acid cytotoxicity and the formation of gallestones. Variations in the ABCB4 gene sequence cause a wide range of biliary diseases, among which, Progressive Familial Intrahepatic cholestasis type 3 (PFIC3) is the most severe. PFIC3 is characterized by early onset of cholestasis that leads to cirrhosis and liver failure before adulthood. The only effective therapy for PFIC3 patients is liver transplantation. More than 500 distinct disease-causing ABCB4 variants have been reported. A challenge is to find pharmacological treatments for the severe forms of these diseases.
ABC transporters share a common basic architecture. Deleterious variants of these transporters affect amino acids important for structure and/or function. My research project is based on a tight interplay between theoretical and experimental approaches to investigate and attempted to correct the potential impact of either nonsense or missense variations identified in patients. Missense mutations are either located in homologous positions of a highly conserved and functionally critical motifs of ABCB4 nucleotide binding domains (NBD1/NBD2) or in transmembrane domains of ABCB4.
We show that all the mutants induce a defect that is rescued by a treatment with specific CFTR modulators both in terms of expression, localization and activity. This proof of concept suggests that CFTR modulators might constitute an efficient targeted pharmacotherapy approach for selected PFIC3 patients carrying mutations that impairs ABCB4 PC secretion activity.

Keywords: ABC transporter, genetic liver disease, PFIC3, CFTR modulator, bile secretion

31- Role of Gli1+ cells in chronic liver diseases

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Introduction: Gli1, a transcription factor of the Hedgehog signaling pathway is a marker of mesenchymal stem cell-like cells in multiple organs including the liver. We aimed to characterize Gli1+ cells in normal liver and determine their contribution to wound healing in distinct models of liver disease. Methods: Single-cell RNA sequencing was used as a first approach to define Gli1+ cells in normal liver. Genetic lineage tracing and ablation of Gli1+ cells was achieved in homeostatic conditions and in models of biliary disease (DDC and BDL) and of MASH. Cell interactions were assessed in cell cultures and organoids. Results: In normal liver, Gli1 mainly demarcated a subpopulation (approximately 12%) of portal fibroblasts characterized by their close apposition to cholangiocytes and Gene Ontology terms related to extracellular matrix and epithelial cell proliferation. Both in the biliary and MASH models, Gli1+ portal fibroblasts were activated and accumulated at sites of fibrogenesis predominantly in portal tracts, notably as multilayers around native bile ducts and around the ductular reaction. Their proliferation was most intense in response to biliary injury and was stimulated by cholangiocyte secretome in culture. The formation of cholangiocyte organoids was boosted by co-culture Gli1+ portal fibroblasts. The genetic depletion of Gli1+ cells caused an inhibition both of liver fibrosis and of the ductular reaction, one week after the onset of biliary injury induced either by DDC or BDL, even though cholestatic injury persisted. Conclusions: Gli1 demarcates a small subpopulation of portal fibroblasts immediately adjacent to bile ducts which promote liver fibrosis and ductular reaction both in biliary and non-biliary models of liver disease. The targeting of these cells could provide an antifibrotic strategy notably in biliary diseases when the cholestatic trigger persists.

Keywords: liver fibrosis; biliary fibrosis; metabolic dysfunction-associated steatohepatitis

32- Identification of mesenchymal cells responsible for liver fibrosis in mice by single-cell RNA sequencing (scRNAseq)

Wenrui GAP

UMRS 938, CRSA, Team Metabolic and biliary, fibro-inflammatory diseases of the liver (J. Gautheron)

Background: Liver fibrosis is a consequence of all chronic liver diseases which causes ~2,000,000 deaths per year worldwide and is characterized by the accumulation of extracellular matrix produced in excess by myofibroblasts (MFs). In the liver, MFs originate from either hepatic stellate cells (HSCs) or portal fibroblasts. Most studies have focused on HSC-derived myofibroblasts (HSC-MFs), however we have uncovered portal fibroblasts with mesenchymal stem cell features (PMSCs) that can generate highly proliferative and proangiogenic myofibroblasts (PMSC-MFs). We have thus postulated that portal MFs (PMFs) play a major role in fibrosis progression. This project aims at defining the transcriptome demarcating PMFs, including PMSC-MFs, in mouse liver fibrosis.

Methods: Two mouse models of liver fibrosis will be used: CCl4 intoxication, that causes a post-cytolitic fibrosis, and Abcb4 KO mice as a biliary model of fibrosis. Cell preparations obtained from the bilio-vascular tree of normal and fibrotic livers will be enriched in mesenchymal cells by depleting cells expressing the lineage markers (Lin) of cholangiocytes (EpCAM), endothelial cells (CD31) and hematopoietic cells (CD45 and CD11b) using cell sorting. scRNAseq analysis of Lin negative cells will be performed.

Results: We assessed Sirius Red staining and Col1a1 mRNA expression by qPCR analysis of to make sure that hepatic fibrosis was comparable between the two models. We modified the protocol previously described for normal liver to obtain cell preparations from bilio-vascular tree of fibrotic livers suitable for scRNAseq analysis, i.e. high cell viability (>80%), no cellular debris and no hepatocyte contamination.

Conclusion: We have set up the experimental conditions that allows us to isolate cells of interest for scRNAseq analysis. scRNAseq analysis are currently underway. Our results will allow us to identify specific markers for PMFs that we intend to target for depletion to limit hepatic fibrosis.

Keywords: liver fibrosis, portal myofibroblasts, scRNAseq

33- Alteration of Kupffer cell homeostasis during ulcerative colitis

Dicken FARDOL
U1166 Eq 5

Background. In inflammatory bowel diseases such as ulcerative colitis (UC), increased gut permeability allows microbial-derived endotoxins to enter the portal circulation and reach the liver where it may impact on hepatic functions. Liver resident macrophages, known as Kupffer cells (KC), are located in hepatic sinusoids where they can sense, notably through their Toll-like receptors (TLRs), the endotoxins liberated in the portal blood. However, the role of KCs in the response to gut-derived signals in the context of UC remains ill defined. Here, we aim to study the role of KCs in gut/liver communications during colitis.
Methods. We used a common model of UC by administrating dextran sodium sulfate (DSS, 3% in drinking water) to mice. Hepatic immuno-inflammatory and metabolic parameters were assessed in response to UC.
Results. Our results show that UC triggers systemic and liver inflammation. Increased numbers of hepatic neutrophils and monocytes were observed and we noticed an elevation in inflammatory gene expression. In addition, KC numbers increased following a proliferation burst that coincided with the onset of UC symptoms. RNA sequencing revealed that KCs were activated during UC and this activation was mitigated when KCs were invalidated for Myd88, an adaptor protein that controls signaling downstream of most TLRs. While UC led to the dysregulation of keys genes involved in hepatic cholesterol and bile acid metabolism together with increased liver and plasma cholesterol levels, these alterations remained in animals lacking Myd88 specifically in KCs.
Conclusion. Our data suggest that UC alters liver immune homeostasis, including KCs numbers and their activation status. However, UC-associated alterations in cholesterol metabolism do not depend on KCs activation. Thus, we now aim at deciphering how KCs exposure to gut-derived endotoxins alters their functions.

Keywords: Colitis Liver Kupffer Macrophage

34- UNRAVELING THE MULTIFACETED ANTITUMOR EFFECTS OF COLD ATMOSPHERIC PLASMA ON CHOLANGIOCARCINOMA

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UMRS938 CRSA - Equipe Gautheron

Cholangiocarcinoma (CCA) is a rare tumor of the bile ducts characterized by a poor prognosis and a rich desmoplastic stroma. As the efficacy of systemic palliative chemotherapies remain quite limited, it is mandatory to develop new therapeutic options against CCA. In this outlook, cold atmospheric plasma (CAP) shows promises in oncology. Generated from the partial ionization of a gas, CAP generates reactive oxygen and nitrogen species that exert deleterious cellular effects leading to cell death or dysfunction.
Human cell lines of CCA and of its microenvironment, namely cancer-associated fibroblasts (CAFs) and tumor-endothelial cell (TECs), cultured in 2D or 3D (spheroids), were treated directly with CAP. In tumor cells, immunogenic cell death (ICD) induction was evaluated in vivo by establishing different vaccination assays and in vitro by measuring the release of DAMPs in the extracellular environment. Concerning the stromal compartment, alterations in phenotype and cell functions were analyzed by live-imaging microscopy in vitro.
We showed that CAP induces antitumor effects that can be direct (i.e tumor cell death) and indirect (i.e stromal cell dysfunctions, activation of immunosurveillance). As proof of the direct antitumor effects, we demonstrated that CAP-triggered oxidative stress decreases tumor cell viability and led to the release of ICD key messengers which stimulate the tumor-surrounding immune cells and promote antitumor immunity, as we demonstrated in vivo. Interestingly, CAP also exhibited indirect antitumor effects by reducing CAF activation, impeding their migration, and inhibiting TEC angiogenic profiles.
CAP opens perspectives for local treatment of CCA. In order to enhance the translational relevance of the technology for the patients, the plasma source has been miniaturized to deliver the plasma in situ via an endoscope (patented). In close collaboration with clinicians, feasibility and safety studies of the endoscopic plasma probe in pigs are in progress.

Keywords: Cholangiocarcinoma - Cold Atmospheric Plasma - Tumor Stroma - Immunogenic Cell Death

35- Tumoral impact of HBSP on the intrahepatic immune cells during liver carcinogenesis

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CRSA, team "Biliary and metabolic, fibro-inflammatory diseases of the liver"

Background: HBV infection is a significant risk factor for Hepatocellular carcinoma Paradoxically, the expression of HBSP resulting from alternative splicing of HBV transcripts has been shown to reduce liver inflammation and fibrogenesis in transgenic mice.
Aim: Characterize the impact of HBSP on immune cell infiltration during liver carcinogenesis.
Materials&Methods: Allografts of stable HBSP-expressing Hep55.1c and antisense control cells were performed in the subcapsular space of Wild-type (WT) and TgHBSP C57BL/6 male mice-livers. After 3-4 weeks, tumor growth and the tumor immune microenvironment (TIME) were evaluated using calipers and FACs. Besides, coculture studies included THP-1 derived macrophages (THP-1m) and TNF-α-treated-HepG2-NTCP cells expressing or not HBSP (Transwell®). The indirect impact of cell-cell interactions was studied by RT-qPCR analyses (iNOS, CD86, CD206, IL10, CCL22 genes).
Results: In allografted WT mice, tumor volume significantly increased compared to control cells. TIME analysis showed a differential recruitment of dendritic cells (higher) and NK cells (lower). Furthermore, the M1-like phenotype of tumor associated macrophages (TAM) was reduced, while no differences were observed in LB, LT or NKT frequencies. Tumors growth was not modified when HBSP was expressed in non-tumoral region (TgHBSP mice). In vitro, THP-1 cells were well differentiated into THP-1m using PMA treatment (100ng/mL), as demonstrated by changes in phenotype (shape and adhesion). The coculture of THP-1m with HepG2-NTCP cells increased only CCL22 expression on THP-1m. TNF- α treatment (80ng/mL) impaired both iNOS and CD206 expression in these immune cells. Interestingly, HBSP expression in HepG2-NTCP cells restored CD206 expression, associated with a reduction of IL10 expression in TNF-α-treated cells. Finally, HBSP in hepatoma cells led to an overexpression of CCL2 in treated-THP-1m-cells.
Conclusion: Orthotopic allografts confirmed the pro-tumoral effect of HBSP related to less M1-like TAM polarization. The indirect crosstalk study showed a few impacts of HBSP on macrophage activities. Direct cell-cell interactions are being explored using 3D models.

Keywords: HBSP, Hepatitis B virus, Hepatocellular carcinoma, tumor immune microenvironment, orthotopic allografts

36- The development of a quick, easy to use and reliable cognitive test for the diagnosis of minimal hepatic encephalopathy

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Background and aim: Covert hepatic encephalopathy (CHE) is a complex and multifactorial complication of chronic liver diseases (CLD). Other factors of brain injury (FBI) may cause neurocognitive impairment independently form the liver condition, making the differential diagnosis difficult using available cognitive tests (PHES, ANT, and CFF). The aim of this study was to search for quick and reliable cognitive tools enabling to identify patients with CHE, taking into account the presence of FBI.
Methods: Multidisciplinary evaluation of patients with CLD referred to our outpatient clinics for differential diagnosis of CHE: neuropsychological assessment, blood sample with biomarkers, electroencephalogram (EEG) and brain MRI (MRI) with spectroscopy. Three cognitive tests were evaluated: a visuoconstructive test, a privative verbal fluency test (PVFT) and the analysis of the voice using machine learning algorithms. Patients were classified using two methods: the first separated patients with or without CHE (two-class model), and the second took into account the presence of FBI (4-class model: CHE with/ without FBI; no CHE with/without FBI).
Results: Thirty patients went through the visuoconstructive test, statistical differences were found in the 4-class model, mostly between patients with and without FBI. Sixty-one patients went through the PVFT, differences were found in in both classifications: patients with CHE had lower scores, even more when they also had FBI. The machine learning analysis of voice recordings included 28 patients and was able to separate patients in the 2-class model (accuracy > 96%) and in the 4-class model (accuracy > 89 %).
Conclusions: These preliminary results are encouraging, the visuoconstrucitve test can screen for CHE and FBI, the PVFT is really quick and seem to be more sensitive for CHE than the ANT or PHES, and the voice recording analysis displays very promising results and can represent a quick and reliable tool for differential diagnosis of CHE.

Keywords: Neuropsychology, Cirrhosis, Hepatic encephalopathy, Neurology

37- Role of Intermediate Protein Nestin in Glioblastoma Cell Proliferation, Migration and Invasion

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PCMC, Sandrine Etienne-Mannevilke

Nestin is a class VI intermediate filament expressed in neuronal progenitor cells during mammalian CNS development. Following neuro- and gliogenesis, it is replaced by other intermediate filament proteins such as glial fibrillary protein for mature astrocytes. However, Nestin can be re-expressed in adults in the context of wound healing and pathological conditions. Notably, higher grade gliomas express increased levels of Nestin which correlates with lower patient survival. Glioblastoma multiform (GBM) is the most prevalent and aggressive brain tumor in adults and current treatment options are still highly inefficient mainly due to the particularly invasive phenotype of GBM. In this Ph.D project, I am studying the role of Nestin in glioblastoma cell proliferation, adhesion, migration and invasion using the commercial GBM cell line U251. The aim of the project is to identify how nestin is implicated in mechanotransduction and mechanosensing processes between the cell and the surrounding ECM and how those processes are altered in GBM cells. For this purpose, Nestin knock-out U251 cell lines are generated by CRISPR-Cas9 and compared to Nestin siRNA and shRNA knock-down cell lines.

Keywords: Cytoskeleton, Migration, Proliferation, Invasion, Glioblastoma

38- Cellular and molecular features of the aberrant vascularization and treatment resistance in clear cell renal cell carcinoma

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Background: The hypoxic tumor stroma is characterized by a chaotic vascularization in a remodelled extracellular matrix (ECM). In clear cell renal cell carcinoma (ccRCC), mutation of the tumor suppressor protein Von Hippel-Lindau leads to a pseudo-hypoxic context promoting tumor invasion, vascularization and ECM remodelling. Accordingly, ccRCC vasculature displays a higher degree of complexity than the vascular network reported in other solid tumors. We recently characterized aberrant endothelial structures presenting specific features of dimensions and shapes. Since more than 70% of the ccRCC patients display poor or no response to anti-angiogenic treatments using tyrosine kinase inhibitors (TKI), we suggest that the microvessel architectural complexity may influence the ccRCC clinical outcome. The goal of this study is therefore to deeply characterize the involvement of the ccRCC vasculature in tumor development and response to treatments.
Materials/methods: This study is conducted on both cohort of ccRCC patients and in vitro 3D model of vascularized microtumors (VMT).
Results: Analysis of ccRCC patient cohort allowed us to: 1/ identify 3 groups of patients based on the predominant architecture of the microvascular network; 2/ suggest an impact of the density of the aberrant structures on treatment resistance and patient outcome. Spatial transcriptomic analysis of ccRCC aberrant vascular structures revealed specific gene signatures involved in endothelium development, vasomotion and ECM remodelling pathways. Using the in vitro VMT model which accurately recapitulates tumor angiogenesis in the early stages of ccRCC development, we demonstrated that the aberrant vascular structures 1/ require strong paracrine and junctional interactions between VEGF-secreting tumor cells and endothelial cells and 2/ display distinct sensitivities to TKI treatments.
Conclusion: This project aims to provide knowledge on the cellular and molecular mechanisms specific to the ccRCC aberrant vascular structures and will provide interesting clinical perspectives for developing new therapeutic targets.

Keywords: ccRCC, tumor angiogenesis, transcriptomic analysesa

39- Effect of Selected Antithrombotic Agents on the Expression of Procoagulant Properties of Cancer Cells and Endothelial Cells Exposed to Cancer Cell Derived Microvesicles

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Procoagulant fingerprint related tissue factor (TF) expression is part of the "biological identity" of cancer cells. Cancer cell derived microvesicles expressing TF (TF-CaCe-dEV) lead to activation of endothelial cells which in turn amplifies hypercoagulability in the tumor microenvironment. The aim of the study is to explore the effect of antithrombotic agents and quercetin on the procoagulant potential of pancreatic cancer cells (BXPC3) and breast cancer cells (MCF7) as well as its effect on endothelial cells (HUVEC) exposed to CaCe-dMV from BXPC3 and MCF7 cells. Pancreatic cancer cells (BXPC3), breast cancer cells (MCF7) and primary human umbilical vein endothelial cells (HUVEC) were pretreated with clinically relevant concentrations of antithrombotic agents (apixaban, enoxaparin, or tinzaparin) and quercetin (300 μM). Subsequently, according to a standardized procedure developed by our group, cells were exposed to normal human platelet poor plasma and coagulation was triggered by addition of calcium chloride. The thrombin generation process was studied with a Calibrated Automated Thrombogram (CAT) assay. Crystal violet was used to assess cell viability. Tinzaparin and quercetin significantly reduced the viability of BXPC3 and MCF7 cells as compared to the control experiment and resulted in significant prolongation of the lag-time by 1.16 and 1.12-fold, respectively, while no significant changes were observed with enoxaparin and apixaban. On the other hand, the exposure of MCF7 cells to tinzaparin, apixaban and quercetin significantly increased the lag-time in the range of 1.2 to 1.6-fold. The lag-time of thrombin generation induced by HUVEC pre-treated with quercetin and exposed to BXPC3 CaCe-dEVs was significantly longer and the peak was significantly lower as compared to those obtained by HUVEC non-treated with quercetin exposed to BXPC3 CaCe-dEVs, while no significant changes observed with apixaban. Pretreatment with either quercetin or apixaban did not significantly modify the effect of HUVEC exposed to MCF7 CaCe-dEVs. Tinzaparin and quercetin decrease the viability of cancer cells, while the exposure of cancer cells to tinzaparin, apixaban or quercetin results in decrease of their procoagulant potentials. However, quercetin effectively prevents endothelial cell activation and possible TF expression upon exposure to a potent stimulus such as CaCe-dEVs from aggressive pancreatic cancer cells (BXPC3).

Keywords: Tissue factor, microvesicles, antithrombotic agents, breast cancer, pancreatic cancer

40- Caracterisation and classification of non small cell lung cancer immune tumor microenvironement

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Background The tumor microenvironment (TME) of non-small-cell lung cancer (NSCLC) is highly complex and heterogeneous. It comprises various cell types including tumor cells, immune cells, cancer-associated fibroblasts (CAFs), and endothelial cells. Importantly, immune cells, especially lymphocytes, can organize in tertiary lymphoid structures (TLS), associated with a good prognosis and response to immunotherapy. On the contrary, patients with no immune cell infiltration or no organization in TLS have a poor clinical outcome. The aim of my PhD project is to decipher the cellular and molecular mechanisms leading to immune exclusion or defective immune cell organization in lung cancer.
Materials and methods : We analyze a retrospective cohort of 219 patients (operated in 2018 at Cochin Hospital), for which we have the clinical data. To characterize the TME, we have set-up a first panel of 6 antibodies (Akoya Opal 6-Plex with the LEICA BOND RX), to quantify the presence of high endothelial veinules, B and T lymphocytes, TLS maturation status, cell proliferation and tumor cells. We're also optimizing a second panel for staining vessels and CAFs.
Results : The first step of my PhD project is to perform a classification of NSCLC patients, based on the density of intratumoral immune cells and TLS. All the cohort has been stained with the first panel and analyses are ongoing, using HALO AI software.
Conclusion : Once the quantification of the staining is done, we will be able to correlate the type of immune infiltrate with other characteristics of the TME using the second panel, and to compare the transcriptomic profiles of the TME of patients with high (with or w/o TLS) or low immune infiltrates using spatial transcriptomics (Visium, 10X GENOMICS). We anticipate identifying specific signatures of tumor cells, CAFs and endothelial cells that may explain immune cell densities and localization.

Keywords: Tumor Microenvironment IHC TLS CAF NSCLC

41- Bone metastases of breast cancer: hormone receptors expression alterations

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Background: Breast cancer has been identified as a leading global health challenge, representing a dominant cause of cancer-related death in female population. Once a cancer is diagnosed, more than 70% of patients will eventually develop bone metastases, with additional risk augmentation among hormone-positive malignancies. Bone metastases are likely to disrupt the physiological bone turnover, leading to increased fragility and higher incidence rate of pathological fractures' occurrence associated with the significant health-related quality of life extenuation. Due to demanding bone biopsy performance, biopsies of bone secondary deposits are not performed routinely in everyday clinical practice, implying the continuation of therapy regimens according to the primary carcinoma's phenotype - generally resulting in poor therapy response.
Aim: The aim of this study was to identify hormone receptors' expression transition from primary breast carcinoma to bone metastases.
Materials and methods: For the purpose of this study we have examined bone tissue samples of 95 female breast cancer patients who developed bone metastases. The bone biopsies were conducted at the referent centre for bone tumors, Institute for orthopaedic diseases "Banjica", and analysed at the Institute of Pathology, Faculty of Medicine, University of Belgrade. Estrogen and progesterone expressions' assessment according to Allred score was performed and afterwards compared with the primary breast cancer's expression. Patients were divided into three categories according to menopausal status. Data was analysed using IBM SPSS statistics, by application of descriptive statistics to the whole sample, as well as McNemar, t-test and Mantel-Haenszel test for suitable variables. Statistical significance was considered at the level of $p<0.05$.
Results: Estrogen receptor change was detected in 23,16%%, while progesterone's in 47,37% of patients. Changes were more pronounced in pre-menopausal and peri-menopausal when compared to post-menopausal women, among whom 75% peri-menopausal women have shown a modification of interest. Progesterone receptors' alteration was found to be statistically significant in premenopausal and perimenopausal women ($p=0.021$, $p=0.018$).
Conclusion: There is a significant change of hormone-receptors' expression between primary breast carcinoma and bone metastases, most clearly present in the peri-menopausal women.

Keywords: bone metastasis, breast cancer, estrogen, progesterone, therapy resistance

43- Intracellular trafficking in epithelilal polarity and cancer: identification of novel molecular factors at the Golgi complex

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Epithelial polarity plays a pivotal role in tissue and organ function, and its disruption is associated with a spectrum of human diseases, including cancer. While aberrant intracellular trafficking of membrane proteins has been implicated in tumor progression, a comprehensive understanding of its role in cancer development remains incomplete. To bridge this gap, we utilized the Retention using Selective Hook (RUSH) assay to investigate modifications in intracellular trafficking of membrane-associated proteins during epithelial-mesenchymal transition (EMT).
EMT, a reversible cellular process integral to cancer progression, involves epithelial cells transitioning into mesenchymal cells. We examined the intracellular trafficking of various membrane-associated proteins in MDCK cells under fully polarized conditions and upon EMT induction by TGF-β. Chimeric constructs, including GFP fused to the GPI-signal attachment of Folate Receptor alpha (FRα) for apical GPI-anchored proteins (GPI-APs), Podocalyxin/GP135 (PCX) as an apical transmembrane, and basolateral transmembrane EphA2 proteins, were employed. Our findings revealed distinct kinetics of exocytosis, including ER exit, Golgi arrival, and exit, for the aforementioned cargoes. We observed significant differences in overall kinetics between fully polarized and EMT-induced cells, particularly regarding apical cargoes been faster as to compare to the basolateral protein. Using super-resolution microscopy, we observed that GPI-FRα and PCX and EphA2 departed from the Golgi complex via distinct tubular or vesicular-like structures, respectively, irrespective of their membrane association and cellular states. Notably, upon EMT induction by TGF-β, we observed an increase in the number of exocytic events accompanied by longer tubular structures compared to the fully polarized state. Furthermore, our study demonstrated that the apical post-Golgi transport of both GPI-FR and PCX in fully polarized MDCK cells relies on microtubules, while the post-Golgi transport of basolateral cargoes, such as EphA2, depends on actin. These findings prompted further exploration of the interplay between actin and microtubules in cargo budding from the Golgi complex and their subsequent intracellular trafficking to the polarized apical surface and the cell surface of EMT-induced MDCK cells. Recently, through GFP-Trap followed by interactomic analyses, we have identified the molecular factors that elucidate the molecular mechanism and dependency of both actin and microtubules (MTs) in the intracellular trafficking of basolateral and apical membrane proteins in fully polarized epithelial cells.

Keywords: Epithelial Polarity, Trafficking, Cancer, Golgi

44- Molecular characterization of intraductal papillary mucinous carcinomas

MAS

Centre de recherche Saint Antoine - Equipe "instabilité des microsatellites et cancer"

Background: Intraductal papillary mucinous neoplasms (IPMN) are the main cystic precursor lesions of pancreatic ductal adenocarcinoma (PDAC). IPMN with an associated carcinoma (IPMC) represent 10% of resected PDACs. Pathologically, two pathways of carcinogenesis are described: the intestinal and non-intestinal pathways, resulting in IPMC of the colloid and tubular subtypes, respectively. Molecular features associated to these lesions are poorly defined. We conducted the first multi-omic analysis dedicated to IPMCs.

Patients and method: We set up an international cohort of IPMC resected between 2004 and 2019. DNAs & RNAs were extracted from FFPE tumor samples and analyzed by high throughput sequencing (whole exome and 3'RNAseq).

Results: 170 patients were included, among which 38 (22%) colloid IPMCs. At the transcriptomic level, a specific signature associated with the intestinal phenotype was identified and correlated with the "classical" signature previously described. Tubular IPMCs were associated with overexpression of immune checkpoint molecules (PD1, CTLA4, TIGIT). At the genomic level, intestinal IPMCs were associated with GNAS variants (65% vs. 13%, $p<0.001$), and had a significantly lower frequency of variants in KRAS (46% vs. 80%, $p<0.001$) and other classical PDAC associated variants (TP53, SMAD4, CDKN2A). A variant impacting BRCA1/2 or other genes of the homologous recombination repair (HRR) pathway was identified in 40% vs 19% of intestinal and non-intestinal IPMCs, respectively ($p=.005$).

Conclusion: Our study represents the largest molecular characterization dedicated to IPMC reported to our knowledge. Our results highlight the distinct profile of lesions associated with the intestinal pathway, associated with a specific transcriptomic signature as well as a higher frequency of variants impacting the HRR pathway. Tubular IPMCs were more closely related to cPDAC and associated with overexpression of immune checkpoint molecules. These specificities suggest potential therapeutic implications for these patient subgroups.

Keywords: PDAC, IPMN, IPMC

45- HRV-TRACKER : proof of concept for a tool to detect respiratory stress and respond with an olfactory stimulation

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Aversive respiratory sensations are regrouped under the term of “dyspnea”. Dyspnea represents a physical pain and a psychological distress with a day-to-day life fear of dying. Therapeutical opportunities to correct dyspnea exist, however they are not always available and sometimes not sufficient. In these cases, the dyspnea is called “persistent” and need new therapeutical approaches to be alleviated. In this context, an olfactory stimulation (OS) could represent a good candidate. Indeed, some studies showed that using menthol could reduce the sensation of respiratory discomfort. Such specific effects for OS could be explained by the close link between the olfactory system and brain regions implicated in emotions comparing to other sensory modalities.
In clinic, to adapt to the omnipresent aspect of dyspnea, an OS should be delivered at any moment. Consequently, we propose to develop a device that identifies respiratory stress and subsequently diffuses an OS. The first step toward the conception of this device is the development of an algorithm able to identify the respiratory stress and to decide whether or not deliver an odor. Therefore, we used the raise of the autonomous nervous system sympathetic branch during a stress event that can be captured by the Heart Rate Variability (HRV) metrics computed from the Electrocardiogram signal. After that, an inference model based on SVM is learned from HRV metrics in order to decide when to deliver, or not, the OS. To test our algorithm, we induced experimental respiratory stress in 30 healthy subjects while diffusing a pleasant, unpleasant and without OS while recording their ECG signal.
Our results show that our algorithm manages to identify respiratory stress and takes the decision to deliver odor during the stress episodes.

Keywords: respiration, HRV, dyspnea, machine learning

46- Ventilatory variability during sleep in patients with chronic obstructive pulmonary disease

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Background: Chronic obstructive pulmonary disease (COPD) is the 3rd leading cause of death worldwide. Management of COPD exacerbations in hospital is associated with higher mortality, poorer quality of life and greater recurrence of subsequent readmissions. The study of ventilatory patterns during sleep, could make it possible to identify biomarkers associated with the onset of an exacerbation, its progression, and the risk of recurrence. Ventilatory variability is physiological but when it is excessive or minimal it indicates respiratory dysfunction. During sleep, breathing is controlled solely by automatic command, with the loss of cortical influences and physical activity. The brainstem 'pacemaker' neurons provide the respiratory rhythm, but the objective ventilation observed depends in addition on the mechanical filter of the respiratory effectors. Thus, the variability measured during sleep is an indicator of the correct functioning of the central control and the neuromuscular network of respiration. Normal non-random respiratory variability is thus observed during sleep, changing according to sleep stage (small in deep slow wave sleep than in REM sleep). Our physiological hypothesis is that changes in respiratory variability during sleep are evidence of potential dysfunction of the effectors of ventilatory mechanics.
Material and methods: The study of breathing during sleep in healthy subjects and in stable and unstable COPD patients will make it possible to establish norms for ventilatory variability during sleep, to measure the impact of control and effectors on this variability, and thus to find an early, sensitive, and specific biomarker associated with effector dysfunction. The discovery of a pattern of ventilatory variability associated with a worsening of COPD would provide a better understanding of the pathophysiological mechanisms underlying its onset.
Results and conclusion: For clinical practice, the aim of this work is to reduce the need for hospitalization and ensure better management of patients with COPD

Keywords: COPD, sleep, exacerbation, respiratory variability

47- Breathing and posture in the practice of wind instruments

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Background: In wind instrument practice, breath control is essential for sound production. It requires a "non- respiratory" use of muscles primarily involved in breathing. These muscles also contribute to maintaining posture, resulting in a coupling between the ventilatory and postural systems. Beyond this structural aspect, the posturo-ventilatory coupling involves neurological control, including cortical structures. Breathing is an automatic process with its generator located in the brainstem. However, human breathing can be voluntary, as observed in the practice of wind instruments. In this case, it is governed by the cerebral cortex. This area also controls posture and environmental visualization. Thus, this coupling utilizes cognitive resources, and playing an instrument demands even more. This could disturb the use of these resources for posturo-ventilatory coupling through attentional competition. This raises a question that must be considered in the specific context of musical practice. How does each musician adapt their posture, breathing, and thus the coupling between the two during musical performance ?
Material and methods: To answer this question, we will use different approaches. Firstly, we will interview music professors. These interviews aim to understand how instrumentalists perceive the potential link between posture and breathing. Thematic analysis will be used to interpret their speech. Secondly, we will conduct experiments on the instrumentalists. We will use motion capture to measure their thoracic volume and the movement of their postural segments. These parameters will be used to quantify the posturo-ventilatory coupling thanks to signal processing techniques : TLA (Time-Locked Average), PLV (Phase Locking Value). Then, we will use acoustic modeling to compute aerodynamics parameters (flow rate) and thus correlate them to the coupling parameters.
Objectives: The final objectives are to provide new pedagogical tools to music teachers and to bring out a deepening of fundamental knowledge of this coupling, potentially leading to clinical advancements.

Keywords: Breathing, Posture, Acoustics, Wind Instruments, Music

48- Posturo- respiratory coupling improvement by upper airways stabilization in awake OSA patients

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Patients with obstructive sleep apnea syndrome (OSAS) do not snore while being awake due to enhanced cortical respiratory drive and postural adaptations known to stabilize upper airways. Both cortical and postural compensations can be assessed through posturo-respiratory coupling (PRC) which is altered in OSAS and could be responsible for functional alteration of postural alignment and postural control. Mandibular advancement device (MAD) is known to stabilize upper airways and release the brain from cortical compensation. Therefore, we hypothesize that wearing MAD relieve patients from both cortical and postural compensation for upper airway stabilization consistent with improvement of PRC.
18 patients were included to assess postural parameters using biplanar radiography and PRC using time-locked average body motion during breathing cycle. We first compared results between rest breathing with and without MAD condition. Then between cognitive loading with and without MAD. All results were compared to a paired group of healthy subjects.
MAD improved thoracic kyphosis, costal ribs inclination and restored physiological correlation between lumbar spine and pelvic incidence. MAD also improved breathing pattern and restored a PRC strategy of ankle joint. MAD reduced changes of breathing pattern, postural stability and PRC induced by cognitive loading. These results support the causative nature of the relationship between breathing-related postural disturbance and cortical and postural mechanisms to maintain upper airway stability and emphasize the necessity to consider day-time functional alterations in the present sleep disorder.

Keywords: Respiratory Neurpohysiology, OSAS, Biomechanics, Rehabilitation

49- Changes in the shear modulus of the diaphragm after ventilatory task in humans

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Background, Motivation and Objective. The diaphragm is a primary respiratory muscle that is prone to dysfunction such as in mechanically ventilated patients in the intensive care unit (ICU). Ultrasound shear wave elastography (SWE) enables the quantification of the viscoelastic properties of muscles in vivo including the local shear modulus (μ) through the temporal-dependent changes in shear-wave speed. Previous works support that muscle mechanical properties are altered after exercise. Whether diaphragm loading is associated with acute changes in mechanical properties has never been investigated.
Methods. Ten volunteers with normal pulmonary and neuromuscular function were studied. Diaphragm SWE was assessed using a linear transducer (SL 10-2) driven by an ultrasound scanner (Aixplorer, Supersonic Imagine, France) on the zone of apposition of the right costal hemidiaphragm. A 10-s SWE sequence was used for the assessment of μ during apnea after normal expiration before and immediately after a 20 min ventilatory task against inspiratory loading at 40% of maximal inspiratory pressure. Average diaphragm μ was extracted from shear modulus.
Results/Discussion. Ten subjects were considered for statistical analysis. μ was significantly reduced after the inspiratory task (13.20 (10.20 – 14.40) versus 9.52 (6.43 – 11.6); $p<0.05$)
These preliminary findings support the capability of SWE to evaluate acute modification of diaphragm mechanical properties after a ventilatory task. The quantification of diaphragm μ using ultrasound SWE in vivo may contribute to a better understanding of the mechanical behavior of this muscle. Further research in ICU with mechanically ventilated patients could improve the understanding of various pathological conditions and patient's management strategies.

Keywords: shear wave elastography ; ultrasound; diaphragm; shear modulus; ventilatory task

50- Effect of Repetitive Magnetic Stimulation on respiratory function after cervical spinal cord injury

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Background: Traumatic cervical spinal cord injuries (cSCI) damage respiratory pathways leading to life-threatening respiratory insufficiency. Inflammation is known to play a role in limiting neural regeneration after injury, and this refrain respiratory recovery. Repetitive Magnetic Stimulation (rMS) has been shown to reduce inflammation at the spinal level. In my PhD project, we are investigating whether rMS has a therapeutic effect of on respiratory function by reducing neuro-inflammation, in a mouse model of cSCI.

Material and methods: Nine adult Swiss male mice underwent hemi-contusion at the C3-C5 level (C3HC, n = 7) or laminectomy (n=2) and received either high-frequency (10 Hz) rMS or sham treatment for 10 days (9 trains of 100 biphasic pulses, separated by 30 s intervals between trains delivered at 80% MO (percentage of maximum output of the stimulator), 900 stimulations per protocol). Animals were divided into 4 groups:(1) laminectomy + sham rMS (n = 1); (2) laminectomy + rMS (n = 1); (3) C3HC + sham rMS (n = 4); (3) C3HC + rMS (n = 3). Respiratory function was assessed using whole-body plethysmography before and after C3HC and after intervention and provide amplitude (tidal volume, Vt) and respiratory frequency (Bf). Moreover, diaphragm activity was measured by in-situ electromyography (EMG). All spinal cord samples were collected for Immunofluorescence experiments.

Results: From the seven mice which underwent C3HC, four had reductions in VT at 7 days post-C3HC compared to baseline (3.7 ± 0.8 vs. 6.3 ± 0.2 µL/g, -41%, p=0.01), and control values (laminectomy) (6.7 ± 0.1µL/g) and could be used as good model in the study to test rMS. As a compensatory mechanism, breathing frequency (Bf) increased in these mice compared to baseline (193 ± 20 vs. 166 ± 23, +22%, p=0.07). One mouse received rMS treatment and exhibited a restoration of tidal volume (7.0 vs. 2.8 µL/g) that was greater than spontaneous recovery as measured in the sham rMS group (4.5 ± 0.2 vs.3.7 ± 0.5 µL/g, n = 3) although this result cannot be statistically analyzed due to the small number of animals.

Conclusions: This is a very pilot result of ongoing investigation that needs to be confirmed by a greater number of animals in each group. Moreover, diaphragm EMG and neuroinflammation at the spinal cord level (astrocyte, macrophage, microglia, and chondroitin sulfate proteoglycan) will be analyzed and correlation with changes in respiratory variables will be investigated in regard to treatment groups.

Keywords: spinal cord injury; hemicontusion model; respiratory function; repetitive Magnetic Stimulation (rMS)

51- Impact of age on long-term cardiovascular effects after external exposure to low doses of cesium 137 (lung-heart axis)

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People living in areas contaminated by nuclear accidents are exposed to chronic low-dose (LD) radiation, the consequences of which have yet to be fully assessed. However, several epidemiological studies show the development of pulmonary and cardiovascular alterations in these populations. In addition, higher morbidity and mortality rates have been recorded in elderly subjects. These results seem to show a correlation between age and the onset of cardiovascular pathologies in these populations. However, further studies are needed to define the underlying biological mechanisms.
The aim of this thesis project is therefore to demonstrate the long-term effects of exposure to low dose radiation on the vascular system of the heart and lung, regarding the age. In this project, male C57BL/6J mice aged 2 and 15 months are exposed to whole-body external gamma irradiation at single doses of 100, 250 and 500 mGy, mimicking the cumulative doses of external exposure. Mice were studied 24 hours, 15 days, and 3 months post-irradiation. Pulmonary arterial pressure and cardiac function were assessed by echo Doppler and Millar probe. Tissue remodeling, notably pulmonary and cardiac fibrosis, and vessel neo-muscularization in the lung will be assessed by histology. We will quantify the resident progenitors that participate in the vascular remodeling of the lung. The vascular network will be reconstructed in 3D using the iDISCO clearing method. Finally, the calcium and inflammatory signaling pathways will be studied using cellular and molecular analysis.
Preliminary results show impaired cardiac function and a significant increase in pulmonary arterial pressure from 250 mGy, followed by a significant increase in pulmonary vessel muscularization from 500 mGy. These initial results show that exposure to LD (<500mGy) induces molecular and functional alterations leading to vascular pathologies affecting the lung-heart axis. Finally, this study provides information on a possible radiation related risk of cardiovascular disease in the <500mGy dose range and identifies a potential dose threshold.

Keywords: Cardiovascular, low doses, age, gamma radiation

52- Neuropilin1 protects kidney from fibrosis through resident fibroblasts in mice

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Background: Renal fibrosis is the common end point of Chronic Kidney Disease (CKD), the hallmark of which is the deposition of pathological extracellular matrix by myofibroblasts. Experiments with lineage tracing in mice and single-cell RNAseq in humans revealed that resident fibroblasts are the major sources of myofibroblasts during CKD. Neuropilin 1 (NRP1) is a membrane-bound coreceptor for class 3 semaphorins and for specific isoforms of vascular endothelial growth factor. Importantly, NRP1 plays a critical role in fibrosis progression in various pathophysiological conditions. In the kidney, NRP1 is expressed in resident fibroblasts of the renal interstitium. We therefore tested the possibility that NRP1 could participate in a cell-autonomous manner in the development of renal fibrosis.
Material and Methods: We used the myelin protein zero-Cre (P0-Cre) to invalidate the expression of Nrp1 constitutively in resident fibroblasts of the kidney. Nrp1-ko mutants and wildtype littermates were subjected to two well-characterized models of renal fibrosis: the folic acid (FA) nephrotoxicity model and the unilateral ureteral obstruction (UUO) model. In separate experiments, we used AngII administration through an osmotic minipump to generate heart fibrosis.
Results: We found that Nrp1-ko mutants displayed proliferative defects that affected renal recovery after acute kidney injury. Accordingly, the renal function and structure of Nrp1-ko mutants were significantly more impaired than those of their wildtype littermates in FA-induced renal disease. The role of Nrp1 in fibrosis was confirmed in UUO. Our lineage tracing experiments showed that renal and cardiac interstitial cells derived from the same P0-Cre positive progenitors. We found that after continuous angiotensin II infusion, the fibrotic lesions are more pronounced in the hearts of Nrp1-ko mice compared to their wildtype littermates.
Conclusion: Our data suggest that NRP1 activation is a protective mechanism against the progression of fibrosis, and this mechanism appears to be common in the kidney and heart.

Keywords: Neuropilin1, renal fibrosis, resident fibroblasts

53- Is GDF15 involved in primary hyperaldosteronism to reduce hypokalemia?

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Introduction : Primary hyperaldosteronism is a syndrome characterized by a high level of aldosterone, a hormone secreted by adrenal glands. This hormone acts on kidneys by stimulating the reabsorption of sodium and the secretion of potassium. Patients present hypertension and hypokalemia. Previously, the laboratory identified a growth differentiation factor, GDF15 and a membrane protein, the pump H-K-ATPase type 2 (HKA2) which work together to reduce potassium renal loss by increasing the number of type A intercalated cells (AIC) in cases of dietary potassium deprivation.
Material and methods°: To investigate the potential role of GDF15 in primary hyperaldosteronism, we will develop a murine model of this syndrome by treating WT mice with a single intramuscular injection of deoxycorticosterone pivalate (DOCP), an analog of aldosterone. Mice were placed in metabolic cages for measurements of water and food intakes, urine excretion. We also measured the kalemia. Furthermore, we isolated outer medullary collecting ducts (OMCD) at 16 and 28 days post injection for AIC counting. Results and conclusions°: Our results demonstrate a significant increase of urinary GDF15 levels. Secondly, we observed a significant increase of AIC proportion 16 days post DOCP injection. Further experiments concerning GDF15 knock-out mice are required to conclude about the role of GDF15 in primary hyperaldosteronism.

Keywords: Primary hyperaldosteronism, GDF15, potassium, type A intercalated cells

54- SGLT2 inhibitor does not slow down dent disease progression in our KI mouse model

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BACKGROUND: Dent's disease is a rare hereditary disorder affecting the proximal tubule. It is characterized by urinary loss of low-molecular-weight proteins, calcium, glucose, phosphate... It is caused by mutation of the CLCN5 gene encoding a protein called ClC-5, which plays a role in endocytosis. To study the mechanisms involved in the evolution of this disease, the laboratory has created a knock-in mouse model carrying a ClC-5 mutation. This mouse displays the main clinical features of the patient. Over time, this model develops fibrosis and inflammation in the kidneys. It also shows a decrease in glomerular filtration rate, which reflects renal function. There is no curative treatment for this disease. SGLT2 inhibitors are a new class of drugs with good results in various chronic kidney diseases with or without diabetes. They have a nephroprotective and cardiovascular effects.
MATERIALS AND METHODS: we treated our KI mice with the inhibitor in their diet for 8 months to slow disease progression. At 10 months, the kidneys were harvested for histological and molecular analysis. Fibrosis and inflammation are quantified by histological and pcr staining. By WB we analyze NGAL protein a marker of renal damage.
RESULTS: Unfortunately, treatment did not reduce fibrosis and inflammation. Nor did we observe any decrease in NGAL expression in the kidneys.
DISCUSSION: Treatment alone does not appear to alter the renal phenotype. Some clinical trials have shown that the efficacy of SGLT2 inhibitors is greatly enhanced in combination with another treatment: inhibitors of the renin angiotensin system. In the future, we may try to treat our mice again with this double treatment.

Keywords: kidney fibrosis treatment

55- Role of SerpinE2 in Chronic Renal Disease

Li LIU

UMRS 1155 - Common and Rare Kidney Diseases

Renal diseases are recognized as a global public health concern. Glomerulonephritis (GN) is a common cause of end-stage renal disease worldwide, characterized by damage to the glomerular basement membrane (GBM), the mesangium, and/or the capillary endothelium, resulting in proteinuria. GN is also characterized by tubulointerstitial fibrosis leading to a reduced glomerular filtration rate. Despite pathophysiological advances, current treatments for GN are non-specific and partially successful and new treatments are needed. SerpinE2, or PN-1 is a member of the Serpin superfamily of proteins, known to have central regulatory roles throughout the mammalian body, and is the most potent inhibitor of thrombin. Our study aims to characterize the expression and function of PN-1 in GN using both in vitro and in vivo approaches. In mouse models of GN induced by nephrotoxic serum (NTS) or folic acid (FA), PN-1 expression was found to be elevated, correlating with disease severity. Moreover, PN-1-deficient mice exhibited worsened renal function and increased inflammation and fibrosis compared to wild-type mice, indicating a protective role for PN-1 in GN. We propose a series of experiments to elucidate the mechanisms underlying PN-1's protective effects. We plan to investigate PN-1's modulation of inflammation, thrombosis, and fibrosis in GN through in vitro studies on cultured cells and in vivo experiments using PN-1-deficient mice and conditional knockout models. Additionally, the possibility of using lentiviral delivery to upregulate PN-1 expression in the kidney as a potential therapeutic strategy will be explored. Overall, this study aims to shed light on the role of PN-1 in GN pathogenesis and its potential as a therapeutic target for mitigating kidney injury. By elucidating the mechanisms through which PN-1 exerts its protective effects, this study could pave the way for the development of novel treatments for GN and other renal diseases.

Keywords: SerpinE2, glomerulonephritis, thrombosis, fibrosis, inflammation

56- Inhibition of the Cannabinoid Receptor CB1: A New Therapeutic Target in Chronic Kidney Diseases

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Chronic kidney disease (CKD) involves the replacement of renal tissue with fibrosis, progressively impairing its function. One therapeutic approach proposed by our team to slow the progression of fibrosis is the inhibition of cannabinoid receptor type 1 (CB1). We have previously demonstrated that renal fibrogenesis is prevented by pharmacological or genetic inhibition of CB1 in experimental models of Unilateral Ureteral Obstruction (UUO) and Ischemia-Reperfusion (I/R-CKD).
The aim of this study is to explore the relative contributions of different cellular types to the development of CKD in the I/R-CKD model. In this study, we focus on two key cellular types involved in the fibrosis process: tubular cells and myofibroblasts.
To this end, we employed a dual approach. In vivo, we used genetic inhibition of CB1 specifically in myofibroblasts using Cnro.P0-Cre transgenic mice. Concurrently in vitro, primary murine myofibroblasts derived from these lines were isolated and cultured. For tubular cells, we utilized a transgenic mouse model, Cnro.Pax8.Lc1, allowing specific and inducible CB1 inhibition in the tubules.
Our analyses using RT-qPCR and RNAscope have validated the transgenic mouse models. Cnr1 expression was specifically invalidated in the myofibroblasts in Cnro.P0-Cre mice and in the tubular cells in Cnro.Pax8.Lc1 mice. The suppression of CB1 in tubular cells exacerbated the disease, leading to a significant increase in serum creatinine levels and worsening of interstitial fibrosis and tubular atrophy. In Cnro.P0-Cre mice, although no difference in creatinine levels was observed, histological analyses showed a significant reduction in renal fibrosis and tubular atrophy while acute tubular lesions are still present. In vitro, using Live Imaging techniques, we have demonstrated that the deletion of Cnr1 in myofibroblasts leads to an increased proliferation of these cells compared to myofibroblasts that express Cnr1.
Our study has shown that specific deletion of Cnr1 in myofibroblasts prevents the development of fibrosis but does not improve renal function during experimental fibrosis. Conversely, specific deletion of Cnr1 in renal tubules is detrimental.

Keywords: Chronic kidney disease (CKD), Type 1 Cannabinoid Receptor, Myofibroblast, tubulars cells, Ischemia-Reperfusion

57- HSP27 promotes activation of parietal epithelial cells and crescent formation in crescentic glomerulonephritis

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Background. Crescentic glomerulonephritis (CrGN) is the most severe form of kidney disease. CrGN is characterized by a pathological accumulation of parietal epithelial cells (PEC) forming the cellular “crescent” in the urinary space leading to end stage renal disease in 30-50% of cases. While current treatments consist of immunosuppressive therapies, none of them directly targets molecular pathways associated with crescent formation and PEC activation. Heat Shock Protein 27 (HSP27) is a stress-induced chaperone protein largely known for its anti-apoptotic and pro-proliferative effect in cancer. Here, we aim to investigate whether HSP27 is involved in PEC activation and crescent formation.
Material & Methods. To explore the role of HSP27, we used a nephrotoxic serum (NTS)–induced crescentic glomerulonephritis preclinical model and biopsies from patients diagnosed with CrGN. In vitro, we used primary cell cultures of PEC, either with or without HSP27 knockdown, to examine migration, proliferation, and the expression of activation markers (CD44, EGF receptor activation…)
Results. HSP27 is largely overexpressed in PEC within glomerular crescents in both patients with CrGN and in kidneys from NTS-treated mice. Targeting of HSP27 with the antisense oligonucleotide (OGX-427) or with pharmacological inhibition (Ivermectin) results in reduced development of crescents in the NTS model. In vitro, silencing of HSP27 in PEC induces a reduction in cellular migration and activation (measured by CD44 expression). These data suggest that HSP27 could promote crescents formation by promoting PEC activation and proliferation. To better understand, we explored the EGFr pathway known to be involved in glomerular epithelial cells activation. Silencing of HSP27 leads to a reduction in the activation of the EGF receptor, explaining at least part of the results obtained.
Conclusion. Taken together, our data highlight a role for HSP27 in the development of crescent lesions and pave the way for innovative therapeutic approaches for CrGN patients.

Keywords: Heat Shock Protein 27 (HSP27), Crescentic glomerulonephritis (CrGN), cellular crescent, Parietal Epithelial Cells (PEC)

58- The role of the Connexin 43 in expérimental tubulopathy

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Context: We have recently reported that the expression of the gap junction protein Cx43 was abnormally elevated, or de novo expressed in injured renal cells, after renal aggression in different models of experimental chronic kidney disease (CKD). However, our previous studies have mainly focused on the role of Cx43 in endothelial cells or podocytes during the progression of hypertensive or experimental glomerulonephritis. We have also observed a de novo expression of Cx43 in renal tubular cells following obstructive nephropathy. The aim of this study is to assess the role of tubular Cx43 in renal tubulopathy.
Method: We are investigating the role of Cx43 in experimental renal tubulopathy using the following approaches. First, we generated mice with a specific deletion of Cx43 at the tubular level. These mice were subjected to unilateral ureteral obstruction (UUO) (Cx43tub-del UUO mice) and sacrificed on day 10. We used these same mice in a second folic acid CKD model to assess renal function and Cx43-mediated mechasnisms contributing to renal fibrosis. Primary tubular cells were also cultured to evaluate and validate these mechanisms.
Results : Cx43tub-del UUO mice showed decreased tubular Cx43 expression compared to controls after UUO (WT UUO) after 10 days of UUO. Masson's Trichrome staining showed preserved renal structure in these mice. Renal damage was also limited in Cx43tub- del UUO mice, evidenced by a decrease in renal injury markers such as KIM-1 and N-GAL. Furthermore, specific deletion of Cx43 in the tubular compartment limited the inflammatory response and further renal fibrosis. Similar results were observed in FA model with Sirius Red staining that showed a significant decrease on interstitial renal fibrosis. In addition, renal function was improved in mice with specific cx43 invalidation in the tubule. The study of the mechanisms leading to renal fibrosis has also shown a possible involvement of the YAP Hippo pathway.
Conclusion: Our results show that tubular Cx43 plays a major role in the alteration of the tubules and further in the progression of tubulointerstitial nephropathy. Further research is needed to determine how tubular Cx43 mediates its deleterious effects during renal tubulopathy progression.

Keywords: Cx43 - inflammation - fibrosis - CKD

60- Consequences of maternal obesity on postnatal cardiac development

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Obesity is a major public health problem, and its growing prevalence is contributing to an increase in the incidence of cardiovascular disease. The overweight epidemic affects pregnant women, and the uterine environment affects organ development. Notably, children of obese mothers have higher rates of cardiovascular events and mortality. However, little is known about postnatal cardiac development and the impact of early dysmetabolism on the mechanical and electrical functions of the heart. The adult cardiomyocyte (CM) is a highly polarized, rod-shaped cell. Its longitudinal ends form contacts between adjacent cells through various junctional proteins constituting the intercalated disc. The disorganization of these junctions is a mechanism common to the various cardiac pathologies, both genetic and acquired, which promote the development of arrhythmias and dysfunction of the cardiac pump function. Our aim is to decipher the effect of maternal obesity on postnatal cardiac development in rats, and to understand the mechanisms involved. To achieve these objectives, biochemical and omic studies are carried out on the offspring's heart at 3 post-natal developmental stages (P5, P20 and P60). Cardiac function is studied using echocardiography. Our in vivo results show that maternal obesity induces major alterations in cardiac function in the offspring at adulthood (P60), notably eccentric remodeling leading to heart failure. Our ex vivo results show that hearts are smaller and that cardiac remodeling is accompanied by cardiomyocyte hypertrophy. Changes in the expression of intercalated disc components are also observed, including delayed maturation of gap junctions. In conclusion, these preliminary data indicate that a prenatal obesogenic diet induces major cardiac dysfunction in the offspring, and suggest a link with abnormalities in the postnatal organization of cardiomyocyte structure.

Keywords: Cardiomyocyte, postnatal organization, cardiac development, dysmetabolism

61- Study of the cardiac sodium channel in a mouse model of Scn5a haploinsufficiency towards a novel approach of therapy

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INTRODUCTION – Brugada syndrome (BrS) is a hereditary heart rhythm disorder that significantly raises the risk of sudden death due to ventricular fibrillation. About 20% of BrS cases can be attributed to a mutation in SCN5A, which encodes the alpha-subunit Nav1.5 of the cardiac sodium-channel, which carries the sodium current INa. These mutations result in a loss of Nav1.5 function, causing a decrease in INa. In the Scn5a+/- mouse model, a sodium-channel dysfunction phenotype is observed, characterized by abnormal conduction (prolonged PR and QRS intervals), a 50% reduction in INa, and an increased vulnerability to arrhythmias.
AIM – This study aimed to evaluate the effects of a N-peptide surexpression, known for its ability to enhance INa, in Scn5a+/- mice. This approach was explored as a potential new therapy.
METHODS – We utilized AAV9-systemic injection to overexpress the expression of the N-peptide in the hearts of Scn5a+/- mice, in order to assess their vulnerability to arrhythmias caused by programmed electrical stimulation (PES). We also investigated the impact of the N-peptide on the levels of endogenous Nav1.5 expression using molecular biology and biochemistry.
RESULTS – The N-peptide successfully brought the PR and QRS intervals back to normal in Scn5a+/- mice. The PR interval was measured at 46.3 ± 0.9 ms in Scn5a+/- mice compared to 40.4 ± 0.8 ms in WT mice ($P<0.0001$), but with the N-peptide, it was reduced to 42.6 ± 0.9 ms ($P<0.05$). Similarly, the QRS interval in Scn5a+/- mice was 15.2 ± 0.4 ms, while in WT mice it was 13.2 ± 0.4 ms ($P<0.01$), but with the N-peptide, it decreased to 13.6 ± 0.4 ms ($P<0.05$). Additionally, the N-peptide appeared to offer protection against induced arrhythmic events caused by PES. Total premature beats in Scn5a+/- mice were 1.3 ± 0.4, but with the N-peptide, it decreased to 0.2 ± 0.2 ($P<0.05$) like WT mice. Although there were no significant differences in total Nav1.5 expression (RNA/protein) between injected and non-injected mice, the N-peptide restored the expression of Nav1.5 at the plasma membrane (Nav1.5/N-cadherin ratio = 0.50 ± 0.08, n = 11 in Scn5a+/- mice vs 1 ± 0.15, n= 11 in WT mice, $P< 0.05$ and 0.94 ± 0.13, n = 11 in injected Scn5a+/- mice, $P< 0.05$).
CONCLUSION – The use of N-peptide in gene therapy has the potential to reduce both conduction problems and heightened susceptibility to arrhythmias in Scn5a+/- mice. While further research is required to validate and delve deeper into our findings, this could offer a hopeful approach to treating individuals with sodium-channel dysfunction like BrS.
Keywords: SCN5A, intracardiac pacing, arrhythmia, ECG, gene therapy

62- Characterization of Purkinje-like cardiac cells derived from iPS cells from patients with Purkinje-induced idiopathic ventricular fibrillation

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UMRS1166 - équipe 1

Introduction Purkinje-induced idiopathic ventricular fibrillation (IVF), initially called short-coupled Torsades de Pointes (scTdP) is a form of IVF triggered by Purkinje ectopy responsible for unexplained sudden cardiac death in young people. This entity is often associated with isolated short-coupled (<350 ms) premature ventricular complexes (scPVC) which can degenerate into ventricular fibrillation and lead to death. The treatments available today are quinidine or the implantation of a cardiac defibrillator. According to the literature, 15% of patients with scTdP have a family history of sudden cardiac death (SCD), suggesting that this arrhythmia may have a genetic origin. However, little is known about the underlying mechanisms of Purkinje arrhythmogenicity and its genetic determinants. This project will focus on patients with Purkinje-induced IVF or scPVCs having a family history of SCD or cardiac arrest/syncope.
Objective The aim of this work is to create a human cellular model of Purkinje-related IVF using iPS-derived cardiomyocytes in order to characterize them in vitro and identify the mechanisms underlying arrhythmogenicity.
Method Blood samples will be collected from patients diagnosed with Purkinje-related IVF, who have at least one kindred with SCD or scPVCs. Peripheral blood mononuclear cells will be isolated and reprogrammed into iPS cells, which will then be differentiated into Purkinje-type cardiac cells using a cocktail of specific drugs.
Results Blood cells from 3 patient are being reprogrammed. Once their quality as pluripotent stem cells has been verified, they will be differentiated into Purkinje-like cells using a cocktail of drugs and further characterized using electrophysiological and molecular techniques.
Conclusion The results of this project will provide new genetic and mechanistic insights into a major cause of sudden cardiac death in young people, which could lead to new therapeutic targets.

Keywords: Idiopathic ventricular fibrillation ; inherited arrhythmias; iPS cells; Purkinje system

63- Validation of deep learning models to detect pathological repolarization disorders on the ECG and the risk of ventricular arrhythmias (DEEPECG4U Clinical)

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Background and Aims Torsades de Pointes (TdP) are potentially fatal ventricular arrhythmias promoted by prolonged ventricular repolarization [1]. A prolongation of the heart rate-corrected QT interval (QTc) is an electrocardiographic (ECG) biomarker used to predict an increased risk of TdP. Limiting ECG analysis to that of QTc is not predictive enough, as the information contained in ECGs is much richer in highly hierarchical patterns, beyond manual measurements of single features. Deep learning is particularly adapted to automatically detecting patterns. We have recently shown that ECG analysis using a DenseNet, a type of convolutional neural network (CNN), identifies ECG features that are more discriminating in predicting the type of LQT and the risk of TdP, beyond QTc [5]. The objective of this doctoral project is to prospectively validate these CNN models in real life conditions, in 5000 patients in 13 different centers of the APHP hospitals.

Methods In the context of this project, we have already developed different DenseNet-like CNN models, capable of identifying from raw ECG data patients with LQT and discriminating their underlying type, as well as predicting drug-induced TdP events [5]. These models have been trained using an existing dataset on drug intake footprints [2], and validated in external cohorts (congenital LQT, patients with TdP events, drug intake footprints, etc) [5].

Results We expect these models to display greater accuracy than the standard methods applied to ECG, namely measurement of QTc duration. If our models are validated, we will start by implementing them in a local cloud-like application, that will be accessible and used by the participating clinical centers.

Conclusion The automated analysis of these ECGs by the CNN models allows saving considerable time and access to delocalized rhythmological expertise within the various hospital departments and in the city and ultimately improve prevention and reduce the occurrence of TdP and eventually sudden death.

Keywords: predictive medicine, ventricular arrhythmias, artificial intelligence

64- Effect of endothelial cell organisation on hiPSC-CM maturation in a cardiac micro-tissue model

Gabriel FRIOB

UMR 8256 - Team CARTHER "Stem cells, cardiovascular physiopathology and biotherapies

Over the past decade, the development of robust protocols for the generation of cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) has considerably facilitated the study of genetic cardiomyopathies. One of the major limitation of this model is the lack of mature contractile function of hiPSC-CMs compared to adult human cardiomyocytes. Numerous studies have shown that the function of hiPSC-CMs can be improved by using multiple cardiac cell types in 3D co-culture to mimic the in vivo cell environment. However, the potential impact of the relative spatial organization of the different cell types inside the cardiac microtissue is still poorly understood. The aim of this study is to evaluate the effect of endothelial cell organization on hiPSC-CM function within a cardiac microtissue model. After derivation of hiPSC-CMs from hiPSCs using a cardiac differentiation protocol, a 3D co-culture model called a "spheroid" is established by self-aggregation of different cardiac cell types (70% hiPSC-CM + 15% cardiac fibroblasts + 15% endothelial cells). Two different spheroid models with the same composition but with different organization (Self-organized: endothelial cells homogeneously distributed or Constrained: endothelial cells are concentrated to form a core at the center of the spheroid) will be functionaly compared by assessing their contractility and kinetics of calcium transients. Contractile analysis shows a significant increase in the amplitude of contraction of spheroids with the constrained organization (core of endothelial cells) to the self organized spheroids (homogeneous distribution of cells). Differences in calcium kinetic parameters such as the time to reach the calcium peak or the time for calcium transient decay are also observed between the two conditions, demonstrating the positive effect of preforming an endothelial cell core on the function of this 3D model. The development of this model highlights the important role of cell organization in the enhanced function of hiPSC-CMs enabled by 3D co-culture of cardiac cells.

Keywords: hiPSC-CMs ; cardiac micro-tissue model ; endothelial cells ; cell organization

65- Targeted mRNA sequencing helps to classify variants affecting splicing in Hypertrophic Cardiomyopathies

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Introduction: Hypertrophic cardiomyopathies (HCM) are inherited cardiac diseases with an autosomal dominant transmission, among which MYBPC3 is the main causal gene responsible for haplo-insufficiency. RNA splicing variants in MYBPC3 appears to be a prevalent cause of HCM, however RNA analysis is not developed for this diseases because of cardiac tissue unavailability. Thus, these variants are often classified as Variant of Unknown Significance (VUS) and can't be use for clinical purposes. Objectives: We aim to propose an optimized enrichment method to detect splicing aberrations in MYBPC3 cDNA causing cardiomyopathies from blood cells mRNA. Method: We selected 26 variants (16 intronics and 10 exonics) detected on DNA sequencing and predicted to affect splicing (SpliceAI, SPiP). polyA+ RNA purified from venous blood cells (PAXGene®) of variant carriers was retro-transcribed, captured with optimized design (KAPA HyperCap®) and sequenced on NextSeq550. A specific bio-informatic pipeline was developed to automatically detect splicing events. Results: The gene MYBPC3 was very well covered and interpretable (RPM~6809, Mean depth~2947X). We detect a splice aberration for 19/26 (73%) of cases, consistent with their respective predictive score, among which 6 (32%) creates a novel junction; 8 (42%) modifies the proportional usage of annotated junctions and 5 (26%) leads to the retention of the entire intron. Then, we perform bio-informatic screening that was able to detect all pathogenic events, including intron retention even when no abnormal junction is associated. Conclusion: Targeted mRNA sequencing from blood cells allows functional identification of splice variants with the perspective to helps for splice variant classification, thus improving the yield of molecular diagnostic in cardiomyopathy patients. This method could also be used for screening, even as first approach, thanks to a specific bio-informatic pipeline and be expanded to other cardiomyopathy genes.

Keywords: Hypertrophic Cardiomyopathy, Splice Variant, Molecular diagnostic

66- Role of P-Protein on Vascular Leakage Associated with Acute Circulatory Failure

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College-de-france , team Role of Matrix Proteins in Hypoxia and Angiogenesis

Introduction
Vascular leakage is a major feature of the post-resuscitation syndrome. Transcriptomic analysis of patients circulating monocytes identified the p-protein gene, coding for the ligand of a receptor involved in vascular biology, as associated with massive vascular leakage after cardiac arrest. In accordance, plasma levels of P-PROTEIN closely correlated with the level of vascular leakage in an independent cohort of patients. In a model of resuscitated cardiac arrest in mice, we confirmed an important vascular leakage in all the organs and a concomitant massive expression of circulating P-PROTEIN. Modulating P-PROTEIN activity strongly affected the survival in the model, with better a return of spontaneous circulation (ROSC) and a better survival in mice injected with rP-Protein.
Objectives
To test the hypothesis that P-PROTEIN may play a role during cardiac arrest induced vascular leakage.
Method
We first set up a model of resuscitated cardiac arrest in mice, with a no-flow and a low-flow time of 8 min each. Survival and were then compared after cardiac arrest between p-protein-KO, WT, and mice injected with recombinant mouse P-PROTEIN (rmP-PROTEIN), as well as vascular leakage using i.v. injected fluorescent dextrans.
Results
We confirmed an important vascular leak in all organs after the return of spontaneous circulation (ROSC) and an induction expression of circulating P-PROTEIN levels at one-hour post-ROSC, in WT mice. Intra-venous injection of 10 µg rmP-PROTEIN at the time of resuscitation strongly reduced the vascular leakage reflected by extravasation of fluorescent dextrans. Survival was also significantly affected by modulating P-PROTEIN activity. We demonstrated a beneficial effect of rmP-PROTEIN injection, with 87.5% ROSC in WT mice injected with rmP-PROTEIN vs. 66.7% controls and an improved survival in rmP-PROTEIN injected mice vs controls ($p<0.0001$). The proportion of mice achieving a ROSC was on the contrary strongly reduced in P-protein -/- mice compared to their WT littermates.

Keywords: permeability, vascular leakage, cardiac arrest, circulatory failure

67- Role of 12-KetoLCA in Atherosclerosis

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U1166, Team5

Atherosclerosis has been one of main factors for global premature mortality and disability. Cholesterol is considered one of the primary culprits in the development of atherosclerosis since one century ago, and lipoprotein, especially low density lipoprotein (LDL) plays important roles in contributing to the development of atherosclerosis. As the metabolites of cholesterol, bile acids have been known that can be involved in the immunity system and cholesterol regulation. Previous data in the team has shown that 12-KetoLCA is highly correlated with secondary bile acids related with Tregs and Th17 regulation such as 3-oxoLCA and isoalloLCA. Hence, this project aims to investigate the effects of 12-KetoLCA on the process of atherosclerosis and its potential immunological roles.

Keywords: Bile acid, Atherosclerosis, Microbiota, Hyperlipidemia

68- Tolerogenic vaccination as an immunotherapy targeting T cells in Alzheimer's disease and other tauopathies

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Alzheimer's disease is now the leading cause of dementia, affecting more than 46 million people worldwide. A great deal of research is currently highlighting the involvement of immune mechanisms in the course of the disease. In addition to innate neuroinflammation mediated by glial cells, there is increasing evidence of the key role played by T lymphocytes and the peripheral-central immune crosstalk in the pathophysiology of AD and other tauopathies. This opens up new therapeutic possibilities based on immunomodulatory approaches. Previous work by the team on a model of tauopathy suggests that Tau pathology may trigger detrimental T cell responses promoting deleterious neuroinflammation and cognitive deficits. In contrast, our recent data show that broad-spectrum amplification of Tregs improves cognitive functions and reduced neuronal inflammation, thus supporting the therapeutic potential of T-cell-targeted immunomodulation in Tauopathies. Our team has developed a strategy known as tolerogenic vaccination as an innovative immunotherapy designed to induce immune tolerance specific to an antigen. We have shown that this tolerogenic vaccination induces a long-term protection in a model of food allergy to ovalbumin and protects against the development of paralytic disorders in a model of autoimmune encephalitis. We propose here to apply this innovative strategy to the treatment of tauopathies in order to induce control of Tau-reactive T cells in the periphery. We will first examine in vitro the tolerogenic impact of these vaccines and their ability to inhibit the activation of Tau-specific T cells derived from cervical lymph node of Thy-Tau22 mice. We will then evaluate in vivo the therapeutic efficacy of our vaccines in the Thy-Tau22 model and characterize the underlying immunomodulatory mechanisms induced by the vaccination. We should provide proof-of-principle of the feasibility and efficacy of tolerogenic vaccination as an innovative immunotherapy targeting the T response to selectively attenuate the Tau-specific response in Alzheimer's disease.

Keywords: Immunotherapy ; Tau ; Adaptive immunity ; Neuroinflammation ; Tolerogenic vaccination

69- Alterations in gut microbiome-immune interactions in multiple sclerosis

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Background: Previous studies have reported perturbed gut microbiota homeostasis in multiple sclerosis (MS). This shift has been linked to changes in humoral response and mutual interaction with bacteria, particularly for IgA whose concentrations appear to be increased in the central nervous system during active MS. However, the gut microbiome composition as well as microbiome-IgA interplay during progression of the disease from early-onset Clinically Isolated Syndrome (CIS) to late-onset MS has not been clearly defined. Hence, the objective of this study was to compare the free and IgA-binding gut microbiome composition, interaction and function of healthy individuals to that of CIS and MS patients in effort to establish microbial signatures that are related to disease progression from early to late stage. Methodology: We recruited a cohort of healthy donors (n =28) as well as CIS (n=11) and MS patients (n=25) from whom faecal samples were collected. Whole microbiome as well as IgA-sorted bacteriome was then identified using both 16S ribosomal ribonucleic acid (rRNA) and shotgun sequencing. This was followed by general microbiome analysis which involved examining microbial diversity and composition, strain-level profiling and functional analysis Results: We show that bacterial and fungal composition, abundance and interaction profile differ between healthy individuals and CIS patients and MS patients. Network analysis revealed increased IgA+ bacterial clustering in CIS individuals as compared to healthy subjects. Increased strain-sharing between IgA+ and IgA- fractions in healthy controls as compared to CIS was also inferred. Functional analysis of IgA+ bacteria indicated enrichment of amino acid biosynthesis, peptidoglycan biosynthesis and peptidoglycan maturation pathways in CIS patients as compared to healthy subjects. Conclusion: Our study highlights potential alterations in microbial ecology in of both free and IgA-interacting bacteria in CIS and MS. This presents a foundational basis for further investigating microbe-immune interactions as a crucial role-player in early-onset MS pathogenesis. We acknowledge that this could be performed through the adoption of a larger and longitudinal study cohort so as to obtain a more comprehensive representation of these mutualistic interactions in patients with MS.

Keywords: Microbiome, IgA-binding, Multiple Sclerois, Interkingdom interactions, Strain-level analysis

70- Management of immune checkpoint inhibitor myotoxicity

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Immune-checkpoint-inhibitor (ICI)-associated myotoxicity involves the heart (myocarditis) and skeletal muscles (myositis), which frequently occur concurrently and is highly fatal. We report the results of a strategy that included identification of individuals with severe ICI-myocarditis by also screening for and managing concomitant respiratory muscle involvement with mechanical ventilation, as well as treatment with CTLA4-fusion protein abatacept and the Janus-kinase inhibitor ruxolitinib. Forty cases with definite ICI-myocarditis were included with pathological confirmation of concomitant myositis in the majority of patients. In the first 10 patients, using recommended guidelines, myotoxicity-related fatality occurred in 60%, consistent with historical controls. In the subsequent 30 cases, we instituted systematic screening for respiratory muscle involvement coupled with active ventilation and treatment using ruxolitinib and abatacept. Abatacept dose was adjusted using CD86-receptor occupancy on circulating monocytes. Myotoxicity-related fatality rate was 3.4%(1/30) in these 30 patients vs.60% in 1st quartile($p<0.0001$). These clinical results are hypothesis-generating and need further evaluation.

Keywords: Myocarditis; myositis; immune checkpoint inhibitors; cancer

71- Preclinical evaluation of a Treg-targeting immunomodulatory treatment in a mouse model of Alzheimer-like tau pathology

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Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by progressive loss of memory and cognitive functions. Its main neuropathological hallmarks are the extracellular aggregation of amyloid beta peptides, intraneuronal aggregation of abnormally phosphorylated Tau proteins, and chronic neuroinflammation mediated by microglia and astrocytes. Besides neuroinflammation, increasing evidence highlight an instrumental role of peripheral immunity and peripheral-central immune crosstalk in AD pathophysiology. Our previous studies in a mouse model of AD-like amyloid pathology evidenced a beneficial role of regulatory T cells (Tregs), which modulate the rate of disease progression and onset of cognitive deficits, at least partially by controlling the development of detrimental microglial responses. Our recent data further supported that Tregs fine-tune the balance of reactive astrocyte subtypes in AD-like amyloid pathology. In parallel, in the THY-Tau22 mouse model of AD-like Tauopathy we evidenced that Tau pathology is associated with detrimental T-cell-mediated processes that contribute to promote detrimental neuroinflammation and cognitive deficits. Considering the unique capacity of Tregs to inhibit both CD4+ and CD8+ T cell responses, our data raise the hypothesis that amplifying Tregs may allow controlling Tau-driven T-cell-mediated detrimental processes in AD and other Tauopathies. We thus evaluated the impact on disease progression of an optimized immunomodulatory treatment aimed at selectively amplifying Tregs in the THY-Tau22 mouse model. Our data support that such treatment i) restores cognitive functions, ii) modulates functional profiles of astrocytes and microglia, iii) without significantly altering pathological Tau protein deposition. Our study supports the therapeutic potential in Tauopathies of Treg-targeting immunomodulatory approaches.

Keywords: Alzheimer's Disease ; T cell immunity ; Immunotherapy ; Neuroimmunology ; Neuroinflammation

72-Fusobacterium nucleatum-directed IgG and IgA seroreactivities are associated with poor prognosis in melanoma and lung cancer

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Background: Intestinal dysbiosis is associated with reduced benefit to cancer immunotherapy. While intratumoral pathobionts such as Fusobacterium nucleatum are associated with tumourigenesis and immune escape, Akkermansia muciniphila restore T cell memory responses during PD-1 blockade and predict clinical benefit. We postulated that immunomicrobiome profiling, including antibody responses, may predict cancer prognosis with checkpoint inhibitors.

Material and methods: Serologic reactivities (IgA, IgG) against four prevalent commensals were measured at baseline by bacterial flow cytometric analysis in 788 patients with stage III-IV cancer (lung, kidney, melanoma, colorectal) including two independent cohorts of melanoma, with or without immunotherapy, and healthy donors. Staining indices were considered as high versus low according to the median value of each cohort, or as continuous variables. The prognostic value of antibodies was estimated using a multivariable Cox model calculating the hazard ratio for high versus low values of each antibody adjusted for the patient baseline characteristics.

Results: A. muc- and F. nuc-specific IgA and IgG staining indices decreased and increased in lung and melanoma patients compared with HD, respectively. In addition, F. nuc-specific IgA fluctuated with chemotherapy and immunotherapy in opposite directions. Importantly, elevated baseline A. muc-specific IgG responses were associated with favorable prognosis in stage III melanoma with or without ipilimumab, as well as with responses to PD1 blockade in stage IV kidney cancers. In contrast, pre-existing F. nuc-specific IgG responses were associated with unfavorable evolution in two independent cohorts of stage III melanoma and, when considered in association with high F. nuc IgA, in stage IV NSCLC under anti-PD-1/ PD-1 therapy.

Conclusions: Lung and melanoma patients harbor baseline increased F. nuc and decreased A. muc- antibody reactivities, that have an unfavorable prognostic value with or without immunotherapy. Prospective validation of this tool and development of prophylactic strategies against oncobacteria or exploit the immunogenic ones are awaited.

Keywords: cancer, immunotherapy, Fusobacterium nucleatum, prognostic biomarkers, gut microbiota

73-Clinical and histological significance of complement terminal pathway activation and intra renal immune response in C3 glomerulopathy

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Background: C3 glomerulopathy is a rare kidney disease resulting from an overactivation of the complement alternative pathway. Although there is also evidence of terminal pathway activation, its occurrence and association with clinical and histological presentation are unknown.
Methods: A French cohort of 42 patients diagnosed with C3 glomerulopathy was retrospectively studied. Extensive characterization of histological parameters was performed using the histological index for C3 glomerulopathy. Kidney C5b-9 staining was performed as a marker of terminal pathway activation and intra-renal immune response was characterized through transcriptomic analysis of 847 immune related genes.
Results: Eighty-eight percent of biopsies presented with significant C5b-9 deposits in glomeruli (n=37/42). Biopsies were grouped according to the magnitude of C5b-9 deposits: 36% (n=15/42) with no or low deposits, 36% (n=15/42) with intermediate deposits and 28% (n=12/42) with high. Except for age distribution, the 3 groups did not present with significant clinical difference at the time of kidney biopsy. High C5b-9 group was characterized by a significant higher histological chronicity score (p=0.005). We identified lower outcome-free survival in high C5b-9 group compared to the 2 others (p=0.001). In multivariable analysis, high glomerular C5b-9 remained a significant predictor of poor kidney prognosis after adjustment on parameters associated with disease prognosis. One third of the 847 studied immune gene were upregulated in C3 glomerulopathy biopsies compared to controls. Unsupervised clustering on differentially expressed genes identified a group of kidney biopsies enriched in high glomerular C5b-9. This group was moreover characterized by a high immune and fibroblastic signature and showed high chronicity score on histological examination.
Conclusions: Intra-renal terminal pathway activation is associated with specific histological phenotype and disease prognosis in C3 glomerulopathy and seems to associate with intra-renal immune response.

Keywords: Immunology, glomerulonephritis, complement

74-Mesenchymal stromal cell (MSC) therapy of bladder tissue damage after radiotherapy

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Chronic Radiation Cystitis (CRC) is a pathology induced by pelvic irradiation. CRC is characterized by chronic inflammation of the bladder, occasionally progressing to fibrosis,
accompanied by symptoms including pain and bleeding. Mesenchymal stromal cells (MSCs) could be a therapeutic option, due to their function in modulating chronic inflammation and fibrosis after irradiation. Previous studies have demonstrated that MSC therapy is an alternative treatment for interstitial cystitis and hemorrhagic cystitis.

To study MSC treatment of CRC, a rat model of radiation cystitis was developed. The irradiation of the entire bladder with a total dose of 40Gy is performed using a Small Animal Radiation Platform (SARRP). To potentiate the effect of treatment, repeated intravenous injections of MSC are administered before the onset of CRC (between 4.5 and 5.5 months after irradiation). Physiological, histological, and transcriptomic analyses are performed during the progression of CRC.

At 8 months post-irradiation, histological studies of the inner wall of the bladder revealed that treatment with MSCs induced a reduction in hyperplasia. Irradiation would induce proliferation of urothelial stem cells without differentiation into superficial cells, leading to loss of impermeability.Treatment would restore tissue homeostasis, enabling impermeability to be maintained. Transcriptomic analysis suggests that HGF and EGF transcripts may contribute to urothelial repair by MSCs. At 12 months post-irradiation, irradiation increase vascular lesions whereas treatment with MSCs appears to reduce these lesions. At 14 months, Irradiation induces disorganization of collagen I and III fibers, leading to rigidification of the bladder wall. At the functional level, at 6, 8 and 12 months, irradiation increased the number of micturitions, while treatment reduced the number of micturitions
after irradiation.
MSC therapy could limit CRC progression by rebalancing tissue homeostasis. MSC therapy could be an alternative to conventional treatments of CRC.

Keywords: Radiation cystitis; Mesenchymal Stromal Cell

75-Multi-OMICS signature of human tissue regulatory T cells

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Purpose: A better understanding of tissue regeneration still remains a public health challenge, and the role of regulatory T cells (Treg cells) in this context has been highlighted in murine models. This specific Treg population promotes tissue regeneration while limiting fibrosis by releasing molecules such as amphiregulin (AREG), a ligand for the epidermal growth factor receptor (EGFR). However, their characterization is challenging due to their distinct profile compared to circulating Treg cells. Tissue Treg cells appear to undergo gradual differentiation and functional specialization within the local tissue microenvironment to fully develop their phenotype. Potential markers for this population have been identified in mice, such as ST2, the receptor for IL-33, which seems to orchestrate Treg cells infiltration into injured tissues. However, their presence and function in humans remain uncertain, thus other markers such as CCR8 and BATF are being investigated, but the underlying mechanisms are still to be understood. A multi-OMICS approach to characterize this population across various human organs would help establish a specific signature for tissue Treg cells and enhance our understanding of their role in tissue regeneration.
Methods: To achieve this goal, we performed phenotypic analysis using flow cytometry and transcriptomic analysis through single-cell RNA sequencing. We successfully characterized tissue Treg cells in various human organs, including the spleen, muscle, visceral adipose tissue (VAT), colon, and small intestine. Additionally, we conducted single-cell RNA sequencing on CD4+ T cells from healthy muscle and blood.
Results: Our findings revealed the presence of Treg cells in all examined tissues of healthy donors, with varying proportions and phenotypic differences, including varying FOXP3 expression levels, across different tissue types. Single-cell RNA sequencing analysis revealed the presence of muscle-specific CD4+ populations compared to blood, and highlighted transcriptomic differences between circulating and tissue Treg cells. A specific signature for muscle-resident Treg cells is being established, and the expression results of known Treg markers confirm a difference between these two populations, also observable by flow cytometry.
Conclusion: In conclusion, we have demonstrated the presence of tissue Treg cells across various healthy human tissues, exhibiting distinct phenotypic and transcriptomic profiles compared to circulating Treg cells.

Keywords: Treg cells, single-cell RNA sequencing

76-Membrane Traffic and Pathogenesis

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Tunneling nanotubes (TNTs) are tubular membranous conduits representing a novel direct way of communication between distant cells, shown in vitro transferring genetic material, mitochondria and different pathogens. However, their physiological relevance is unclear because their existence in vivo has not yet been shown explicitly. We hypothesize that TNTs could represent an early feature of cell-to-cell communication in the developing nervous system which predates classical synaptic transmissions, and be instrumental for the emergence of functional mature neuronal networks. I am currently investigating the presence and functionality of TNTs in the developing cerebellum, spinal cord, retina of chick embryos and cerebellum of mice by a multidisciplinary approach.
Using the iOn genetic switch, a gene editing tool based on the PiggyBac transposon system; I am producing chick and mouse nervous system organs which the mitochondria of juxtaposed cells, as well as their membrane, is labeled with distinct colors, enabling us to identify the TNT membrane and follow mitochondria transfer between neighbor cells. Slices from these models are imaged using multi-color confocal microscopy allowing an estimate of mitochondrial transfer in TNTs. Lineage tracing and mosaic analysis, by this somatic transfection, will enable us to assess the functionality of TNTs.

Keywords: Cell communication, Neuroscience, Molecular Biology

77- Study of the impact of activated neutrophils on the blood-brain barrier in Epilepsy

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Epilepsy, a neurological disorder marked by recurrent seizures and inflammation, implicates neuroinflammation, possibly stemming from peripheral inflammation and leukocyte migration across the blood-brain barrier (BBB). Polymorphonuclear neutrophils (PMNs), crucial innate immunity components, mediate inflammation-induced damage. Despite elevated IL-8 levels attracting and activating PMNs, the precise characterization and impact on BBB of PMN in epilepsy remains insufficiently elucidated.

Blood samples from epileptic patients assessed systemic inflammation via cytokine levels and the neutrophil-lymphocyte ratio (NLR). Flow cytometry focused on membrane markers (CD11b, CD62L, CD54, CXCR1, CXCR4) to delineate activation states: steady (CXCR4+, CD62+), activated (CXCR4+, CD62Llow), hyperactivated (CXCR4high, CD62Llow). We also explored "epileptic" PMNs impact on BBB disruption by exposing cerebral endothelial cells (CECs) to PMN secretomes, evaluating CEC permeability via X-celligence technology, and assessing junction protein expression through immunofluorescence.

Investigations unveiled chronic systemic inflammation with elevated NLRs and pro-inflammatory cytokines. Flow cytometry showed membrane marker shedding, with activated PMNs significantly elevated, correlating with clinical data. Patient PMNs secretomes induced CECs permeability, with heightened junction protein loss upon exposure to activated E-PMN secretomes.

This project assesses PMN phenotypes in epileptic patients, revealing abnormal PMN activation. This exploration facilitated a disease score's development, aiding in reclassifying patients potentially unaware of seizures. Recognizing BBB disruption as an epileptogenic factor, this project lays groundwork for strategies targeting drug-refractory temporal lobe epilepsy.

Keywords: Epilepsy / Neutrophils / Bood Brain Barrier/ Neuroinflammation

78-Influence of the serotonergic system on the development of microglia

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Microglial cells, also known as brain-resident macrophages, play a major role in the development and maturation of neuronal circuits. During the early postnatal weeks, microglia undergo transcriptomic and morphological changes that are crucial for their optimal functioning. A potential factor acting on the regulation of microglia maturation is serotonin (5HT), a neuromodulator which is also involved in the development and refinement of neuronal circuits.
Indeed, it has been shown that microglia express receptors for serotonin, notably the 5-HT2B subtype, and that the lack of the microglial 5-HT2BR in mice leads to morphological defects in microglia and neuronal connectivity anomalies reminiscent of autism spectrum disorders (ASD).
We aim to understand how serotonin regulates the maturation of microglia and some of their functions that are critical for the proper establishment of neuronal circuits.
In order to explore this regulation, we first quantified the morphological contacts between 5-HT axons and microglia throughout the postnatal development of mice. We found that those contacts increase between postnatal day 3 and 15.
We are now assessing the morphology and the transcriptomic signature of microglia at P15 in two types of neonatal alterations of this communication: a genetic mouse model lacking the 5-HT2B receptor specifically in microglia, and a pharmacological model of chronic activation of the 5-HT2B receptors.
Our first results support the hypothesis that 5-HT plays a role in microglial development, and our goal is now to identify 5-HT-dependant microglial genes and investigate their role in microglial and neuronal maturation.

Keywords: Microglia, Serotonin, Neuro-immune interaction, Neurodevelopment, ASD

79-Targeting dendritic over-inhibition to alleviate cognitive deficits in Down syndrome: the role of Martinotti inhibitory interneurons

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Introduction:
Overinhibition of cortical circuit was proposed as an important pathophysiological mechanism underlying intellectual disabilities and cognitive deficits in Down syndrome (DS). Negative allosteric modulators (α5-NAMs) targeting the α5 subunit of the GABAA receptors (α5-GABAARs) have been shown to improve cognitive deficits in DS mouse models. α5-GABAARs are selectively expressed at inhibitory synapses between specific somatostatin-positive interneurons, the Martinotti cells (MCs) and distal dendrites of pyramidal neurons (PN) in the cortex and their homologous in the hippocampus. The aim of this project is to understand which molecular mechanisms underlie alterations of dendritic inhibition at MC-PN synapses in the Dp(16)1Yey mice modelling DS.
Methods:
Dp(16)1Yey mice were crossed with GAD-67-GFP mice, line X98 (X98), The Jackson Laboratory, stock #006340 expressing GFP in MCs. MCs from the prefrontal cortex of Dp(16)1Yey and WT mice were dissociated, purified by FACS, and sequenced using bulk-RNAseq. In parallel, α5-GABAARs and their associated proteins Radixin and Gephyrin were labelled by immunohistochemistry on mouse brain sections of Dp(16)1YeyxGAD67-GFPX98 mice. Confocal and super-resolution microscopy were used to analyze their co-colocalization.
Results:
We will here reveal analyses from bulk-RNAseq of MCs from the prefrontal cortex of WT and Dp(16)1Yey mice showing the differentially expressed genes in these GABAergic interneurons involved in dendritic inhibition. Immunolabelling of α5-GABAARs, Radixin and Gephyrin allowed us to show the synaptic and extra synaptic localisation of the receptors at the MC-PN synapses.
Conclusion:
This work will enable us to understand the molecular mechanisms involved in dendritic inhibition at MC-PN synapses in DS and reveal changes in the synaptic localisation of α5-GABAARs . The next step will be to test whether modulation of dendritic inhibition can counteract those molecular changes observed in Dp(16)1Yey mice by treating the mice with α5-NAMs.
Keywords: Down syndrome, Martinotti cell, 5-GABAARs,α 5-NAMs, Dp(16)1 YeyxGAD67- GFPX98 mice

80- Role of schizophrenia-associated exportin 7 in the development and function of cortical neurons

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Schizophrenia (SZ) is a severe and persistent psychiatric disorder influenced by genetic factors. A recent exome-sequencing study of schizophrenia identified 10 genes as having an exome-wide significant excess of rare damaging coding variants. Of these variants, protein truncating variants (PTVs) of the XPO7 gene conferred the highest SZ risk. XPO7 encodes exportin 7, a protein that gates nucleocytoplasmic transport. XPO7 is expressed in neurons, but its role in the brain is completely unknown. We developed a new mouse model of XPO7 knock-down in cortical neurons to study the role of XPO7 in neuronal development and function, and thereby obtain mechanistic insight into the aetiology of SZ.
Using in utero electroporation of shRNA at embryonic stage E14.5, we knocked down XPO7 in pyramidal neurons of layers II/III of the frontal cortex. We then investigated the effects of decreased XPO7 expression on neural progenitor proliferation, neuronal excitability and synaptic function.
We first observed that, although most XPO7 KD neurons migrated correctly, some displayed abnormal localization in lower cortical layers. The proliferation of neural progenitors was increased upon XPO7 KD, potentially contributing to localization defects of these neurons. Furthermore, we used patch-clamp to study of physiological properties of neurons that had correctly reached layer II/III of the cortex and showed that XPO7 KD causes neuronal hyper-excitability. XPO7 KD neurons also displayed a reduction in the frequency of miniature synaptic excitatory and inhibitory currents, indicating decreased synaptic input and possibly reflecting a decrease in the number of synapses. Finally, we also noted a slower deactivation of NMDA receptors-mediated currents, reflecting a gain of function.
These findings underscore the crucial role of XPO7 in regulating neuronal proliferation, neuronal excitability, and synaptic function, and may provide insight into SZ pathogenesis. Further experiments will clarify the underlying molecular pathways.

Keywords: Cortical development, Neuronal properties, Synapses, Proliferation, Migration

81-Comparison of neuroinflammation in and around the lesion between two different mouse models of cervical spinal cord injury

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Background: Traumatic cervical spinal cord injuries (cSCI) damage respiratory pathways leading to life-threatening respiratory insufficiency. Inflammation is known to play a role in limiting neural regeneration after injury. We aimed to determine the expression level of chondroitin sulfate proteoglycans (CSPGs), ones of the most axonal growth inhibitory molecules, in two different models of SCI, either from a cervical hemisection model (HS, routinely used to study respiration in SCI) or from a cervical hemicontusion model (HC, closer to human pathophysiology of cSCI).
Material and methods: Twelve adult WT Swiss male mice underwent unilateral cervical SCI either by (1) HS at C2 level (n = 6) or (2) or HC at the C3-C5 level (n = 6). Spinal cord samples at the level of the lesion and around it (spinal segments above or below it) were obtained after transcardiac perfusion for immunohistochemistry analyses using Neurotrace™ and biotinylated wisteria floribunda agglutinin (WFA).
Results: Extend of injury were similar between HS and HC groups (85 ± 5 % and 74 ± 8 %, respectively). In spinal cord segments around the lesion level (above or below), the total number of neurons was similar in both groups (HS: 799 ± 104 vs. HC: 636 ± 102, p=0.19). CSPGs labelling was present both at the level of the lesion and around it. Interestingly, around the lesion site, a greater number of neurons surrounded by CSPG was found in the HS than in HC (75±5 vs. 57±4 respectively, $p < 0.05$).
Conclusions: In both models, inflammation evaluated by CSPGs expression was not only present at the level of the lesion but also around it. In addition, we found a high level of CSPGs expression in the area surrounding neurons around the site of the lesion in both models, which may explain the limited synaptic plasticity observed following some regenerative therapies in SCI. CSPGs surrounding neurons could be a good marker of neuroinflammation for future studies.

Keywords: spinal cord injury; hemicontusion model; hemisection model; neuroinflammation; chondroitin sulfate proteoglycans (CSPGs)

82- Oxaliplatin-induced peripheral neuropathy: molecular and anatomical characterisation of intra-nerve vessels in the acute phase

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CIRB

Many cancer treatments have side effects, such as the peripheral neuropathy caused by Oxaliplatin, a platinum salt. Symptoms of peripheral neuropathy induced by Oxaliplatin causes intense pain in the vast majority of patients and may lead to discontinuation of chemotherapeutic treatment. In addition, Oxaliplatin is used in a wide range of cancers, particularly when first-line therapy does not work anymore. Understanding the mechanisms underlying the occurrence of peripheral neuropathy is therefore a public health issue. While the main studies have focused on the nervous component of the disease, none has succeeded in finding an efficient therapeutic treatment. Interestingly, peripheral nerves are highly vascularised to ensure proper homeostasis and thereby a good conduction of the intra-nervous signal. To study peripheral nerve vascular component involvement in the disease, the team has developed an acute mouse model of this neuropathy. Initial results from the team's suggest that Oxaliplatin induces symptoms by causing vasoconstriction of the nerve vessels, and more precisely by increasing the vascular smooth muscle contraction pathway. Moreover, the injection of vasodilators acting on smooth muscle cells significantly reduces symptoms as attested by behavioral tests. The main aim of my thesis is to gain a better understanding of these mechanisms highlighted by the team by characterising the intra-nerve vessels at molecular and anatomical level in our acute model. This will bring us closer to validating this hypothesis and could open up new avenues of treatment using vasodilators in acute peripheral neuropathies induced by oxaliplatin. To do this, I developed a dissociation protocol for my nerve to isolate endothelial and mural cells from these vessels in order to perform single-cell sequencing. I'm now concentrating on carrying out all the necessary controls before sequencing. I am also using immunostaining and transparisation to determine the proportion of different vessels making up the intra-nerve vessels.

Keywords: Intra-nervous vessels - hypoxia - vasoconstriction - Oxaliplatin - peripheral neuropathy

83-Toxicity of Aß peptide (intracellular pool) in Alzheimer's disease and new therapeutic approaches development

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Introduction:
Alzheimer's disease (AD) is a neurodegenerative disease impacting memory and cognitive function. The so-called "amyloid cascade" hypothesis suggests that the accumulation of Aβ peptides leads to amyloid plaques formation, triggering inflammation and subsequent brain lesions. While monoclonal antibodies have been used to target Aβ aggregates, their size limits efficient crossing of the blood-brain barrier (BBB) and targeting intracellular sites where Aß peptides also accumulate. VHHs, small camelid single domain antibodies, offer interesting alternative due to their compact size (15 kDa) and dual benefits of antibody binding activity and small molecules properties. This project aims to assess the efficacy of VHHs in targeting extracellular and intracellular Aβ deposits and to evaluate their therapeutic potential in an AD mouse model.
Materials and Methods:
A specific VHH, R3VQ, recognizing Aβ, was developed and optimized. Various R3VQ formats (monomer, dimer, fused to human Fc region) were designed and evaluated in vitro for binding capacity and therapeutic effects. In vivo studies in APP-PS1-KI mice assessed their binding and diffusion capabilities.
Results:
Different R3VQ formats demonstrated binding activity to Aβ 1-42 (both non-fibrillized and fibrillized forms) via ELISA tests. Thioflavin assays indicated R3VQ ability to inhibit Aβ 1-42 fibrillization over time. Immunohistochemistry on APP/PS1 KI mice revealed R3VQ's labeling of intracellular and extracellular Aβ deposits. In vivo tests demonstrated broad diffusion and specific binding of R3VQ variants in brain tissue after stereotaxic injections.
Conclusion:
This study is anticipated to evaluate the preclinical efficacy of R3VQ in AD treatment. Those tests will lead us to two enhanced immunotherapy strategies in preclinical models: 1) use of ultrasound to transiently open the BBB to facilitate diffusion of R3VQ through the BBB, and 2) use of an AAV vector to express R3VQ directly in the brain cells.

Keywords: Alzheimer's disease, nanobodies, VHH, anti amyloïd

84-Rod-derived cone viability factor 2 for the treatment of inherited retinal degeneration

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Background: Retinitis pigmentosa (RP) is characterized by progressive rod then cone degeneration, leading to blindness in severe cases with no therapeutic option. The nucleoredoxin-like 1 (NXNL1) gene codes for two isoforms: a short form, rod-derived cone viability factor (RdCVF), promoting cone aerobic glycolysis and a long form (RdCVFL) modulating oxidative damage. NXNL2, a paralogue of NXNL1, also encodes two isoforms with similarities in photoreceptor protection. We investigated whether both Nxnl1 and Nxnl2 are required for photoreceptor survival in mouse retina. We also studied the benefit of combined both short forms in photoreceptor protection by using the rd10 mouse model of RP.
Methods: Nxnl1-/-Nxnl2-/- mice were generated. Their retinal phenotype was investigated using electroretinogram (ERG) and optical coherence tomography (OCT). The cone density was measured by labeling cones in flat-mounted retinas.
Rd10 mice were subretinally injected with AAV products at PN14.Visual acuity was tested by PhenoSys quantitative optomotor response (qOMR) system 1 month post injection.
Results: At 3 months of age, the thickness of outer nuclear layer (ONL) consisting of over 95% rod photoreceptors showed 9.09% reduction ($p=0.0001$) in Nxnl1-/-Nxnl2-/- ($n=17$) compared to wild type (wt) mice ($n=20$); the rod-driven scotopic ERG showed 31.01% ($p<0.0001$) and 29.04% ($p<0.0001$) decrease in a- and b-wave amplitude in Nxnl1-/-Nxnl2-/- ($n=17$) compared to wt mice ($n=20$). At 12.5 months of age, the cone density in Nxnl1-/- Nxnl2-/- mice was reduced by 23.15% ($p=0.0080$, $n=9$) compared to wt mice ($n=11$).
Rd10 mice injected with a combination of AAV-RdCVF and AAV-RdCVF2 showed an increased visual acuity ($p=0.0032$, $n=3$) compared to the group injected with AAV-GFP ($n=3$).
Conclusions:
Both Nxnl1 and Nxnl2 are essential for photoreceptor survival in mice. The combination of RdCVF and RdCVF2 promoted vision rescue in rd10 mice, showing clinical potential for RP. Further investigation will decipher the underlying mechanism of RdCVF2 in cone rescue.

Keywords: photoreceptor protection, gene therapy

85-Setting up a retinal organoid model for RDH12-related Leber Congenital Amaurosis

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Background:
Leber's congenital amaurosis is a severe form of retinal dystrophy that has an early onset. Different causative genes have been identified including the RDH12 gene that codes for a retinol dehydrogenase enzyme in the photoreceptors of the retina. It also plays an essential role in the removal of toxic aldehyde compounds produced by light exposure. Despite the severe phenotype displayed in certain patients carrying mutations in the RDH12 gene, experimental mouse models, where RDH12 is absent, do not exhibit a comparable phenotype. As the mouse model presents some limitations, we develop an in vitro model, called retinal organoids, developed from induced pluripotency stem (iPS) cells derived directly from patient cells to understand the impact of mutations in the RDH12 gene in humans.
Material and methods: Three iPS cells lines are derived from patients carrying different mutations on the RDH12 gene. Differentiation of the iPS cells into retinal organoids is done by using a 2D followed by a 3D culture protocol. Then retinal organoids are characterized by immunostaining. Isogenic clones are generated by the CRISPR-spCas9 HDR strategy delivered by nucleofection.
Results: These retinal organoids from RDH12-patients show a distorted retinal lamination. There is also a loss of photoreceptors and reactive gliosis during retinal degeneration. Isogenic control clones are selected with the right correction and the absence of off-target and genetic abnormalities.
Conclusions: These retinal organoids seem to reflect a phenotype that has been shown in some patients where the retinal architecture is distorted. Comparison to a larger cohort of RDH12-patients can be interesting to confirm this phenotype observed in retinal organoids.

Keywords: Leber's congenital amaurosis, RDH12, retinal degeneration, retinal organoids, iPS cells

86-Reconstruction of the eye geometry by inverse optical design

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Background: This poster discusses the use of off-axis aberrometry for estimating the parameters of a numerical eye model by solving an inverse problem. Aberrometry measures the optical aberrations of the eye, but it has traditionally been limited to on-axis measurements in clinical ophthalmic applications. Off-axis measurements, which provide useful insights on peripheral vision performance, are more challenging to measure and interpret, but may help to discriminate between systems yielding similar on-axis aberrations, and hence contribute to solving this inverse problem. However, estimating the shape and positions of the eye's optical components from aberrometry measurements presents a significant experimental and mathematical challenge.

Material and Methods: We have designed and built a compact off-axis aberrometer intended for clinical trials, based on a traditional on-axis Shack-Hartmann aberrometer with an added off-axis (wide field) scanning arm and telescope relay system specified for scanning a -15°/+15° field on the retina.
Light from a laser diode passes through the pupil at the desired angle and is focused on the retina, backscattering light into the instrument, which is in turn analyzed by a Shack-Hartmann wavefront sensor. Measured wavefront maps hence carry the information of optical aberrations resulting from the curvatures, conicities, refractive indices, tilts and offsets of the underlying optical components of the eye.
We have also developed an optical simulation module for the measured wavefront maps at various illumination angles, which will be used for solving our inverse problem.

Results: - Reconstruction of parameters of a model eye with statistical elements (repeatability, estimation of uncertainties) from experimental data.
- Simulation of off-axis aberrations from model eye parameters.

Keywords: off-axis aberrometry, personalized eye models, inverse problem

87-Anatomical pathways and molecular mechanisms associated with blue light-induced photophobia

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BACKGROUND: Photophobia, a light-inducing sensory disturbance, is prevalent in neurological and ophthalmological disorders. Chronic light exposure (especially blue/violet light) is suspected to play a deleterious role in the epidemiology and the evolution of photophobia. The pathophysiological mechanisms of photophobia due to blue light are still poorly understood. This project aims to develop a preclinical photophobia model induced by chronic blue light exposure and investigate the associated molecular and cellular changes from the eye to the brain.
M&M: The model is obtained by exposing adult C57BL/6 mice to blue (460nm peak, condition) or yellow (570nm peak, control) light for 9hours daily over a week. Light sensitivity is assessed before and during exposure using a dark/light box. Post-exposure, cornea, retina, trigeminal ganglion, and brain are collected for RNAseq and immunohistochemistry analysis (Iba1, cFOS, GFAP staining). Statistical analysis is performed.
RESULTS: Our preliminary results from five mice exposed to 7days of blue light reveal a clear light aversion. Before exposure, mice stayed in the light box for 100seconds on average over a 5-minute period. After exposure, mice developed a clear photophobia, shown by a significative decrease (25%) of the average time spent in the light box and an increase (13%) in the darkness. Further validation with additional animals and comparison to yellow light-exposed mice is planned. Immunohistochemistry protocols for GFAP, cFOS, Iba1 staining are established for nerve damage, neuronal activation, and inflammatory responses. RNAseq analysis is ongoing to decipher molecular changes associated with blue light photophobia.
CONCLUSIONS: A photophobia model induced by blue light has been developed, with validated staining protocol in naive mice. This model will help to depict the transcriptomic signature and neuroanatomical abnormalities/plasticity along the corneal and retinal pathways to the brain in mice developing photophobia. Further, this project could lead to the development of new therapeutic approaches to photophobia.

Keywords: blue light, photophobia, pain pathways

88-Metabolic function of MLKL, the mediator of necroptosis, in adipose tissue

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Background and aims: Obesity is a complex metabolic condition characterized by chronic inflammation in adipose tissue, leading to disruptions in homeostasis and endocrine function. The role of programmed cell death, particularly necroptosis mediated by RIPK (Receptor interacting protein kinase) 1, RIPK3, and MLKL (Mixed lineage kinase domain like), has emerged as a key factor in obesity-related complications, including type-2 diabetes and metabolic-associated steatohepatitis (MASH). This study aims to elucidate the specific functions of MLKL within adipocytes in the context of obesity, shedding light on its metabolic implications. Methods: A mouse model with adipocyte-specific Mlkl deletion (MlklAdi-KO) was generated using the Cre-lox system. Both wild-type (WT) and MlklAdi-KO mice (8 weeks-old) were fed with a high-fat diet to induce obesity. Metabolic parameters were assessed using metabolic cages, while insulin resistance was evaluated through oral Glucose Tolerance Test (oGTT) and Insulin Tolerance Test (ITT). Liver histology and quantification of intrahepatic triglycerides were performed to assess steatosis. Transcriptomic analysis on visceral adipose tissue was conducted to examine pathways affected by Mlkl deletion. Results: MlklAdi-KO mice exhibited reduced weight gain compared to WT, indicating a metabolic regulatory role of MLKL in adipose tissue. oGTT and ITT revealed that MlklAdi-KO mice had less glucose impairment and insulin resistance compared to WT counterparts. Additionnally, MlklAdi-KO mice showed improved glucose tolerance and insulin sensitivity compared to WT mice. Histological analysis revealed decreased steatosis in MlklAdi-KO mice, accompanied by lower levels of intrahepatic triglycerides. Serum analysis indicated reduced levels of liver enzymes and LDL cholesterol in MlklAdi-KO mice. Furthermore, adipose tissue analysis demonstrated decreased expression of pro-inflammatory cytokines and markers in MlklAdi-KO mice. Transcriptomic analysis revealed alterations in lipid metabolism gene expression associated with Mlkl deficiency. Conclusion: Our findings underscore the significant role of MLKL in the development of insulin resistance and metabolic disorders associated with obesity. These insights may pave the way for novel therapeutic interventions in conditions lacking effective treatments.

Keywords: Adipose tissue; Insulin resistance; Mlkl

89-Deciphering inherited retinal dystrophies through generation of 3D stem cell-based retinal organoids

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Current models of neurodegenerative diseases derived from human induced pluripotent stem cells (iPSCs) have the capacity to recapitulate cellular and molecular processes leading to the disease, creating novel avenues to model and understand the pathogenicity. Regarding retinal disorders, iPSC technology is relevant for inherited retinal dystrophies such as retinitis pigmentosa (RP) that affect more than 1.5 million people worldwide. Retinas of small-animal models of RP can be used to study some aspects of the pathology, but they do not phenocopy all the features of the human disease. Thus, patient-specific iPSC-derived retinal cells provide new insights into the molecular and cellular mechanisms of specific genetic mutations. One of the major genes underlying this disorder is rhodopsin (RHO), coding for the light-absorbing molecule that initiates the signal transmission cascade in rod photoreceptors. Several attempts have been made to classify RHO mutations on the basis of their clinical manifestation and their biochemical and cellular behaviors. To understand RHO causative mutation pathogenicity, we are ambitioning to model RP caused by two specific RHO mutations, using specific RP patient iPSC lines. To achieve this objective, we have generated iPSC lines from RP patients carrying RHO mutations and we plan to: 1) derive isogenic control iPSC lines by CRISPR/Cas9 editing; 2) direct the differentiation of RHO mutated-iPSCs into retinal organoids and 3) characterize photoreceptor dystrophy mechanisms related to each RHO mutation. The study of the phenotype of iPSC-derived photoreceptors should allow us to identify the molecular and cellular mechanisms underlying the pathogenicity of each specific RHO mutation in a human context. The development of such iPSC lines carrying different RP mutations will also help for developing successful treatments using gene-editing technology.

Keywords: Retina, induced Pluripotent Stem Cell; Organoid; Genome editing; Hereditary retinal dystrophy

90-Delivery of Cas9 ribonucleoprotein to the retina of mice with Retinitis Pigmentosa

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Retinitis Pigmentosa (RP), the predominant cause of genetic blindness, is inherited in an autosomal dominant manner (adRP) in 35% of cases. Mutations in the RHO gene, which encodes Rhodopsin, account for the majority of adRP cases. In those case, gene addition therapy is hindered by the toxic effects of mutant Rhodopsin. Instead, "Suppression and Replacement" strategies, independent of specific mutations and employing CRISPR/Cas9 targeting RHO combined with cDNA delivery, have demonstrated efficacy in animal models harboring the most prevalent mutation observed in US patients, linked with a mild phenotype.
In an attempt to investigate the applicability of this strategy to model with a more severe phenotype, we aim to perform this approach on an adRP-mouse characterized by one of the most recurrent mutations in Europe and Asia.
We first conducted in vitro guide RNA (gRNA) screening on the RHO gene. The optimal gRNA was administered in vivo via dual-AAV encoding Cas9 and gRNA in adRP-mice. Next-generation sequencing (NGS) showed modest 0.6% editing efficiency on the whole retina.
To enhance editing efficiency, a non-viral approach was explored, injecting naked Cas9 ribonucleoproteins (RNPs) into adRP mouse retinas, revealing a total of 2% editing via NGS analysis.
In evaluating safety, Cas9 RNP injection induced retinal damage, signifying in vivo RNP toxicity.
Our preliminary results offer insights into AAV vs RNP-associated gene editing and in vivo toxicity for adRP treatment. These findings suggest the need for optimizing delivery strategies, notably in degenerating models, to increase editing efficiency and decrease toxicity.

Keywords : Gene therapy ; Retina ; CRISPR/Cas9 ; AAV ; Mouse model

91-Functional alterations in the gut microbiota are associated with the inflammatory and digestive phenotype of patients with A20 haploinsufficiency.

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Introduction. Haploinsufficiency A20 (HA20) is a monogenic disease associated with loss-of-function of the TNFAIP3 gene. Patients present with periodic fever, and almost 40% of them have digestive disorders including diarrhea. The gut microbiota shapes the metabolic and immune responses of the host depending on its composition and its ability to metabolize gut-derived metabolites. Consequently, dysbiosis and disruption of metabolites derived from the gut microbiota could favor the inflammatory and digestive phenotype of patients with HA20.

Objective. To investigate the composition of the gut microbiota and the profile of bile acids, SCFAs and tryptophan-derived metabolites in HA20 patients.

Methods. Stools from 17 French HA20 patients and 22 healthy subjects were collected. The intestinal microbiota was analyzed by 16s sequencing. Fecal bile acids, SCFAs and tryptophan metabolites were analyzed by high-performance liquid chromatography-mass spectrometry. Results were stratified according to stool consistency defined by the Bristol Stool Scale (BSS).

Results. HA20 patients displayed decreased bacterial diversity and a significantly different gut microbiome composition from healthy subjects ($p=0.026$). Bile acid profile was abnormal suggesting a deficit in the deconjugation and transformation of bile acids by the gut microbiota ($p=0.01$, respectively). HA20 patients also had significantly higher levels of fecal fatty acids than healthy subjects, suggesting an absorption deficit ($p=0.025$). Bile acid and SCFA profiles were significantly more impaired in patients with higher BSS ($p=0.016$ and $p<0.006$, respectively).

Conclusion. Patients with HA20 may have a specific dysbiosis compared with healthy subjects, which could induce the changes observed in metabolites. Increased toxic bile acids in the gut could be involved in the digestive symptoms of HA20 disease. In addition, deficient absorption of anti-inflammatory SCFAs may contribute to the pro-inflammatory state of HA20 patients. These changes are greater in patients with diarrhea, suggesting a causal relationship between intestinal dysmetabolism and the digestive disorders of HA20 disease.

Keywords: Gut microbiota, Inflammatory bowel disease, A20 haploinsufficiency

92-Quantitative Cardiac Magnetic Resonance Imaging Biomarkers in Systemic Lupus Erythematosus: A Systematic Review and Meta-Analysis

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Background: Cardiovascular complications are a major cause of morbidity and mortality in patients with SLE. CMR has the potential to detect and characterize subclinical cardiovascular involvement in this setting. However, individual study population sizes remain small and CMR findings vary substantially between studies. The aim of this systematic study was to assess quantitative cardiac magnetic resonance (CMR) imaging biomarkers and their clinical correlates in systemic lupus erythematosus (SLE).
Materials and Methods : Electronic databases were systematically searched from inception until November 2023. All studies reporting CMR imaging data in SLE patients were included. Risk of bias was assessed using the Newcastle-Ottawa Quality Assessment Scale. CMR findings of SLE patients were compared to that of matched controls. Features associated with CMR biomarkers were collected in a qualitative analysis.
Results: A total of 64 studies were included in the systematic review pooling 3,304 individuals including 1,870 SLE patient. Of these, 19 case-control studies were included in the comparative meta-analysis (1,576 individuals, including 884 SLE patients). When compared to controls, left ventricular (LV) ejection fraction and indexed end-diastolic volume were significantly altered in SLE patients (62% vs. 64%, p=0.001; 77 vs. 71 ml/m2, p=0.006). Late gadolinium enhancement (LGE) extent was significantly higher in SLE patients (LGE mass/total LV mass: 3.5% vs. 1.1%, p=0.009). Native T1 and T2 relaxation times were significantly higher in SLE patients (native T1 [1.5T]: 1005 vs. 982 ms, p=0.02; native T1 [3T]: 1277 vs. 1150 ms, p<0.001; T2 [all fields]: 58 vs. 51 ms, p<0.001). Three studies found an association between disease activity and T2 relaxation times. Two studies identified an association between clinical outcomes and CMR parameters.
Conclusions: While CMR-assessed ventricular function and volumes only slightly differed in SLE patients when compared to controls, myocardial tissue characterization parameters were significantly altered and associated with disease activity and outcomes.

Keywords: cardiac magnetic resonance; systemic lupus erythematosus; meta-analysis; systematic review.

93-Impact of the MDW biomarker on the time to introduction of antibiotic in patients with suspected sepsis in the emergency room: retrospective before-and-after study

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Introduction: Sepsis recognition and antibiotics initiation in the emergency department (ED) are often delayed. The MDW (monocyte distribution width) is a blood count parameter that has demonstrated its value as a sepsis biomarker. The objective of the study is to demonstrate an improvement in the time to introduction of antibiotics in septic patients, after implantation of MDW in the ED.
Methodology: This is a before-and-after study on retrospective data in patients consulting the emergency room for a suspected infection, evaluating the impact of the use of MDW in the management of septic patients (SOFA score ≥ 2 and confirmed infection). The period before ran from January 1st to April 16th 2023 and the period after the introduction of the MDW automate, from May 15 to October 22, 2023. The time for administration of antibiotics was compared between the 2 periods.
Results: so far, 2529 patients with a suspicion of infection were selected – 793 in first period and 1736 in second period. From those, 166 and 180, respectively, have a sepsis. The median age is 74 years-old (IQR 62-86) and 35.3% (122) are woman. Predominant infection sources are pulmonary (50.6%, n=175) and urinary (22%, n=76). 274 patients (79.2%) had an antibiotic, 39% in first and 40.2% in second period. The median time to antibiotics was 5.4 hours (IQR 3.5-7.7) and 4.88 hours (IQR 2.5-7.1), respectively (p = 0.87). Sepsis bundle was completed in 64 (18.5%) patients with a median time to bundle of 10.2h (IQR 6.8-13) and 12.8h (IQR 8.9-17.6), respectively (p = 0.87). There is no difference in mortality in both periods (9.9% and 8.1%, p=0.22).
Conclusion: The introduction of MDW in the ER does not seem to reduce the time to antibiotics. We are waiting on concluding data collection to start multivariate analysis

Keywords: sepsis, biomarkers, antibiotics, MDW, mortality

94-Mathematical for adipocytes size distribution: Study of equilibria

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Obesity is nowadays a global public health issue [1]. It is defined as an excess of lipids stored that harms health. The adipose tissue is in charge of this storage via its main cells : adipocytes. These cells present a singular property: their size varies from 10µm up to 150µm of diameter (which implies a large volume variation). In addition the observed size distribution is bimodal, presenting 2 characteristic sizes (around 30µm and 130µm with an inter-individual variability). Identifying the underlying mechanisms of this bimodality as well as the consequences on the dynamics of adipose tissue is a crucial issue to apprehend obesity.

In this work we reproduce adipocyte size distributions to better understand the origin of its particular shape. The model we consider proposes a simple mathematical explanation of the adipose tissue size distribution bimodality [2], assuming the size of adipocytes only depends on the amount of lipids stored in. We consider a system of Ordinary Differential Equations (ODE) that aims at describing adipocyte size taking into account lipid fluxes. In this system each equation describes a cell lipid content evolution over time. The extracellular lipid amount over time is also described, and constitutes the coupling term. However, the variability within the cell population is not described in the initial model. So the modeled size distributions are not realistic (they are made of one or two Dirac distributions).

Making the assumption that size distributions result from a system at equilibrium, we study the steady state of the ODE system previously described. By simulation, we assessed the hypothesis of intrinsic variability of cells, varying within the population a few key parameters. Some parameters are now specific to the cell. We show that it allows us to qualitatively reproduce the size distribution measured in rats or human adipose tissue. Also, we demonstrate the plausibility of perspectives including adipogenesis will be presented.

Keywords a mono-stable profile for the majority of cells. These results will be discussed and **:** Adipocytes, Mathematical model, Bimodality, Equilibria, Obesity

95-Restoring Immune Fitness in obese suffering patients

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According to forecasts by the World Atlas of Obesity, 4 billion adults will suffer from obesity by 2035. In addition to numerous cardiometabolic and hepatic complications, obesity can lead to cancer, joint disease and even neurodegeneration. Obese patients suffer from immune dysregulation, which contributes to the development of these so-called non-communicating diseases (NCDs). It is important to study the underlying signaling pathways and mechanisms, in order to explain the increased susceptibility to obesity-associated pathologies and severe infections. Immune checkpoint molecules (ICPs) are involved in the regulation of multiple functions of innate and adaptive immunity. Many dysimmune mechanisms are associated with increased and deregulated expression of ICPs, as is the case, for example, in cancers or severe infections.
It has already been demonstrated that obese patients have weakened immune profiles.
We already have the EU-FP7-METACARDIS cohort, with clinical and metabolomic data on 2,500 patients, from which an initial link between diet and dysregulation of ICPs can be established.
In a second phase, we are planning a nutritional intervention starting in October 2024 on almost 800 patients, testing a “pro-immune” diet against a standard diet.
The aim is to distinguish, using qPCR and flow cytometry, a difference in ICPs expression in patients from different groups. Ultimately, we aim to be able to offer patients personalized diets to restore a physiological immune profile.

Keywords: Obesity; Immune-Checkpoints; Bioinformatics; Metabolomics

96-The ground reaction force analysis in standing position to observe the postural control impairments after chemotherapy

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Background: Neurotoxic chemotherapy can cause disturbances in postural control and potentially increase the effort required to maintain balance. The aim of this ancillary analysis was to assess the effects of neurotoxic chemotherapy on ground reaction force (GRF) parameters during standing in women with gynecological cancer.
Material and methods: 33 women aged 48.18 ± 9.94 years treated with taxanes for breast cancer participated in two assessments: before and after neurotoxic chemotherapy. The postural control was measured on a force platform (Kistler instrument) in a standing reference condition (eyes open on rigid surface) and with sensory disturbances (eyes closed, unstable surface, vibration on Achilles tendons) and in dual task. Mediolateral (ML), anteroposterior (AP) and vertical (V) GRF was recorded during 30 s at 100 Hz. V GRF was normalized to the body weight. Wilcoxon statistical test was applied to compare the ML, AP and V peak forces between the two assessments. A Benjamini-Hochberg correction was applied.
Results: Compared to baseline, the maximum peak values were increased in all conditions tested after chemotherapy for ML and V GRF components ($p < 0.030$), except those perturbed by vibration (Table 1). No difference was observed for AP GRF.
Conclusions: The GRF peak force analysis highlighted the impairments in postural balance after chemotherapy. Greater postural adjustments were required to maintain balance after chemotherapy, indicating a deterioration in postural control. Chemotherapy-induced somatosensory deficits may explain the greater instability in the somatosensory impaired conditions, in the ML and V GRF, as well as the unchanged postural control in the vibration conditions. In view of the postural control difficulties identified, appropriate supportive care could be considered to help maintain balance during and after chemotherapy treatment.

Keywords: postural control ; neurotoxic chemotherapy ; ground reaction force

97-Integrating Patient-Centered Assessment: Enhancing Audiology Care through the COSI Questionnaire in France with French NLP Modelling

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Aims :This study focuses on the challenges associated with assessing the effectiveness of hearing aids, which can be difficult to interpret using traditional methods. It emphasizes the transition towards patient-centred care in audiology and using Patient-Reported Outcome Measures (PROMs) to evaluate care quality. This study aims to assess the efficacy of the Client Orientation Scale of Improvement (COSI) in enhancing the classification of patient-reported outcomes in audiology care. By incorporating PROMs such as the COSI questionnaire, we aim to better understand the specific needs and experiences of hearing-impaired individuals. Additionally, we seek to validate the COSI questionnaire in the French context and highlight the need for data-driven categorization methods to improve patient care.
Methods : We analyzed a dataset comprising 700,000 open-text survey responses from the COSI questionnaire. Expert knowledge was utilized to label 1\% of the total dataset. This was then employed to train a text classification model using CamemBERT, a Natural Language Processing (NLP) model explicitly tailored for French text. A notable accuracy of 97\% was attained during this pre-training phase, ensuring the reliability of subsequent text classification tasks. The trained model was then leveraged to label the remaining 693,000 responses. Analysis of audiograms, speech tests, age, sex, and open-text survey responses was conducted to gain insights into patients' needs and experiences. Furthermore, the analysis focused on individuals with symmetric hearing loss. Dimensionality reduction techniques, particularly t-Distributed Stochastic Neighbor Embedding (t-SNE), were utilized to examine the contributions of audiograms, speech tests, and the COSI questionnaire to the pure-tone average (PTA) hearing loss categories.
Results : Our study highlights the significant benefits of incorporating the COSI questionnaire in assessing patient needs, surpassing the limitations of relying solely on audiograms and speech tests. By integrating the COSI, we achieved a more nuanced understanding of individual hearing difficulties and rehabilitation goals. Conclusion : This study underscores the critical role of incorporating PROMs, exemplified by the COSI questionnaire, in audiology care. By leveraging PROMs, healthcare providers gain valuable insights into hearing-impaired individuals' unique needs and experiences,facilitating personalized treatment approaches. Furthermore, the high text classification accuracy achieved in this study (97\%) indicates the robustness of the classification model, supporting its potential for widespread application in patient care

settings. Moving forward, validating and refining the COSI questionnaire in the French context and developing data-driven categorization methods are essential steps towards optimizing patient outcomes in audiology care.
Keywords: COSI, Natural Language Processing, PROM

98-Automated radiolabelling of [68Ga]Ga-EMP100 targeting c-MET for clinical PET-CT imaging

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The transmembrane receptor c-MET plays a key role in cell growth and migration, particularly in cancer. Its measurement in the clinic by immunohistochemistry has limitations. PET-CT offers an alternative, allowing in vivo visualisation of c-MET positive lesions throughout the body. Radiopharmaceuticals targeting c-MET are under development, including [68Ga]Ga-EMP100, a cyclic oligopeptide with high affinity for c-MET. The aim of this project was to automate the radiolabelling of [68Ga]Ga-EMP100.
The Gaia automated system and single-use reagents were used. After manual optimisation to adjust pH, temperature and heating time, automation was used to determine the optimal peptide dose and the effect of excipients. The criteria of radiochemical purity (>95%), molar activity (>10GBq/µmol) and yield (>50%) guided this optimisation, resulting in the production of three batches that met current standards.
The experiments revealed an optimal pH range (3.25 to 3.75) and stabilisation temperature of 90°C, which allowed almost complete incorporation of gallium-68 in 10 min. Experiments with different amounts of peptide identified the minimum amount required for an adequate yield. The addition of ascorbic acid and ethanol improved the purity of the final product. The three batches produced using these parameters all met the defined quality specifications, with stability verified over 3 hours.
In conclusion, the optimisation of the radiolabelling conditions for [68Ga]Ga-EMP100 enabled the production of compliant batches, paving the way for its use in PET-CT for clinical imaging of c-MET. This success is an important step towards the integration of new diagnostic solutions in oncology.

Keywords: Radiopharmaceutical, [68Ga]Ga-EMP100, c-MET, PET-CT imaging

99-Differential Hearing Restoration in the DFNB9 Mouse Model through AAV Gene Therapy with Human and Mouse cDNA

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DFNB9 is a form of genetic hearing loss, caused by mutations in the OTOF gene, which encodes for otoferlin protein. Otoferlin triggers the final steps of synaptic exocytosis, ensuring rapid vesicular neurotransmitter release at inner hair cells (IHC) ribbon synapses. My team recently reported that delivery of murine Otof cDNA in Otof-KO models, restores hearing in a sustained manner providing hopes for the treatment of DFNB9 patients (Akil et al., 2019). For translational application of gene therapies, the human protein must also be tested in the Otof-KO mouse model. Therefore, we assessed whether the same degree of auditory rescue could be obtained using human Otof cDNA. We administered the therapy at two different time points, before (P2) and after the hearing onset (P15). Mice were injected with a dual-AAV system carrying one of the two cDNAs, murine or human. And the hearing function was evaluated at different time points, by auditory brainstem responses, startle reflex, and pre-pulse inhibition. In addition, after evaluating the hearing, mice were euthanized, and their cochlea was extracted for immunohistochemistry analysis. We confirmed the correct expression and localization of both proteins by immunohistochemistry, and at both time points. Interestingly, we found functional differences in recovery. More specifically, we found higher hearing thresholds, decreased amplitude, and increased latency of wave 1 of auditory brain stem responses (ABRs), when administering the human Otof sequence. In addition, we did not observe any recovery at the central level, as indicated by an impaired startle reflex and absence of the pre-pulse inhibition response. These results probably mean that there is an intrinsic functional deficit of the human OTOF protein when expressed in the murine cellular environment. We are currently performing in-vivo and ex-vivo electrophysiological recordings to understand how the observed differences may affect central mechanisms and plasticity.

Keywords: Gene Therapy Deafness Otoferlin Mouse

100-Hearing rehabilitation and tinnitus treatment, study of an extended-wear hearing aid

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Background: The ageing of the population is a major public health issue. In addition to longer life expectancy, the issue of "ageing well" is now on the agenda. Age-related hearing loss, a symptom of ageing, is associated with an increase of social isolation, symptoms of depression, cognitive decline and mortality. Regular use of hearing aids by hearing impaired persons appears to be an important way of slowing these phenomena. However, age-related loss of autonomy (cognition, fine motor skills) may limit the use of hearing aids (loss, complex handling, etc.) and therefore reduce their benefits. The aim of our study is to compare the quality of life of hearing-impaired patients with a loss of autonomy using two hearing aids with completely different ergonomic characteristics: conventional hearing aids and extended-wear hearing aids.
Materials and methods: Prospective, multicentre, international, interventional and crossover study in voluntary participants. The study will be conducted in three phases: A = no intervention (2 weeks); B = intervention 1 (4 weeks); C = intervention 2 (4 weeks), the order of intervention being randomised. 30 patients will be included in this study: 15 patients with mild cognitive impairment and 15 patients with dexterity impairment linked to motor and/or visual impairment. The primary endpoint will be the HUI3 score.
Expected results: Improvement in the HUI3 score with a permanent wear hearing aid compared with a conventional hearing aid. The inclusion of patients is expected for the beginning of June 2024.
Additional study: To investigate the impact of an extended-wear hearing aid on tinnitus-related discomfort using uninterrupted auditory stimulation. Study of pre- and post-fitting scores on questionnaires studying the impact of tinnitus.

Keywords: Hearing loss, autonomy loss, tinnitus, extended-wear hearing aid, hearing rehabilitation

101-Micro-CT analysis of rodent temporal bones: Identifying optimal species for otological research

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Backgrounds. Choosing the right model animal is crucial for studying ear diseases and finding effective treatments. The majority of otological research involves rodents such as guinea pig, gerbil, rat and mouse. The aim of the study was to compare the anatomy of temporal bones using micro-CT across four rodent models (guinea pigs, gerbils, rats, and mice) and humans, to define which is the more appropriate according to the area of expertise.

Material and methods. Middle and inner ear structures of human, and rodents were measured, analyzed and compared. A 3D reconstruction model was also produced.

Results. The main characteristics (size, access and orientation) of the tympanic membrane, ossicular chain, cochlea, posterior labyrinth and facial nerve were described and discussed.

Conclusion. Micro-CT analysis of rodents can guide researchers in selecting the most suitable model based on specific anatomical interests such as the tympanic membrane, ossicular chain, or cochlea. Our findings highlight the strengths and limitations of each species, providing essential insights that could enhance the precision and applicability of otological studies.

Keywords: three-dimensional anatomy, imaging, animal model, middle ear anatomy, cochlea

102-Implication of a bacterial quorum sensing molecule in inflammation and intestinal barrier function in inflammatory bowel disease

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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 has anti-inflammatory properties on immune cells, notably via a bitter taste receptor (TAS2R) and a protective role on the integrity of tight junctions in intestinal epithelial cells under inflammation, but this mechanism remains unknown. Our aim is to evaluate the involvement of TAS2Rs in the protective effects of 3-oxo-C12:2-HSL on barrier function in intestinal epithelial cells.

We use the Caco-2/TC7 cells, a human intestinal epithelial cell line, cultured on semi-permeable filters with induction of an inflammatory condition by adding cytokines, in the presence of 3-oxo-C12:2.

Our results show that 3-oxo-C12:2-HSL attenuates paracellular hyperpermeability and MCP-1 chemokine secretion induced by pro-inflammatory cytokines (IFNγ/TNFα) in Caco-2/TC7 cells, confirming a protective role on the epithelial barrier. Among TAS2Rs that can be activated by 3-oxo-C12:2-HSL, we show that TAS2R13 is one of the most highly 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.

This project will further our understanding of the impact of a bacterial quorum sensing molecule on intestinal barrier function in IBD.

Keywords: Inflammatory bowel disease, microbiota, intestinal barrier, quorum sensing, bitter taste receptors, tight junctions

104-Role of NADPH oxidases in the dialogue between tumor cells and tumor-associated macrophages during breast tumor progression

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Background : Macrophages represent the majority population of the tumor microenvironment, and their polarisation is largely influenced by environmental factors. They can present a state of differentiation M0, M1 (pro-inflammatory profile), or M2, (anti-inflammatory / immunosuppressive profile). In the context of cancer, we talk about tumor-associated macrophages (TAMs), 70% of which have an M2-type phenotype, playing an active role in tumor progression. In this perspective, the role of NADPH oxidases (NOX) in the polarisation of macrophages, particularly pro-tumor macrophages (M2), has been demonstrated.
Moreover, triple-negative breast cancer (TNBC) patients have a very poor prognosis and no current breast cancer treatment targets the tumor microenvironment.
Therefore, the main objective of this project is to inhibit tumor progression by targeting the dialogue between macrophages and tumor cells, using NOX inhibitors.
Material and methods: We studied the role of NADPH oxidases, using NOX inhibitors, in the dialogue between breast tumor cells, aggressive (MDA-MB-231, SUM-159) or not (MCF-7), and macrophages (M0, M1, M2) or TAMs. TAMs were generated by the addition of TNBC conditioned media.
Results: First of all, we showed that TNBC conditioned media influenced macrophage polarisation, by increasing the specific markers M1 (CD80, TNF-α) and M2 (CD206, PPAR-γ) and thus generating TAMs. Then, after demonstrating that NOX 1, 2 and especially 4 are preferentially expressed in M2, we demonstrated that NADPH oxidase inhibitors (VAS3947 and 2870) were able to (1) inhibit ROS production in macrophages (M0, M1 and M2), and (2) decrease the marker CD80 in M1, and markers CD206 and PPAR-γ in M2, as well as in TAMs.
Further experiments are currently in progress to confirm the role of NOX in the macrophages differentiation on the dialogue between breast tumor cells and TAMs. In addition, the influence of the conditioned macrophage media on the phenotypes of different breast cancer cell lines has been investigated.
Conclusion: This project will enable the development of a new therapeutic strategy by targeting the differentiation of TAMs within the tumor microenvironment, through the action of NADPH oxidase inhibitors, with the aim of reducing tumor aggressiveness.
Keywords: Triple negative breast cancer, tumor-associated macrophages, NADPH oxidases.

105-Preventing HIV integrase inhibitors-induced adipocyte dysfunctions using GLP1/GIP analogs

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Background: Adipose tissue (AT) is an endocrine organ playing a critical role in whole-body energy homeostasis through its energy storage and secretory capacities.
In some people living with HIV (PLWH), excessive AT accumulation could result from treatment with an HIV integrase inhibitor (INSTI), especially dolutegravir. We have demonstrated that INSTI exposure can alter differentiating adipose stromal cells (ASC) and fully differentiated adipocytes in vitro, ultimately resulting in an unhealthy AT expansion characterized by adipocyte hypertrophy, fibrosis, and insulin resistance. Despite their detrimental effect on AT, INSTIs are mandatory for the care of PLWH. We therefore consider novel therapeutic strategies to counteract weight gain and the metabolic side effects of INSTIs.
Glucose-Dependent-Insulinotropic-Polypeptide (GIP) and Glucagon-Like-Peptide-1 (GLP-1) are hormones released in the intestine in response to nutrient intake. They stimulate insulin release by pancreatic beta cells, thereby participating in glucose homeostasis. Interestingly, GLP-1 analogs have also shown great potential for reducing fat mass. Mounting evidence indicates that the combined administration of GIP and GLP-1 has a synergistic effect by significantly increasing insulin response and reducing fat mass. Therefore, the beneficial role of GLP-1 and GIP analogs represents an innovative strategy that is important to be assessed in the context of HIV infection and antiretrovirals.
Methods: Fully differentiated white adipocytes from human ASC were exposed or not to dolutegravir ± Liraglutide (GLP1 analog) or tirzepatide (dual GIP/GLP1 analog) for 5 days.
Results: We found that in dolutegravir-treated adipocytes, liraglutide and tirzepatide prevented dolutegravir-induced elevated lipid accumulation and the expression level of markers linked to lipogenesis. Also, they reduced dolutegravir-induced mitochondrial dysfunction and fibrotic gene expression. Finally, Liraglutide and Tirzepatide preserved insulin sensitivity in accordance with a higher expression of the insulin-sensitizing hormone, adiponectin.
Conclusion: Altogether, we show here that dolutegravir promotes adipocyte dysfunctions that can be prevented by GLP-1/GIP analogs. These data are very promising for the reversion of INSTI-induced adipose alterations.
Keywords: Adipose tissue, HIV integrase inhibitors, adipocyte dysfunction, GIP/GLP1 analogs