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Centre Louis Jeantet
Route de Florissant 77
CH-1206 Genève

MedLem16 meeting, Thursday 17th November 2016
www.unil.ch/medlem/

Organizing & scientific committee:
Dr Olivier Gaide, Dr Maxime Pellegrin, Prof. Aurélien Thomas and Dr Natacha Turck

Scientific Program

09.00 – 09.30	Registration & welcome coffee
09.30 – 09.45	Welcome to the MedLem **Dr Olivier Gaide**
09.45 – 10.15	Keynote 1 *"Bioinformatics resources to describe and understand complex biological responses. The example of inflammatory processes"* **Dr Lydie Lane**
10.15 – 11.15	Elevator Pitches Session 1. *"Role of MIF/CD74 signaling pathway in the development of pleural mesothelioma"* **C. D'Amato-Brito** 2. *"T cells respond directly to Toll-like receptor 4 signal to enhance autoreactivity in animal model of type 1 diabetes"* **M. Alibashe** 3. "Tumor Necrosis Factor -TNFR2 interaction regulates MDSC suppressor function in pleural tuberculosis" **L. Chavez-Galan** 4. *"Bioinformatics support for investigating glycan-binding proteins in inflammation"* **D. Alocci** 5. *"Infection prediction for aneurysmal subarachnoid hemorrhage patients at hospital admission: a panel combining serum amyloid A and clinical parameters"* **L. Azurmendi**
11.15 – 11.45	Keynote 2 *"Physiopathology of ocular diseases: therapeutic innovations"* **Prof. Francine Behar-Cohen**
11.45 – 13.30	Poster Session & Lunch

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13.30 – 15.15	Selected Oral Session 1. *"Big medicine, big data, big ethics"* **Prof. B. Elger** 2. *"Immunoglobulins are new immune players in WAT progression to senescence"* **Dr F. Gilardi** 3. *"Putting together neuroinflammation, untargeted metabolomics and data mining in biomarker discovery for neurodegenerative diseases"* **Dr V. González-Ruiz** 4. *"HDL from chronic kidney disease stage 5 exhibits superior oxidative stress protection in vitro and an increase in sphingosine-1-phosphate content"* **Dr M. Frias**
15.15– 15.45	Keynote 3 *"3D Neural tissues derived from human embryonic stem cells and iPS cells as in vitro models for neurotoxicity studies"* **Prof. Luc Stoppini**
15.45 – 16.00	Poster & Pitch Presentation Award Ceremony
16.00 – 16.30	Concluding message & goodbye drink **Prof. Dominique Soldati-Favre** (vice-dean for research of the Faculty of Medicine at the University of Geneva)

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Talks abstracts

Role of MIF/CD74 signaling pathway in the development of pleural mesothelioma

[1]D'Amato-Brito C., [1]Serre-Beinier V., [1]Cipriano D., [2]Colin D., [2]Germain S., [2]Seimbille Y., [2]Robert J., [2]Triponez F.

University Hospitals and University of Geneva[2], University of Geneva[1]

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine implicated in acute and chronic inflammatory diseases. MIF is overexpressed in various tumors. It displays a number of functions that provide a direct link between the process of inflammation and tumor growth. Our group recently identified the MIF-receptor CD74 as an independent prognostic factor for overall survival in patients with malignant pleural mesothelioma.In the present study, we compared the levels of expression of MIF and CD74 in different human mesothelioma cell lines and investigated their physiopathological functions in vitro and in vivo.Human mesothelioma cells expressed more CD74 and secreted less MIF than non tumoral MeT5A cells, suggesting a higher sensitivity to MIF. In mesothelioma cells, high MIF levels were associated with a high multiplication rate of cells. In vitro, reduction of MIF or CD74 levels in both mesothelioma cell lines showed that the MIF/CD74 signaling pathway promoted tumor cell proliferation and protected MPM cells from apoptosis. Finally, mesothelioma cell lines expressing high CD74 levels had a low tumorigenic potential after xenogeneic implantation in athymic nude mice.All these data highlight the complexity of the MIF/CD74 signaling pathway in the development of mesothelioma.

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T cells respond directly to Toll-like receptor 4 signal to enhance autoreactivity in animal model of type 1 diabetes

[1]Alibashe M., [1]Brioudes E., [1]Parnaud G., [1]Barbieux C., [1]Lavallard V., [1]Berishvili E., [1]Bosco D., [1]Berney T.

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Background

TLR4 is a transmembrane receptor of the innate immune system that recognize LPS of Gram-negative bacteria. Its stimulation induces pro-inflammatory responses and modulates adaptive immunity. Our aim is to determine the role of TLR4 in the activation and proliferation of T cells in the onset of autoimmune diabetes, using the non-obese diabetic (NOD) mouse model.

Methods

Spontaneously diabetic NOD mice, were treated with CLI-095, a cyclohexene derivative that inhibits TLR4 signalling, before onset of autoimmune diabetes. Onset of diabetes was detected by glycaemia and insulitis analysed by histology. In vitro, activation and proliferation of NOD T cells stimulated with LPS were assessed by FACS and ELISPOT. IL-2 and IFNγ secretions were measured by ELISA.

Results

TLR4 blockade markedly decreases infiltrative insulitis, delays the median age of diabetes onset (21.5-week vs 17-week), and decreases in vitro NOD T cell activation, cell divisions and antigen-specific proliferation ($p=0.0046$). Moreover, TLR4 stimulation increase diabetogenic T cells proliferation ($p<0.001$), IL-2 secretion ($p=0.0302$), IFNγ-secreting cells ($p=0.015$) and the secretion of IFNγ ($p<0.05$).

Conclusion

TLR4 blockade inhibits T lymphocytes activation and proliferation, leading to a decreased insulitic infiltrate and a delayed diabetes development. Furthermore, we demonstrated that autoreactive T-cells are able to directly respond to TLR4 stimulation which may contribute to the destructive mechanisms leading to the loss of the insulin-producing β-cells.

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Tumor Necrosis Factor -TNFR2 interaction regulates MDSC suppressor function in pleural tuberculosis

[1]Chavez-Galan L., [2]Vesin D., [3]Ryffel B., [3]Quesniaux V., [2]Garcia I.

University of Geneva[2], University of Orleans[3], University of Geneva [1]

Background: TNF is essential for host protection in tuberculosis (TB). Myeloid-derived suppressor cells (MDSC) accumulate in pleural effusions of TB patients and suppress T cell proliferation. In chronic inflammation, TNF blocks MDSC differentiation. Our aim is to evaluate the role of TNF pathway in MDSC accumulation in a murine model of pleural TB infection.

Methods: Mycobacterial pleurisy was generated by intrapleural cavity injection of Mycobacterium bovis BCG Pasteur. MDSC were isolated from the pleural effusion of wild-type (WT), TNF knock-out (TNF-KO), and transmembrane TNF knock-in (tmTNF-KI) mice. Isolated MDSC were co-cultured with lymphocytes. Phenotypical characterization and proliferation were assessed by flow cytometry.

Results: Pleural cavity of infected mice showed an accumulation of cells that phenotypically resembled MDSC. Whereas WT and tmTNF KI MDSC were able to suppress T cell proliferation, TNF KO MDSC were unable to restrain T cell proliferation. The TNF receptor interacting with tmTNF was explored by co-culturing MDSC from WT or tmTNF KI mouse with splenocytes from TNFR1 or TNFR2 KO mice. We find that proliferation of T cells deprived of TNFR1 were suppressed by WT and tmTNF MDSC while division of T cells deprived of TNFR2 were not suppressed, suggesting that the suppressive function was dependent on TNFR2.

Conclusion: MDSC accumulate in the pleural cavity and by cell-to-cell contact exert suppressive activity on T cells to reduce the inflammatory process. Our data demonstrate that TNF is involved in MDSC activity and tmTNF-TNFR2 interaction plays a critical role in MDSC suppressive function during pleural mycobacterial infection.

Bioinformatics support for investigating glycan-binding proteins in inflammation

[1]Alocci D., [1]Mariethoz J., [1]Lisacek F.

Swiss Institute of Bioinformatics[1]

A number of reports highlight the initiation and regulatory roles of glycan-binding proteins such as C-type lectins, sialic acid-binding immunoglobulin-like lectins and galectins in inflammation, as recently reviewed in [1]. However, corresponding information especially regarding glycans is scattered in the literature and/or in databases with limited connectivity. Glycans are complex molecules sharing binding patterns as the well-known blood groups. The actual state of art is still requiring a full manual work to identify possible interesting patterns. To step forward, we develop bioinformatics tools that extract and/or match known patterns to compare the binding potential of full structures mapped in glycomics experiments. These tools are linked to proteomics and glycomics databases and designed to help scientists piecing together fragmented information through intuitive interfaces [2]. We will demonstrate their use through visual examples.

References:

[1] Schnaar RL (2016) Glycobiology simplified: diverse roles of glycan recognition in inflammation. J. Leukocyte Biology 99(6):825-838.

[2| www.expasy.org/glycomics

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Infection prediction for aneurysmal subarachnoid hemorrhage patients at hospital admission: a panel combining serum amyloid A and clinical parameters

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Purpose: Aneurysmal subarachnoid hemorrhage (aSAH) is associated with higher risk of mortality/morbidity. Nosocomial infections that present in at least 30% of the patients, are the main causes of outcome worsening and death. We hypothesize that a panel of clinical parameters (GCS, WFNS, Fisher, and age) and inflammatory blood biomarkers (serum amyloid A (SAA), C-reactive protein (CRP), neopterin (NP), and white blood cells (WBC)) could increase the capacity of individual markers to dichotomize the patients at risk of infections.

Methods: The present study included 104 patients (69 infected/35 non-infected) from two independent cohorts. Biomarker concentrations were evaluated from plasma samples at hospital admission with commercial ELISAs, and receivers operating characteristic curves were used to assess their performance. The most accurate panel combination was obtained using Panelomix.

Results: At hospital admission, the most sensitive parameters for the stratification of patients at risk of infection were SAA and the WFNS. To reach 100% SP (95% CI, 100–100), SE values of 26.9% (95% CI, 15.9–38.1) and 31.9% (95% CI, 21.7–43.5) were obtained respectively. Moreover, the combination into a panel of SAA, WBC, WFNS, and age significantly improved the SE to 64.3% (95% CI, 50–78.6) when comparing to the 32% of WFNS alone (p=0.004).

Conclusions: At hospital admission the predictor panel including SAA, WBC, WFNS, and age appears as a promising tool for predicting infections that will develop during hospitalization. This could lead to a better management of patients, faster administration of antibiotherapy, less number of infections, and consequently an improvement in their associated outcomes.

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Big medicine, big data, big ethics

Elger B.

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Modern medicine produces big data in various settings. After providing three typical examples of Big Data research in the health sciences, an overview is given about the most recent discussion of ethical issues concerning Big Data research. Swiss research ethics regulations as well as relevant position statements and guidelines, including from the Institute of Medicine (IOM) will be analyzed.

Immunoglobulins are new immune players in WAT progression to senescence

[1]Gilardi F., [2]Giordano Attianese G., [3]Trang K., [3]Naldi A., [3]Winkler C., [3]Baruchet M., [3]Toffoli B., [3]Caputo T., [3]Desvergne B.

Universtiy of Lausanne[2], University of Lausanne[3], University of Lausanne - Center for Integrative Genomics[1]

Epigenetics that conveys flexibility to the genome in response to environmental and nutritional inputs, participate to the regulation of the senescence process in many mammalian tissues. We investigated how chromatin remodeling events contribute to very early phase of white adipose tissue (WAT) aging, when systemic body decline is still negligible. WAT from 3 and 12 months old mice was collected as representative of young- and middle-adulthood, respectively. Functional enrichment analyses on differentially expressed genes showed alteration in carbohydrates/amino acids metabolism and in extracellular matrix related pathways, as expected. The genome-wide profiling of histone marks and RNA Pol II recruitment revealed a general decrease of H3K9Ac in the WAT of 12Mo mice, accompanied by a deep remodeling of Pol II recruitment on both TSS and gene body. Most importantly, ChIP-seq analysis highlighted a significant remodeling of a number of genes belonging to immunity-related pathways. However, no classical signs of WAT inflammation were detected, except for an increased number of NK and T reg cells, accompanied by lymphatic vessel hyperplasia. Unexpectedly, we also found high levels of membrane associated IgG deposition in 12Mo WAT, likely due to their retention by the FcRIV isotype (the human orthologue of Fc?RIIIa) that we found to be specifically expressed at the membrane levels of adipocytes. The activation of FcRIV-IgG complexes, by inducing IL33, could contribute to the recruitment of NK and T reg cells. This perturbation of the inflammatory/anti-inflammatory balance could represent one of the first steps of WAT senescence.

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Putting together neuroinflammation, untargeted metabolomics and data mining in biomarker discovery for neurodegenerative diseases

[1]Jeanneret F., [1]González-Ruiz V., [2]Sandström J., [2]Tschudi-Monnet F., [2]Zurich M.-G., [1]Boccard J., [3]Rudaz S.

Université de Lausanne[2], Université de Genève [3], Université de Genève[1]

Neuroinflammation is a key event in the first stages of neurodegenerative processes such as Alzheimer's or Parkinson's diseases, and can be studied using xenobiotics exposure in appropriate in-vitro models. In that context, metabolomics untargeted data acquisition based on high-resolution mass spectrometry (HRMS) constitutes a promising approach for obtaining a large-scale coverage of the sample contents – even unexpected molecules– at once. Subsequent data analysis can reveal latent information hidden in the large volume of data and drive biomarker discovery. Moreover, retrospective investigations of the data can be performed without the need to reprocess the physical samples.

In the present work, inflammation was induced in 3D aggregating rat brain cell cultures by paraquat exposure. The influence of different factors (i.e. cells maturation, paraquat dose and exposure time) were studied simultaneously. A generic HILIC UHPLC method was developed to improve the separation of polar metabolites, and coupled to a Q-TOF HRMS detector to conduct untargeted data acquisition. A dedicated chemometric tool (AMOPLS) was used to extract relevant information from the multifactorial data generated.

The developed LC-MS method provided good analytical performance, with adequate separation and detection of representative polar low molecular weight compounds. Data analysis revealed that all of the three studied factors contributed to significant metabolic alterations, while specific biomarkers could be related to neuronal differentiation or inflammation. Biological interpretation will be improved by ongoing efforts to extend the library of standards for bringing definitive metabolite annotation to a larger scale.

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HDL from chronic kidney disease stage 5 exhibits superior oxidative stress protection in vitro and an increase in sphingosine-1-phosphate content

[1]Frias M., [2]Brulhart-Meynet M.-C., [3]Thomas A., [4]Frej C., [4]Dahlback B., [5]Stenvinkel P., [1]James R., [5]Brinck J.

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Background

Chronic kidney disease (CKD) exacerbates the risk of death due to cardiovascular disease (CVD). Modifications to blood lipid metabolism which manifest as increases in circulating triglycerides and reductions of high density lipoprotein (HDL)-cholesterol are thought to contribute to increased risk. In CKD patients, higher HDL-cholesterol levels were not associated with reduced mortality risk. Recent research has revealed numerous mechanisms by which HDL could favourably influence CVD risk. In this study, we compared plasma levels of sphingosine-1-phosphate (S1P), HDL-associated S1P (HDL-S1P) and HDL-mediated protection against oxidative stress between CKD and control patients.

Methods

HDL was individually isolated from 20 CKD patients and 20 controls. Plasma S1P, apolipoprotein M (apoM) concentrations, HDL-S1P content and the capacity of HDL to protect cardiomyocytes against doxorubicin-induced oxidative stress in vitro were measured.

Results

CKD patients showed a typical profile with significant reductions in plasma HDL-cholesterol and albumin and an increase in triglycerides and pro-inflammatory cytokines (TNF-alpha and IL-6). Unexpectedly HDL-S1P content ($p=0.001$) and HDL cardioprotective capacity ($p=0.034$) were increased significantly in CKD patients. Linear regression analysis of which factors could influence HDL-S1P content showed an independent, negative and positive association with plasma albumin and apoM levels, respectively.

Discussion

The novel and unexpected observation in this study is that uremic HDL is more effective than control HDL for protecting cardiomyocytes against oxidative stress. It is explained by its higher S1P content which we previously demonstrated to be the determinant of HDL-mediated cardioprotective capacity. Interestingly, lower concentrations of albumin in CKD associated with higher HDL-S1P.

Posters abstracts

Molecular characterization of subretinal fluid in Central Serous Chorioretinopathy patient: a proteomic and metabolomics case study

[1]Dor M., [2]Bararpour N., [2]Kowalczuk L., [2]Matet A., [2]Daruich-Matet A., [2]Dirani A., [2]Behar Cohen F., [2]Thomas A., [1]Turck N.

University of Lausanne[2], University of Geneva[1]

Using proteomic and metabolomics analyses, this study aimed at comparing the subretinal fluid (SRF) composition from one patient with central serous chorioretinopathy (CSCR) to SRF from two patients with rhegmatogenous retinal detachment (RRD) and at establishing correlations with physiopathogenic hypotheses.

Amongst the 291 proteins identified in the SRF, 128 were significantly different between CSCR and RRD (77 down-regulated; 51 up-regulated). The highest up-regulated proteins in CSCR SRF participated in glycolysis and gluconeogenesis, and the highest down-regulated were involved in cellular adhesion. Pathways related to inflammation and immune response as alternative complement pathway, liver X receptor/retinoid X receptor (related to lipid transport and macrophage activation) and farnesoid X receptor/retinoid X receptor (related to bile acid metabolism) pathways were also differently regulated. In the CSCR SRF, 76 metabolites were significantly differentially regulated (43 down-regulated; 33 up-regulated). The most significantly altered pathway was associated with protein digestion and absorption. Choline pathway, glycolysis and gluconeogenesis, steroid hormone metabolism and bile acid biosynthesis emerged as differentially regulated pathways by both analyses. Combined analyses revealed pathways involved in metabolic, neurological, psychological and cardiovascular diseases, and in several cellular functions: cell death and survival, cellular movement, inflammatory and immune response.

The CSCR SRF showed signature of cardiovascular and endocrine systems, which are clinically associated with the disease. Steroid, bile acid and complement activation gave indications for the worse visual prognosis of RRD compared to CSCR. Finally, molecular characterization of SRF is a valuable source of information, particularly for deciphering mechanisms of macular diseases.

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Epigenetics impact on the onset of obesity-related inflammation in white adipose tissue

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Human obesity is accompanied by chronic inflammation, also referred as metaflammation, which affects primarily metabolic organs such as white adipose tissue (WAT). Notably, the mechanisms underlying the onset of metaflammation are still poorly understood. Here, we aim to elucidate the role of epigenetics and metabolite alterations in the initiation of metaflammation in obese WAT.

Mice were fed with a high fat diet (HFD) for 1, 8 and 20 weeks. All analyses were performed in two functionally different fat depots: subcutaneous (sc) and visceral (v) WAT, the latter being expected to show a higher rate of macrophage infiltration upon HFD. The histological characterization showed, as expected, that Crown-like structures appear only in vWAT starting from 8 weeks. Accordingly, markers of activated macrophages were increased in vWAT after 8 and 20 weeks of HFD. PCA analysis, combining gene expression data and measurements of plasmatic insulin, leptin and resistin, showed that vWAT inflammation is evolving with time, as indicated by the separation between the 8 and 20 weeks groups. We next evaluated the genome-wide landscape of RNA Pol II binding and histone marks (H3K4me1, H3K27Ac). Pre-processing analysis assessed the quality and strength of the immunoprecipitations. Finally, metabolomic profiling demonstrated differentially expressed features altered significantly in 8 weeks HFD vWAT. Regulated putative metabolites mainly belong to diradylglycerols, glycerophosphocholines, amino acids/ peptides and ceramide.

Collectively, our preliminary results show that an active inflammatory response is induced selectively in vWAT of HFD-treated mice and this process is accompanied by a number of metabolite alterations.

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Characterisation of pre-clinical mesothelioma models for therapeutic and non-invasive molecular imaging strategies development

[1]Serre-Beinier V., [2]Germain S., [1]D'Amato-Brito C., [2]Senoner I., [1]Triponez F., [2]Colin D.

Thoracic Surgery Unit, University Hospitals and University of Geneva, Geneva, Switzerland[1], MicroPET/SPECT/CT Imaging Laboratory, Centre for BioMedical Imaging (CIBM), University Hospitals and University of Geneva, Geneva, Switzerland[2]

Malignant Pleural Mesothelioma (MPM) is an asbestos-related cancer and one of the most resistant and aggressive cancer. MPM is primarily treated by surgery, but the best current palliative treatment for patients allows a median survival of only 9 to 18 months. There is therefore an urgent need to develop new therapeutic and non-invasive imaging monitoring strategies of this cancer.

We are developing a new pre-clinical drug discovery workflow consisting in reliable 3D MPM cell culture, chicken chorioallantoic membrane (CAM)-based transplantation and mice orthotopic models. We are also evaluating molecular imaging strategies of the CAM and mice models.

Human MPM cancer cells were tested for their potential to grow in vitro in 3D, in the CAM and when injected subcutaneously or intrapleurally in athymic mice. Epithelioid and sarcomatoid phenotypes were analysed by western blotting quantifications of Epithelial-to-Mesenchymal markers. Imaging potentials of Computed Tomography, Magnetic Resonance and [18F]FDG, [18F]FLT or [18F]FMISO-Positron Emission Tomography were assessed.

On side of its high relevance in the discovery of new treatments and monitoring strategies of the MPM, these models demonstrate a high 3R (Replace, Reduce, Refine) potential of relevance in other cancer studies. We demonstrate that the CAM model is a good preliminary model and could partially replace mice. Molecular imaging technics which are non-invasive, allow reducing the number of animals used thanks to its ability to monitor tumour growth. Finally, molecular imaging methods increase the translational power of drug discovery studies as they rely on clinically-used technics.

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Coadministration of ticagrelor and ritonavir: Toward prospective dose adjustment to maintain an optimal platelet inhibition using the PBPK approach

[1]Marsousi N., [1]Daali Y., [2]Fontana P., [3]Rudaz S., [1]Samer C., [1]Desmeules J.

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The aim of this study is to adjust the dose of ticagrelor in case of co-treatment with ritonavir to achieve the same pharmacokinetic and pharmacodynamic profiles as administered alone, using physiologically-based pharmacokinetic (PBPK) approach. A model for ticagrelor was created using Simcyp® software (v14, Certara) based on in-vitro and in-vivo parameters. A clinical study was then conducted on 20 male healthy volunteers. During the first session, a single dose 180 mg of ticagrelor was administered to the volunteers and obtained pharmacokinetic data were fitted into the model for optimization. A simulated trial in presence of a single dose 100 mg of ritonavir allowed evaluating the impact of CYP3A inhibition on the concentration-time profile of ticagrelor and predicting the adjusted dose of ticagrelor in the presence of ritonavir. The predicted dose (45 mg) was co-administered with a single 100 mg dose ritonavir in the same volunteers during the second session of the clinical trial. Pharmacokinetic profiles as well as antiplatelet effect of ticagrelor were measured at both sessions. The Area Under the Curve (AUC0-24h) of 45 mg ticagrelor co-administered with ritonavir in session 2 was successfully predicted with the model and was comparable at two sessions. The platelet inhibition was nearly complete in both sessions with a mean Platelet Reactivity Index of 11.3% ± 2.8% (CI 95%) in session 1 vs. 15.7% ± 4.3% (CI 95%) in session 2 using the VAsodilator-Stimulated Phosphoprotein (VASP) assay and no bleeding or other adverse events were observed thanks to this dose adjustment.

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Anti-Apolipoprotein A-1 autoantibodies as active modulators of atherothrombosis

[1]Pagano S., [2]Carbone F., [3]Burger F., [3]Roth A., [4]Bertolotto M., [5]Pane B., [6]Spinella G., [6]Palombo D., [7]Pende A., [7]Dallegri F., [2]Satta N., [2]Virzi J., [2]Fontana P., [2]Mach F., [2]Montecucco F., [2]Vuilleumier N.

Geneva University Hospital [2], San Martino hospital[5], Geneva University-HUG[1], Genoa University Hospital[4], University of Genoa[7], San Martino Hospital[6], Geneva University Hospital[3]

Introduction: Humoral autoimmune-mediated inflammation plays a role in atherogenesis, and potentially in atherothrombosis. Anti-apolipoprotein A-1 (apoA-1) IgG have been reported to represent emergent mediators of atherogenesis trough Toll-like receptors (TLR) 2,4 and CD14 signaling. Aims: to investigate if and how anti-apoA-1 IgG could promote atherothrombosis by modulating the expression and activity of Tissue Factor (TF), a key coagulation pathway regulator involved in atherothrombosis. Methods: serum levels of anti-apoA-1 IgG were measured by ELISA. Atherothrombosis features were determined by immunohistochemical TF staining of human carotid biopsies derived from patients with severe carotid stenosis undergoing elective surgery (n=176), and on aortic roots of different genetic backgrounds mice (ApoE-/-; TLR2-/-ApoE-/- and TLR4-/-ApoE-/-) exposed to passive immunization with anti-apoA-1 IgG. On human-monocytes-derived-macrophages (HMDM) the anti-apoA-1 IgG-induced TF expression and activity was analyzed by FACS and chromogenic assays in presence of different pharmacological inhibitors. Results: Significant associations were retrieved between anti-apoA-1 IgG circulating levels and intraplaque TF expression in human carotid biopsies. On HMDM, anti-apoA-1 IgG induced a TLR2,4/CD14-dependent increase in TF expression/ activity, involving NF-kappaB and AP-1 transcription factors, after JNK activation. In ApoE-/- mice, anti-apoA-1 IgG passive immunization significantly enhanced intraplaque TF expression when compared to control IgG. This effect was lost in both TLR2-/-ApoE-/- and TLR4-/-ApoE-/- backgrounds. Conclusions: These results extend previous findings by demonstrating that anti-ApoA-1 IgG are associated with higher propensity to atherothrombosis, and could per se induce TF expression in human macrophages, supporting a possible causal link between anti-ApoA-1 IgG and atherothrombosis.

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The vascular wall of intracranial aneurysm prone to rupture contains less collagen and smooth muscle cells

[1]Morel S., [1]Diagbouga M., [1]Sutter E., [2]Corniola M., [2]Gondar R., [2]Jaegersberg M., [2]Braunersreuther V., [1]Bochaton-Piallat M.-L., [2]Schaller K., [2]Bijlenga P., [1]Kwak B.

University of Geneva[1], Geneva University Hospital[2]

Background: Intracranial aneurysm (IA) is a disease of the vascular wall corresponding to a local outpouching of the artery. Its prevalence is 2-3% of the general population. IAs are mostly quiescent and asymptomatic, but their rupture leads to severe brain damage or death. Presently, physicians lack adequate tools to safely determine whether an IA is at risk of rupture. Our goal is to use the aneurysm 3D-shape to characterize disease status.

Methods: IA domes, ruptured (N=10) or not (N=22), have been collected in the framework of the AneuX project at the Geneva University Hospitals. Domes were fixed in Formol, embedded in paraffin, sectioned and stained. Results are expressed as median (Interquartile range).

Results: Mean age of patients at time of surgery (years: 56+/-12 and 55+/-10) and proportion of woman (78% and 73%) were not different between ruptured and unruptured IAs. Aneurysm characteristics on angiographical images are not different between ruptured and unruptured IAs. Histological analysis of aneurysmal wall composition revealed that ruptured IAs have a significantly lower content of smooth muscle cells (0.12(0.01-0.29) vs. 0.26(0.17-0.42), $p<0.05$) and collagen (0.11(0.07-0.24) vs. 0.31(0.19-0.53), $p<0.05$) in comparison with unruptured IAs, whereas the elastin content was not affected (0.34(0.11-0.45) vs. 0.40(0.09-0.55), ns). Interestingly, the collagen content in the aneurysm wall of ruptured IAs was not affected by the thickness of the wall.

Conclusion: In conclusion, our results show that the IA wall prone to rupture presents a lower smooth muscle cell and collagen content, independently of the wall thickness.

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The CALIPHO group: Bioinformatics and experimental pipelines to uncover functions of uncharacterized human proteins

[1]Mary C., [2]Group C.

Faculty of Medicine of the University of Geneva [1], Faculty of Medecine/Swiss Institue of Bioinformatics[2]

The completion of the human proteome annotation was achieved by the UniProt consortium in 2008. The CALIPHO (Computer Analysis and Laboratory Investigation of Proteins of Human Origin) group aims to use both bioinformatics and experimental pipelines to improve our current knowledge on the human proteins. In this frame, CALIPHO has developed nextProt, a knowledge platform on human proteins. It strives to be a comprehensive resource that provides a variety of types of information on human proteins coming from high throughput experiments.

According to the data implemented in neXtProt, about 2'000 of the 20'325 human proteins are still lacking functional characterization (Gaudet et al. 2013).

The CALIPHO group uses the experimental pipeline to validate hypotheses coming from bioinformatics analysis, focusing on three main types of proteins: mitochondrial proteins, proteins involved in ciliogenesis and enzymes. It has led to the characterization of four proteins: the protein C2orf62 involved in actin organization and ciliogenesis (Bontems et al. 2014), the mitochondrial protein C11orf83 required for bc1 complex assembly (Desmurs et al. 2015) and the two enzymes DERA (human desoxyribose aldolase) (Salleron et al. 2014) and APIP (5'-methylthioribulose-1-phosphate dehydratase) (Mary et al. 2012). CALIPHO is working in close collaborations with Swiss and European groups performing studies in structural biology, model organisms, system biology, and human medicine. These collaborations are essential to support our effort of characterization of the human proteins.

In the search of biomarkers for thyroid associated orbithopathy (TAO)

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Introduction: Tears are known as lubricating the eyes and ensuring nutrition and protection of the surrounding ocular tissues. However, its production and composition is dependent on various stimuli, including ocular and systemic diseases. Here, we propose that, tears could represent an innovative source of biomarkers in thyroid-associated orbitopathy (TAO) disease.

Materials and Methods: Schirmer's test was adopted to collect tears from TAO (N =20, 3 males, mean age (±SD): 46.0 years (±13.0)) and healthy patients (N =18, 8 males, 45.4 years (±18.7)). Independent isobaric proteomics experiments were carried out and analyzed on a linear trap quadrupole (LTQ) Orbitrap Velos Pro. Easyprot software was used to obtain protein identification and quantification (2 unique peptides, 1% FDR, ratio<0.66 or >1.5, p-value<0.05). Pathways were analyzed using Ingenuity pathway analysis software (6.7 version).

Results: 646 proteins were identified and 62 were considered as differentially expressed 27 up-and 35 downregulated. Interestingly, among them, the acute phase response signalling and glycolysis pathways were mainly represented. In parallel, the levels of 10 cytokines were measured (ProInflammatory panel, Mesoscale) with ELISA, and 4 (IL-6, IL-10, IL-12, TNF-α) were found significantly upregulated in tears of TAO patients.

Conclusion: These results confirm that tears are a suitable source to discover biomarkers for TAO disease. Moreover, the emergence of proteins involved in the glycolysis, associated to inflammation, could bring new general knowledge about TAO and also shed light on a disease that still remains not well characterized.

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MedLem[16]

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Linking obesity, metabolic disorder and inflammation via metabolomics and epigenetics

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Obesity has been recognized as an important risk factor in onset of metabolic syndrome which is extensively associated with dyslipidemia, insulin residence, hypertension, non-alcoholic liver disease and type-2 diabetes. This study aims at comprehensively dissecting the epigenetic and metabolomic response in the fat tissue of a mouse model of Diet-Induced Obesity in order to identify the alterations which play a causal role in the onset of inflammation. Untargeted metabolomics was performed using a UPLC-HRMS (Thermo Scientific Q Exactive Plus MS) to compare differentially expressed metabolites in sub-cutaneous and visceral white adipose (scWAT, vWAT) tissue comparing high fat diet (HFD) and chow diet (Control) mice at 1, 8, and 20 weeks . After normalisation, discriminate metabolites were determined using supervised learning approaches, including 'pamr' packages into R (http://cran.r-project.org) and web-server metaboanalyst (http://www.metaboanalyst.ca/). In addition, univariate analysis was performed by applying non parametric Wilcoxon rank-sum test to determine the significant pathology-related metabolites and their expression level in two groups. Although metabolomics analyses are still ongoing, promising preliminary data have been obtained. Briefly, our results demonstrate that several metabolites are meaningfully deregulated with respect to the diet status as well as the type of adipose tissue, i.e. visceral and sub-cutaneous. Interestingly, the associated biological pathways linked to the biological function are recognized to be involved in adipogenesis.

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Metabolomic imaging mass spectrometry of high-risk metastasizing uveal tumors classified upon BAP1 mutational status

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Uveal melanoma is the most common primary intraocular tumor of the adult. Mutations in BAP1 gene on chromosome 3p21.1 are detected in more than 80% of metastatic tumors. The mortality of uveal melanoma is strongly linked to liver metastasis. Gene expression studies, pangenomic studies (MLPA, aCGH, aSNP) and mutational analysis in uveal melanoma revealed that these patient with an adverse outcome express dedifferentiated, stem cell-like gene profile (class II), have a monosomy 3 and 8q gains as well as BAP1 mutations. Genome profiling paired with metabolomic fingerprinting revealed strong association between metabolic traits and loci. In this project Matrix -assisted laser desorption ionization (MALDI) imaging mass spectrometry (IMS) is efficiently served to demonstrate in situ molecular signature and to provide a new information on biological processes associated with UM.

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UNTARGETED OMICS-BASED DISCOVERY OF EARLY ISCHEMIA INDUCED CHANGES IN Ex Vivo RAT HEART MODEL

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Myocardial ischemia is a complex state characterized by reduced supply of oxygen and nutrients as well as inadequate removal of cellular metabolites. Early myocardial ischemia (EMI) is the time period during the initial hours from the onset of injury that lacks histopathological findings and molecular description defined by a standardized panel of immunohistochemical markers. EMI is a precursor to a variety of ischemic heart diseases and a suspected cause in most sudden cardiac death (SCD) cases. In legal medicine, each year several SCD cases remain unexplained (10% in our center) because these cases lack specific finding. Improving current post-mortem methods to diagnose EMI will allow better assistance of the public prosecutor and the families of the victims. In this study we investigated biomolecular changes in an ex vivo rat model of EMI by performing global untargeted screening with matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI IMS) and multivariate data analysis. Cardiac tissue exposed to ischemia for 15 min, 30 min, 1h, 2h, and 4h was analyzed by MALDI IMS for multiple compound classes. The results achieved with MALDI IMS were compared to histological and immunohistochemical evaluation of the same samples. The identification of new, specific markers of EMI will not only improve the post mortem diagnosis in cases of SCD but and may also lead to the detection of potential targets for therapy.

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Neutrophils contribute to ischemic limb perfusion and modulate systemic monocyte phenotype and Th17/Treg ratio in a mouse model of peripheral artery disease

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Objective

Atherosclerotic peripheral artery disease (PAD) is characterized by stenosis/obstruction of leg arteries leading to decreased muscle perfusion and patients' walking capacity impairment. PAD affects millions of people worldwide and its prevalence is constantly increasing. Pathophysiological mechanisms underlying PAD remain largely unknown. In the present study, we assessed the role of neutrophils on walking capacity, limb perfusion, and systemic inflammation in a PAD mouse model.

Methods

Atherosclerotic C57BL/6 ApoE KO mice underwent right common iliac artery ligation to generate PAD. Mice were then treated with either a neutrophil-depleting antibody (EXP) or isotype (Ctrl) every two days for 5 weeks. Walking capacity (running treadmill test), limb perfusion (Laser Doppler), and inflammatory cells balance were analysed by flow cytometry at the end of experience.

Results

Walking capacity remained significantly and equally impaired in the two groups. Ischemic limb perfusion significantly decreased by 20% in EXP compared to Ctrl ($p<0.05$). Pro-inflammatory (Ly6Chigh) to anti-inflammatory (Ly6Clow) monocytes ratio was increased in EXP compared to Ctrl ($p<0.05$). Th1 (CD4+ IFN?+) to Th2 (CD4+ IL4+) ratio did not significantly differ, while Th17 (CD4+ IL17+) to Treg (CD4+CD25+Foxp3+) tended to be higher in EXP ($p=0.08$ vs Ctrl).

Conclusion

Neutrophils favour ischemic limb perfusion, and shift systemic monocytes as well as Th17/Treg ratio towards a less pro-inflammatory phenotype. Our results suggest a protective role for neutrophils in PAD.

Comparative proteomic analysis of sub-retinal fluid and vitreous humor in rhegmatogeneous retinal detachment

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Rhegmatogenous retinal detachment is the most common type of retinal detachment that is separation of the neurosensory retinal from the retinal pigment epithelium by sub-retinal fluid (SRF) defects. In this study, SRF and vitreous humor (VH) proteome have been utilised to establish a qualitative proteome analysis to investigate the origin of SRF to have better understanding in rhegmatogenous retinal detachment./ Collection of VH and SRF of three patients (age range from 68 to 71) have been examined. We employed off-gel fractionation and immunodepletion to optimise the protein profiling. List of proteome were subjected to gene ontology (GO) enrichment analysis using WebGestalt and Wikipathways. Dynamic network for GO network was analysed using a Cytoscape plug-in, ClueGo./ We obtained 934 VH proteins and 842 SRF proteins and 691 proteins were in common in between VH and SRF. In SRF a high percentage of proteins are related to biological processes such as hexose metabolic process and platelet activation. VH proteins are related to the platelet activation, but also to the neuro projection morphogenesis and negative regulation of endopeptidase. Interestingly, additional SRF proteins have biological process of mRNA splicing via spliceosome. Although immunodepletion has masked most abundant proteins, the issue of low abundant protein identification remained unresolved./ Lack of robust SRF proteome dataset impeded precise understanding of the SRF. Along with VH proteome dataset, a high confidence list of human plasma proteome has been investigated to study the origin of SRF, and we propose that SRF is highly likely to be originated from VH.

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Evaluation of CYP450 and transporters expression and activity in HepaRG cell line in different conditions.

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HepaRG cell line is able to differentiate to both hepatocyte-like and biliary-like cells. Previous works have shown that confluent HepaRG cells start to differentiate when adding 2% DMSO in the culture medium. However, DMSO is well known to induce cell death.

In order to optimize CYP450 activity while decreasing cell apoptosis, we tested different culture conditions for differentiation (DMSO concentration 0-2%, ± EGF/HGF. CYP activity was assessed using a cocktail approach by LC-MS/MS. The expression of hepatic enzymes and transporters involved in drug disposition was assessed with Nanostring® technology.

While differentiation was induced by DMSO, cell viability was significantly decreased when adding DMSO up to 2%. DMSO increased significantly the activity of CYP3A4, 2B6 and 1A2. The addition of growth factors was found to have a negative impact on cell differentiation and thus CYP activity, but significantly improved cell viability. There was a good correlation between CYP activity and expression except for CYP1A2. In all conditions tested, CYP2D6 showed a weak activity and expression levels were undetectable. UGT1A1 and UGT2B7 transcripts were found at appreciable levels and were influenced by DMSO concentration, as well as hepatic transporters. Efflux transporters MRP2, MRP3 and MDR1 (P-gp) levels were high, whereas BSEP, BCRP and MRP1 levels were low. The uptake transporter OCT1 was largely expressed and OATP1B1, 2B1, OCT3, OAT2 expression were found in acceptable levels. On the contrary, NTCP and OATP1B3 transcripts were undetectable. Differentiation medium containing 1.5% showed similar viability compared to the reference MIL720 (Biopredic®) with slightly lower CYP450 activities.

SERUM LEVELS OF ANTI-APOLIPOPROTEIN A-1 IGG ARE ASSOCIATED WITH LONG-TERM DISABILITY AND CEREBRAL LESION VOLUME IN ACUTE ISCHEMIC STROKE PATIENTS

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Objective- Autoantibodies to ApolipoproteinA-1 (anti-ApoA-1 IgG) were shown to predict major adverse cardiovascular events and promote atherogenesis. Their potential relationship with clinical disability and ischemic lesion volume after acute ischaemic stroke (AIS) remains unexplored. In this study, we included 76 patients admitted for AIS and we investigated whether baseline serum anti-ApoA-1 IgG levels could predict AIS-induced clinical disability and AIS-related ischaemic lesion volume.

Methods- Clinical disability was assessed by the modified Rankin Scale. Ischaemic lesion volume was assessed by Computed Tomography. We evaluated the possible pro-apoptotic and pro-necrotic effects of anti-ApoA-1 IgG on human astrocytoma cell line (U251) using flow cytometry.

Results- High levels of anti-ApoA-1 IgG were retrieved in 15.8% (12/76) of patients. Increased anti-ApoA-1 IgG levels at baseline were independent predictors of worse mRS (β=0.364; *p*=0.002; adjusted odds ratio [OR] 1.05 [95% CI 1.01-1.09]; *p*=0.017) and CT-assessed ischaemic lesion volume (β=0.333; p<0.001; adjusted OR 1.06 [95% CI 1.01-1.12]; *p*=0.048) at 3 months. No difference in baseline clinical, biochemical and radiological characteristics was observed between patients with high versus low levels of anti-ApoA-1 IgG. Incubating human astrocytoma U251 cells with anti-ApoA-1 IgG induced a dose-dependent necrosis and apoptosis *in vitro*.

Conclusion- our study showed that anti-ApoA-1 IgG serum levels determined at AIS onset may be associated with poorer clinical recovery and worse brain lesion volume 3 months after AIS. These observations could be partly explained by the deleterious effect of anti-ApoA-1 IgG on human brain cell survival *in vitro* and may have clinical implication in the prediction of poor outcome in AIS.