Directory

Institute

Pathophysiology

Metabolism & Nutrition

Circulatory system, Hemostasis, Pneumology, Dermatology, Diabetes, Metabolism/Nutrition, Endocrinology Gastroenterology, Hepatology, Uro-Nephrology, Osteoarticular system

- April 2017 -
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Introduction
1 – Fields covered

The life science Institute *Pathophysiology, Metabolism & Nutrition* (PMN) covers a wide spectrum of research in physiology, experimental medicine and human diseases. The fields covered include heart and vessels, lungs, endocrine organs, liver, kidney, skin, joints and bones, and organs involved in nutrient processing, from all aspects of nutrition, from the control of food intake and nutritional behavior to digestive processing, control of substrate use and storage. The diseases in question frequently show common biological mechanisms and often lack of suitable treatments designed on a pathophysiological basis.

2 – Major scientific challenges in biology and medicine

- **Public health care challenges**

Among diseases within the PMN scope, cardiovascular, respiratory, metabolic and nutritional diseases with their devastating complications represent a major public health care challenge. Diabetes, hyperlipidemia, obesity, renal insufficiency lead to cardiovascular disease, the major cause of mortality in industrialized countries and usually develop in relation with atherosclerosis.

Coronary artery disease, stroke and chronic heart failure are responsible for 75% of cardiovascular-related deaths. They represent 29% of deaths in France, almost equaling deaths due to cancer. Thrombotic diseases are very prevalent, arterial thrombosis (ischaemic diseases) and venous thrombosis (thrombo-embolic disease) are the world's leading cause of death. The prevalence of constitutional haemorrhagic diseases is limited, but their social and economic impact is significant, as in the case of haemophilia.

Respiratory diseases (asthma, chronic obstructive pulmonary disease, COPD; pulmonary fibres) affect millions of people in France and their incidence is increasing. COPD alone already represents the third-largest cause of death in Europe (sixth in the world).

The prevalence of diabetes is 7.4% in France in the age range 20 to 79 (IDF 2015). The increasing prevalence of diabetes parallels that of obesity, which raised to 15.8% among men and 15.6% among women in France (age range 30 to 69, INVS 2013). Regarding the visceral obesity the prevalence reaches 41.6% and 48.5% respectively. Diabetes is among the leading causes of blindness, end stage renal disease, non-traumatic limb amputation in adults and coronary heart disease. Type 2 diabetes is a multifactorial disease that shows heterogeneity in many respects and is often a manifestation of a much broader underlying disorder often referred to as the metabolic syndrome, an operational paradigm that includes hyperinsulinaemia, dyslipidaemia, hypertension, visceral obesity, hypercoagulability and microalbuminuria. Metabolic syndrome also leads to various non-vascular complications, including steatohepatitis, cirrhosis or arthrosis. The metabolic epidemics and its cardiovascular complications although world-wide have been most pronounced in non-European populations, as shown by studies from Native American and Canadian communities, Pacific and Indian Ocean island populations or populations throughout Asia.

Paradoxically, malnutrition is also a major threat to global human health and survival. Recent estimates indicate that nutritional deficiencies account for 3 million child deaths each year in less-developed countries while progress toward designing effective life-saving interventions is currently hampered by serious gaps in our understanding of nutrient metabolism in the human. Denutrition is further observed in 40% of patients suffering from chronic diseases, in 30 to 50% of hospitalized patients of all pathologies and is an independent factor for morbidity/mortality. Overall, there is a need for an increased research effort focusing on nutrition and its disorders, including the interrelationship with the environment, the human microbiome, digestive physiology, nutritional behaviors or food security.

Diseases of the bones and joints are also a concern for the French, particularly due to ageing of the population. On their own they represent half of chronic diseases in people over 65 and are a major cause of invalidity (arthrosis is the second largest handicap factor in men, the fourth in women).
Among the over 50s, one woman in four and one man in eight will be affected by osteoporosis during their lives.

Skin diseases include a proportion of allergic complaints (atopic dermatitis, contact eczema, occupational dermatoses, photo-allergies, urticaria and skin accidents due to oral administration of a drug (toxicodermatitis)) and a proportion of chronic inflammatory disorders (psoriasis, atopic dermatitis, pelada, etc.). Among this latter group, psoriasis, affects between two and three million people in France and is associated with a significant change in the quality of life, often leading to a severe social handicap. The impact of this dermatosis on the quality of life is as significant as that caused by asthma, diabetes or chronic cardiac ischaemic diseases. The social cost of psoriasis is therefore considerable. Ageing of the population is increasingly frequently accompanied by chronic vascular complaints of the lower limbs. Their treatment is complex and should be multi-disciplinary, ideally as part of a care network led by dermatologists specialised in the field of cicatrisation.

Other diseases that are in the scope of the Institute are frequent and/or carry high morbidity/mortality rates. Basic and clinical research is required to progress in our understanding of the mechanisms involved in cardiac, vascular, respiratory, renal, endocrine, digestive, dermatologic and osteoarticular disorders.

- **Scientific challenges**

Diseases implicating heart and vessels, lungs, endocrine tissues, kidney, liver, skin, bone and joints and the digestive tract, although organ-specific, cannot be considered independently of the numerous interactions with the whole organism and the environment. Transversal aspects of physiology and pathology will be emphasized within the scope of the PMN Institute. Underlying fundamental research and biological issues that need to be addressed correspondingly cover a large field of disciplines requiring strong links between institutional partners, as well as with industrial partners. All these diseases share: 1- an incomplete knowledge of the genes involved in their etiology, a situation which is rapidly changing thanks in particular to the new genomic approaches, 2- an insufficient basic knowledge of gene and protein functions in target organs as well as their interaction with the environment; 3- incomplete understanding of pathophysiological mechanisms of diseases expressed within corresponding tissues, i.e. of mechanisms of initiation and progression of disease processes; 4- the general insufficiency of available treatments and preventive strategies based on a better understanding of the mechanisms of common diseases; 5- the understudied strategies of cell therapy, which could benefit to many of these diseases; 6- the importance of discovering new biomarkers that would be useful for diagnostic, prognostic and treatment guidance.

1) **Gene/protein-function studies in physiology and interactions with environment**

The availability of gene sequences encoding for molecules of unknown functions emphasizes the need for extensive gene-function studies and for the characterization of tissue distribution of newly identified genes. As part of this task, the development of new models to empower these studies is required. Other emphasis is required on development biology, on studies of ageing mechanisms, on comparative genomics with the goal of better understanding of human gene and protein functions, on integrative physiology to decipher signalling and metabolic pathways and interactions at the whole organism level, on the understanding of gene interactions and gene networks that impact on individual cell and tissue functions, on epigenetics and metagenomics to study gene interactions with the environment. The sequence of an increasing number of individual human genomes also paves the path toward in depth understanding of human gene and post-transcriptional diversity in cell, tissue and organ physiology. With few exceptions, the miRNA target genes and the mechanism of target suppression are currently unknown because reliable experimental methods for comprehensively identifying the miRNA targets have yet to be developed.
2) Disease-initiating mechanisms and mechanisms of disease progression in common diseases.

Mechanisms that trigger destructive processes within target tissues are seldom identified. Other than certain rare monogenic diseases involving key genes in cell function, common diseases usually develop on a multigenic susceptibility background associating “normal” gene variants, often affecting quantitative traits (intermediate phenotypes) that contribute to the clinical phenotype observed, most often interacting with environmental factors as part of a multifactorial process. As triggering factors remain elusive in most cases, new hypotheses in disease initiation should be tested, possibly stochastic events in initiation process or early external factors within perinatal or prenatal development. Genetic and molecular epidemiology can help to isolate specific risk factors, especially to identify subjects at risk of sudden death, a major problem in industrially developed countries. Furthermore, striking changes in the incidence of major multifactorial diseases will need to be addressed. Delineating initiation and progression events in common diseases is a challenge that applies at three levels: genetic susceptibility, cell pathways involved in disease and the role of environmental factors. Within a given genetic background, some genes concur at initiating the disease process while others control disease progression that directly impact on the age at disease onset following a preclinical phase. Seemingly, some environmental factors trigger the disease process while other modulate disease progression.

3) Treatments based on mechanisms of diseases

In many diseases within the PMN scope, current treatments remain insufficient for different reasons, depending of the field covered. Some treatments remain symptomatic or palliative (e.g. treatments of chronic heart disease, substitutive therapies in endocrine diseases, dialysis in end stage renal diseases, organ transplantation in renal, heart, lung, liver or gut failures). In other examples, pathophysiological treatment that remain non-specific (e.g. immunosuppressive treatments in immune/inflammatory diseases) induces severe side effects. Novel strategies aimed at controlling the immune/inflammatory response should be developed (small molecules targeting inflammatory pathways, monoclonal antibodies vaccination). In a third set of diseases, preventive strategies are available but only target biomarkers that relate with a risk factor associated with the disease process (e.g. treatment of obesity to prevent metabolic and cardiovascular diseases) but in most cases the risk factor that is amenable to treatment is only part of the susceptibility that underlies the pathological process (e.g. atherosclerosis in case of cardiovascular diseases). Finally, major ischemic diseases (heart, brain, limbs, kidney) suffer from the lack of treatments able to protect tissues for ischemic sequelae.

4) Strategies for cell replacement

Among the aforementioned palliative therapeutic strategies, organ transplantation have developed since the mid fifties. They have now been generalized in many field of medicine (e.g. renal transplantation imply a favorable risk to benefit ratio as compared to dialysis, heart or liver transplantation are the only feasible strategies in corresponding organ failures). However, transplantation still face the lack of organ donors, significant side effects relating with long term immunosuppression and the complexity of surgical procedures. Evolution of organ replacement strategies faces the need for new strategies to provide cells or organs amenable to transplantation in human diseases. An underlying emphasis will be in study of organ development and molecular mechanisms involved, the study of stem cell biology and strategies to develop artificial or in vitro-engineered tissues and organs. Embryonic and adult stem cell transplantation as a potential means of regenerating injured tissues is currently receiving a great deal of interest. To exploit this as a viable therapy, methods need to be developed for harvesting and expansion of stem cells in sufficient quantities. This in turn implies greater knowledge of the pathways controlling replication and maturation of stem cells. Genomic and proteomic methods are ideally suited to provide these new
insights. Standardised experimental animal models that reproduce human disease are required for translational research. Furthermore information regarding the safety of cells based therapy in patients is needed.

- **Technological challenges**

  To achieve insight into physiology and pathophysiological processes in the fields covered by the PMN institute, technological challenges are multifold. The human genome opens the need for better understanding of gene regulation and interaction with the environment, both in physiology and in pathology. Beyond high-throughput DNA sequencing and transcriptomics, epigenetics and metagenomics will need to be developed to get insight into both organ development and physiology and into mechanisms of common diseases. A parallel challenge is, at the other extreme of the spectrum from fundamental research to applied medicine, the need for setting up clinical phenotyping platforms including sensory platform in an effort towards an improved classification of common diseases that, as aforementioned, lack identified aetiologies on which medical classifications may rely on.

  Specific challenges:

  1) Development of improved models to apply the advances of genetics, genomics transcriptomics and proteomics to the study of gene and protein functions, and creation of tools (platforms) enabling genomic analysis of epigenetic programs and changes in individual or small groups of cells in integrated contexts

  2) Development of appropriate facilities for study of both large animals models and small model organisms where teams of researchers and clinicians can address molecular, physiological and pathological questions by studying underlying mechanisms in integrated contexts. This includes the development of advanced imaging techniques (e.g. cell imaging, high-resolution imaging, multiphoton microscopy, small animal imaging).

  3) Development of translational research networks at the crossroad of research institutes and hospitals with the aim of developing biomarkers and innovative therapeutic strategies.

  4) Optimize the use of the important human bioresources (blood, plasma, DNA, tissue samples) available and currently being constituted in the fields covered by the PMN Institute, and facilitate their exploitation using advanced technology.

  5) Establishment of resource centres centralising interactive data sets such as that (Standards based Infrastructure with Distributed Resources, SIDR) set up by the CNRS/INIST to better collect, annotate, exploit and harness qualitative and quantitative data sets from different sources to be used in modelling and systems biology approaches for understanding basic biological mechanisms and pathophysiological regulations.

3 – ITMO PMN in numbers

- 353 research teams
- 1,500 researchers
- 1,130 hospital practitioners
- 15,800 publications a year
Team distribution by domain

- The teams are distributed in the following domains:
  - Osteoarticular system 9%
  - Pneumology 8%
  - Cardiovascular system and Hemostasis 18%
  - Dermatology 2%
  - Nephrology 6%
  - Hepatology 4%
  - Gastroenterology 4%
  - Endocrinology 7%
  - Diabetes/Metabolism/Nutrition 42%

Geographical distribution

- About 39% of ITMO PMN teams are based in the Paris area.
- Teams are affiliated to 34 universities (5 in Paris) and 11 Grandes Ecoles (4 in Paris).
Some spots display focused and specific research activities:
- metabolism/nutrition in Bordeaux, Clermont-Ferrand, Dijon and Lyon
- cardiovascular and metabolic diseases in Toulouse
- diabetes in Lille
- cardiometabolism in Nantes

In the Paris area, the teams are spread out on 30 spots. Some of them are focused on specific field:
- cardiovascular in Georges Pompidou European Hospital
- cardiometabolism in Pitié Salpêtrière Hospital
- diabetes in Cochin.
- metabolism/nutrition in Jouy-en-Josas
- hepatology/gastroenterology in Bichat Hospital

Other research infrastructures

Clinical Investigation Centres (CIC)
Set up by the Ministry of Health through the DGOS (Department for the supply of healthcare) and Inserm (the National Institute for Health and Medical Research), the CICs are clinical research infrastructures dedicated to the organisation, coordination and realization of physiology, pathophysiology and/or therapy protocols with the aim to increase knowledge of diseases, their prevention and treatments. The CICs’ activities are always closely linked to the University Hospital research programs. Actually there are 54 CICs spread out in France. 26 are connected through 8 national thematic networks, 3 of them displaying the following themes: “Thrombosis”, “Cardiovascular diseases” and “Hepatology and Gastroenterology”. More than 40% of protocols developed in CIC networks relate to one of the domains covered by ITMO PMN.

Networks for excellence in clinical research
The national infrastructure for French clinical research F-CRIN approved networks for excellence in clinical research to lead original and internationally appealing scientific programmes. These programmes have targeted themes with major potential for development and benefit from renowned collective scientific and methodological expertise with a strong capacity for research. Out of the 8 approved Networks, 5 are specialized to the following themes: “Thrombosis”, “Obesity”, “Cardiovascular diseases”, “Chronic kidney diseases” and “Autoimmune and autoinflammatory diseases”.

Human Nutrition Research Centers (HNRC)
The Human Nutrition Research Centers were set up to develop research on clinical nutrition in healthy human and out patients, to provide specific facilities regarding both investigation tools and specific food conditioning. 4 centers were created in Lyon, Clermont-Ferrand, Nantes and Paris (Ile de France). Each of these centers is a combination of several research units from INRA, INSERM, Universities and clinical units from University Hospitals with specific facilities.
Launched in 2009 by the French Government, the Investments for the Future programmes are strategic initiatives which aim to boost French competitiveness by investing particularly in research and higher education. This strong financial support to research, higher education and innovation aimed at promoting excellence and the development of high-level projects and clusters and strengthening France’s capacity for innovation.

Among others, the main programmes in the fields of the PMN institute are listed below and detailed in the following figure:

- Equipment of excellence, EQUIPEX (very high quality scientific facilities)
- Laboratories of Excellence, LABEX (internationally visible labs)
- Research Hospitals, IHU (centers of excellence in research, care, training and technology transfer in the health field)
- Cohort (long-term funding for cohorts with underlying health issues)
- Preindustrial Biotechnology Demonstrators (allowing faster achievement of the proof of commercial concept)
- National Infrastructures in Health and Biotechnology
- Hospital University research in Health, RHU (supporting translational health research projects or clinical research project)
**RHU:**
- BIOART-LUNG 2020 5 M€
- FIGHT-HF 9 M€
- CARMA 9 M€
- CHOPIN 8,3 M€
- iLite 8,5 M€
- iVASC 8,5 M€
- iMAP 9 M€
- MARVELOUS 5,5 M€
- PreciNASH 8 M€
- STOP-AS 6,6 M€

**IHU:**
- ICAN 45 M€
- LIRYC 45 M€
- MixSurg 67,3 M€

**Promising IHU:**
- OPERA
- CESTI

**Cohorts:**
- CKD rein 4 M€
- E4N 7,9 M€
- CONSTANCES 35 M€

**Demonstration project:**
- MetaGenoPolis 19 M€

**National Infrastructures:**
- Biobanques 17 M€
- F-CRIN 18 M€
- MetaboHub 10 M€
- ECELLFRANCE 12 M€

**Labex:**
- EGID 18 M€
- INFLAMEX 9 M€
- LipStic 8 M€
- LERMIT 19 M€
- Mitocross 5,5 M€
- Hepsys 3 M€

**Equipex:**
- IVTV 2,7 M€
- LIGAN 8 M€
- MUSIC 3 M€
- RE-CO-NAI 13 M€
- ImaginExBioMed 6,8 M€
- HEPATHER 10 M€
Circulatory system
Daniel Henrion

Cardiovascular Mechanotransduction (CarMe)

Université d’ Angers
Inserm U1083 CNRS UMR 6015
Daniel Henrion
Angers

Key facts
Team
• Researchers : 12
• Technicians : 5
• Postdoc fellows : 2
• PhD Students : 10
Translational approaches
• Patents : 2
• Clinical research grants : 4
• Industry partnerships : 4
International research links
• Germany, Gret-Britain, USA,
Spain, Canada, Hungary

Keywords
• blood flow
• ischemia/reperfusion
• GPCRs
• endothelium
• mechanotransduction
• Microcirculation
• limb ischemia
• confocal microscopy
• resistance arteries
• bio-computing
• Local blood flow
• electrophysiology

Study of small resistance arteries mechanotransduction in ischemic diseases (limb and heart ischemia, hypertension, diabetes, obesity)

Research Brief :
Resistance arteries are located upstream capillaries, are crucial to the delivery of blood to vital tissues at relevant flow and pressure. Disorders of these small arteries can raise capillary pressure and cause downstream organ damage such as that seen in diabetes, neurovascular disorders or kidney disease. We aim a) to define how structure and function of small arteries change in ischemic disorders associated with ageing and the related risk factors and aa) to identify the specific changes in pathways involved in resistance artery homeostasis leading to the identification of novel targets/biomarkers for intervention and disease prevention.

We have 3 specific objectives:
1- investigate flow-mechanotransduction in resistance arteries in order to better define the pathways involved with a special focus on the mechanosensitive channels and on the mechanosensitive receptors.
2- determine in resistance arteries the mechanism of remodelling involved in ischemic disorders
3- investigate the mechanisms involved in ischemia-reperfusion injury and to bring forward new strategies to prevent its occurrence.

Finally, ischemic disorders are investigated using mouse models of ischemia/reperfusion in healthy and diseased ageing and in human vessels from patients with severe limb ischemia and healthy volunteers.

Methodologies Used :
Arteriography and myography for resistance arteries (in vitro function)
In vivo microcirculatory function (Laser-Doppler flow metry, arteriography...)
Molecular biology of resistance arteries and mitochondria
Electrophysiology (patch-clamp on tissue slices, microelectrodes on xen. oocytes)
Confocal microscopy (fixed tissues and real-time)
Molecular modeling and dynamics, bioinformatics

Publications


Vascular response to pressure and flow

Acute and chronic vascular response to pressure and flow determine a proper tissue perfusion. A desequilibrium between pressure- and flow-dependent tone and wall structure is involved in cardiovascular disorders. From www.bnmi.fr (team 2: CarMe)

Purinergic signalling in myogenic tone.


Vascular effects of the angiotensin II type 2 receptor

From Caillon et al., Cardiovasc Res. 2016;112(1):515-25
**Bioinformatics**

- Functional genomics
- Bioinformatic analysis
- Next generation sequencing
- Microarray analysis

**Research Brief**

Clinical and basic research demonstrates that the endothelium plays a crucial role in mediating homeostasis and is involved in virtually every disease, either as a primary determinant of pathophysiology or as a victim of collateral damage. The endothelium is involved in the maintenance of normal organs and vascular structure and function. It may be thought as an organ by itself. Following its abnormalities in function would provide a tremendous opportunity to inform about the status of the disease progress. As it is widely distributed and easily accessible, it may be regarded as critical target in the fight against some cardiovascular ischemic diseases.

Our project aims to improve endothelium knowledge and how endothelium interacts with its microenvironment. We are interested in understanding endothelial machinery in the control of vessel maintenance and function in different pathological settings:

- Role of endothelial dysfunction in heart failure with preserved ejection fraction
- Role of endothelial dysfunction in critical hind limb ischemia
- Endothelial cells, blood-brain barrier dysfunction in cerebrovascular disease
- Retinopathy and vascular lesions
- Role of endothelial cell in thrombosis? example of myeloproliferative neoplasms

Wnt/Frizzled, Hedgehog and JAK2 signaling pathways are specifically studied in endothelial dysfunction.

**Methodologies Used**

- Molecular and cellular biology
- Cell culture: vascular cell proliferation, directional migration, cell velocity, videomicroscopy, 2-D and 3-D, adhesion, NETs, angiogenesis models, hypoxia models; Platelets, megacyclopiesis Biochemistry
- Vessel imaging: microscanner (microCT), confocal microscopy, 3D image reconstruction
- Experimental animal models: conditional knock-out mouse models, model of hindlimb ischemia, infarctus and ischemia reperfusion, oxygen induced retinopathy, corneal angiogenesis, Thrombosises models

**Publications**

1. Renault-Marie-Ange, Vandierdonck Soizic, Chapouly Candice, Yu Y., Qin G., Metras Alexandre, Couffinhal Thierry, Losordo D. W., Yao Qinyu, Reynaud Annabel, Jasprood-Vinassa Beatrice, Belloc Isabelle, Desgranges Claude and Gadeau Alain-Pierre (2013). Gill regulation of myogenesis is necessary for ischemia-induced angiogenesis, Circulation Research, 113(1), 1148-1158
5. Sewduth Raj Nayan, Jasprood-Vinassa Beatrice, Peghaire Claire, Bats Marie-Lise, Sewduth Raj Nayan, Jeannings Cyril, Jasprood-Vinassa Beatrice, Couffinhal Thierry, Duplaa Cécile and Dufourcq Pascale (2015). Fzd7 (Frizzled-7) Expressed by Endothelial Cells Controls Blood Vessel Formation Through Wnt/beta-Catenin Canonical Signaling, Arteriosclerosis, Thrombosis, and Vascular Biology, 35(8), 2369-2380
Neutrophil extracellular traps in patients with JAK2V617F positive myeloproliferative neoplasms

Endothelial polarization

3D visualisation of mouse cerebral vascularisation (microscanner)
Stéphane Germain

Role of Matrix Proteins in Hypoxia and Angiogenesis

Université de Paris 06
(Université Pierre et Marie Curie)
Inserm U1050 CNRS
Alain Prochiantz
Paris

Our goal is to understand how endothelial cells respond to hypoxia in order to identify new specific markers of hypoxia-induced angiogenesis and new potential therapeutic targets in cancer and ischemic cardiovascular diseases

Research Brief:

Biological events that permits an organism to maintain tissue viability in hypoxia remains poorly understood. How hypoxic endothelial cells integrate chemical signals with mechanical cues from their local microenvironment to protect vascular integrity during ischemic insult and/or induce functional capillary networks that exhibit specialized form remains an open question. A key role of hypoxia in regulating endothelial function is nevertheless established and growing evidence shows that angiogenesis, blood vessels formation by sprouting or growth of preexisting vessels, can be triggered by hypoxia, both during development and in pathological conditions. Our efforts have recently been focused on characterizing the role of Lysyl Oxidase-like 2. Thrombospondin-1 and Angiopoietin-like 4 in regulating angiogenesis and vascular integrity. The complementary technical expertise of the members of the team together with the established collaborations with clinicians (Pathology, Urology, Cancer and Biochemistry departments, Hopital Saint-Louis and HEGP) led to the definition of angptl4 mRNA as an accurate marker for primary ccRCC diagnosis. Altogether, our studies aimed at better understanding of the complex interplay between endothelial cells and soluble growth factors and mechanical factors from the extracellular matrix will certainly have significant implications for understanding the regulation of developmental and pathological angiogenesis driven by hypoxia.

Methodologies Used:

Multidisciplinary approach combining gene discovery approach for complex human diseases
- Cell culture, cell biology: vascular cell proliferation, adhesion, migration, cell velocity, videomicroscopy, 2-D and 3-D angiogenesis models, normoxia, hypoxia
- Gene/protein structure function analysis (molecular biology, transcriptomics, extracellular matrix proteomics, transfection, mutagenesis)
- Animal models, transgenic mice, cardiovascular functional exploration, cancer, metastases
- Vascular development (animal models, zebrafish studied by confocal, second harmony, bi-photon, and electron microscopy)

Publications


The interaction of HSPGs with endothelial transglutaminase-2 limits VEGF165-induced angiogenesis

Online Cover This week features a Research Article that shows that transglutaminase-2 prevents heparan sulfate from potentiating signaling by a specific VEGF isoform, thereby attenuating blood vessel formation. The image shows retinal vascularization in a mouse deficient in transglutaminase-2.
Catherine Llorens-Cortes

Neuropeptides Centraux et Régulations Hydrique et Cardiovasculaire
-Central neuropeptides in the regulation of body fluid homeostasis and cardiovascular functions

Collège de France
Inserm U1050
Alain Prochiantz
Paris

Our work is to identify new therapeutic targets (enzymes involved in the metabolism of (neuro)vasoactive peptides or their receptors) involved in water balance and cardiovascular functions control. The synthesis of compounds acting on these targets leads to the development of therapeutic agents.

Research Brief:

BRAIN RENIN-ANGIOTENSIN SYSTEM (RAS). We showed in the brain RAS that aminopeptidase A (APA) generates angiotensin III (AngIII) from AngII and that brain AngIII exerts a tonic stimulatory effect on the control of blood pressure (BP) in hypertensive animals. In coll. with the team of B. Roques (U640), we designed the first specific and selective APA inhibitor, EC33 and we showed that the inhibition of brain APA decreases BP. Brain APA constitutes a potential therapeutic target for the treatment of hypertension. We produced a new APA inhibitor, RB150 able, after administration by oral route, to cross the intestinal, hepatic and blood brain barriers, to block the activity of the brain RAS and to normalize BP in hypertensive animals. We pursue the preclinical development of RB150 with Quantum Genomics. APELINERGIC SYSTEM. We isolated an orphan receptor which was shown to be the receptor of a new peptide, apelin. We demonstrated that apelin and its receptor are expressed together with vasopressin (AVP) in hypothalamic neurons. We showed that the icv injection of apelin in lactating rats decreased the activity of these neurons and the systemic secretion of AVP, resulting in aconiguous diuresis. Apelin is a natural inhibitor of the anti-diuretic effects of AVP. We showed that in rats and humans, apelin and AVP are regulated in opposite manners by osmotic stimuli. In addition, apelin decreases BP, improves cardiac contractility. Apelin controls water balance and cardiovascular functions.

*Methodologies Used:

Molecular modeling and molecular biology: 3D model of enzyme or GPCRs and site-directed mutagenesis studies - Screening of chemical libraries - Pharmacological studies of GPCRs stably expressed in eukaryotic cells: binding, cAMP production, [Ca2+]I mobilization, internalization followed by confocal microscopy - Neuroanatomical studies: immunohistochemistry, in situ hybridization - Purification of peptides by HPLC and radioimmunoassay - Enzymatic studies: expression of recombinant enzymes, purification, Western-blot analysis, enzymatic activity - Physiological studies: measurement of vasopressin release, drinking behavior, diuresis, plasma and urinary electrolytes - Vessel vasoreactivity - Blood pressure - Cardiac function

Publications


Chantal Boulanger

Endothelial Physiopathology and Biomarkers of Vascular Diseases

Université de Paris 05
(Université Rene Descartes)
Inserm UMR 970
Alain Tedgui
Paris

**Associating molecular and integrated physiology to decipher new avenues in the field of endothelial dysfunction**

**Research Brief**: Cardiovascular diseases are an increasing social and economical burden. An initial step is the loss of vasculo-protective functions of the endothelium. Thus, we need to decipher the mechanisms regulating endothelial dysfunctions to identify new therapeutic targets in vascular diseases. In addition, early detection of dysfunctional endothelial cells will help stratify cardiovascular risk and pharmacological treatment of asymptomatic subjects.

In the past decade we have pioneered research on the release of membrane vesicles (microparticles or microvesicles) from dysfunctional endothelial cells. We have demonstrated that circulating endothelial microparticles (EMP) are potentially useful clinical indicators of dysfunctional endothelium and a prognostic marker of cardiovascular mortality. But extracellular release of membrane vesicles is not only a sign of cell injury, these vesicles are also a new mediators affecting the function of target cells. Indeed we have demonstrated that EMP are paracrine signals for vascular repair in ischemic diseases. In addition, microparticles promote pro-inflammatory and pro-angiogenic responses in human atherosclerotic lesions.

Our current research integrates new research avenues in the field of endothelial dysfunction. We investigate:

1/ the role of autophagy in endothelial activation,
2/ role of micro-RNA packaging in endothelial microvesicles and exosomes in atherosclerosis
3/ the endothelial consequences of erythrocyte activation

**Methodologies Used**: Flow cytometry for cell and microvesicle analysis
Tunable resistive pulse sensing
Endothelial cell culture (murine, human)
Fluorescence microscopy
Myograph for studying isolated blood vessel reactivity
Original murine models with specific endothelial deletion

**Publications**


**Key facts**

**Team**
- Researchers : 5
- Technicians : 5
- Postdoc fellows : 2
- PhD Students : 2

**Translational approaches**
- Patents : 0
- Clinical research grants : 6
- Industry partnerships : 1

**Keywords**
- Rare arterial diseases
- Mitral Valve Prolapse
- Genetics
- Fibromuscular dysplasia
- Gene expression
- Exome sequencing studies
- Genetic association studies
- Knock-out mouse model

**Biological Resources**
- DNA collection and cohort of patients and families with arterial fibromuscular dysplasia
- DNA collection and cohort of patients and families with vascular Ehlers Danlos syndrome
- DNA collection and cohorts of patients with rare inherited vascular disorders
- DNA collection and tissue collection of patients with cardiac valvular diseases (mitral valve prolapse)

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**Xavier Jeunemaitre**

**Genes and rare arterial diseases**

Université de Paris 05  
(Université Rene Descartes)  
Inserm U970  
Alain Tedgui  
Paris

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**Integrated translational research based on several unique patients cohorts, the constitution and exploitation of DNA and tissue biobanks, the use of the most recent genetic technologies to identify new disease-causing genes and variants, the creation and characterization of cellular and mouse models**

**Research Brief**:

Our team aims to identify causative genes and understand mechanistic basis of several rare arterial diseases. We are interested in rare forms of hypertension (Pseudohypoaldosteronism, type II: PHAII) and Fibromuscular Dysplasia: FMD) and rare vascular diseases : vascular Ehlers-Danlos Syndrome (vEDS) and inherited forms of aortic aneurysms (TAA). We have also high interest in understanding the genetics and the biology of mitral valve prolapse (MVP) for which we have recently identified several genetic risk loci.

We apply three complimentary strategies to achieve these goals:

1) **High throughput genetic and genomic approaches**, which are exome sequencing and genome-wide association to families and large cohorts of patients recruited at the Hypertension Department and the National Reference Centre for Rare Vascular Diseases.

2) **Molecular and physiological investigation in CRISPR-Cas9 engineered cells and animal models of genes involved in the regulation of hypertension and vascular tone**: WNK pathway, KLHL3-CUL3 ubiquitin ligase complex and collagen 3 alpha 1 gene COL3A1, mutated in vEDS.

3) **Clinical investigation and complications follow-up search of circulating biomarkers and vascular tone assessment for vEDS and FMD patients**.

**Methodologies Used**:

- Human genetic studies : genome-wide association and linkage studies, families and population based cohorts, Exome and targeted sequencing
- Mouse models : transgenesis, gene inactivation, tissue-specific inactivation, in vivo blood pressure monitoring, metabolic cages, arterial myograph, creation of original mouse models
- Tissue characterization : Immunohistochemistry, In situ hybridisation, Confocal microscopy imaging, mRNA quantification, Western blotting, RNA-seq, chromatin interaction
- Cellular models : classical cellular characterization, cell trafficking, inhibition by siRNA and shRNA, original cellular models (SDHB inactivation), ubiquitination process, BRET imaging.
Publications


Jean-Sébastien Silvestre
Philippe Menasché

Regenerative therapies for cardiac and vascular diseases

Paris S University
Inserm UMR 970
Alain Tedgui
Paris

The team spans a fully integrated spectrum encompassing basic, preclinical and translational research to develop efficient approaches of cell and non-cell-based strategies to circumvent the adverse remodeling occurring in patients with cardiovascular ischemic diseases

Research Brief:

The team is based on the complementary expertise contributed by a group (Dr JS Silvestre) experienced in deciphering of signaling pathways involved in post-ischemic tissue remodeling and a group (Pr P Menasché) with a long-standing experience in the preclinical, translational and clinical aspects of stem cell research. Together, we form an ideal platform of expertise and technical know-how, ranging from the basic features of cell injury, regeneration and remodeling to the clinical applications of cell-based therapies complying with the increasingly stringent regulatory requirements. The background of the group members (both basic scientists and practising clinicians) as well as their respective expertise allows the team to cover a spectrum of activities from the mechanisms of postischemic tissue remodelling and regeneration at the molecular level to the development of therapeutic strategies to mimic and boost these processes. Through the use of tools ranging from molecular biology methods to small and large animal models, the team spans a fully integrated spectrum encompassing basic, preclinical and translational research. Our main objectives are to decipher the molecular and cellular mechanisms involved in post-ischemic tissue remodeling and to develop efficient approaches of cell-based strategies to circumvent the adverse remodeling occurring in patients with cardiovascular diseases.

Methodologies Used:
- Pathophysiological models of postischemic tissue remodeling: hindlimb ischemia induced by right femoral artery ligation and cardiac ischemia induced by occlusion of the proximal left anterior descending coronary artery
- Vessel growth analysis by high definition microangiography, immunohistochemistry and laser Doppler imaging to analyze flow recovery
- Transthoracic echocardiography to follow non-invasively systolic and diastolic ventricular function
- Transthoracic echo-guided injection
- Flow cytometer: FACS sorter, Image stream

Key facts
Team
- Researchers: 3
- Technicians: 3
- Postdoc fellows: 2
- PhD Students: 4

Translational approaches
- Patents: 2
- Clinical research grants: 1
- Industry partnerships: 2

International research links
- USA, Germany, United Kingdom

Biological Resources
- Neonatal model of cardiac regeneration
- In vitro model of cardiac cell differentiation
- Pathophysiological models of postischemic tissue remodeling

Publications


Immune cell stimulation is among the earliest responses detectable in the injured cardiac tissue and plays an instrumental role in the coordination of multiple processes governing cardiac remodeling. In animal models, the number, type and activation state of the different subclasses of inflammatory cells dictate their impact on cardiac repair leading to either positive or deleterious cardiac remodeling. (From Zlatanova et al, Front Cardiovasc Med, 2016)

The evolution of the stem cell theory for heart failure

The recent big bang in the evolution of the stem cell theory suggests that therapeutic cells rather act as reservoirs of a wide array of bioactive entities that trigger multiple and synergic endogenous repair pathways. Abbreviations: BM: bone marrow, PB: peripheral blood; AT: adipose tissue; iPSCs: induced pluripotent stem cells; ESCs: embryonic stem cells; SCs: stem cells. (From Silvestre JS/P Menasché, Ebiomedicine, 2015)
Christian Stockmann

INFLAMMATORY VASCULAR REMODELING AND MICROENVIRONMENTAL HOMEOSTASIS

Université Paris Descartes
Paris 5
Inserm U 970
Alain Tedgui
Paris

We define inflammation-driven vascular remodeling as central interconnection between cancer progression, organ fibrosis and physiological tissue regeneration!

Research Brief:

The infiltration of inflammatory cells into hypoxic tissue microenvironments and subsequent formation of new blood vessels (angiogenesis) or remodeling of the existing vasculature are central features of physiological wound healing responses as well as the pathophysiological processes of tumor angiogenesis and tissue fibrosis.

We aim to

(i) decipher how inflammatory cell-driven remodeling of the vasculature affects these processes

(ii) develop therapeutic strategies to target deleterious effects as well as to exploit the beneficial impact of the inflammatory response and vascular remodeling.

Methodologies Used:

- transgenic mouse models
- in vivo models of cancer, organ fibrosis and tissue regeneration
- in vivo, real time imaging of angiogenesis and tissue hypoxia
- Flow cytometry
- immunohistochemistry

Publications


Chahrazade Kantari-Mimoun, Ewelina Krzywinska, Magali Castells, Ralph Klose, Anna-Katharina Meinecke, Ursula Lemberger, Milos Goykovic, Katrin Schröder, Christoph Oesterreicher, Joachim Fandrey, Helene Rundqvist, Christian Stockmann (2017). Boosting the hypoxic response in myeloid cells accelerates fibrosis resolution and regeneration of the liver in mice, Oncotarget. 10(18632), 14749

**Key facts**

**Team**
- Researchers : 1
- Technicians : 1
- Postdoc fellows : 1
- PhD Students : 0

**Translational approaches**
- Patents : 2
- Clinical research grants : 0
- Industry partnerships : 2

**International research links**
- USA
- Japan
- Austria, Sweden, Germany

**Keywords**
- tumor microenvironment
- innate immunity
- hypoxia
- angiogenesis
- fibrosis
- Flow cytometry
- transgenic mouse models
- Histology

**Biological Resources**
- conditional in vivo deletions in innate immune cell subsets of Hypoxia-inducible factors, von Hippel Lindau Protein, VEGF
Inflammation-driven vascular remodeling as central connection between cancer, tissue regeneration and fibrosis
Antonino Nicoletti

**Immunopathology and immunomodulation of cardiovascular diseases**

Université de Paris 07 (Université Denis Diderot)
Inserm U1148
Didier Letourneur
Paris

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**Key facts**

**Team**
- Researchers: 3
- Technicians: 4
- Postdoc fellows: 1
- PhD Students: 4

**Translational approaches**
- Patents: 3
- Clinical research grants: 0
- Industry partnerships: 1

**Keywords**
- Atherosclerosis
- Immunology
- Flow cytometry
- Microscopy

**Biological Resources**
- Various strains of mice: CD31 KO, CD31 Tg, ApoE KO, actin GFP Tg, OT2 TCR Tg, LTßR KO, Qa-1KO

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**Research Brief:**

It is nowadays recognized that a crucial immune pathogenetic component contributes to atherogenesis. Our team works on the immune mechanisms and on the signaling pathways involved in atherogenesis. Our projects encompass molecular aspects as well as integrative pathophysiology and aim at discovering new prognostic, diagnostic, and therapeutic tools.

Two main research programs are currently developed:

1) The adventitial lymphoid neogenesis concentrates the main actors of the adaptive immunity. We wish to characterize these lymphoid structures and understand their pathogenic role.

2) The CD31 is an inhibitor of inflammation and we have discovered that it can be shed on activated lymphocytes. We are setting up molecular strategies able to restore the CD31 signaling.

**Methodologies Used:**
- Polychromatic flow cytometry
- 6D microscopy
- Experimental models
- Cell immunobiology

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**Publications**


Ana Maria Gomez

Calcium Signaling and Cardiovascular Physiopathology

Université de Paris 11
(Université Paris Sud)
Inserm UMR-S 1180
Ana Maria Gomez
Chatenay Malabry

Our team has the internationally recognized expertise in cardiac excitation-contraction coupling in physiologic and pathologic conditions and in molecular basis of arrhythmia. We are one of the rare teams that combine simultaneous recordings of patch-clamp and confocal microscopy in cardiomyocytes.

Research Brief:
Cardiovascular diseases remain the leading cause of death in developed countries. Last stage of cardiac pathologies, heart failure (HF) is major cause of morbidity and mortality. HF patient’s prognosis is very poor, and about 50% die suddenly as consequence of arrhythmia. This is the consequence of misunderstanding of the mechanisms responsible of contractile dysfunction and arrhythmogenesis. In addition of activating cardiac contraction, Ca²⁺ is recently emerging as a key factor in transcription regulation (excitation-transcription coupling) and in arrhythmia development.

Our project aims to elucidate the adaptive and maladaptive mechanisms involved in HF and arrhythmia, focusing on compartmentalized intracellular Ca²⁺ signals, through the Ca²⁺ release channel, the ryanodine receptor (RyR) and the molecular pathways involved in its malfunction. We will focus on a cAMP-directly activated protein named Epac and in the hormone aldosterone, whose expressions are increased in HF, but also in the cardiotoxic effects of anticancer therapies. We have shown a major role of Epac and aldosterone in Ca²⁺ handling. We will analyze their role in HF and arrhythmia. Moreover, mutations in the RyR are responsible of lethal arrhythmia. We have shown functional consequences of a mutation in the C terminal portion of the RyR. Our project is to analyze a new mutation in the N-terminal portion of the channel.

Methodologies Used:
- Electrophysiology: patch-clamp and lipid bilayers
- Confocal Microscopy (alone or coupled to patch-clamp)
- Superresolution Gated STED
- Biochemistry, molecular and cellular biology.
- Transgenic animal breeding
- Holter telemetry

Publications


**Grégoire Vandecasteele**

**Cyclic Nucleotide Signaling and Cardiovascular Pathophysiology**

University Paris Sud  University Paris Saclay  
Inserm UMR-S1180  
Ana-Maria Gomez  
Châtenay-Malabry

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**Research Brief:**

Heart failure (HF) is the only cardiovascular disease that is increasing in prevalence in Europe and the USA. Most cases of HF are caused by diseases of heart muscle that result in pathologic hypertrophy (“remodeling” at the ventricular chamber level) and contractile dysfunction. The majority of HF in patients under 70 years of age reflects impaired systolic function resulting from dilated cardiomyopathy. Beta-adrenergic receptor/cAMP cascade is centrally involved in the pathophysiology of HF, as demonstrated by the correlation between elevated norepinephrine and mortality and the beneficial effect of beta-blockers in this pathology. However, such medications are effective in only 40-50% of HF patients. In the recent past, our team has crucially contributed to the understanding that physiological cAMP signaling is confined in specific subcellular domains and suggested that drawbacks of HF treatments are due to their bypass of compartmentalization. The goal of our team is to provide an in-depth analysis of cAMP signaling in pathologic hypertrophy and to define defective cAMP signaling events that underlie HF. Since HF is associated with anomalies of the vasomotor tone, we also explore the organization of the cAMP signaling cascade in vascular smooth muscle.

**Methodologies Used:**

Our studies are conducted in rat, mouse and humans. Experimental approaches combine assessment of cardiac function in vivo (echocardiography, ECG) and at the organ level (Langendorff perfused heart), and single cell (patch-clamp, fluorescence imaging) and biochemical studies. A major focus is placed on cAMP phosphodiesterases (PDE) and protein kinase A because our previous work has demonstrated that these enzymes play a key role in the organization of the intracellular cAMP cascade. Through the development of molecules that activate specific cardiac PDE isoforms, our project will attempt to provide new treatments of HF acting on localized cAMP signaling to improve heart function and clinical outcomes.

**Publications**


Cyclic nucleotides metabolism in cardiac myocytes.

The major phosphodiesterases (PDEs) expressed in cardiac myocytes are indicated, together with their subcellular localization in relation to their role in regulating hypertrophic growth and excitation-contraction coupling. AC: adenylate cyclase; AKAP: A-kinase anchoring protein; CaMKII: Ca2+/calmodulin-dependent kinase II; cAMP: cyclic adenosine monophosphate. Adapted from Bobin et al. Arch Cardiovasc Dis. 2016.

Cyclic nucleotide metabolism in vascular endothelial and smooth muscle cells.

Cyclic nucleotide metabolism in vascular endothelial and smooth muscle cells. For each cell type, the main pathways leading to cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) synthesis and the major phosphodiesterase (PDE) families involved in their degradation are indicated. GPCR: G-protein coupled receptor; Gs: heterotrimeric G-protein stimulating AC; NO: nitric oxide; NOS: NO synthase; pGC: particulate guanylate cyclase.

Gene therapy with phosphodiesterases to treat heart failure

The figure illustrates one of the current research project of the team supported by the European Research Area Network (ERA-Net) on Cardiovascular diseases and involving three other european teams (FO Levy, Norway; V Nikolaev, Germany; E Hirsch, Italy). Our goal is to test whether augmenting the activity of a cAMP phosphodiesterase (PDE) in the heart by gene therapy with adeno-associated virus (AAV) is beneficial in heart failure.
Jean-Sébastien Hulot

Biology and pharmacology of cardiovascular remodeling

Université de Paris 06
(Université Pierre et Marie Curie)
Inserm UMR1166
Stéphane Hatem
Paris

Alliance of high-level basic research and translational medicine

Research Brief:
Heart Failure (HF) remains a leading cause of mortality and morbidity in Europe. Our general aims are to understand the molecular and cellular mechanisms involved in the transition to heart failure and to identify relevant targets to reverse the adverse remodeling process or alternatively promote myocardial tissue repair.

During the last years, the team has consequently set up animal and cellular models to study ischemic heart failure (the most prevalent form of HFREF) as well as cardiac hypertrophy, an adaptive cardiac response to stress (particularly hemodynamic overload) that progressively leads to heart failure (and mimics some stages of HFP EF). In these murine models of heart failure, we have notably identify a new population of adult stem cells that reside in the myocardium and are identified by the expression of PW1/Peg3 gene. We found that these cells are involved in the fibrotic remodelling of the myocardium in response to stress, thus identifying a new target to limit injury-induced adverse remodelling.

More recently the team has developed innovative tools based on human induced pluripotent stem cells to further model cardiac disorders in a dish. This human cellular platform allows to perform pharmacological investigations, model mono- or multigenic forms of cardiomyopathy, investigate underlying pathological pathways and perform direct intervention (genome editing) to correct or introduce punctual genomic changes and perform functional analyses.

Methodologies Used:
Human cellular models of cardiac disorders using patient-specific hiPSC
Targeted genome editing using TALENS and/or CRISPR/Cas9
Gene transfer in the cardiovascular system using AAV and adenovirus;
Calcium signalling and calcium sources in cardiovascular cells;
Experimental mouse models for heart failure

Publications


Benard L, Oh JG, Cacheux M, Lee A, Nonnenmacher M, Matasic D, Kohlbrener E, Kho CW, Pavoine C, Hajjar RJ, Hulot JS (2016). Cardiac Stim1 silencing impairs adaptive hypertrophy and promotes heart failure through inactivation of mTORC2/Akt signaling, Circulation. 133(1458),


Endogenous Cardiac Stem Cells

PW1 expression identifies cardiac adult stem cells with fibrogenic potential

Human Models of Cardiac Diseases

A heart-shape colony of human iPS cells

Cardiomyocytes Generated from hiPSC

Human iPS-cell derived cardiomyocytes model cardiac diseases in a dish
David-Alexandre Tregouët

Genomics & Pathophysiology of Cardiovascular Diseases

Université de Paris 06
(Université Pierre et Marie Curie)
Inserm UMR_S 1166
Stéphane Hatem
Paris

Our team combines expertise from clinicians-scientists, molecular biologists, cellular biologists, and statisticians and bioinformaticians to fully dedicated to the genetics of the whole spectrum of cardiovascular diseases without any distinction on their prevalence or degree of familiality.

Research Brief:
Our research program is focused on the epidemiological, clinical, molecular and functional genomics of cardiovascular diseases (CVD) with a strong emphasis on cardiomyopathies, arrhythmias, coronary artery disease and venous thrombosis. This research is built on important bioresources (DNA, RNA, cells, patient samples) assembled from a large collections of patients, families and population-based individuals. In addition, we have access to a local high-throughput genomic platform to discover novel molecular targets and generate new hypotheses on the causal mechanisms leading to CVDs, - a strong expertise in bioinformatics and biostatistics required to deal with the considerable amount of data generated by high-throughput technologies, - a group of molecular and cellular biologists to develop functional studies based on cellular, tissue and animal models, and - numerous collaborations that we have established with national, European and International partners.
We are also conducting clinical trials to assess whether the combination of easy-to-identify clinical markers and/or genetic variants can help to derive stratified risk-score and guide the therapeutic decisions.

Methodologies Used:
- Molecular and cellular biology
- Murine and zebrafish models of inherited CVDs
- High throughput microarray and sequencing technologies
- Epidemiological Genomics of populational and clinical cohorts
- Bioinformatics and Biostatistics

Publications


Matteo Mangoni  Stéphanie Barrère - Lemaire

Cardiac Physiopathology and Cardioprotection

Université de Montpellier
Inserm U1191  CNRS UMR5203
Jean-Philippe Pin
Montpellier

We developed worldwide unique mouse models to investigate the mechanisms underlying dysfunction of heart automatically. We dissect the signaling mechanisms involving the death-receptor apoptotic pathway in ischemia-reperfusion injury.

Research Brief:
Our research aims to develop strategies to protect the heart from dysfunction of impulse generation and ischemic injury. We aim to understand the mechanisms underlying the genesis and regulation of heart rate, an important determinant of morbidity and mortality. We were the first to develop in vitro electrophysiological studies on mouse sino-atrial (SAN) and atrioventricular (AVN) cells associated to confocal live imaging of calcium. Our strategy is based on the use of genetically modified mouse models to identify the contribution of the ion channels and intracellular calcium release in the generation and regulation of cardiac automaticity associated with in vivo experiments using telemetry on freely moving mice. We also aim to understanding the mechanisms underlying cell death and the cardioprotective effect of postconditioning protocols to prevent reperfusion injuries after infarction. We have evidenced the importance of the Fas-dependent pathway and the major role of the adaptator protein DAXX. The inactivation of the extrinsic pathway is associated to a large reduction in infarct size. Our therapeutical strategies are based on inhibiting reperfusion-induced apoptosis. We aim now at dissecting the mechanisms of Postconditioning protocols and the molecular determinants of the apoptotic cascade related to death receptors activation. Translational clinical studies on cardioprotection are also developed.

Methodologies Used:
- Patch clamp recording
- Confocal imaging of intracellular Ca²⁺ dynamics
- Cardiac exploration ex vivo (isolated heart) and in vivo (echography, telemetry)
- Genetically-modified mouse models of cardiac automaticity and impulse conduction

Mouse models of ischemia-reperfusion injury

Publications


Keywords
- cardiac arrhythmias
- apoptosis
- ischemia-reperfusion injury
- electrophysiology
- pacemaker activity
- patch clamp recording
- confocal imaging of intracellular Ca²⁺ handling
- genetically-modified mouse models of dysfunction in cardiac pacemaking
- cardiac exploration ex vivo (isolated heart) and in vivo (echography, telemetry)
- mouse models of ischemia-reperfusion injury

Biological Resources
- in vivo and in vitro models of heart disease
- surgical models
- genetic models

Key Facts
Team
- Researchers: 5
- Technicians: 1
- Postdoc fellows: 2
- PhD Students: 2

Translational approaches
- Patents: 2
- Clinical research grants: 1
- Industry partnerships: 1

International research links
- Germany, Austria, Italy, England, USA, Thailand
Mouse models of sino-atrial node dysfunction available in our research team. Models are generated by inducing constitutive or conditional loss-of-function in ion channels involved in the generation of sino-atrial pacemaker activity and atrioventricular conduction.

**Targeting of GirK4 channels rescues sino-atrial dysfunction**

Cav1.3 knockout (KO) mice show sinus bradycardia and 2nd degree atrioventricular block. Girk4 KO mice present with normal heart. Crossing these mouse lines produces Cav1.3/Girk4 KO animals with normal heart rate. When Cav1.3 KO mice undergo an intraperitoneal injection of the GIRK pore blocker tertiapin (represented bound to the channel pore) normalization of heart rate is observed. Girk4 targeting could be a future therapeutic approach for dysfunction of heart automaticity.

**Cardioprotective cellular pathways to fight Ischemia-reperfusion injury**

Our objective is to develop strategies that prevent cellular apoptosis following ischemia-reperfusion injury. We target the apoptotic extrinsic pathway down-stream the FAS receptor and stimulate the cardioprotective pathways.
Sylvain Richard

Ion channels and Calcium Homeostasis in Cardiac and Vascular Muscles

Université de Montpellier
Inserm U1046 CNRS UMR_9214
Jacques Mercier
Montpellier

**Research Brief**: Thanks to complementary expertise in cellular and in vivo electrophysiology, pharmacology, functional imaging, and cell biology, we aim at identifying novel concepts and pharmacological targets in cardiovascular diseases (Figure 1). We study Excitation-Contraction coupling during heart failure (HF) with cardiac remodeling leading to reduced/preserved ejection fraction; HTAP, hypertrophy, and resistance to senescence associated with telomeric stability.

Among different drugs currently studied, we seek to identify and understand the beneficial effects of antiarrhythmics. At the vascular level, we study the role of Na+ (Nav channels) and Ca2+ homeostasis in the contraction of vascular myocytes (VSMCs). We focus on hypoxia and mechanosenstivity, especially in hypertension and vasomotion. Another major axis of our research concerns intrinsic alterations of VSMCs exhibiting similarities with tumor cells in idiopathic PHA (Figure 3). Functional and genetic studies are carried out on cells from PHAi patients. These myocytes have high capacity for proliferation and resistance to senescence associated with telomeric stability.

*Methodologies Used*: Broad expertise and unique plat-form to study *from organ function in vivo to cell function, and back. Wide range of biological models, techniques, methodologies.

**Biological Resources**
- Rodent models: Heart failure with reduced/preserved ejection fraction; hypertrophy, HTAP, ischemia-reperfusion
- Ca imaging

Research Brief:

- Ischemia-reperfusion
- Hypertrophy
- Conduction disorders
- Sodium handling
- Calcium handling

**Publications**
Figure 1

Environmental factors
- Hypoxia, oxidative stress, injury

Autocrine/paracrine factors

Cell response
- Cell contraction/growth/apoptosis

Physio-pathological changes
- Vascular remodeling/heart failure/fibrosis/arythmias...

Figure 2

Figure 3
The team is leader in basic fundamental research on lipid peroxidation signalling in vascular cells, animal models for atherosclerosis and transplant vasculopathy and translational studies on circulating biomarkers for the follow-up of coronary patients.

Research Brief:

Low density lipoproteins (LDL) in the vascular wall play a key role in the early stages of atherogenesis. Oxidized lipids participate in the development of lesions by promoting local inflammatory response, smooth muscle cell migration and proliferation, extracellular matrix production and plaque remodeling. Oxidized lipid properties depend on their local concentration, nature or uptake by scavenger receptors (CD36, LOX-1, SRA...), which contribute to fragilize the lesions, promote plaque erosion or rupture and finally lead to athero-thrombosis events.

Our team aims at deciphering the mechanisms evoked by oxidized lipids, implicated in the balance cell survival/death. This includes the ceramide/sphingosine-1-phosphate rheostat, which constitutes a key-mechanism for neoangiogenesis, endoplasmic reticulum stress, and cytosolic calcium dysregulation. We investigate the role of antioxidant and antiapoptotic defense systems such as Nrf2, autophagy and mitophagy, and the modification of cellular proteins by lipid peroxidation products which alter their function and contribute to apoptosis.

Our objectives are to (i) characterize the role of oxidized lipids in neoangiogenesis, vascular aging, calcification of advanced plaques, and the protective role of autophagy, (ii) developing innovative computational vascular medical imaging techniques for visualizing vascular hemodynamics and wall shear stress, and (iii) identifying new non-invasive biomarkers for the coronary patient follow-up.

Methodologies Used:


Publications:


Expression of hydroxynonenal-adducts in human atherosclerosis lesions

Mitophagy monitoring in oxidized LDL stimulated human VSMC
Frank Lezoualc'h

Signalling and pathophysiology of heart failure

Université de Toulouse, Paul Sabatier
Inserm UMR-1048
Angelo Parini
Toulouse

Our team is specialized in the functional characterization and signaling of the cyclic AMP-binding Epac, Carabin and monoamine oxidase-A in the cardiovascular system. Our aim is to identify new therapeutical targets for the treatment of heart failure.

Research Brief:

Our team entitled “Signalling and pathophysiology of heart failure” associates clinicians and researchers sharing a common scientific background and interest in understanding the molecular and cellular mechanisms involved in Heart Failure (HF). The final objective is to identify relevant targets to prevent or reverse HF.

We have identified new signaling pathways involved in cardiac hypertrophy and failure. These signaling events involve the cAMP-binding proteins Epac and Carabin that couple membrane receptors to pathological cardiac remodeling. It also involves the metabolism of catecholamines by monoamine oxidase-A (MAO-A) as a source of reactive oxygen species (ROS) in HF and cardiac aging. Our goal now is to dissect the signaling pathways of Epac/Carabin/MAO-A in order to understand how these proteins influence cell fate. We will analyze their target genes and epigenetic mark during cardiac remodeling. Our efforts also aim at better understanding the importance of MAO-A/ROS axis in the development of HF associated with aging.

Our approach is multidisciplinary: we are seeking pharmacological modulators of these therapeutic targets (in silico screening and HTS) and develop mouse lines and gene therapy vectors to identify their role in the myocardium. Our methodologies combine cell culture, cell imaging, biochemical assays, mass spectrometry, molecular biology (RNA Seq, ChiP-Seq, ..) and experimental models of heart failure.

* Methodologies Used:

Molecular and cellular biology methods (PCR, immunocytochemistry, cell culture (neonatal cardiac myocytes, adult cardiac myocytes, cell lines), infection and transfection.

Biochemistry (Immunoblot, immunoprecipitation, affinity precipitation assay, 2-D gel)

RNAseq & ChIPseq

Calcium imaging

Experimental animal models (conditional knock-out mouse models, models of cardiac hypertrophy and failure)

Publications


Epac signalling leads to pathological cardiac remodeling and heart failure

Beta-adrenergic receptors (B-AR) activate Epac which induces Ca2+ dysregulation via the ryanodine receptor (RyR) leading to arrhythmia. Epac also regulates the activity of transcription factors (TF) which are involved in pathological cardiac remodeling.

Deleterious effect of MAO-A on mitochondrial damage, cardiomyocyte death and heart failure.

MAO-A-generated oxidative stress triggers p53 activation leading to down-regulation of peroxisome proliferator-activated receptor-gamma coactivator-1a (PGC-1a), a master regulator of mitochondrial biogenesis. On the other hand, MAO-A-generated oxidative stress impairs lysosome function and acidification leading to autophagic flux blockade and altered mitochondrial quality control.
Jean-Michel Senard
Céline Galés

Molecular and clinical determinants of cardiac architecture

Université de Toulouse 3
( Université Paul Sabatier)
Inserm 1048
Angelo Parini
Toulouse

The translational projects of the team are based on original methods for sympathetic nervous system activity recording and pharmacology of related GPCRs

Research Brief:
The physiologic response of an organ basically relies on a complex interplay between the different cell types structuring its tissue. At the cellular level, the response arises from the plasma membrane through different receptors, channels, pumps? that integrate and process the extracellular stimuli (chemical, mechanical). Alteration of the plasma membrane response is the hallmark of a number of pathological conditions. Now, how the plasma membrane and the overall cell architecture behave in such pathologies and what is the impact on the surface organization and functions of the proteins inserted in the plasma membrane still remain poorly understood. This is however of major interest in pharmacological medicine since most marketed drugs target these plasma membrane proteins.

In this context, the research program of our team focuses on understanding the relationship between the architecture of cardiac tissue (more precisely of the cardiac contractile cells) on the function and pharmacology of the heart and but also on the brain.

Our specific interests are subdivided in three main projects all around the notion of heart architecture:
Ø Identification of the molecular determinants of adult cardiomyocyte morphology & cardiac tissue organization
Ø Characterization of the Heart / Brain axis
Ø Exploration of cardiac cell surface GPCR architecture as a molecular basis underlying biased agonism efficacy of ligands (pharmacology of GPCR).

Methodologies Used:
Muscle and renal sympathetic nerve activity (animals and humans)
Blood pressure and heart rate variability
Cardiovascular phenotyping including histomorphology
Bioluminescent resonance energy transfer applied to GPCRs study

Publications


BRET biosensor sensing G protein activation

Basics of biosensors for G protein activation sensing using BRET technology

Nanoscale imaging of normal cardiomyocyte

Electron microscopy picture showing the organization of the lateral membrane of normal cardiomyocyte depicting the periodic presence of crests. Inset: subsarcolemmal mitochondria under the crests.
Marie-Christine Alessi

Nutrition, Obesity and Thrombotic risk (NORT) Thrombosis biomarkers - Vascular and Haemostasis dysfunction during obesity

Université de Aix-Marseille 2 (Université de la Méditerranée)
Inserm UMR 1062 INRA UMR 1260
Marie-Christine Alessi
Marseille

Thrombosis is a major clinical problem. Through the study of large populations and rare diseases we aim to identify hereditary and non-hereditary components that contribute to haemostasis and thrombosis. Our major goal is to identify relevant biomarkers and new therapeutical targets.

Research Brief:
The formation of thrombi at sites of vessel lesions is a major clinical problem. Thrombosis results from the interaction of genetic and environmental risk factors. Progress in this field requires the identification of specific hereditary and environmental risk factors in affected individuals and of the design of new antithrombotic therapies. Emerging technologies are beginning to allow the unbiased characterization of variation in genes, RNA, proteins and metabolites associated with thrombotic conditions. These approaches will lead us to identify genes, epigenetic and metabolites variations that could be biomarkers themselves or will point to circulating markers of thrombosis for further exploration. Furthermore, by studying rare inherited diseases causing platelet dysfunction or low platelet counts, we aim to identify new pathways involved in thrombosis. Our second objective is to investigate the relationships between haemostasis/thrombosis and environment. We will examine how nutrition and obesity can affect thrombosis and the cardiovascular pathophysiology. Several projects have been implemented to identify metabolic players involved in thrombotic disorders induced by lipids and micronutrients intake. During obesity, expansion of ectopic fat (epicardial and perivascular depots) may exert adverse lipotoxic, prothrombotic, and proinflammatory effects. Using noninvasive imaging we will unveil the direct myocardial and vascular targets of ectopic adipose tissue action.

Methodologies Used:
- Cohort evaluation / Genotyping plateforms
- Mass spectroscopy (Metabolomics) / Magnetic Resonance Imaging (collaborative work)
- Rat model of metabolic syndrome / Murine models of thrombosis and obesity
- Cell cultures, flow chamber, microscopy
- Biological evaluation (cytometry, qPCR, ?)

Publications

Cuisnet T, Morange PE, Quilici J, Bonnet JL, Gachet C, Alessi MC (2011). Paraoxonase-1 and clopidogrel efficacy., Nature medicine. 17(9), 1039; author reply 1042-1044


**Agnès VINET**

LaPEC

University of Avignon
Equipe d’Accueil EA4278
Agnes VINET
Avignon

**Research Brief:**

The translational project of the Laboratoire de Pharm-Ecologie CardioVasculaire (LaPEC, EA4278) focuses on vascular and myocardial dysfunctions, with associated links to prevention and rehabilitation in cardiac-metabolic diseases through physical exercise and/or nutrition. The implication of the inflammatory status and the nitric oxide (NO) channel in the genesis of the oxidant stress is central to these projects. Specific focus is also addressed on the effect of adipose tissue, its different phenotype and localization, and its related inflammation and oxidative stress on cardiovascular function. The potential outcomes of our research fall within the scope of a better appraisal and therapeutic efficiency through:

- the identification of at risk populations at an early stage of vascular and myocardial dysfunction;
- the revelation of precursor signs impacted by exercise and/or nutrition, allowing better pharmacological targeting, working to the objective of synergistic and potentially additive effects. These actions ultimately support an overall public health approach which is also designed to limit costs linked to treating these pathologies.

**Methodologies Used:**

- Resting and stressed (dobutamin or exercise) echocardiography in humans and animals (Vivid Q, GE and Vevo, VisualSonic)
- Resting and stressed (exercise) Vascular ultrasonography in humans and animals (Vivid Q, GE and Vevo, VisualSonic)
- Laser Doppler in humans and animals (Perisoft and Pericam, Perimed)
- Isolated heart (Langerdorf)
- Myocardial ischemia-reperfusion
- Isolated artery (aortic, mesenteric)
- Biochemical assays (Western blot analysis, immunohistochemistry, ELISA)
- Cell culture

**Publications**

Translational approach in cardiometabolic disease
Saïd Bendahhou
Ion channels pathophysiology
Université Côte d’Azur
CNRS UMR7370
Jacques Barhanin
Nice

The team investigates the role of ion channels in non excitable tissues to shed lights on rare disorders.

Methodologies Used:
- Electrophysiology
- Imaging
- IPS reprogramming and differentiation
- protein analysis
- Transcript analysis

Publications


Hypercoagulability, arterial stiffness and ageing

Université de Lorraine
Inserm U1116
Patrick Lacolley
Vandoeuvre-les-Nancy

Interaction and integration of basic and clinical research in the cardiovascular biology field based on the combination of expertise on vascular stiffness, coagulation and original imaging tools

Research Brief:
The team aims at deciphering vascular stiffening and hypercoagulable phenotypes as well telomere dynamics and inflammation in arterial ageing. It has combined three approaches, knockout murine models to inactivate genes coding for molecular targets, in vitro perfused arterial segments, and cell cultures to show that vascular smooth muscle cells and integrins are key players in mechanotransduction and arterial stiffness, thrombin generation within the vessel wall and vascular mechanisms of ageing. The translational strategy has focused on the characterization of arterial ageing phenotypes, providing an improved understanding of the connections between the vessel wall and thrombin generation, and the selection of biomarkers for cardiovascular pathologies.

Our research on the process of early vascular ageing has generated basic work highlighting the major role of vascular smooth muscle cell (VSMC) plasticity in arterial stiffening and thrombosis. Our work has contributed to identifying new molecular mechanisms of arterial stiffening and its complications such as dissection, aneurysm and fibrosis of the vascular wall as well as atherothrombosis.

Methodologies Used:
Methods to measure parameters of arterial stiffness and thrombin generation in vivo and in vitro: echotracking, pulse wave velocity, calibrated automated thrombography (whole blood, plasma)
Appropriate animal models (pharmacology, transgenic)
Primary cultures of human, rat and mouse vascular smooth muscle cells (cyclic stretch, siRNA, confocal and second harmonic generation microscopy)
Flow cytometry for extracellular vesicles, calcium and cell phenotypic markers
Telomere length measurement (Southern blots of the terminal restriction fragments)
Single photon emission computed tomography and hybrid imaging with X-ray computed tomography and 18F-fluorodeoxyglucose positron emission tomography
Cohorts, pharmacological trials, genetics

Publications

Lacolley P, Regnault V, Nicoletti A, Li Z and Michel J-B. (2012). The vascular smooth muscle cell in arterial pathology: a cell that can take on multiple roles., CARDIOVASCULAR RESEARCH. 95(), 194-204


SRF-related decreases in contractile proteins and cell-ECM attachment increase arterial elasticity

Top: mechanical properties of carotid arteries from control (CTL SMKO) and smooth muscle-specific knockout of serum response factor (SRF SMKO) mice; distensibility?arterial pressure (AP) curves (left) and incremental elastic modulus (Einc)-wall stress (WS) curves (right).

Bottom: Alteration of contractile and cytoskeletal proteins. Carotid sections stained with antibodies against smooth muscle alpha-actin (SMA; red) and myosin heavy chain (SM-MHC), beta-actin, alpha5 integrin (green).

Prothrombotic phenotype of spontaneously hypertensive rat arteries

Top right: FeCl3-induced thrombus formation in carotid arteries from 12-week-old spontaneously hypertensive rats (SHR) or Wistar rats.

Bottom left: Thrombin generation curves in a Wistar platelet-free plasma pool triggered with 50 pmol/L tissue factor (TF) or with 2 mm rings from thoracic aortas of 5-week-old or 12-week-old SHR or Wistar. Bottom right: Thrombin generation curves at the surfaces of vascular smooth muscle cells (VSMCs) or endothelial cells (ECs) from 12-week-old SHR or Wistar.

Baseline leukocyte telomere length and leukocyte telomere attrition versus atherosclerotic plaques

Adjusted baseline leukocyte telomere length (LTL) (kilo base pairs) versus the presence of carotid atherosclerotic plaque (CAP) at baseline (BL) and follow-up (left); Adjusted LTL attrition (base pair/year) versus the presence of CAP at baseline and follow-up (right). Values are mean ± SEM. No/no: absence of CAP in both BL and follow-up examinations; no/yes: presence of CAP only at the follow-up examination; yes/yes: presence of CAP in both BL and follow-up examinations.
Bruno Levy
Shock, heart failure: cardiovascular mechanisms and treatment
Université de Lorraine
Inserm U1116
Patrick Lacolley
Vandoeuvre-les-Nancy

Key facts
Team
• Researchers : 9
• Technicians : 3
• Postdoc fellows : 2
• PhD Students : 6

Translational approaches
• Patents : 6
• Clinical research grants : 6
• Industry partnerships : 3

International research links
• Europe, United States, Asia

Keywords
• metabolic syndrome
• septic shock
• mineralocorticoid receptor
• heart failure
• cohorts
• biomarkers
• omics

Leadership position in the field of heart failure clinical trials, mineralocorticoid receptor antagonist as major advance in heart failure treatment and first demonstration of the role of TREM-1 in septic shock

Research Brief :
The main objective is to elaborate personalized strategies mechanistically targeted at cardiovascular (CV) ageing for improving the health care of age-related CV diseases, especially acute and chronic heart failure (HF). More specific projects are as follows:
1- identifying bioprofiles of HF and CV aging, with a special focus on: systemic biomarkers of fibrosis and inflammation (see Figure), telomeres considered as a major determinant of age-related CV, and cross phenotyping with functional and molecular biomarkers from CV imaging (PET, MRI and echography),
2- developing and testing bioprofile-guided therapies of HF, CV ageing and frailty, on a multi-organ scale and including bridge-to-recovery strategies for acute HF.

Methodologies Used :
1. The EMPHASIS-HF was performed in HF patients with reduced ejection fraction (HFREF) and mild symptoms. After this landmark trial, MRA was assigned a Class 1 level A for the treatment of HF.
2. When given after myocardial infarction, a small peptide patented by unit members (INSERM transfert) and modulating the activity of TREM receptors, is able to prevent from an excess in inflammatory response and thereby, to limit the deleterious left ventricular remodeling.
3. The prevention and treatment of HF by mineralocorticoid receptor antagonists are clearly enhanced in obese patients and in an experimental model of obesity, thanks to metabolic and anti-inflammatory effects.

Publications
Theragnostic strategies targeted on inflammation / fibrosis

Mechanistic pathways
Anti-fibrotic
Inflammation modulation
Prevention of inflammation amplification
Inflammation resolution

Therapeutic intervention
Sche-CT-1
EOM
DNA, RNA, microRNA
Preclinical, cell lines, ex vivo for PET
TRIUM-I
Resonance, C6028
Gastrointestinal, 188Re-Lu2 for PET

Biomarkers / Bioprofiles
Sal-3, CT-1
DNA, RNA, microRNA
Preclinical, cell lines, ex vivo for PET

Cohorts - Biobanks - Biomarkers
STANDESAS
HOMAGE
FIBRO-TARGETS
EMPHASIS
PHIC ARMPHIBIOT
PHRC-Hypo Echo
FAST-MI

Inflammatory cells
Inflammation/toxic effects

For PET imaging:
- 18F-FDOPA
- 11C-BRP
- 188Re-Lu2
- 186RbOxidative
**Key facts**

**Team**
- Researchers: 32
- Technicians: 8
- Postdoc fellows: 3
- PhD Students: 36

**Translational approaches**
- Patents: 2
- Clinical research grants: 12
- Industry partnerships: 2

**International research links**
- Belgique
- Angleterre
- Etats Unis

**Keywords**
- cardiovascular calcification
- uraemia
- calcified aortic valve disease
- pulse pressure
- bone remodelling
- molecular biology
- cardiovascular exploration
- bone cell evaluation
- animal model of chronic kidney disease

**Biological Resources**
- primary cell culture
- secondary cell culture
- wild type and knock-out mice
- biological samples (animal and human)

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### Said Kamel

**Pathophysiological mechanisms and consequences of cardiovascular calcifications: role of cardiovascular and bone remodelling**

Université d’Amiens  
(Université de Picardie - Jules Verne)  
Inserm U 1088  
Said Kamel  
Amiens

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**Research Brief:**

Cardiovascular calcifications (CVC) are frequently encountered in the general population. They are associated with a high cardiovascular risk. They are observed with a much greater prevalence in patients with chronic kidney disease (CKD), with diabetes and also in patients with inflammatory diseases such as rheumatoid arthritis. The work done during last few years have allowed us to go along several important research pathways. We are pursuing our work following several of these research lines, in particular the role of the calcium-sensing receptor and that of the uremic toxins, the hemodynamic consequences of CVC, and the identification of novel markers able to predict these soft-tissue calcifications. We are currently evaluating the role of pro-inflammatory mediators in the pathogenesis of CVC, by using experimental models and performing clinical investigations. Our research will focus on the development of innovating therapeutic strategies. The consequences of our research efforts, based on cell culture models, animal models and human investigation, should be a better understanding of the molecular mechanisms which are responsible for CVC. In addition, our research work should permit an easier detection and more adequate follow-up of the calcification as well as the identification of novel therapeutic targets, with the final goal to improve the care of patients with CVC, in the presence or absence of CKD, who carry a major cardiovascular risk.

*Methodologies Used:*

- Cell culture, cell migration assays (Boyden’s Chamber), molecular biology including MicroRNA
- In vitro mineralization assays, osteoclast differentiation
- Cranial window technique, isolated cerebral micro-vessel preparation
- Echocardiography, pulse wave velocity, cardiac hemodynamics
- Ex vivo vascular exploration, histomorphometry

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**Publications**


Calcified aortic valve stained with von Kossa in a model of chronic kidney disease Apo E-/- mice.

**Association between metformin and vascular calcification in type 2 diabetic patients**

Association of antidiabetic drugs with below-knee arterial calcification. A. The histogram represents the frequency of each antidiabetic drug. The black bars indicate patients treated by metformin alone or in combination with other antidiabetic drugs. The white bars indicate patients treated by other antidiabetic drugs without metformin. B. Univariate logistic regression with specific focus on pharmacological antidiabetic therapy.

**Calcifying matrix vesicles produced by vascular smooth muscle cells.**

Interaction between matrix vesicles produced by vascular smooth muscle cells and type I collagen. Immunofluorescence was assayed using Alexa555-labeled type I collagen (Col I) and Alexa488-labeled Annexin A6 (AnxA6) antibodies. Non-permeabilized MOVAS-1 cells were cultured in the absence (Ctrl) or presence of 4 mM phosphate (Pi) for 8 days without or with an inhibitor of vascular calcification. Scale: 10 μm. Boxes highlight the inset region (x3).
Nuclear receptors in the metabolic syndrome

Université de Lille 2 (Droit et Sante)
Inserm - CHU de Lille UMR 1011 Institut Pasteur de Lille UMR 1011
Bart Staels
Lille

We combine a strong background in the field of nuclear receptors and metabolism, and a unique scientific environment and up-to-date technological platforms.

Research Brief:
The organism senses its energy status through the close communication of several organs which integrate multiple endocrine (hormones, cytokines) and metabolic (glucose, free fatty acids..) signals. Dysregulation of the tight control of metabolism leads to dyslipidemia, insulin resistance and obesity which predispose to the development of cardiovascular complications and atherosclerosis. We aim to better understand the metabolic functions of nuclear receptors (NRs), with a major focus on FXR, PPARs, Rev-erba and RORa, and to define the potential benefit of pharmacological agents acting via these NRs on human health.

The role of these NRs will be investigated by comparing total- or organ-specific deficient or over-expressing mice of these NRs to wild-type mice with respect to basal metabolic parameters, energy expenditure, gene and protein expression and pharmacological response. Cellular and molecular approaches will also be used to identify the molecular mechanisms at the basis of the identified physiological functions. Since NRs are potential pharmacological targets, the use of existing as well as the identification of novel synthetic ligands will allow us to study the biological effects of these compounds. Finally, the role of these NRs in human physiology will be investigated by analysis of tissue biopsies from subjects suffering from metabolic disorders. It is expected that these approaches will uncover new therapeutic strategies for the treatment of metabolic diseases.

Methodologies Used:
In vivo mouse phenotyping, Cell culture (cell lines or primary cells), Molecular biology approaches (transfection, quantitative PCR, Western-blot, Chomatrin Immunoprecipitation (ChiP), Gene silencing, DNA micro-array technology, ChiP-seq technology?), Immuno-histochemistry

Publications


Non-Alcoholic Fatty Liver Disease (NAFLD): modulation by PPARs

Bile acids signal via FXR and TGR5

Transcriptional control of metabolic pathways by circadian oscillators
Key facts

Team
• Researchers : 15
• Technicians : 6
• Postdoc fellows : 0
• PhD Students : 15

Translational approaches
• Patents : 2
• Clinical research grants : 7
• Industry partnerships : 5

International research links
• ERANET : Netherlands, Poland, Belgium, Spain - OTHER: Finland, Germany

Translational approaches
• PhD Students : 15
• Postdoc fellows : 0
• Technicians : 6
• Researchers : 15

Research Brief:
Our cardiovascular research focuses on 3 aspects: vascular protection, treatment of aortic stenosis and improvement of cardiac contractile function/reduction of heart failure. This research is translational, performed both in experimental/pre-clinical models and in humans (healthy volunteers and patients). Our vascular research concerns protection of vascular endothelial cells against injury or dysfunction induced by risk factors (hypertension, diabetes) or cardiovascular diseases (myocardial infarction, heart failure, septic shock etc.). Pharmacological targets currently evaluated include in particular protein tyrosine phosphatase 1B, soluble epoxide hydrolase, and dopamine receptors.

Regarding aortic stenosis, our work is based on the Rouen discovery and development of transcatheter aortic valve implantation (TAVI): we attempt to uncover new mechanisms and new pathways for prevention or slowing of aortic stenosis development. We also address the links between endothelial dysfunction and aortic stenosis. This research is performed within the frame of the FHU REMOD-VHF and RHU STOP-AS both placed under the leadership of our group.

Finally, our cardiac research concerns the evaluation of new treatments of diastolic dysfunction or heart failure, and the cardiac consequences of aortic stenosis or its reversion. In particular, we focus on the benefits of lymphangiogenic therapy in heart failure, within the frame of an ERA-NET project under our leadership.

Methodologies Used:
- Experimental and clinical cardiac and vascular imaging (echocardiography, echo-tracking, tonometry, tissue Doppler, Holter).
- Magnetic resonance imaging for small animals.
- In vitro vascular functional evaluation (arteriograph, myograph).
- Experimental models of cardiovascular diseases in rats and mice (myocardial infarction, heart failure, hypertension, aortic stenosis, insulin resistance etc.).
- Evaluation of oxidative stress.
- Culture aortic valve cells.
- Evaluation of cardiac lymphatic network & lymphangiography.

Publications


Non invasive, dynamic, echo-tracking based imaging of human radial artery diameter, allowing evaluation of changes in vascular tone and thus of endothelial-dependent dilatation and endothelial dysfunction.

Transcatheter Aortic Valve

Aortic Valve designed for Transcatheter Aortic Valve Implantation (TAVI)

Cardiac lymphatics network

Immunohistochemical imaging of cardiac lymphatic network (rat left ventricle)
Research teams with secondary association to PMN Institute
Alain Cohen Solal
Biomarkers and Heart Failure

Université de Paris 07
(Université Denis Diderot)
Inserm UMR-S 942 CHU Lariboisière-St Louis
Alain Cohen Solal
Paris

Our team is unique as it has mounted a large local, national et international networks on biomarkers in heart diseases, mainly heart failure. It performs translational research in small animal models of heart failure where the new biomarkers are tested (tissue location and functional significance).

Research Brief:
Our team, composed of clinical and basic science searchers, works at discovering and validation new biomarkers in cardiac diseases, mainly heart failure. We use biobank data in our lab with samples coming from national and international collaborators connected in a network (GREAT). We also performed translational research as our biomarkers as tested in small heart failure animal models, especially in terms of tissue location and functionality.

Methodologies Used:
- Biobank
- International multicenter registries and trials
- Translational research (small animal models of models of heart failure)
- Echocardiography, exercise testing
- Immunohistochemistry, PCR, western blots, Elisa, tissue engineering

Publications


Identification of molecular determinants of cardiovascular diseases

Université de Lille 2 (Droit et Sante)
Institut Pasteur de Lille UMR1167
Philippe Amouyel
Lille

**Research Brief:**
The research project of our group is translational with the aim to find new biomarkers of left ventricular remodeling post-infarction and heart failure. The team has expertise on coordinating recruitment of patients with cardiac disorders, clearly phenotyped for left ventricular remodeling post-infarction (REVE 1 (n=266) and REVE 2 (n=246) studies) or heart failure (PTHF (n=60) and INCA (n>2000) studies). These clinical studies allow recuperating plasma and serum samples that are used for differential proteomic and transcriptomic (miRNA and lncRNA) analyses. We have developed techniques allowing access and detection of plasma "deep" proteome. We have the expertise in discovery and validation of targets from proteomic (SELDI-TOF, 2D-DIGE, multiplex, ELISA) and miRNAomic (arrays, Q-RT-PCR).

Two approaches are currently developed: 1) a clinical approach with the purpose to develop clinical diagnostic applications for which we analyzed all the data obtained by system biology and; 2) a molecular approach with the purpose to understand the mechanisms underlying the targets (proteins, post-translational modified proteins, miRNA, lncRNA ) modulation in the pathologies studied. The discovery of new biological factors involved in the different cardiovascular pathologies would help to a better stratification of patients at risk.

**Methodologies Used:**
- Tissues, cells and plasma/serum (depleted for major proteins) proteomic.
- 2D-gel electrophoresis, SELDI-TOF, mass spectrometry
- Arrays and Q-RT-PCR for miRNAs and lncRNAs, ELISA, multiplex assays
- Primary culture of neonatal rat cardiomyocytes, human smooth muscle cells (aorta), macrophages
- Laser-microdissection laser, cells and tissues imaging

**Publications**


Activity of team 2

Translational Research on Molecular Determinants of Cardiovascular Diseases by Omics

**Objectives:** Find new biomarkers for diagnosis and prognosis of left ventricular remodeling post-myocardial infarction and heart failure

**From Bench**
- Experimental models
- ELISA, Mu Nolox
- Cohorts of patients

**To Bedside**
- mRNAs, miRNAs, protein analysis
- Laser capture microdissection
- Subsies from samples

Team 2, UMR1167 - Inserm-Lille 2, CHU Lille

Major results in HF

Discovery of new biomarkers of remodeling post-myocardial infarction

**Phosphoproteomics of 2-month MI hearts**
- Decrease of phosphorylated troponin I (P-TnI)

**Validation in rat**
- Low level of P-TnI

Analysis of long non-coding RNA in plasma
- Circulating lncRNA, LIPCAR, predicts survival in patients with heart failure

**LIPCAR Project**

LIPCAR, a prognostic marker of heart failure with impaired ejection fraction (LIPCAR-HF)

Consortium of 5 countries: France (Lille), Germany (Hannover), Italy (Milano), Poland (Warsaw), Spain (Pamplona)

Evaluation of LIPCAR combined or against circulatory biomarkers of myocardial fibrosis, cardiomyocyte stress/injury, and inflammation in heart failure (HF) patients and controls

New molecular phenotyping of patients who are at greater risk for adverse outcome in heart failure with reduced (HFrEF) and mid-range (HFP EF) ejection fraction

European Research Area Network on Cardiovascular Diseases (ERA-CVD)
The research is original at the national and international levels, both in the fields of heart viral diseases and enterovirus infections. In France, there are only two research groups involved in enterovirus persistent infections and with its will and effort to study these infections in humans.

Research Brief:
Human enteroviruses (EVs), and specifically Coxsackievirus B (CVB), are a common cause of acute cardiac infection and myocarditis in children and young adults. In 10% of the cases, the acute infection evolves to persistence, inducing a chronic myocarditis. This pathology will lead in 9% of the patient to a dilated cardiomyopathy (DCM 7 cases/100 000 inhabitants), which is the second leading cause of cardiac transplantation. The involvement of these forms in DCM is supported by the detection of viral RNA and VP1 capsid protein in 35% of the cardiac samples of end stage patient suffering from idiopathic myocarditis. Molecular mechanisms triggering the switch from the acute to the chronic myocarditis and DCM are still unknown, therefore limiting the development of specific therapeutic strategies against EV-induced chronic heart diseases. A better understanding of the molecular mechanisms implicated in EV persistence of viral forms in human cardiac tissues and could stimulate the development of new therapeutic strategies in acute and chronic cardiac infections, such as DCM, caused by EV.

Methodologies Used:
We used for the first time a new technology allowing broad viral detection in clinical samples that couples broad-range PCR amplification to electrospray ionization-time of flight mass spectrometry analysis (PCRMS).
We developed NGS approaches to quantify major and minor persistent viral population in heart tissues.
We developed an original CVB3 induced chronic myocarditis in DBA/2J mice model.

Publications


Expression of viral proteins of 5' deleted viral forms

A. In vitro translation assay of CV-B3 replicons B. Transfection of FL and deleted viral RNA carrying emGFP into HCM. Cells were fixed 24H and immunofluorescent assays were performed. Blue: Nucleus, Red: VP1, Green: emGFP.

Pathophysiology of viral cardiac infection

A. Immunofluorescent staining of dystrophin in the heart tissue sections of infected DCM patients (DCM+ EV+), uninfected EV DCM patients (DCM+ EV-) and controls (DCM- EV-). Bar scale=50μm. The white rectangle is displayed in B. B. Serial sections of cardiac tissues of infected DCM patients (DCM+ EV+) were analyzed by in situ hybridization of the viral RNA (left, blue: nucleus, red: RNA) and immunofluorescent staining of dystrophin (right, green).
Hemostasis
DENIS Cécile
Integrative hemostasis: from fundamental aspects to hemorrhagic disorders

Université Paris Sud : Paris
11
Inserm UMR1176
Denis Cécile
Le Kremlin-Bicêtre

Our team strength resides in the integrative approach applied to the study of hemostatic proteins with techniques ranging from enzymology to the use of dedicated murine models and patients samples.

Research Brief:
The research portfolio of the team is tailored around the patho-physiological aspects of haemostasis, with particular emphasis on hemorrhagic disorders related to coagulation and platelet defects. Four main topics can be distinguished: 1) pathogenesis of congenital or acquired Von Willebrand disease (VWD); 2) pathogenesis of hemophilia; 3) pathogenesis of (inherited) platelet disorders; 4) role of VWF beyond hemostasis.

In general, these topics are addressed in a similar fashion: there is a focus on fundamental & clinical aspects of the disease, we further delineate clearance pathways of hemostatic proteins, and novel therapeutic strategies are explored and tested in in vivo models. For instance, we continue to focus on the pathology of VWD-type 2B. Recently, we identified that VWD-type 2B results in an unexpected thrombocytopenia, while an increased clearance of VWF/platelet complexes plays a role in the thrombocytopenia that characterizes these patients. By using in vitro & in vivo approaches we are deciphering the molecular basis of these findings. Furthermore, we use our mouse model for hemophilia A to get more insight into the functional defects that is associated with several mutations in factor VIII. This model is also be used to test a series of candidates that might be useful for the specific treatment of patients with mild/moderate hemophilia A. We also continue our work on filaminopathy A as a cause of platelet dysfunction.

Methodologies Used:
In vivo thrombosis models in mice
Platelet function analysis
Culture of CD34+ cells
Bleeding assays in mice (tail clip assay, tail vien transection)
Surface Plasmon Resonance (Octet)
Coagulation assays/ Thrombin Generation Time
Hydrodynamic injection
Stable cell transfection
Immunofluorescence staining (Classic or Duo-link)
Perfusion assays in flow
ELISA
Production and purification of recombinant proteins

Publications


Pascale Gaussem

Innovatives Therapies in Haemostasis IThEM

Université de Paris 05
(Université Rene Descartes) Faculté de Pharmacie de Paris
Inserm UMR-S1140 CHU Paris
Pascale Gaussem
Paris

**UMR_S1140 is recognized for its expertise in haemostasis, in management of antithrombotic treatments and in development of innovative biotherapies (in vitro platelet and vascular cell production).**

**Research Brief:**

Theme 1 Vascular therapy research is designed to understand how immature cells form blood vessel in pathophysiological conditions. The objectives are focus on both the cellular and molecular basis of vasculogenesis to characterize and study the implication of adult stem cells with vasculogenic potential, and the development of strategies to optimize the beneficial effects of cell therapy protocols. Clinical studies focus on the vascular remodelling in fibrosis.

Theme 2 Platelets: from birth to function addresses novel strategies based on flow conditions to produce human platelets generated from its precursors and analyses new mechanisms potentially involved in the regulation of platelet functions, with a focus on cyclic nucleotide pathway, which is also the target of antiplatelet agents.

Theme 3 Haemostasis and response to antithrombotic drugs conducts a transversal program on antithrombotic agents from the molecular basis of coagulation to animal models and clinical studies in targeted populations (elderly, children). The objectives are 1- to optimize the management of antithrombotic agents and their reversal; 2- to better understand the relative contribution of factors that influence inter-individual variability in the response to antithrombotic agents, including genetic and non-genetic factors and 3- to develop innovative in vitro tests to manage patients in emergency haemorrhagic situations.

*Methodologies Used:*
Preclinical models of angiogenesis, thrombosis, bleeding, and intravital microscopy
Cell biology: cell culture in 2D and 3D scaffold (primary (endothelial), stem cells(endothelial progenitors, megacaryocytes,mesenchymal), cell transfection, in vitro angiogenesis assays, flow experiments, immunocytochemistry, production of recombinant proteins
Molecular biology, RT-qPCR, Flow cytometry, biochemistry (WB, ELISA, signalling)
Immunohistochemistry, confocal microscopy...

**Publications**

Silvestre JS, Smadja DM, Lévy BI (2013). Postschismic revascularization: from cellular and molecular mechanisms to clinical applications., Physiological reviews. 93(4), 1743-802


Pneumology
Patrick Berger
Bronchial remodeling

Université de Bordeaux
Inserm U1045
Marthan Roger
Bordeaux

The project of the team associates human and animal studies in a multidisciplinary approach (physiologists, chest physicians, radiologists, physicists, pharmacologists, and paediatricians) with strong interconnection between the team and the clinical investigation center in the hospital.

Research Brief:
Asthma and chronic obstructive pulmonary disease (COPD) are very frequent inflammatory diseases that are characterized by different patterns of bronchial remodelling. However, characteristics and localization of the increased in Bronchial Smooth Muscle (BSM) mass are different. In COPD, there is a BSM cell hypertrophy which is only present in distal bronchi whereas in asthma, there are both BSM cell hypertrophy and hyperplasia within the entire bronchial tree. Anyhow, BSM remodelling has been associated with a poor prognosis, high morbidity, and deterioration of lung function. As a consequence BSM remodelling should be a target of innovative treatments.

The general aim of this project is therefore to understand, evaluate and treat bronchial remodelling. The specific aims are to further unravel the mechanisms of bronchial remodelling in both asthma and COPD as well as to develop new non invasive tools to assess bronchial remodelling in vivo.

Methodologies Used:
For this purpose, the research project will combine clinical, functional, radiological data obtained in vivo with histological, functional, cellular, and molecular data obtained in vitro.

Publications


**Axis 1**

**Axis N°1 : Remodeling / asthma**

- Bronchial remodeling in asthma

**Axis 2**

**Axis N°2 : Remodeling / COPD**

- Bronchial remodeling in COPD

**Axis 3**

**Axis N°3 : Imaging or airway remodeling**

- CT imaging / 4D : dynamic study of bronchial wall

**Imaging of bronchial remodeling**
Christelle Guibert
Pathophysiology of pulmonary and systemic circulation

Université de Bordeaux
Inserm U1045
Roger Marthan
Bordeaux

Our team works on a real interface between cardio-vascular and pulmonary diseases and is composed of multidisciplinary researchers with various trainings (scientists, physicians, pharmacists). Our aims are to perform translational research with clinical trial when relevant.

Research Brief:
The main scientific scope of the team relates to biology of the pulmonary and systemic circulation. Regarding pulmonary circulation, we focus our research on (1) pulmonary hypertension (PH) (cellular and molecular mechanisms associated to vascular remodeling and reactivity as well as pharmacological treatments) and (2) impact of environmental factors (airborne pollution and hyperoxia). Regarding systemic circulation, our studies focus on actin cytoskeleton remodelling (i.e. podosomes in endothelial cells exposed to various factors such as VEGF and TGFbeta, key players of remodeling). By addressing vascular pathophysiology on pulmonary and systemic circulations, our team works on a real interface between cardio-vascular and pulmonary diseases. Our team is composed of multidisciplinary researchers with various trainings (scientists, physicians, pharmacists).

Specific objectives of the team are the following:
1. To address the role of Stretch-activated channels (SAC) and intercellular communications (connexins) in PH
2. To develop an animal model of bronchopulmonary dysplasia associated to PH in newborns and to address the role of SAC and intercellular communications in connection with theme 1
3. To address the impact of particulate pollution on the pulmonary circulation
4. To address systemic vascular remodelling (role of podosomes and connexin 40)

Methods Used:
Biological material and main methodologies used:
(1) Freshly isolated vascular cells, cultured cells and tissue (pulmonary arteries) for molecular biology (PCR, qPCR), cellular biology (electron microscopy, Western Blot, siRNA, FACS, tests for migration, proliferation and apoptosis), immunohistochemistry, patch-clamp and fluorescent imaging (calcium, reactive oxygen species (ROS))
(2) Vessels (arterial rings, pressurized and cannulated small vessels) from animal models and human tissue (reactivity, electron paramagnetic resonance for measurement of ROS)
(3) Animal models of pulmonary hypertension and/or transgenic animals for in vivo experiments
(4) Human tissue (pulmonary arteries and/or lung from adult and fetus)

Publications


Summary of signaling pathways associated to stretch in pulmonary arterial smooth muscle cells.

Signaling pathways in normal rats (left), rats suffering from chronically hypoxic pulmonary hypertension (middle) and rats suffering from pulmonary arterial hypertension induced by monocrotaline (right) (from Gilbert G. et al., Cardiovasc Res, 2014).

Connexin 43 immunofluorescent labeling (red).

Connexin 43 labeling is shown on a pulmonary arterial cross section (left) and on the endothelial side of an opened vessel (right). Nuclei are labelled in blue and autofluorescence of external and internal elastic lamina are in green. Labellings were observed with confocal microscope (Nikon TE2000). CML, smooth muscle cells, CE, endothelial cells.

Remodeling of intrapulmonary arteries (IPA) from newborn rats following 15 days of hyperoxia (90 %).
Marc HUMBERT

Pulmonary Arterial Hypertension: Pathophysiology and Novel Therapies

Université Paris Sud : Paris 11
Inserm UMR S 999
HUMBERT Marc
Le Plessis-Robinson

On the basis of a nationwide Registry, a unique biobank, experimental models and highly competitive scientists, our group focuses on molecular pathways causing pulmonary vascular remodeling, identifying targets to foster drug development and tests novel treatments for Pulmonary Hypertension (PH).

Research Brief:

Pulmonary arterial hypertension (PAH) describes a group of devastating diseases, comprising idiopathic and associated forms, causing breathlessness, loss of exercise capacity and death due to elevated pulmonary artery pressure and subsequent right heart failure. PAH is defined by an elevation of the mean pulmonary artery pressure above 25mmHg at rest without elevation of the pulmonary capillary wedge pressure. Extensive pulmonary artery remodeling with loss of vessel patency is the underlying pathomechanism. The main scope of our Research Team relates to PAH pathophysiology and clinical management. Deciphering of the mechanisms of lung vascular remodeling and identification of novel molecular targets to alleviate and ultimately cure PAH is the main objective of this proposal. Our group will study a number of molecular pathways causing pulmonary remodelling in human and animal models of pulmonary hypertension (PH) on the basis of a nationwide web-based Registry, a biobank, and highly competitive scientists: Dr Sylvia COHEN-KAMINSKY, Dr Christophe GUIGNABERT, Dr Alice HUERTAS, Dr Frédéric PERROS, and the medical and surgical team of the Referral Center for PH at Bicêtre Hospital (AP-HP) and Hôpital Marie Lannelongue. Our main goal is to identify targets for therapy, foster drug development based on these targets and test novel treatments in order to alleviate and cure PAH, a large burden to mankind.

Methodologies Used:

- System flow cytometry MacsQuant
- EnVision 2103 plate reader
- FlexStation 3
- Q-PCR system in real time StepOne +
- PCR thermal cyclers
- ChemiDoc system multiple detection: fluorescence, colorimetry, densitometry, chemiluminescence
- Nanodrop
- Q-PCR system in real time StepOne +
- PCR thermal cyclers
- Electrophysiology
- Microscopy
- Laser Microdissection Microscope
- Hypoxia chambers for rodents
- Hemodynamic laboratory for rodents

Publications


Typical histologic lesions encountered in lungs from patients with Pulmonary Arterial Hypertension

(A) Tertiary lymphoid tissue (top); interstitial inflammatory infiltrates (bottom). (B) Hypertrophy/hyperplasia of the media; perivascular lymphocytic infiltrates (top); Fibrosis of the adventitia (bottom). (C) Concentric intimal fibrosis (nonlaminar or laminar); fibroblasts and myofibroblasts accumulation, combined with hypertrophy of the media and fibrous broadening of the adventitia and perivascular inflammation. (D) Plexiform lesions.
Marina Pretolani

Airway inflammation and remodeling in chronic obstructive lung diseases

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Paris

Keywords
• Asthma
• Airway inflammation
• Airway remodeling
• Lung transplantation
• Bio-markers
• Cohorts
• Primary human respiratory cells
• Mouse models
• Cell functions
• Immunodetections

Biological Resources
• Cohorts of patients with asthma and chronic obstructive pulmonary disease (COPD): clinical data and biological samples
• Cohort of patients undergoing lung transplantation: clinical data and biological samples
• Ex vivo cultured human bronchial epithelial cells from asthmatics, from patients with COPD and from lung transplant recipients
• Ex vivo cultured airway smooth muscle cells from patients with asthma
• Murine models of asthma and of bronchiolar epithelium injury and repair

Publications


Tools for studying clinical and physiopathological aspects of asthma onset and progression

Studies on asthma are based on the use of the multicenter, longitudinal COBRA cohort. This cohort provides extensive information concerning natural history, biological and risk factors involved in disease onset, progression and response to treatment. It is also an important source of biological samples required for mechanistic studies and for setting-up proof-of-concept trials to test novel therapeutic tools.

Abbreviation: BAL, bronchoalveolar lavage

Patient profiling approach of severe asthma

Severe asthma is an heterogeneous disease, manifesting in several distinct clinical and histological phenotypes that develop through a variety of physiopathological mechanisms. A better characterization of these different phenotypes involves the use of wide panels of hallmarks of inflammation and remodeling, the characterization of cellular and molecular mechanisms involved in these processes, as well as the identification of novel biomarkers of disease onset and progression.
Innate Immunity and anti-infective pulmonary defenses

Jean-Michel Sallenave

Université Paris Diderot Paris 7
INSERM U1152
Marina Pretolani PARIS

We are interested in the molecular and cellular mechanisms involved in pathogen (eg viruses such as Influenza, bacteria such as Pseudomonas aeruginosa) recognition and in the response to environmental and manufactured agents (i.e nanoparticles).

Research Brief:
It is now accepted that lung mucosal tissue confer important properties to the immune system, both at homeostasis and during infectious situations. At the mucosal surface, epithelial cells and alveolar macrophages interact, eg through surfactant, regulatory cytokines and antimicrobial molecules, to ensure a non-inflammatory regulatory and tolerogenic phenotype. After infection, this brake is released, and these cells participate in the network to organize pro-inflammatory responses and adaptive immunity to contain microbial aggression and to insure return to haemostasis. Our group is particularly interested in the innate mechanisms of defense and its dysregulation, which could explain the pathophysiology of lung chronic and acute inflammatory disorders.

Our main models of study focus on two aspects:
A) a therapeutic one, which aims to understand the basic mechanisms of host responses against:
1) Pseudomonas aeruginosa infections, an opportunistic pathogen in nosocomial infections, as well as in cystic fibrosis and in exacerbations of chronic obstructive pulmonary diseases (COPD).
2) Lung infections by Influenza virus, a pathogen responsible for acute infections leading to seasonal flu or pandemic episodes, but also present during exacerbations in asthma, cystic fibrosis, COPD or lung fibrosis.
B) a prophylactic one, which aims to increase immune responses against these pathogens, by choosing adjuvant formulations able to break the mucosal tolerogenic milieu.

*Methodologies Used:
-PCR
-Cell culture
-FACS
-ELISA
-PAGE analysis and Western Blot
-in vivo injections and instillation techniques (lung)
-immunological techniques

Publications


Antimicrobial expression and functions at the lung mucosal interface

A) The lung alveolar-capillary barrier at haemostasis: antimicrobial molecules (AMMs, SLPI, elafin) are protective against infections and provide a tolerogenic phenotype at local lymph nodes. B) During infection, AMM expression increases in epithelial cells and generate chemotactic signals for inflammatory cells. C) When the alveolar-capillary barrier is disrupted, AMM can protect tissue destruction with their anti-protease activity. D) AMMs are also important for tissue repair.

Nanoparticles as modulators of lung infections

Nanoparticles can influence/modulate lung responses towards infections. Our team is deciphering the molecular and cellular mechanisms by which nanoparticles (silica, silver...) can modulate the lung responses against bacteria (Pseudomonas aeruginosa...) and viral (Influenza) infectious agents.
Myriam Polette

Plasticity of the airway epithelium in normal and pathological conditions

Université de Reims
Champagne-Ardenne
Inserm UMR-S 903
Myriam POLETTE
Reims

Combine biological and clinical approaches to identify predictive or severity-associated biomarkers of respiratory diseases and to test novel therapeutic strategies to restore a functional airway epithelium

Research Brief:
The plasticity of the airway epithelium plays a major role in inflammatory diseases such as chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF) as well as in cancers. The hallmark of these chronic inflammatory respiratory diseases is the transdifferentiation of epithelial cells leading to an epithelial remodeling: basal and secretory cell hyperplasia, squamous metaplasia, alteration of ciliated cell differentiation. Epithelium remodeling prevents the restoration of the epithelial functional integrity. In addition, inflammation maintains epithelial remodeling, which eventually leads to severe and irreversible respiratory insufficiencies. These abnormalities of epithelial repair can also promote the genesis of pre-neoplastic and neoplastic lesions. In this context, dedifferentiation processes associated with an epithelial-mesenchymal transition are involved in the metastatic progression of Non-Small-Cell Lung Carcinoma (NSCLC) and Head and Neck Cancer (HNC). We recognized two research axes to investigate cell plasticity: epithelial transdifferentiation in the remodeling of the airway epithelium in CF and COPD; epithelial dedifferentiation in the tumor progression of NSCLC and HNC. The two main objectives: (1) to identify the molecular actors impacting respiratory diseases among the ones involved in epithelial cell differentiation and functionality; (2) to correlate these actors to the severity of the pathology and propose consistent therapeutic solutions.

Methodologies Used:
- Cell models
- Cell and molecular biology
- Microscopy (Video, confocal, calcium signaling,...)
- Histology and immunohistochemistry

Publications


Research strategies

Airway epithelium remodeling and ciliogenesis alteration in CF and COPD

a. Histological sections showing goblet cell hyperplasia (up) and narrowing of small airways (bottom) in COPD. b. Detection of primary cilia in basal cells in normal or remodeled epithelium. c. Successive steps of human airway epithelial regeneration in an in vitro model of air-liquid interface culture. d. CF epithelial cells reconstitute in vitro a remodeled epithelium, higher and exhibiting ciliated cell differentiation default.

FHIT maintains an epithelial phenotype in lung cancer cells

a. Loss of FHIT expression in invasive lung cancer cells (up); FHIT overexpression in invasive lung cancer cells restores an epithelial phenotype (bottom). b. FHIT silencing induces expression of EMT-associated genes. c. FHIT-regulated tumor invasion is RTK dependent. d. EMT induced by FHIT inhibition requires an EGFR signaling pathway.
Vincent Sapin
Translational approach to epithelial injury and repair
Université Clermont Auvergne
CNRS UMR6293 Inserm 1103
Chantal Vaury
Clermont-ferrand

Team working on epithelial injury and repair by using translational approaches.

Research Brief:
Following exo- and endogenous attacks, the attainment of the epithelial barrier integrity is an element found in human pathologies. The ability to repair such an epithelial attack conditions the evolution of these clinical events. Located at the intersections of many metabolical and inflammatory processes, the receptor for advanced glycation endproducts (RAGE) and its pathway could be of primary importance in this situation. The team has begun to demonstrate it on models of epithelial amniotic and pulmonary aggression encountered in 2 frequent pathologies (premature rupture of amniotic membranes (PROM) and acute respiratory distress syndrome (ARDS)). Considering the complexity of the possible "RAGE ligand/isofrom" combinations associated with the pathological activation of this pathway, it's essential to identify the importance of these different combinations and to determine if new ligands could be involved in PROM and ARDS. Using pharmacological and molecular approaches, we will identified abnormally modulated pathways that could be associated with the arising of both pathologies. Then, as the interaction of the epithelium with the cells of its near environment is a strong determinant of such aggression, the project aims to demonstrate the importance of RAGE pathway in cellular communications. Finally, availability of a mouse KO for RAGE will also allow us to study, in vivo, such impacts. Our results must permit to obtain diagnostic, prognostic and therapeutic advances.

*Methodologies Used:*
- Cloning
- Cell tranfection
- Promotology studies and reporter gene
- Microscopy
- Western-blot
- qPCR
- Multiplex assay
- Elisa
- Crispr CAS9

Publications


Transcriptomic analyses of atRA effects on human fetal membranes

Transcriptomic analysis was performed on amnion and chorion explants and primary amniocytes treated with atRA or its vehicle (DMSO) for 24 h (n=12). Volcano plots of the results obtained for each condition (a). Red and blue dots represent each probe up- or down-regulated after applying a restriction to the fold change (FC). Cellular pathways identified as most meaningful after Genomatix® analysis of atRA-regulated genes in amnion and primary amniocytes are shown in (b).

Bedside chest radio and CT scans of 2 characteristic patients with nonfocal (A, B), and focal (C, D)

(A) Lung injury scoring shows a significant injury on Days 1, 2, and 4 in injured animals as compared with sham control animals (n=76?8 for each time point). (B?F) Representative hematoxylin and eosin-stained sections at ×20 original magnification of sham and injured animals at all time points after acid aspiration. (B) Sham; (C) Day 0, injured; (D) Day 1, injured; (E) Day 2, injured; and (F) Day 4, injured.
Dermatology
Our team has a leading position in translational research from bench to bedside and our research is focused on the interplay between the skin immune system and epidermal cells in human, allowing a better understanding of inflammatory skin disorders and skin lesions associated with systemic diseases.

**Research Brief:**
The team is focusing its research on the immune mechanism involved in skin inflammatory diseases, in particular Vitiligo, the most common skin depigmenting disorder. Based on a strong clinical research program dedicated to this pathology in the Dermatology department (Bordeaux Hospital), National Reference Center for Rare Skin Diseases, and the support of the "Vitiligo European Task Force" international group, we have developed basic and translational human research studies exploring both the innate and the adaptive immune responses in vitiligo. This approach has led to the identification of the role of the Type I Interferon signature in disease initiation and the involvement of memory T cells expressing CXCR3. Our main goal is to better understand the link between immunity and melanocyte loss in vitiligo to identify new therapeutic targets. We explore the role of inflammatory cytokines on the function, survival and adhesion of melanocytes, the cell responsible for pigmentation. Part of our research is also focused on depigmentation occurring as a side effect in patients receiving immunotherapies. Moreover, our research is translated to pigmentation disorders affecting inflammatory skin diseases, such as psoriasis or scleroderma. Our team is part of the "Fédération Hospitalo-Universitaire" ACRONIM, Bordeaux University, and works in collaboration with the Immunology unit (CNRS, UMR 5164) on the pathogenesis of systemic diseases.

**Methodologies Used:**
1. Isolation and expansion of blood and skin immune cells for: Phenotyping: multiparametric analyses by flow cytometry, immunohistochemistry,immunofluorescence studies / Functional assays: cell proliferation and survival (CFSE, MTT), apoptosis (annexin V, caspase 3, tunel assay)
2. Impact of soluble inflammatory factors on epidermal cell cultures: Primary cultures of melanocytes prolifération and survival (CFSE, MTT), apoptosis (annexin V, caspase 3, tunel assay)
3. Identification of biomarkers in serum and skin samples of patients: ELISA, multiplex ELISA/ Real-time RT-PCR analysis

**Publications**


Our Immuno-dermatology team is focusing its research on human depigmenting skin inflammatory disorders, in particular vitiligo. Our main goal is to understand the role of the skin immune system in this disease to identify new therapeutic targets. We are also extending our research to depigmentation associated with other skin or systemic inflammatory diseases. Our work is done in close collaboration with the dermatology department of Bordeaux Hospital. National collaborations have been developed.

**Immunology-dermatology project overview**

To understand the interplay between the skin immune system and epidermal cells that lead to melanocyte loss, in vitro and ex vivo studies are performed on human tissues to characterize:
- the phenotype and function of skin immune cells (T cells, dendritic cells)
- the impact of inflammatory soluble factors on skin epithelium: keratinocytes and melanocytes
The description of autoantibodies to citrullinated proteins, of which we have identified the tissue targets and contributed to demonstrate the pathophysiological importance, has revolutionized the early diagnosis of Rheumatoid Arthritis and allowed the development of worldwide used diagnostic tests.

Research Brief:
Located within the biological research area of Toulouse Purpan Hospital, UDEAR laboratory is dedicated to the study of joint, skin and eye chronic diseases. Its goal is to highlight new diagnostic tools and therapeutic targets for these diseases, but also to understand at the fundamental level the physiological regulation of the corresponding organs. The laboratory is composed of 4 multidisciplinary groups. Two of them are dedicated to the study of terminal differentiation of the epidermis and its defects in skin diseases. More specifically, they investigate the molecular bases of epidermal barrier dysfunction, and the pathophysiology and genetic basis of Ichthyoses and Atopic Dermatitis. A group improves the diagnosis and studies the pathophysiology of Rheumatoid Arthritis. It focuses on the involvement of the disease-specific anti-citrullinated protein autoantibodies, their role in macrophage activation and the characterization of their tissue targets. A fourth group, who joined UDEAR in January 2016, is interested in the terminal differentiation of the corneal and conjunctival epithelia, and in the genetics and pathophysiology of Keratoconus and diseases of ocular development.

The laboratory uses human sample collections, and is linked to 2 national hospital reference centers dedicated to rare genetic skin diseases and to Keratoconus, respectively. It is relied on a large network of academic and industrial collaborations, at a local, national and international level.

Methodologies Used:
Cytology, immunohistology, solid phase immune complex reconstitution, cell sorting, cell culture, flow cytometry, protein separation (chromatography, electrophoresis), protein analysis (Western blot, ELISA), RNA interference, confocal microscopy, electron microscopy, new generation sequencing, quantitative RT-PCR, transcriptomics, proteomics, genome wide association study, exome sequencing, recombinant protein production, knockout mouse models.

Publications


Clavel C, Cecatto L, Anquillet F, Serre G, Sebbag M. (2016). Among human macrophages polarized to different phenotypes, the M-CSF-oriented cells present the highest pro-inflammatory response to the rheumatoid arthritis-specific immune complexes containing ACPA., Ann Rheum Dis. 75(7), 2184-2191


Involvement of autoantibodies to citrullinated proteins (ACPA) and RF in Rheumatoid Arthritis

ACPA and RF: accomplice in the RA synovium!

IgG ACPA are closely specific for RA. They are the first to appear, can act alone by forming immobilized immune complexes with cit-Fibrin, inducing (7) and maintaining synovium inflammation.

IgM RF are less specific, not always present, appear later, act combined with ACPA by forming macro immune complexes, and strongly amplifying the inflammatory events!

Atopic Dermatitis: a new pathophysiological model
Diabetes
Agnès Lehuen

Immune mechanisms of type 1 diabetes

Université de Paris 05 (Université Rene Descartes)
Inserm U1016
Pierre-Olivier Couraud
Paris

Expertise in human T cell assay and prevention of type 1 diabetes by NKT cells.

Research Brief:

Type 1 diabetes (T1D) is an early onset autoimmune disease caused by the destruction of beta pancreatic cells by the immune system, whereas type 2 diabetes (T2D) is associated to low grade inflammation that contributes to the induction of insulin resistance. The objective of our projects is to decipher the role of specific innate and adaptive immune cells and their molecular pathways in the development of diabetes. These studies are based on newly developed mouse models and the use of patient samples, mainly from our close relationship with the clinical Diabetology Department of the Cochin Hospital.

We are analyzing the role of innate-like T cells, NKT and Mucosal Associated Invariant (MAI) T cells recognizing bacterial ligands, in the physiopathology of these diseases. Since T1D is mediated by pathogenic T cells, we are determining self-epitopes in patients and in humanized mouse models of T1D to develop therapies based on immune-regulation and antigen-specific strategies to induce tolerance toward beta-pancreatic cells. Genetic factors are involved in both types of diabetes, particularly those related to the control of day-night rhythm. This is underlined by the fact that shift work can raise the risk of developing T2D. Our previous work identified the circadian-rhythm related gene Bmal2 (Arntl2) as an interesting candidate in diabetes physiopathology. All these approaches will allow the development of new biomarkers and innovative therapeutic strategies against diabetes.

Methodologies Used:

- Humanized mouse model of type 1 diabetes
- Viral infections of mouse model of type 1 diabetes
- In vivo NKT cell triggering in mouse model of type 1 diabetes
- ELISPOT assay to detect autoreactive T cells (human and mouse)
- Human anti-islet antigen T cell lines

Publications


Lebailly B, He C, Rogner UC (2014). Linking the circadian rhythm gene Arntl2 to interleukin 21 expression in type 1 diabetes, Diabetes. 63(6), 2148-57

Characterisation of autoreactive T cells in mouse models and patients

Detection of insulin specific CD8+ T cells in type 1 diabetic patients (IIFC detection technology)

Biavard C, Diabetes Med 2013
Luce et al, Diabetes Care Med 2013
Luce et al, Diabetes 2013

Development of NTRC humanised mice as a new preclinical model to study type 1 diabetes

NTRC humanised mice
Research Brief:

Type 1 diabetes (T1D) is an autoimmune disease caused by autoreactive T lymphocytes which destroy insulin-producing pancreatic islet beta-cells. Despite this knowledge, neither the diagnosis nor the therapy of T1D targets pathogenic T lymphocytes. Our research projects therefore aim at exploiting these T lymphocytes as disease biomarkers and as therapeutic targets to prevent beta-cell destruction, and at understanding the cross-talk between T lymphocytes and pancreatic beta cells.

The long-term objective is to develop an immune "staging" and intervention protocol in subjects at risk for T1D development, in order to detect and block beta-cell autoimmunity at an early stage. The strategies developed may lead to a paradigm shift in the approach to T1D by identifying and treating the immune disease early, before the appearance of its metabolic consequences. By targeting the mechanisms underlying disease development, such strategies would pave the way to T1D prevention and treatment.

Methodologies Used:
* Human and mouse models
* Cell culturing and T-cell cloning
* Flow cytometry and HLA tetramers
* ELISpot

Publications


Raphaël Scharfmann

Control of pancreatic endocrine cell development

Paris Descartes
Inserm U1016
Pierre-Olivier Couraud
Paris

We have gained expertise in developing assays in reconstituted rodent and human models to define intercellular signals regulating pancreatic beta cell development.

Research Brief:

Type-1 diabetes is caused by an autoimmune destruction of insulin producing beta cells resulting in insulin deficiency. Insulin therapy is unsatisfactory. Thus defining new strategies (cell or regenerative therapies) as basis to cure diabetic patients represents a major challenge. Beta cells develop from pancreatic progenitors that proliferate and next differentiate into functional insulin-producing cells. This is a complex process, each step being controlled by specific signals. Theoretically, beta cell mass can be enhanced by: i) activating the proliferation of pancreatic progenitors; ii) activating their differentiation into beta cells; iii) activating the proliferation of beta cells themselves. During the past years, we developed tools based on rodent models to search for signals controlling each step of beta cell development. We developed strategies to transfer to reconstituted human models, data generated in rodent models. We also developed the first available human beta cell lines (a premiere). We generated new results and hypotheses concerning signals controlling each step of pancreatic development. We also dissected specific forms of neonatal diabetes in Human, which permits to define new treatments for children with neonatal diabetes. We are currently continuing this work which is important on a cognitive point of view, but also to define new approaches to find a cure for diabetes.

Methodologies Used:

Bioassays to define signals regulating beta cell development.
In vitro and in vivo bioassays.
Reconstituted rodent and human bioassays.
Methodologies to develop human beta cell lines.

Publications


Nutrition, Diabetes and the Brain

Université de Lyon 1
(Université Claude Bernard)
Inserm U1213
Gilles Mithieux
Lyon

We uncovered glucose production by the intestine and its paradoxical benefits on energy (food intake, energy expenditure) and glucose (insulin sensitivity, insulin secretion) homeostasis, which is a basis of our research project.

Research Brief :

The project deals with the respective roles of the glucose-producing organs (the liver, kidney and small intestine) in the mechanisms of control of glucose and energy homeostasis. We have recently shown that endogenous glucose production (EGP) by the small intestine exerts, paradoxically, a beneficial role in this homeostasis. It activates a nervous signal, starting from the walls of the portal vein (the so-called portal glucose signal), which initiates centrally a satiety phenomenon, and at the hepatic level, a potentiation of the suppression of glucose production and an increase in insulin sensitivity. This paradigm has allowed us to explain by protein-enriched diets and the rapid amelioration of obesity and diabetes by dietary proteins or fibers and after gastric bypass surgery.

This led us to propose a novel concept of the role of EGP in the control of glucose and energy homeostasis: EGP by the liver should be deleterious, initiating insulin-resistance and further frank diabetes, whereas EGP by the small intestine would be beneficial in energy homeostasis.

To further document this novel concept in the field of obesity and diabetes, we created novel mice models of time-dependent and organ-specific deletion (or overexpression) of glucose-6 phosphatase (the key enzyme of EGP). These models allow us to contrast diabetes with the mirror disease (the Human glucose-6 phosphatase deficiency), for a better understanding of both the epidemic and the rare diseases.

Methodologies Used :

- Microsurgery in rats and mice
- Energy (food intake, energy metabolism) and glucose (glucose tolerance, insulin sensitivity) homeostasis in rodents
- Time-dependent and tissue-specific deletion (or overexpression) of glucose production in mice
- Use of glucose-labeled tracers to quantify whole body and organ-specific glucose fluxes
- Behavioral studies in relation with food intake and anxiety-depression

Publications


GAUTIER-STEIN A, SOTY M, CHILLOUX J, ZITOUN C, RAJAS F & MITHIEUX G (2012). Glucotoxicity induces glucose-6-phosphatase catalytic unit expression by acting on the interaction of hypoxia inducible factor-1a with cAMP-responsive element-binding protein, Diabetes. 60(), 2451-2460


ABDUL-WAHED A, GAUTIER-STEIN A, CASTERAS S, SOTY M, ROUSSEL D, ROMESTAING C, GUILLA H, TOURETTE JA, PLECHE N, ZITOUN C, GRI B, SARDELLA A, RAJAS F & MITHIEUX G (2014). A link between hepatic glucose production and peripheral energy metabolism via hepatokines, Molecular Metabolism. 3(), 531-543

Gut-brain glucose signaling by intestinal gluconeogenesis and associated benefits

describes the gut-brain neural circuit initiated by intestinal gluconeogenesis, from the induction of gluconeogenesis genes up to the central and peripheral benefits in glucose and energy homeostasis. The situation of protein-feeding is illustrated. Comparable chain of processes take place under the action of dietary fibers or after gastric bypass surgery.
Jennifer Rieusset

Organelle communication and diabetes

Université de Lyon 1
(Université Claude Bernard)
Inserm U1060
Hubert Vidal
Lyon

Our major strength is to have a double expertise in both skeletal muscle insulin sensitivity and beta cell function, in order to identify common mechanisms to their metabolic alterations and to propose new and more effective preventive and/or therapeutic targets against type 2 diabetes.

Research Brief :

Our team, managed by Charles Thivolet and myself, focuses on molecular mechanisms of altered insulin action and secretion in type 2 diabetes (T2DM). Among these mechanisms, we focus on the role of two key intracellular organelles: mitochondria and endoplasmic reticulum (ER). Both organelles interact at contact points, called MAM (mitochondria-associated endoplasmic reticulum membranes), in order to exchange both lipids and calcium, 2 metabolites that play a key role in metabolic homeostasis. We recently identified a new role of MAM in the control of insulin action and secretion, as well as organelle miscommunication in liver, skeletal muscle and beta cells of obese and diabetic mice. The general goal of our research program is to better characterize the nature and the physiological significance of MAM in the control of glucose homeostasis and their roles in the pathogenesis of T2DM. More specifically, our specific aims are:

1) To identify the molecular nature of MAM actors and their functional roles,
2) To characterize the physiological significance of MAM in the control of insulin action and secretion,
3) To identify the regulators of MAM and their functional impacts,
4) To validate if the MAM could be a new target for the treatment of T2DM.

Ultimately, our scientific project will clarify the mechanisms by which MAM are involved in the pathogenesis of T2DM and should determine if MAM could be a new target to improve both insulin action and secretion in T2DM.

Methodologies Used :

- Primary cultures of human myotubes, hepatocytes and beta cells of pancreas
- Adenoviral overexpression or invalidation by RNAi of genes in vitro
- Analysis of the structure, density and the functions of mitochondria (electronic microscopy, respiration, ATP synthesis, fatty acids oxidation)
- Analysis of the homeostasis of endoplasmic reticulum (electronic microscopy, real-time PCR, Western blotting)
- Analysis of ER-mitochondria interactions (electronic microscopy, in situ PLA, subcellular fractionation)
- Analysis of insulin signalling (immuno-precipitation, western-blotting)
- Analysis of the mass and functions of beta cells

Publications


The general goal of our research is to better understand the role of ER-mitochondria interactions (known as MAM for mitochondrial-associated membranes) in the control of glucose homeostasis (in insulin action and secretion) and in the pathogenesis of type 2 diabetes.

**Visualization and quantification of ER-mitochondria interactions by in situ Proximity Ligation Assay**

A) incubation of cells or tissues with two different primary antibodies, one directed against the IP3R1 channel in the ER, and another one against a mitochondrial protein (VDAC or CypD) or the chaperone Grp75, B-C) circularization and ligation of connector oligonucleotides of secondary antibodies when proteins are less than 40 nm away, D) rolling circle amplification with polymerase and E) detection of the product with fluorescent or peroxidase-coupled probes.
Anne Bouloumie-Diehl
the stroma-vascular cells of adipose tissue

Université de Toulouse 3
(Université Paul Sabatier)
Inserm UMR 1048
Angelo Parini
Toulouse

Our approaches on human and rodent adipose tissues that combine cell sorting, confocal analyses and primary culture of adipocytes, endothelial cells, immune cells and progenitor cells are unique allowing the study of native cells and their interactions.

Research Brief:
Our research is focused on the cells from the stroma-vascular fraction of the adipose tissue, i.e. endothelial cells, macrophages, T lymphocytes and progenitor cells, in human and rodent models. We study the relative contribution of the distinct cell subsets in the adipose tissue dysfunction linked with obesity and associated pathologies. The clinical and animal studies are combined with cellular (immunoselection/depletion cell sorting, 3D confocal analyses, flow cytometry, primary cultures) and biochemical approaches. The projects are focused on 1) the proliferation, differentiation and reparative potentials of the adipose tissue progenitor cells, 2) the immuno-inflammatory processes in the aging adipose tissue, and 3) the modulatory role of the adipose tissue endothelium in the adipose tissue growth and inflammation.

Methodologies Used:
Immunoselection/depletion cell sorting by the use of magnetic nano- and micro-beads
Flow cytometry and three dimensional confocal analyses of the adipose tissue
Primary cultures of human and murine mature adipocytes, adipose tissue endothelial cells, macrophages, lymphocytes, progenitor cells and preadipocytes.

Publications

Keywords
• diabetes
• senescence
• adipogenesis
• endothelial cells
• stem/progenitor cells
• adipose tissue
• secretome
• inflammation
• flow cytometry
• confocal microscopy
• cell culture
• immunoselection
• molecular biology

Biological Resources
• native endothelial cells, macrophages, lymphocytes, progenitor cells and adipocytes from human adipose tissue
• Conditioned media from endothelial cells, macrophages, lymphocytes, progenitor cells and adipocytes from human adipose tissue
• mRNA from native endothelial cells, macrophages, lymphocytes, progenitor cells and adipocytes from human adipose tissue

Key facts
Team
• Researchers : 4
• Technicians : 3
• Postdoc fellows : 1
• PhD Students : 2

Translational approaches
• Patents : 0
• Clinical research grants : 0
• Industry partnerships : 2

International research links
• Sweden
• Spain
Identification of the human adipose tissue native white and brite progenitor cells

Flow cytometry of the human adipose tissue stroma-vascular cells after collagenase digestion allows the immunoselection of CD45-/CD34+/CD31- progenitor cells that can upon culture accumulate lipids in their multiple lipid droplets (BODIPY) or express the mitochondrial uncoupling protein 1 (UCP1), which are markers of mature white and brite fat cells.

Adipose tissue microenvironment and obesity

Lymphocyte T neighboring of mature adipocytes is increased with obesity. Here the immune cells are labelled in red (CD3) and green (CD45) while the adipocytes (in blue) are recognizable by their round-shaped profile.

Endothelial Notch signaling pathway and interactions with the microenvironment

(A) Dimerization of NOTCH receptor with DLL/JAG ligand, proteolytic cleavage by ADAM family proteases /g-secretase complex, NOTCH intracellular domain (NICD) translocation to nucleus, interaction with MAML/CSL and transcription of target genes. (B) Inflammation suppresses NOTCH in endothelial cells, expression of inflammatory and atherogenic mediators, immune cell recruitment, bi-directional heterotypic communication. (C) Oxidized phospholipids repress NOTCH & promote endothelial activation.
Eric Renard

Determinants and correction of insulin secretion loss in diabetes

Université de Montpellier
CNRS 5203 INSERM U1191
Jean-Philippe Pin
MONTPELLIER

**Key facts**

**Team**
- Researchers : 5
- Technicians : 1
- Postdoc fellows : 1
- PhD Students : 0

**Translational approaches**
- Patents : 1
- Clinical research grants : 6
- Industry partnerships : 5

**International research links**
- Netherlands
- Italy
- USA

**Keywords**
- severe obesity
- artificial pancreas
- insulin
- Diabetes
- islet transplantation
- insulin delivery devices
- continuous glucose monitoring
- closed-loop algorithms
- bariatric surgery
- islet isolation

**Biological Resources**
- plasma samples, islet cells

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**Our team was the first one that historically implanted an artificial beta cell in diabetic humans in 2000 and tested artificial pancreas in ambulatory conditions in diabetic patients in 2011.**

**Research Brief:**

Our research program is based on developing artificial pancreas as a therapy of diabetes and on deciphering the factors determining the occurrence of diabetes in severe obesity. It also includes research on the development of islet transplantation and bio-artificial pancreas.

**Methodologies Used:**
- Pumps for continuous insulin delivery
- Continuous glucose monitoring
- Closed-loop algorithms
- Bariatric surgery
- Islet transplantation

**Publications**

**Spaan NA, Teplova AE, Renard E, Spaan JAE (2014).** Implantable insulin pumps: an effective option with restricted dissemination., Lancet Diabetes Endocrinol. 2(5), 358-60


**Kropff J, Del Favero S, Place J, Messori M, Keith-Hynes P, Toffanin C, Magni L, DeVries JH, Renard E, Cobelli C, Farret A, on behalf of the AP@home Consortium. (2015).** Multicenter outpatient dinner/overnight reduction of hypoglycemia and increased time of glucose in target with a wearable artificial pancreas using modular model predictive control in adults with type 1 diabetes., Diabetes Obes Metab. 17(4), 468-76

**Courbet A, Endy D, Renard E, Molina F, Bonnet J. (2015).** Detection of pathological biomarkers in human clinical samples via amplifying genetic switches and logic gates., Sci Transl Med. 7(27), 289ra83


Components of a wearable model of artificial pancreas

Outpatient trial of artificial pancreas in children

Components of an artificial pancreas for outpatient trial in children
Our team performs studies from molecular to cells and animal model in order to have an integrative view of the dysfunction of adipose tissue and metabolism linked to obesity and diabetes.

**Research Brief:**

The research of our team deals with the pathophysiology of the insulin resistance in obesity and type 2 diabetes (T2D), focusing on the mechanisms involved in adipose tissue (AT) dysfunction in obesity and how this dysfunction is linked with the development of insulin resistance. Our goal is to decipher how stresses and stress responsive pathways that develop in AT during obesity (inflammation, hypoxia, DNA damage) alter its metabolic and endocrine function and to determine if and how these pathways can alter liver functions.

We develop research activities along two related and highly integrated areas:

1. To unravel and characterize novel molecular mechanisms and signaling pathways induced by AT stresses causing metabolic dysfunction of AT and adipocyte insulin resistance.
2. To identify new genetic and epigenetic players controlling liver metabolism and to decipher their implication in insulin resistance. This axis was initiated by Jean-François Louet (CR1 CNRS) who joined the team by the end of 2013 (see below: organization of the team). JF Louet has a solid background in the study of the transcriptional control of liver metabolism by multi-proteins co-regulator complexes, especially the steroid receptor coactivator (SRC) family.

We combine molecular and mechanistic studies using different cell models, studies in preclinical animal models and translational research to evaluate the clinical relevance of the animal and in vitro findings.

* **Methodologies Used:**
- Primary human adipocytes in culture
- Cell signaling and gene expression quantification
- Metabolic studies in isolated adipocytes and muscles and in animals
- Cellular imaging of protein trafficking
- Animal models of obesity and diabetes (KO mice, High-Fat diet and genetically obese mice)

**Publications**


Ceppo F, Berthou F, Jager J, Dumas K, Cormont M, Tanti JF. (2014). Implication of the Tpl2 kinase in inflammatory changes and insulin resistance induced by the interaction between adipocytes and macrophages., Endocrinology. 155(3), 951-64


Key facts
Team
• Researchers : 3
• Technicians : 2
• Postdoc fellows : 4
• PhD Students : 4
Translational approaches
• Patents : 2
• Clinical research grants : 1
• Industry partnerships : 3
International research links
• USA, Canada, UK,
• Germany, Austria, Spain,
• Belgium, Sweden, Denmark
Keywords
• Reprogramming
• GABA
• Diabetes
• Pax4
• Arx
• Mouse
• Molecular Biology

Patrick Collombat
Diabetes Genetics
Université de Nice
Sophia-Antipolis
Inserm U1091
Stéphane Noselli
Nice

We use reprogramming to convert pancreatic cells into insulin-producing beta-cells

Research Brief :
Our group is involved diabetes research. Both Type I Diabetes (insulin-dependent) and Type II (non-insulin dependent) diabetes ultimately result in the selective loss of insulin-producing beta-cells in the endocrine pancreas. The subsequent lack in insulin hormone induces a blood hyperglycemia that may be attenuated by daily injection of exogenous insulin hormone. Nevertheless, due to variations in glycemia, vascular damages, blindness, amputation or even death may occur.

We belong to a JDRF-funded consortium whose goal is to gain further insight into the mechanisms regulating the genesis of the mouse pancreas and apply this knowledge to improve the treatment of diabetes. Toward this aim, using the mouse as a model, we have identified two transcription factors, Arx and Pax4, playing a crucial role in the genesis of the different endocrine cell subtypes, including insulin-secreting beta-cells. Importantly, we showed that the forced expression of Pax4 in alpha-cells is sufficient to induce their continuous regeneration and conversion into cells displaying a beta-cell phenotype.

Aiming to eventually apply these findings to human, we searched for compounds able to induce similar processes. GABA was thus identified and found that it was able to induce alpha-cell-mediated beta-like cell neogenesis in the mouse. The beta-like cells thereby generated were functional and could reverse several times the consequences of chemically-induced diabetes in vivo.

* Methodologies Used :
- Mouse
- Immunohistochemistry
- Molecular Biology
- qPCR

Publications


GABA induces beta-like cell regeneration in mice rendered diabetics
Metabolism
Nutrition
Ramaroson Andriantsitohaina
Oxidative stress and metabolic diseases
Université d’Angers
Inserm UMR1063
Ramaroson Andriantsitohaina
Angers

Key facts
Team
• Researchers : 12
• Technicians : 5
• Postdoc fellows : 2
• PhD Students : 7
Translational approaches
• Patents : 3
• Clinical research grants : 3
• Industry partnerships : 0
Keywords
• oxidative stress
• extracellular vesicles
• metabolic diseases
• polyphenols
• Flow cytometry
• confocal microscopy
• oxygraphy
• myography
• molecular biology

Biological Resources
• Cohorts

Research Brief :
Extracellular vesicles (EVs) and metabolic dysfunctions: interrelationship between metabolic syndrome (MetS) and obstructive sleep apnea (OSA)
The main goal is to predict MetS development and its interrelationship and penetration with OSA with into overt disease as well as define new therapeutic opportunities from the delivery of “specific” vesicle subsets. Thus, the focal point of the project is EVs bringing novelty, and establishing possibly a selling point in view of concepts uniqueness. For disease outcome, the focus will be of common downstream consequences of MetS and OSA such as increased cardiovascular morbidity including peripheral vascular diseases and coronary artery disease.

Therapeutic strategies to fight against metabolic dysfunctions: EVs and nutritional approaches. EVs will be engineered to over-express different therapeutic players (proteins, mRNA or miRNA) by driving the synthesis of the relevant EV-producing cells. A goal will be the selection of specific EV subsets to assess their therapeutic potential in proof-of-concept analyses. We will investigate an optimization of the impact of nutrients from carrot, apple and red wine polyphenol compounds against metabolic diseases. This will be addressed in an integrative manner with regard to the impact of plant genetic variability, culture conditions and conservations on different cells of interest (vascular, adipocytes and hepatocytes) and in experimental models of insulin resistance and obesity.

* Methodologies Used :
? In vivo (echography, laser doppler, telemetry) and in vitro (myography, arteriography, langerdorff) approaches: animal models (pharmacology, knock out).
? Cell culture (primary cells and cell lines) and biology (flow cytometry, confocal microscopy, patch clamp, oxygraphy). Molecular biology (quantitative PCR, Western blot, gene silencing).
? Clinical studies (epidemiology, pharmacology, genetics).

Publications
Stephan Pr. Chevalier

Nutrition, Growth and Cancer

Université de Tours
(Université François Rabelais)
CHU Inserm UMR 1069
Stephan Chevalier
Tours

Our objectives are to establish a rationale for implementing clinical trials using specific lipid nutrients to increase anticancer treatment efficacy, to prevent metastasis, to limit therapeutic relapse in chronic forms of breast cancers, to fight cancer cachexia and allow better tolerance to drugs.

Research Brief:
The research unit has a long-standing expertise in performing research at the interface between nutrition and cancer, and has received international recognition in this field. The team was the first to link diet-related changes in the breast-associated adipose tissue (lipidome) in relation to breast cancer development and metastasis. This finding is highly consistent with the hypothesis that the western diet plays a pivotal role in the development and the progression of several high incidence and mortality-inducing tumor types, including breast and prostate cancers. UMR1069 has also described the potential benefits of the clinical use of lipid nutrients in order to increase anticancer treatment efficiency. Cancer-induced cachexia, a progressive alteration in the nutritional status of patients that drastically affects their survival, is another association between cancer and nutrition being investigated. Specific dietary and pharmacological lipid interventions may have important beneficial effects and clinical applications.

Our scientific project aims at investigating the cellular mechanisms of action of lipids (ether-lipids, cardiolipins, polyunsaturated fatty acids) to regulate cancer cachexia and tumor progression (bed to bench side). The objective is to facilitate the transfer of such fundamental knowledge to patients developing chemo- or hormono-resistant cancers and/or metastases and/or cancer-induced cachexia (bench to bedside).

Methods Used:
Randomized clinical trial in cancer patients, Analysis of energy metabolism in humans and rodents, Mouse and rat models of breast tumors and metastases, Epithelial and endothelial cell cultures (proliferation, migration, invasion, tubulogenesis), Electrophysiology of cancer cells (automated platform development), Lipid biochemistry (chromatography, spectrometry) and chemical synthesis, Bioenergetics analysis of mitochondrial functions, Molecular and cellular tools (cloning, siRNA, PCR and Western blotting, lentivus microscopy), Bright field, phase contrast and fluorescence microscopy.

Publications


Cardiolipin de novo synthesis and remodeling on the matrix side of the inner mitochondrial membrane

CL metabolism is dependent on biosynthesis and remodeling of the fatty acid composition. Liver mitochondria from cachectic animals display lower oxidative phosphorylation efficiency compared to pair-fed animals, due to increased energy wasting processes. There is a positive correlation between CL content and energy wasting intensity. PGPS enzyme was upregulated in liver mitochondria from cachectic rats. PGPS expression was upregulated by TNFa, associated with a specific increase in CL content.

In vivo analysis of the fatty acid (FA) composition of human breast adipose tissue

(A) Detection of multifocal tumor by Magnetic Resonance Imaging, MRI. (B): Forest Plot showing an association between tumor multifocality and fatty acid levels in breast adipose tissue. Low levels of n-3 PUFA such as EPA (20:5n-3) and DHA (22:6n-3), significantly correlate with a higher relative risk (OR, Odds Ratio) of tumor multifocality.

Cancer cells escape from the primary tumor by degrading and migrating through extracellular matrix

Béta4 subunit of NaV channels, expressed in normal epithelial cells, is lost in invasive cancer cells. By contrast, NaV1.5 channel and P2X7 receptor are expressed in highly invasive cancer cells (not in normal cells) and specifically in cholesterol-rich lipid rafts in invadopodia. NaV1.5, colocalized with NHE1 exchanger, promotes its activity of protons extrusion. NaV1.5-NHE1 and P2X7 activate ECM degradation and the fatty acid DHA (22:6n-3) limits these processes through PPARβ regulation.
Control of Iron Metabolism and Iron Associated Diseases

Université de Rennes 1
Inserm U1241
Bruno Clement
Rennes

Key facts

Team
• Researchers : 20
• Technicians : 5
• Postdoc fellows : 2
• PhD Students : 7

Translational approaches
• Patents : 3
• Clinical research grants : 5
• Industry partnerships : 1

Keywords
• iron metabolism disease
• Microbiology
• metals
• microbiota
• parodontitis
• hepatocellular carcinoma
• osteoporosis
• hemochromatosis
• hepcidin
• iron metabolism
• cell biology
• molecular biology
• animal models
• in vitro models
• bio-clinical studies
• microbiology
• ICP-MS
• microbiota

Biological Resources
• Iron overload animal models and stored samples
• Iron overload animal models and correspponding stored samples
• Participation in the hepatology part of the Biological Resource Center of Rennes.
• In vitro hepatic and digestive models
• Database on Rare genetic iron overload diseases with CHU (National Reference Center).

Integration between basic and bio-clinical studies in order to improve knowledge on iron metabolism physiology and diseases, as well as mechanisms involved in their complications.

Research Brief:
Disturbances in iron homeostasis, including iron excess, affect well-being and life expectancy. However, huge differences exist in the phenotype of genetic iron overload disease. Identifying the causes of this variability is a major challenge for the development of methods that would control iron metabolism diseases. In order to get a better knowledge of those mechanisms, with the perspective of identifying targets for the control of iron-related diseases, we are characterizing new factors that may control iron level in plasma ?a hub for iron metabolism?, including:
i) the impact of expression and/or activities of proteins involved in the control of iron concentration in plasma, such as hepcidin, ferroportin and ceruloplasmin, their links with other metals and the impact of chelators;
ii) the relationships between microbiota and iron metabolism to determine how digestive microbiota may modulate iron metabolism, and whether iron overload disease has an impact on bacteria virulence, by studying oral microbiota that is easily accessible and will permit to study the relationships between iron excess, bacteria and lesions by focusing on periodontal diseases.

We are performing an integrative approach with original models and platforms devoted to metal quantification and metabolism in links with physicians (hematologists, rheumatologists, odontologists), the clinical investigation center and the National Reference Center for Rare Genetic Iron Overload Diseases.

Methods Used:
• Human and mouse hepatocytes cultures
• wild type and knock-out mice and rat models exposed or not to stimuli known to impact cellular or systemic iron metabolism.
• Gene expression studies.
• Functional studies allowing analysis of gene function and the impact of mutations.
• Bioclinical studies in patients through clinical, biochemical and genetic parameters.
• Use of databases for correlations between phenotype and genotype.
• Biochemical tools giving access to metabolic studies (stable isotopes and ICP/MS.
• Biofilms models
• Microbiota studies

Publications


Olivier Loréal
LOREAL Olivier.olivier@univ-rennes1.fr - 33+(0)223233865 - https://numecan.univ-rennes1.fr/?page_id=17

ITMO Physiopathologie, métabolisme, nutrition
The CIMIAD team characterizes the iron metabolism with special regards on the place of hepcidin on the iron biodistribution within the body and its relationships with other metals and microbiota, as well as the impact of iron excess on liver, bones and oral health during iron overload diseases.
Ani-SCANs Adelaide University
INRA US1395
Charles-Henri Malbert
Saint-Gilles

Aniscan is the sole facility that operates nuclear imaging in a large animal model either in the anaesthetised condition or in conscious animals. Furthermore, Aniscan get a recognised expertise in chronic vagal stimulation as a minimally invasive alternative to bariatric surgery.

Research Brief:
We aimed to quantify the metabolic alterations induced by diet induced obesity with specific reference to brain-gut axis using nuclear imaging in a large animal model. To do so, we have developed several minimally invasive research tools to investigate receptor occupancy, glucose metabolism and blood flow in the miniature pig. We have build a unique three dimensional brain atlas of the pig together with additional digital ressources mandatory to use and quantify the information issued from the imaging machines. These resources were partially incorporated in AniMate and Pmod softwares. Concomitantly we have acquired a recognised expertise on the manipulation of the brain-gut axis through chronic stimulation of the abdominal vagus. We have demonstrated that, once applied bilaterally on the abdominal vagus, this stimulation was able to reduce food intake and to restore insulin sensitivity in a model of diet induced morbid obesity. This occurs simultaneously at the muscle, liver and brain level. Furthermore, we showed that vagal stimulation was associated with improved limbic connectivity - a feature altered by acquired obesity. Finally, we developed a new stimulating scheme dedicated to the abdominal vagus nerve capable of activation of C type neurons within the limitations of an implantable neurostimulator. This scheme was able to activate the dorsal vagal complex and the majority of its effernts areas with a striking similarity to the brain activation observed postprandially.

Methodologies Used:
- PET imaging using 18F or 68Ga derivatives. Dynamic PET imaging with in line and off line arterial radioactivity measurements.
- Brain SPECT imaging using Tc99m and 123 Iodine derivatives
- Dynamic planar abdominal imaging in conscious pigs for measurement of solids and liquids gastric emptying
- CT based body composition
- Image guided brain surgery including in situ neuronal recordings
- Minimally invasive ultrasound guided surgery for arterial/venous catheter placements and biopsies
- Minimally invasive placement of electrodes on abdominal vagal trunks using laparoscopic surgery
- Indirect calorimetry in anaesthetised animals
- Evaluation of microstructure of the meal and meal preferences using robotic feeders

Publications
Malbert CH (2013). The brain-gut axis: insights from the obese pig model, Bull Acad Natl Med. 197(9), 1683-1699
Clouard C, Meunier-Salaün MC, Meurice P, Malbert CH, Val-Laitelit D (2014). Combined compared to dissociated oral and intestinal sucrose stimuli induce different brain hedonic processes, Front Psychol. 7(5), 861
Ochoa M, Val-Laitelit D, Lalles JP, Meurice P Malbert CH. (2016). Obesogenic diets have deleterious effects on fat deposits irrespective of the nature of dietary carbohydrates in a Yucatan minipig model, Nutr Res. 36(9), 947-954
Multimodal analysis of the brain activity after vagal stimulation

Multimodal analysis of FDG (glucose metabolism) and DATScan (DAT receptor binding) obtained in vagal stimulated versus control obese miniature pigs. Left image - Independent component analysis aims to identify independent components in each image modality as well as the relationships of these independent components across image modalities. Right - We applied a group ICA algorithm to define coherent network components. The width of each line indicates the strength of the relationship.

Hepatic glucose uptake after vagal stimulation

Changes in hepatic glucose uptake (dynamic PET FDG imaging) and hepatic volume (CT imaging) in lean, obese and obese animals with chronic vagal stimulation during 12 weeks. Note the restoration of the insulin sensitivity (expressed as MRglu) obtained by vagal stimulation. Hepatic glucose uptake was calculated in insulin stimulation condition obtained by insulin clamp. This allow to extract the whole body insulin sensitivity together with the hepatic glucose production and uptake.

Effect of various vagal stimulation patterns on brain activity

Statistical parameter mapping (SPM) of the brain FDG uptake obtained in vagally stimulated versus control animals. SPM analysis was performed on pixelwise MRGlu calculated images to take into account absolute variation in the overall glucose uptake. Three modalities of vagal stimulation were compared to control (S1, S2 and S3). S2 and S3 were specifically designed to activate C neurons. Only S3 pattern was able to increase the metabolism of the dorsal vagal and hypothalamic complexes.
Anne Devin

Cell energy metabolism

Université de Bordeaux
CNRS UMR 5095
Bertrand Daignan-Fornier
Bordeaux

Unique combination of competencies in our team that studies cell energy metabolism and dynamics in isolated mitochondria, permeabilized cell and whole cells in a wide range of models: yeast, cultured cells, mouse. Our lab is at the forefront of research on mitochondrial energetics and dynamics.

Research Brief:

Cell energy metabolism includes energy conversion that leads to NADH reoxidation and ATP production. Two cellular pathways are involved in these processes: glycolysis and oxidative phosphorylation (mitochondria). Our laboratory is primarily involved in studying the control and regulation of oxidative phosphorylation during cell proliferation. Indeed, the cellular needs for both ATP synthesis and NADH reoxidation are susceptible to huge variations with rapid kinetics and this requires tight adjustments from the cell. We thus study the mechanisms that allow such adjustments. This is achieved at three levels of integration: the cellular level, the isolated mitochondria level and the oxidative phosphorylation complexes level.

Furthermore, the influence of alterations of mitochondrial dynamics on energy metabolism is studied. Mitochondrial dysfunction is a common cause of disease in both children and adults. Within the cell mitochondria form a dynamic network as a result of balanced fusion and fission. Mammalian mitofusin 1 and mitofusin 2 belong to the GTPase family of proteins and are required for mitochondrial outer membrane fusion. The recent discovery of the role of MFN2 in maintaining the activity of the mevalonate pathway could help to address the great diversity of phenotypes related to the loss of MFN2 through a common metabolic origin.

Methodologies Used:

The Laboratory possesses last generation Oroboros oxygraphs, Hitachi F7000 fluorimeter highly sensitive bioluminometer, spectrophotometers, thermal cycler...

The methodologies used range from molecular biology, western blotting, energy metabolism assessment, cell biology.

Publications

Laurent Martinez

High Density Lipoproteins (HDL) and PI3K Signaling in Atherosclerosis

Université de Toulouse 3
(Université Paul Sabatier)
INSERM UMR1048
Angelo Parini
Toulouse

Our projects aim to identify new molecular mechanisms and lipid signaling pathways involved in HDL-mediated atheroprotection, in order to determine new targets for the prevention and treatment of Coronary Artery Diseases (CAD).

Research Brief:

Atherosclerosis is a chronic inflammatory pathology of the vascular wall, in large part due to the accumulation of macrophages foam cells without adequate removal of cholesterol by High Density Lipoproteins (HDL). Lipid deposits lead to the formation of atherosclerotic plaques that damage the vascular wall and can be further complicated by plaque disruption and thrombosis. Our project, developed around 3 axes, is focused on the metabolic and vascular atheroprotective functions of HDL and on phosphoinositide 3-kinase lipid signaling pathways in atherosclerosis:

1°) HDL-mediated Reverse Cholesterol Transport (RCT).

The protective effect of HDL against atherosclerosis is mostly attributed to their central functions in RCT. In this context, we identified a metabolic sequence in which apolipoprotein A-I (apoA-I) binds to ATP-synthase (F1-ATPase) at the surface of hepatocytes, triggering ATP hydrolysis. The generated ADP interacts with the P2Y13 receptor, which then stimulates HDL uptake. We currently evaluate the relevance of this HDL-uptake pathway in atherosclerosis and its regulation.

2°) New signaling pathways in vascular wall protection.

We have demonstrated that the gamma isoform of PI3K plays an essential role in inflammatory processes of vascular wall. We are now studying PI3K and apoA-I induced-signaling pathway in the prevention of vascular dammages.

3°) Identification of genotypic or biological determinants of HDL levels and functions.

* Methodologies Used :

1/ Mice models of atherosclerosis: atherosclerotic lesions in aortic roots sections and « en face » analysis.
2/ Lipoproteins metabolism: Lipoproteins uptake by cell and liver, plasma lipoprotein analysis, gallbladder cannulation.
3/ Mice models of endovascular lesions: arterial reendothelization and intimal hyperplasia.
4/ Primary cell culture (mice hepatocytes and aortic SMC), fluorescent proteins production and labelling.

Publications


Cedric Moro  Dominique Langin

Obesity Research Laboratory

Université de Toulouse 3 (Université Paul Sabatier)
Inserm UMR1048
Angelo Parini
Toulouse

Our translational approach goes from discovery of novel pathways and regulations in clinical studies to molecular deciphering of the mechanisms in human cell and transgenic mouse models, thus ascertaining relevance of our projects in the context of human obesity and its metabolic consequences.

Research Brief :

The Obesity Research Laboratory works on the consequences of the excess of fat mass observed in obesity and aims at understanding the biological determinants and molecular mechanisms of obesity-related metabolic complications with a special emphasis on type 2 diabetes.

We have studied novel aspects of fatty acid metabolism in adipose tissue and skeletal muscle as well as the links between metabolic, inflammatory and fibrotic pathways and their relationship with lipotoxicity and insulin resistance. Using a bedside-to-bench approach, we have shown that modulation of adipose tissue and skeletal muscle lipolysis impacts on fat oxidation, lipotoxicity and insulin sensitivity. Studies in mice and humans revealed that pharmacological (inhibition of lipolysis, treatment with natriuretic peptides) and lifestyle (physical exercise, low calorie diet) interventions improve adipose tissue and skeletal muscle function as well as whole-body insulin sensitivity. In fat cells, inhibition of lipolysis or activation of fatty acid oxidation shows beneficial effects by limiting fatty acid release into blood circulation and by intrinsic modulation of glucose metabolism. In skeletal muscle, the regulation of intramyocellular triglyceride metabolism by lipases and of fatty acid oxidation by natriuretic peptides may be protective against the deleterious role of excess circulating fatty acids in obesity.

* Methodologies Used :

Our overall objective is now to decipher the cellular and molecular mechanisms associating fatty acid and glucose metabolisms at tissue and whole body levels and to evaluate the therapeutic potential of the inhibition of adipose tissue lipolysis and activation of skeletal muscle and adipose tissue fatty acid oxidation.

These topics are tackled by approaches combining clinical studies in humans (obesity, dietary interventions and physical activity), phenotyping of transgenic mouse models and studies on cellular models (primary cultures of human fat and skeletal muscle cells).

Publications


Coudé M, Badin PM, Vila IK, Laurenis C, Louche K, Marques MA, Bourliet V, Mousiel E, Tavernier G, Ristan AC, Galgani JE, Joannis DR, Smith SR, Langin D, Moro C (2015). Defective Natriuretic Peptide Receptor Signaling in Skeletal Muscle Links Obesity to Type 2 Diabetes, Diabetes. 64(12), 4033


Key facts

Team
- Researchers : 12
- Technicians : 6
- Postdoc fellows : 2
- PhD Students : 3

Translational approaches
- Patents : 2
- Clinical research grants : 2
- Industry partnerships : 3

International research links
- Karolinska Institute (Prof. Peter Arner, Prof. Mikael Ryden)
- University of Graz (Prof. Rudolf Zechn)
- Oslo University (Prof. Arild Rustan)

Keywords
- • dietary intervention
- • physical exercise
- • calorie restriction
- • insulin resistance
- • adipokines
- • inflammation
- • fatty acid metabolism
- • lipolysis
- • skeletal muscle
- • adipose tissue
- • Obesity
- • gene expression profiling
- • lipid metabolism
- • transgenic mice
- • human cell primary cultures
- • Functional genomics

Biological Resources
- • human adipose tissue biobanks
- • primary cultures of human fat and skeletal muscle cells

Publications


Coudé M, Badin PM, Vila IK, Laurenis C, Louche K, Marques MA, Bourliet V, Mousiel E, Tavernier G, Ristan AC, Galgani JE, Joannis DR, Smith SR, Langin D, Moro C (2015). Defective Natriuretic Peptide Receptor Signaling in Skeletal Muscle Links Obesity to Type 2 Diabetes, Diabetes. 64(12), 4033

Overview Obesity Research Laboratory

Exercise / Diet
Pharmacological compounds

Lipolysis

Endocrine & paracrine factors

Browning

Substrate oxidation

Insulin action
Philippe Valet

Adipocyte secretions, obesities and associated diseases

Université de Toulouse 3
(Université Paul Sabatier)
Inserm U1048
Angelo Parini
Toulouse

We are investigating adipokines from molecular studies up to a potential use as pharmacological targets for obesity/diabetes in humans.

Research Brief:
Our research activity focuses on adipocyte bioactive secretions in normal and obese states and their involvement in obesity associated disorders. Metabolic alterations can occur in the way how tissues/organs behave when dealing with physiological adaptation (physical exercise, overweight), loss of response (insulin-resistance, low grade inflammation, ageing, steatosis) or disease (obesity, diabetes, rare diseases, cancer). Every key tissue in regard to energy metabolism (adipose, liver, muscle) is able to release bioactive molecules (adipokines, myokines?) acting locally or through the entire body. Our expertise in the field of adipocyte/myocyte energy metabolism as well as in the study of adipokines led us to identify original candidates involved in the use of energy substrates, to study their regulations, their metabolic actions in normal/altered situations and settle clinical trial devoted to the proof of concept in humans. Our will is to bring new clues to the question of the involvement of such cell secretory products in the dysregulations observed during metabolic diseases. We identify relevant secreted candidates as well as pertinent cellular targets leading to original pharmacological development and, thus, to improve therapeutic approaches of metabolic diseases.

Methodologies Used:
- Lipids and glucose metabolism (in vitro & in vivo)
- Pharmacological studies in vitro and in vivo
- Functionnal genomics in mouse
- Real time NO and H2O2 production in tissues.

Publications


Key facts

Team
- Researchers: 5
- Technicians: 4
- Postdoc fellows: 1
- PhD Students: 2

Translational approaches
- Patents: 5
- Clinical research grants: 2
- Industry partnerships: 4

Translational approaches

We described molecular mechanisms as risk factors within the gut microbiota to intestinal functions i.e. neuroendocrine/immune/lipids responsible for control of metabolism and heart function. From these discoveries we set biotech companies to treat and prevent cardiometabolic diseases

Research Brief:
Determine molecular mechanisms controlling glycemia/dyslipidemia. We demonstrated the first that 1. GLUT2 and the GLP-1 receptor are molecular components of an enteric system, which detects blood glucose, that is connected to the brain via the enteric nerves. It regulates brain GLP-1 signaling and muscle glucose utilization, hepatic glucose production, insulin/glucagon secretion, and vascular blood flow. We defined this new physiological concept: The gut brain anticipatory axis and showed its impairment during diabetes, therefore setting the basis of new therapeutic strategies to treat diabetes/dyslipidemia. In the quest of the regulatory mechanisms we identified that intestinal microbiota produces factors (Metafactors, as lipopolysaccharides and bacterial DNA) responsible for inflammation, insulin resistance, hepatic lipid overload, and adipose tissue development. We are studying the molecular interactions between the Metafactors and the host for the control of glycemia, dyslipidemia, hepatic and vascular diseases. This involves deciphering 1. new metafactors (biomarkers for prediction/diagnosis/stratification), 2. eukaryotic targets of metafactors, 3. the enteric-immune system in the development of vaccine strategies, 4. molecular mechanisms in enterocytes responsible for metafactor absorption 5. The regulatory role of enterococcal hormones (GLP-1) on the anticipatory metabolic reflex. Patents are filed and biotech companies funded (Physiogenex SAS, Vaiomer SAS)

*Methodologies Used:
- State of the art techniques unique in France to study in vivo in the awake free moving mouse glucose (tracers) and vascular homeostasis (ultrasonic probe) simultaneously with brain infusate to respect the integrity of the physiological systems and to address molecular issues.
- Vagus nerve recording.
- Blood hormone biochemistry.
- Metagenomique, bioinformatique, biostatistics metafactors secretome (secreted bacterial product library)
- Accurate immunological phenotyping (FACS, lymphocyte transfer, confocal analyses?)
- Lipidology, enteric cell culture, molecular fluorescent tools for the analysis of lipid handing

Publications


Grasset E, Puel A, Charpentier J, Collet X, Christensen JE, Terce F, Burcelin R (2017). A Specific Gut Microbiota Dysbiosis of Type 2 Diabetic Mice Induces GLP-1 Resistance through an Enteric NO-Dependent and Gut-Brain Axis Mechanism, Cell Metabolism. 25(), 1075

Remy Burcelin

Intestinal Risk factors, diabetes, dyslipidemia

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Angelo Parini
Toulouse

Burcelin Rémy - remy.burcelin@inserm.fr - +33 (5)61325614 - http://www.i2mc.inserm.fr/index.php/fr/equipes-de-recherche/equipe-2
we are studying the molecular mechanisms through which the gut microbial risk factors control enteric functions such as NO production in response to GLP1

16S rDNA Metagenomic analyses

from the sequencing of gut and tissue microbiota we identify bacterial taxons differentially present within tissues.

Intestinal vili from germ free mice and conventional
Adipose tissue plasticity

Université de Toulouse 3
(Université Paul Sabatier)
CNRS ERL 5311  Inserm U 1031
Louis Castella
Toulouse

Key facts

Team
• Researchers : 9
• Technicians : 3
• Postdoc fellows : 1
• PhD Students : 3

Translational approaches
• Patents : 1
• Clinical research grants : 1
• Industry partnerships : 0

Keywords
• regeneration
• redox metabolism
• stroma
• adipose tissue
• cell therapy
• mesenchymal stem cell
• primary culture
• cell expansion
• animal model for cell transplantation
• cytometry
• 3D whole tissue imaging (spectral analysis)

Biological Resources
• transgenic mice
• biobanks of ASC

We are one of the rare team in the world with a double expertise in adipose tissue biology and regenerative medicine.

Research Brief:

Adipose tissue (AT) displays great plasticity and interests a large scientific community working not only on obesity epidemic but also on plastic and reconstructive surgery and regenerative medicine. The discovery that AT hosts a large pool of adipose derived stroma/stem cells (ASC) suitable for cell transplantation largely boosted this field, in which we are one of the world leaders (we published the 1st clinical trial on ASC transplantation in critical limb ischemia). ASC effects are mediated through their multipotent differentiation and mimicry potentials as well as their strong paracrine and immune-modulatory activity. We also showed that ASC egress from AT under immune/inflammatory stimuli suggesting their role in other tissues. Beside ASC, the importance of immune cells in AT physiology makes them a preponderant determinant of AT homeostasis. Recently, we showed that AT hosts a specific endogenous hematopoietic process, that generate immune cells contributing to tissue remodelling after lesion. Our hypothesis is that AT is a reservoir of regenerative and recruitable mesenchymal and immune cells and more particularly that ASC, through their pleiotropic effects, behave as orchestra conductor of stroma controlling proper tissue homeostasis.

* Methodologies Used:
Primary culture,
Cell transplantation,
Cell and 3D whole tissue imaging (spectral analysis),
Cytometry (multistaining analysis, cell sorting),
cell biology,
biochemistry (redox metabolism),
Animal models
molecular biology (microarray, Q RT-PCR...)

Publications


Role of ASC and adipose tissue derived immune cells in repair processes

Adipose Tissue (AT) is a reservoir of both Adipose Stromal Cells (ASC) and hematopoietic stem cells (HSC). ASCs differentiation potentials are controlled at least in part by redox metabolism, and are able to migrate to other organs under specific signals. AT-HSC generate immune cells involved in the control of AT-homeostasis and tissue remodelling after lesion. AT may thus be considered as a reservoir of regenerative and recruitable stromal and immune cells that control tissue homeostasis.
Marie-Paule Roth

Genetics and regulation of iron metabolism

Université Paul Sabatier
Toulouse III
Inserm, Inra UMR1220
Nathalie Vergnolle
Toulouse

Our team is the largest dedicated to the research on iron metabolism in France and its members are internationally recognized for their discovery of several of the key players in the regulation of hepcidin (i.e., BMP6, erythroferrone). Léon Kautz is an ERC-STG 2016 laureate.

Research Brief:
Our main objectives are: (i) to understand the molecular mechanisms necessary to adapt the quantity of iron absorbed through the duodenum to the iron needs for erythropoiesis; (ii) to find and validate new therapeutic targets to prevent iron overload associated with genetic hemochromatosis or beta-thalassemia and to divert iron from bacteria in infections with intra or extracellular pathogens.

Methodologies Used:
- Culture of mouse primary hepatocytes
- Quantitative PCR and expression microarrays
- Western blot
- In situ hybridization
- Immunohistochemistry
- Production of recombinant proteins
- Mouse microsurgery

Publications


Hepcidin expression is regulated by iron signals, erythropoiesis, and inflammation

Our team is characterizing the mechanisms by which high iron stores (via BMP6, HJV, HFE, TFR2, and BMP/SMAD signaling), inflammation (via IL6, STAT3, and activin B) and ER stress (via TMPRSS6) activate hepcidin transcription. We are also investigating how ERFE, which is produced when erythropoietic activity is high, suppresses hepcidin.
**Michele Trabucchi**

**CONTROL OF GENE EXPRESSION**

Université de Nice
Sophia-Antipolis
Inserm 1065
Patrick Auberger
NICE

**Keywords**
- non-coding RNAs
- gene expression
- RNA metabolism
- inflammatory disorders
- epigenetics
- mass-spectrometry
- high throughput sequencing
- in vivo models
- bioinformatics
- epidemiology

**Biological Resources**
- mouse lines

**Research Brief:**
We focused on discovering novel mechanisms of post-transcriptional events controlling gene expression. Particularly, since its creation the team has utilized several newly-emerging technologies to investigate the importance of the expression control and the mode of action of small RNAs in development and physiopathological events of metabolic disorders. We use different high-throughput experimental approaches, including mass-spectrometry and deep sequencing analysis coupled with bioinformatics, mouse models and epidemiological approaches. We have developed a series of important and complex stories to the point that they stand scrutiny at rigorous and prestigious journals, including Nature, Plos Genetics, BMC Medicine, Nucleic Acids Research, Cell Death & Disease, Nature Structural & Molecular Biology, and Nucleic Acids Research. In these papers, we described novel aspects of small RNA-dependent gene expression control, which shed a light of their central role in physiopathological events.

**Methodologies Used:**
- Mass-spectrometry; high-throughput sequencing; in vivo models; bioinformatics, epidemiology

**Publications**


Repetto E, Briata P, Kuziner N, Harte BD, McManus MT, Gherzi R, Rosenfeld MG, Trabucchi M (2012). Let-7b/c enhance the stability of a tissue-specific mRNA during mammalian organogenesis as part of a feedback loop involving KSRP., PLoS genetics. 8(7), e1002823


Ez-Zoubir Amri

Cellular and molecular regulation of fat mass

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CNRS UMR 7277 Inserm U1091
Stéphane Noselli
Nice

Use of unique cellular model, human multipotent adipose-derived stem (hMADS) cells, which differentiate into white adipocytes and convert into functional brown adipocytes

Research Brief:

Obesity reached epidemic proportions with no satisfactory treatment so far. Furthermore weight gain and fat mass redistribution represent a worldwide problem with aging as a larger proportion of the adult population is at risk of developing obesity, osteoporosis and associated diseases. Development of new therapies to control fat mass and its associated diseases, will be of great interest in terms of public health.

The objectives of our research program deal with the regulation of fat mass by two complementary approaches that are i) to favor the recruitment of functional brown adipocytes to enhance energy expenditure and ii) to lower the recruitment of white adipocytes by studying the role of oxytocin.

In contrast to early contention, healthy adult humans possess active brown adipose tissue with a potential for metabolic significance. Identification of factors leading to increased mass/activity of human brown adipose tissue are of great interest for the treatment of overweight/obesity. For this purpose, we set up a unique cellular model, human multipotent adipose-derived stem (hMADS) cells, which differentiate into white adipocytes and are able to convert into functional brown adipocytes. Our first aim deals with the analysis of mechanisms of conversion of human white to brown adipocytes and to identify potential therapeutic targets. Our second aim focus on the oxytocin involvement in the control of fat mass and in its distribution between adipose depots in animal models.

Methodologies Used:
- Molecular and cellular biology
- Cell signalling
- Primary cell culture
- Animal models

Publications


hahdour RA, Giroud M, Vegiopoulos A, Herzig S, Alhaua G, Amri EZ and Pisani DF. (2016). IP-receptor and PPARs trigger the conversion of human white to brite adipocyte induced by carbaprostacyclin, Biochim Biophys Acta. 1861(4), 285

Converion of white to brite adipocyte

Mesenchymal stem cells

PPARγ activation

White adipocyte

Brown/Brite/Beige adipocyte

Oxytocin controls fat and bone mass

Trabecular Bone

Intra abdominal Adipose Tissue
Subcutaneous Adipose Tissue

Oxytocin treatment

- Trabecular Bone
- Marrow Adiposity
- Fat Mass
Adaptive responses to immuno-metabolic dysregulations

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Sophia-Antipolis
Inserm U1065
Patrick Aubberger
NICE

Our goals are to understand the molecular mechanisms implicated in the adaptive responses to metabolic challenges of various tissues and cell types, to identify defects of these responses leading to metabolic disorders, and to explore new pharmacological approaches for metabolic diseases.

Research Brief:
In most industrialized countries, obesity has become epidemic, resulting in a dramatic rise of associated pathologies (e.g. diabetes, cardiovascular disease, asthma, Alzheimer’s disease, and several forms of cancer). Growing evidence supports that the common denominator of these pathologic conditions is obesity induced low-grade inflammation. Our team’s research efforts concentrate on the interplay between immunological and metabolic processes which has recently been defined as immunome¬tabolism; an emerging field of investigation at the interface between the historically distinct disciplines of immunology and metabolism. More specifically, our research focuses on the recently described role of adipose tissue T cells in the development of obesity-associated insulin resistance and type-2 diabetes. In particular, we are investigating the role of the ligand-activated nuclear receptor transcription factor Peroxisome Proliferator-Activated Receptor Beta (PPARbeta) in T cell biology in this context. We use both in vitro and in vivo models to study the role of PPARbeta in T cell proliferation / polarization, metabolism, mitochondria biogenesis/function, and oxidative stress. From these studies, we anticipate to obtain a better knowledge of the cellular and molecular mechanisms implicated in metabolic dysfunctions in obese/insulin resistant patients and to identify new targets for novel therapeutic approaches.

*Methodologies Used:
Mouse transgenics
Molecular and cellular biology
Biochemical and metabolic analyses
Nutritional and environmental interventions in animal models

Publications


Mothe-Satney I, Piquet J, Murdaca J, Sibille B, Grimaldi PA, Neels JG (2016). Proliferator-Activated Receptor Beta (PPAR?) activity increases the immune response and shortens the early phases of skeletal muscle regeneration, Biochimie. in press()


T cells in adipose tissue

T cell staining (CD3) showing adipose tissue from an obese mouse with several adipocytes surrounded by T cells.
Patrick Borel
Human Micronutrition
Aix-Marseille Université
INRA U1260 Inserm U1062
Marie-Christine Alessi
Marseille

Our main objective is to study the impact of lipid micronutrients (mainly vitamins and carotenoids) on etiology of vascular and cardiometabolic diseases. We are the only team able to study both the fate of these compounds from the meal to their site of action as well as their health effects.

Research Brief:
Numerous lines of evidence suggest that lipid micronutrients (LM: mainly fat soluble vitamins, carotenoids and phytosterols) have beneficial effects on several degenerative diseases including cardiovascular diseases. This can be explained by the fact that vitamin E and carotenoids exhibit antioxidant properties, phytosterols diminish cholesterol absorption, and vitamins D, E and carotenoids inhibit inflammation. However the bioavailability of these compounds is very low, it is affected by numerous factors (from the effect of the food matrix to the effect of genetic variations in genes involved in their absorption) and it is very variable among individuals. The objective of the team will be to assess the effects of LM and lipids on metabolic deteriorations that participate in the etiology of vascular and cardiovascular diseases, e.g. obesity, inflammation and insulin sensitivity, taking into account the factors that govern and affect their bioavailability and their metabolism. This will be done thanks to an integrative biology approach that uses complementary models, from in vitro digestion models to clinical studies through cell cultures, wild-type or transgenic animals and metabolomic. These objectives not only meet the INRA strategic research policies, stating that in-depth knowledge is required on the relationships between food, nutrition, prevention and health, but also those of the Food and Nutrition thematic area of the ALLEnvi Alliance and those of the PMN ITMO.

Methodologies Used:
- Animal experiments
- Cell culture
- GC-MS
- HPLC
- Intervention studies on healthy subjects and on insulin resistant subjects (obese, type 2 diabetic and subjects with metabolic syndrome)
- Molecular biology
- Multivariate analysis
- Stable isotope kinetic studies

Publications
Western diet is associated with a decrease in energy expenditure, an increase in energy consumption, and a decrease in micronutrient consumption. The aim of the team is to study the impact of lipid micronutrients and lipids on the etiology of vascular and cardiovascular diseases. Because these micronutrients are poorly absorbed, we will take into account both their bioavailability and their metabolism. We are able to study the fate of vitamins and micronutrients from the meal to the cell nucleus.

We were the first to show that SR-B1 is involved in apical uptake of a carotenoid and extended this result to other carotenoids and vitamins D, E, and K. We also showed that NPC1L1 is involved in vitamin D uptake and that ABCA1 is involved in vitamin E basolateral efflux. Recently, we have shown that CD36 can impact the absorption of vitamin E, D, and K, and are currently investigating the role of ASBT and other ABC transporters. Besides, we investigate transporter molecular functioning.

We are one of the leading teams in the world, working on the impact of lipidic micronutrients on adipose tissue biology and systemic consequences. We demonstrated that active derivatives of vitamin D, carotenoids, or vitamin A modulate gene expression in adipose tissue, leading to improvement of several risk factors such as adiposity, inflammation, energy metabolism or glucose homeostasis. These data pave the way for nutritional preventive approaches in the context of cardiometabolic diseases.
The metabolic syndrome has reached epidemic proportion. Abdominal obesity and insulin resistance play a central role in the development of this syndrome. The team’s goal is to identify nutritional and therapeutic strategies to improve adipocyte homeostasis and prevent or reduce insulin resistance.

The metabolic syndrome (MS) is a cluster of metabolic derangements that are associated with primary disturbance in adipose tissue. The resulting disorders define MS as increased waist circumference, decreased serum high-density lipoprotein, increased serum triglycerides levels, hypertension and fasting hyperglycemia. Insulin resistance and visceral obesity have been recognized as the most important pathogenic factors. MS account for the majority of cardiovascular disease risk in European and US population.

The general aim of the team is to identify nutritional and therapeutic strategies to improve adipocyte homeostasis and to prevent or reduce insulin resistance in patient at risk. The characterization of the effects of micronutrients (Vitamin D, carotenoids, Vitamin C) on adipocytes and adipose tissue gene expression, their metabolic impact as well as the study of their effects on insulin resistance will provide arguments for the role of dietary micronutrients in preventing the development of key features of the MS.

Insulin resistance at the level of adipose tissue results in excess release of free fatty acids that participate to the development of the MS and type 2 diabetes. The effects of micronutrients and natural or newly designed molecules will be studied in two specific processes involved in insulin sensitivity: Glut 4 translocation and insulin receptor maturation. The impact of modifications of these processes on insulin sensitivity will be validated in vivo.

Methodologies Used:
- Cell culture experiments
- Biological evaluation (qPCR, Western blotting, flow cytometry?)
- High throughput screening (micro-array: RNA and miRNA expression; DNA methylation)
- Preclinical models and metabolic phenotyping
- Clinical studies
- Patient follow-up via establishment of cohorts

Publications


Berenguer M, Martinez L, Giorgetti-Peraldi S, Le Marchand-Brustel Y, Govers R (2010). A serum factor induces insulin-independent translocation of GLUT4 to the cell surface which is maintained in insulin resistance., PloS one. 5(12), e15560


Metabolic syndrome is a disorder that affects 20% of the French population and that increases the risk for cardiovascular disease and diabetes.

**Micronutrients modulate the proinflammatory profile of adipose tissue**

Using preclinical mouse model of obesity and high throughput approaches, the team has demonstrated the ability of micronutrients to alter gene expression in adipose tissue. For instance, lycopene reduces proinflammatory cytokine and chemokine expression of adipose tissue explants from mice subjected to a HFD.

**GLUT4 translocation and Insulin receptor maturation**

The team has unique expertises in the study of GLUT4 translocation and in the study of the cleavages that affect insulin receptor. A serum factor was shown to induce insulin-independent translocation of GLUT4 to the cell surface (a). Expression of the antifibrinolytic factor PAI-1 was shown to inhibit furin-dependent maturation of insulin receptor and insulin signaling (b).
Uwe Schlattner

LBFA - Laboratoire de Bioénergétique Fondamentale et Appliquée

Université Grenoble Alpes
Inserm U1055
Uwe Schlattner
Grenoble

Research at LBFA is integrating molecular, cellular, whole organism and clinical research in bioenergetics.

Research Brief:
The main focus of LBFA research is on energy homeostasis and mitochondrial physiology, as well as their dysfunction in human disease. This includes projects on cell signaling, cell compartmentation, efficiency of cellular ATP generation, regulation of cell death, exercise and nutrition. LBFA research is organized in three axes:

(1) "Energy signaling and systems bioenergetics" (U Schlattner) is working on molecular mechanisms in the regulation of cellular energy state and energy homeostasis, in particular structure, function and signaling of AMP-activated protein kinase, topology, dynamics and function of mitochondrial microcompartments, and spatio-temporal dynamics of cellular energetics.

(2) "Mitochondria and cell death" (E. Fontaine) is working on mitochondria, oxidative phosphorylation, cell death (mitochondrial permeability transition), and pathological energy disorders.

(3) "Metabolism, nutrition & exercise" is working on nutritional end exercise effects on metabolic regulation, nutritional status, nutrition of the elderly and nutritional supplements. LBFA also develops and applies integrative approaches, including innovative technologies (e.g. in vivo imaging with intracellular sensors, interactomics) and mathematical modeling within the Federative Structure - Environmental and Systems Biology (BEeSy).

Methodologies Used:
Recombinant protein expression, purification and biochemical/biophysical characterization. Experimental models of nutritional regimes, metabolic or energy disorders in vitro and in vivo (cell culture, mice, rats).
Proteomics (2D-PAGE etc., mass spectrometry) and transcriptomics (RT-PCR, microarrays).
Interactomics (innovative yeast-two-hybrid systems, surface plasmon resonance).
Microscopy (fluorescence, confocal).
Metabolic and metabolite analysis (metabolic cage with gas exchange and movement analysis, cell perfusion, oxygraphy, HPLC).

Publications:
**LBFA interdisciplinary research**

- UFR chimie-biology – pharmacy – APS – medicine
- From molecule to man
  - bench to bedside

**LBFA research axes**

- Energy signaling
  - AMP-activated protein kinase
  - Mitochondrial kinases and ATPases
- Systems bioenergetics
  - Energy homeostasis, mitochondrial, cardiovascular disease
- Mitochondria & cell death
  - Mitochondrial permeability transition
- Mitochondria & cell death
  - Iron homeostasis, mitochondrial, cancer
- Mitochondria & cell death
  - Diabetes and artificial pancreas
- Metabolism: nutrition & exercise
  - Nutrition, muscle & healthy aging
  - Maternal exercise

**SFR Environnemental and Systems Biology**

**Federal Research Structure (BEEsy)**

**Ambitions:**
- Developing the internationally emerging field of systems biology as a transversal axis in life science research at UJF
- Connecting present lines of research and to exploit the traditionally strong interdisciplinary research
- Developing the biology campus at St. Martin d'Hères
- Allowing close proximity between undergraduate education and interdisciplinary research laboratories
Translational research in nutrition and metabolic disease.

Research Brief:
The main objectives of our research program are 1) to understand at the molecular level the adaptive responses to changes in our nutritional environment and 2) to identify the potential defects in these processes that could contribute to the metabolic pathologies. To this aim, we investigate the adaptive mechanisms to metabolic stress and pollutant exposure under different experimental conditions reflecting as much as possible normal life. Based on the significant advances made over the last years, our strategy rely on 5 complementary aresearch programs:

1. Metabolic adaptation and inflammation of fat tissues during overfeeding to understand adipose tissue depots remodeling and to propose strategies to fight obesity and prevent its complications.
2. Adipose stem cells in healthy or pathological adipose tissues to understand the mechanisms initiating adipose tissue alterations and inflammation which lead to the complications of obesity.
3. Metabolic disrupters: impact of environmental pollutants in metabolic diseases to unravel the contribution and the mechanisms of action of pollutants in triggering or amplifying metabolic diseases.
4. Metabolic adaptations in chronic kidney disease to define the determinants of metabolic complications, especially insulin resistance, in chronic kidney disease.
5. Probiotics as a new strategy to fight metabolic diseases to demonstrate that selected bacterial strains can be powerful agents to treat obese and diabetic patients.

Methodologies Used:
- Nutrigenomics and gene expression analysis
- Nutritional interventions in humans and in animal models
- Microbiota and probiotics
- Epigenetics and chromatin organisation study
- Hormone signaling
- Skeletal muscle and adipose tissue cell culture

Publications:


Marie-Caroline Michalski
Philippe Moulin

Postprandial Lipids and Lipoproteins: Regulations and Functional Impacts

Université Claude Bernard Lyon
1 INSA-Lyon
Inserm U1060 INRA UMR1397
Hubert VIDAL
VILLEURBANNE

To facilitate and accelerate translational research by combining genetics, lipidomics and both in vitro and clinical experiments in order to explore lipid metabolism in patients and in healthy controls. To consider the molecular and supramolecular structures of dietary lipids in their effects.

Research Brief:
The main scientific objectives are to understand the mechanisms and the consequences of hypertriglyceridemia by studying both primary and secondary hypertriglyceridemia as well as postprandial hyperlipidemia. The team will focus on: 1) How dietary lipids, through their structure and oxidation, can metabolically impact on intestinal absorption, TGRL composition and lipolysis, and the metabolic fate of lipids in the postprandial phase. The role of specific lipids present in the gut on LPS coabsorption and biology of the gut cell lineage will be considered. 2) How TGRL modified by nutrition and/or altered by abdominal obesity/diabetes or malabsorption play a role in atherothrombotic and inflammatory processes both in the fasting and postprandial phase. The role of oxygenated species derived from DHA on ischemic cardiovascular disease will be studied. 3) How new genetic and epigenetic regulations interfere with TGRL lipolysis. Interactions between LPL/AV/GPIHBP1 on endothelial cells will be studied. Association studies in extreme phenotypes and segregation studies in families with unexplained familial chylomicronemia syndrome will be conducted to identify new genes involved in TG metabolism. Studies considering the role of miRNA in the regulation of lipolysis gene expression will be expended. This project will provide new dietary strategies to prevent the alterations of postprandial lipemia and identify new therapeutic targets for improving treatment of hypertriglyceridemia.

Methodologies Used:
- Nutritional interventions in humans and in animal models (mice and rats).
- Cell cultures Cell biology (transwell inserts).
- Next generation sequencing (386 gene chips) applied in the field of dyslipoproteinemia
- Lipoprotein isolations and platelet aggregation.
- Endotoxemia analysis.
- Lipidomic platform analyses (HPLC, GC, GC-MS/MS, LC-MS/MS).

Publications


Impact of dietary lipid structures on postprandial lipemia and metabolism

Genetics of hyperchylomicronemia: a tool for unraveling new TG-rich lipoprotein regulations

Functional impact of TGRL as vector of oxidized lipids on circulating cells and endothelium
CRNH Rhône Alpes

Human Nutrition Research Center

Université Claude Bernard
Lyon I
Inserrn, inra, CarMen
Julie-Anne Nazare
Lyon, Grenoble, Saint-Etienne

A center of excellence in human nutrition and health from preclinical to clinical research

Research Brief:
The CRNH Rhône-Alpes is a GIP, founded in 1996 and renewed until 2020. Partners are:
Research institutes (INSERM and INRA), Universities (Lyon 1, J. Fourier in Grenoble, J. Monnet in Saint Etienne), Hospitals (Hospices Civils de Lyon, CHU Grenoble and St Etienne).
The Human Nutrition Research Center Rhône-Alpes strives to improve human nutrition and health. It develops research programs in nutrition within the framework of national, european and international research programs, working closely with industrial partners and researchers worldwide.
The association of more than 200 people from 20 hospital services, 3 universities and research units (CarMen INSERM U1060-INRA USC 1362-INSA, INSERM U1042 and U1055, EA 4607 SNA-EPIS) makes it possible to set up studies on priority Public Health matters such as obesity, diabetes, cardiovascular diseases and malnutrition associated with chronic diseases and extreme old age.
The CRNH Rhône-Alpes is involved in all the major fields of nutrition research through very close partnerships with other CRNHs (Auvergne, Ile de France, Ouest) and with the research center of the Paul Bocuse Institute (food behaviour/experimental restaurant).
All these research strengths are associated in the CENS (European Center for Nutrition and Health): a consortium of scientists and clinicians specialized in nutrition, together with industrial partners to address health and societal challenges at an international level.

Methodologies Used:
Nutritional intervention in healthy subjects or patients and metabolic phenotyping (OGTT, clamps for insulin sensitivity determination; Bio impedancemetry, labeled water, DEXA, imaging for body composition determination)
Stable isotopes technology for substrate turnover and food bioavailability determined by mass spectrometry (GCMS, GCIRMS)
Indirect calorimetry, doubly labeled water, actimetry and questionnaires for energy expenditure measurement
Muscle and/or adipose tissue biopsies for genomics and proteomics analyses
Access to MRI and PET MRI

Publications
Borel AL, Pépin JL, Nasse L, Baguet JP, Netter S, Benhamou PY (2013). Short sleep duration measured by wrist actimetry is associated with deteriorated glycemic control in type 1 diabetes., Diabetes care. 36(10), 2902-8
François Leulier

Functional genomics of host/intestinal bacteria interactions

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(Université Claude Bernard) Ecole Normale Supérieure de Lyon
CNRS
Vincent Laudet
Lyon

We have developed an original model to study host/microbiota interaction which has a great potential for functional and mechanistic studies thanks to its simplicity and genetic tractability

Research Brief:
Metazoans establish reciprocal interactions with their commensal bacterial communities (i.e microbiota). Despite recent progress, a clear view of the physiological benefits associated with host/microbiota relationship remains elusive. Hence the molecular mechanisms through which the microbiota exerts its beneficial influences are still largely undefined. Hence, the goals of our research programs are two folds:
(1) Decipher the molecular dialogue governing the mutualistic interaction between intestinal bacteria and their host. To this end, we are using an animal model, Drosophila melanogaster and one of its natural commensal, Lactobacillus plantarum. We are developing a multiscale functional approach to identify the mechanisms that underly their mutualistic relationship by initially focusing on L.plantarum mediated host juvenile growth promotion. Our approaches aim at identifying both the bacterial and host genetic networks required to sustain a mutualistic relationship. We are also translating our discoveries to mammalian models by studying the impact of selected strains of L.plantarum on mice juvenile growth.
(2) Identify and characterize new potential Lactobacilli probiotic strains beneficial to host biology by mean of in vivo functional screens using the Drosophila model and tailored bacterial experimental evolution strategies to generate "optimized" probiotic strains.

* Methodologies Used:
Drosophila and Mouse gnotobiology
Functional genomics
Drosophila and Lactobacilli forward and reverse genetics
Experimental Evolution.
Nutritional manipulation

Publications


**Research Brief:**

Maintaining vascular function is essential in preserving health and healthy aging. Early vascular dysfunction is associated with initiation of atherosclerosis. CVD development and contributes to the occurrence of type 2-diabetes and cognitive decline. Therefore, it is of great importance to identify innovative and scientifically sound dietary strategies to prevent vascular dysfunctions. Evidence from large prospective cohort studies have pointed out that dietary bioactive plant compounds contribute to the cardiovascular protective effects of diets rich in plant foods. However, the actual impact of most of these compounds in human and their mechanisms of action have not yet been fully established. In this context, using a translational approach and combining classical and "omics" methodologies, the research of the NutriVasc team focuses on understanding the role of plant food bioactives in the prevention of vascular dysfunctions, with as major objectives:

1. Provide clinical evidence of the role of bioactive compounds (polyphenols, phytoprostans) in the prevention or delay of vascular dysfunction and unravel the complexity of the underlying cellular and molecular mechanisms of action.
2. Characterize the complexity of the individual exposure to plant bioactive metabolites by the Food Metabolome approach.
3. Identify the main determinants of inter-individual variation in the response to the consumption of plant food bioactives (bioavailability and vascular protective effects).

**Methodologies Used:**

- Randomized controlled trials in humans / Animal models of atherosclerosis and cell culture (endothelial cells, monocytes, macrophages) / Non-invasive assessment of vascular function (endothelial function in micro and macrocirculation, arterial stiffness) / Biological evaluation (biochemical analysis, immuno-histochemistry, histology, flow cytometry) / Molecular and cellular analyses: gene expression (qPCR, transcriptomics), protein expression (Western blotting, proteomics), chemotaxis, monocyte adhesion, transendothelial migration, macrophage polarization / Targeted lipidomics based on LC-MS/MS mass spectrometry to characterize lipid mediators / Metabolomics based on high resolution mass spectrometry to characterize the food metabolome.

**Publications**


Outlines of the research developed in the NutriVasc Team

Identification of biomarkers of exposure - Food metabolome

Determinants of inter-individual variability

Physiological effects and mechanisms of action on vascular function

Relationship between diet, exposure and health

Identification of biomarkers of health responsive to diet

Exemples of key findings

First clinical evidence of the role of flavanones in the beneficial effect induced by citrus foods consumption on vascular function

Hesperidin reduces DBP
Hesperidin contributes to the improvement in microvascular reactivity

Naringin is responsible for the beneficial effect of grapefruit on arterial stiffness

Identification of cellular and molecular targets of dietary polyphenols

Orange juice and hesperidin modulate expression of genes in humans white blood cells

In human and in mice polyphenols affect expression of apoptosis-related cell adhesion and transendothelial migration.
Our team has a strong expertise in studying the mechanisms responsible for the maintenance of protein/amino acid homeostasis during physiological and catabolic states.

Research Brief:
Numerous diseases (cancer, sepsis, ) and aging are frequently associated with a dysregulation of amino acid and protein homeostasis. The main consequence is a catabolic state that strongly contributes to the deterioration of patients health and compromises treatments. The main objectives of the team are to understand the mechanisms involved in maintaining protein/amino-acid homeostasis. The endpoint is to develop pharmacological and nutritional strategies to prevent and/or attenuate protein/amino acid homeostasis dysregulations.

Our work focuses on three complementary research themes studying different aspects of the regulation of the metabolism of amino acids and proteins during several physiological and pathological situations (perinatal nutrition, muscle wasting, food intake disorders, aging, ): -Characterization of the molecular mechanisms involved in adaptation to variations in amino acid availability. -Regulation of tissue protein metabolism: protein synthesis and proteolysis (ubiquitin-proteasome system, autophagy). -Regulation of inter-organ relationships for the use of amino acids.

Methodologies Used:
Cellular and molecular biology 
Gene knock-down and over-expression in vivo and in vitro (electroporation, viral vectors, )
Proteolysis and protein synthesis determination 
Gene expression measurement (qPCR, polysome analysis)
Metabolomics / proteomics
In situ hybridization and immunohistochemistry 
Recombinant protein production and purification 
Biomolecular interaction studies (Surface Plasmon Resonance, Y3H and Y2H, etc.)
Nutritional interventions in humans and in animal models 
Food digestion and digestibility in cannulated animal models
In vivo nutrients fluxes evaluation
Access to human cohorts

Publications


Deval C, Capel F, Lallet B, Polge C, Béchet D, Taillandier D, Attia D, Combaret L (2016). Docosahexaenoic acid-supplementation prior to fasting prevents muscle atrophy in mice, J Cachexia Sarcopenia Muscle. 7(), 587

Protein/Amino Acid homeostasis

Our goal is to understand the mechanisms responsible for the maintenance of protein/amino acid homeostasis during physiological and catabolic states.

Integrative approaches using various models and tools

Regulating the expression of therapeutic transgenes by controlled intake of dietary essential AA

Measurement of the Lactoferrase activity in the liver and pancreas of mice transduced with a vector expressing Lactoferrase driven by a promoter activated by amino acid starvation. Mice were subjected to a control (Ctrl) or a ile deficient diet (Ile). See Chaveroux et al 2016
Fatima Djouadi  Jean Bastin

Inborn mitochondrial disorders: pharmacological therapy and metabolic signaling

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INSERM U1124
Robert Barouki
Paris

Research Brief:
Our objective is to identify drugs or natural compounds which could be beneficial for correction of inborn fatty acid ß-oxidation (FAO) or respiratory chain (RC) defects, a large group of genetic disorders associated to life-threatening presentations, or to milder late-onset phenotypes, without treatment in most cases. We favor a pre-clinical approach performed in patients’ cells (fibroblasts, myoblasts, myotubes) representing different enzymes defects associated to various mutations/genotypes. Our rationale is to test candidate molecules selected for their potential to stimulate or mitochondrial functions, in order to see if exposure to these molecules can improve residual FAO or RC capacities in panels of patients cells. We already proved that bezafibrate, a widely prescribed hypolipidemic drug, can correct mild FAO or RC deficiencies by activation of PPAR nuclear receptors, both ex-vivo in fibroblasts, and through a pilot clinical trial in FAO-deficient patients. Later on, resveratrol, a natural plant polyphenol, was also found to potentially correct mild FAO or RC deficiencies through complex metabolic signaling pathways involving the ERR orphan receptor and the PGC1alpha transcription co-activator. Presently, we focus on screening in patient cells the effects of natural (berberine, quercetin) or synthetic (AICAR, metformin, thiazolidinediones) compounds that potentially target the AMP-activated protein kinase, a major sensor and regulator of energy metabolism.

Methods Used:
Measurements of metabolic fluxes (tritiated palmitate oxidation and oxygen consumption rates) in cultured patients’ fibroblasts and myoblasts/myotubes. Pharmacological screening of selected compounds by dose-response and kinetics studies in control and in patients’ cells. Relation genotype/pharmacological responses, genotype-based pharmacological screening. Determination of substrates’ concentrations (glucose, lactate, NAD/NADH, etc...) and enzyme assays (Krebs cycle, pentose-P pathway, respiratory chain complexes, antioxidant enzymes, etc...) by spectrophotometric and fluorimetric methods in 96-well plates. Quantitative PCR, Western-blot, immunoprecipitation,
Frédéric Bouillaud
Mitochondria, bioenergetics, metabolism and signaling

Université de Paris 05
(Université Rene Descartes)
CNRS UMRB104. Inserm U1016
Pierre-Olivier Couraud
Paris

Our general objective is to study the crosstalk between mitochondrial function, metabolism and diseases by deciphering the molecular mechanisms involved in mitochondrial adaptation to intrinsic and/or environmental insults.

Research Brief:
The aim of the team is to study how mitochondrial bioenergetics constitutes a determining factor for complex phenotypes. We address two different situations:
1) Bioenergetics is known to be the primary target of genetic defects, environmental or endogenous modifying factors; we then analyze how bioenergetic machinery (essentially mitochondria) is affected and subsequently both deleterious impact and potential compensatory responses at the cellular level.
2) Bioenergetics appears modified in pathological states (over-nutrition, obesity, diabetes, cancer?). Firstly, one should substantiate and characterise the qualitative and quantitative bioenergetic changes associated to the pathological state. Then the question of bioenergetic’s role is to be considered: does it constitute an adaptive response, an aggravating factor or could it be directly causative of the pathological state.

Our models are derived from human mitochondrial diseases or are based on modulation of specific genes (Ucp2, Cpt1, Sqr).

We consider that the transport of substrates across the mitochondrial is a critical control point and consider two systems the mitochondrial carrier UCP2 and the carnitine palmitoyl transferase 1 (CPT1).

Our studies on sulfide bioenergetics illustrates the intricacy between toxic, signaling and adaptive components.

Regular collaboration with teams needing to approach mitochondrial bioenergetics often provides additional models.

* Methodologies Used:
Respirometry (Oroboros and Seahorse)
Metabolic fluxes
ROS and membrane potential fluorescent probes
Molecular biology : recombinant DNA & immunodetection
Transgenic models

Publications


Bouillaud F, Alves-Guerra MC, Ricquier D (2016). UCPs, at the interface between bioenergetics and metabolism, Biochim Biophys Acta - Molecular Cell Research. 1863(10), 2443-56

Mario Pende
Cell growth control by nutrients
Paris Descartes
INSERM U1151 U1151
Xavier Nassif
Paris

**Key facts**

**Team**
- Researchers: 3
- Technicians: 2
- Postdoc fellows: 5
- PhD Students: 5

**Translational approaches**
- Patents: 0
- Clinical research grants: 0
- Industry partnerships: 0

**Keywords**
- Growth
- Signal transduction
- mTOR
- Biochemistry
- Cell biology
- Mouse models

**Biological Resources**
- Mouse models

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**Functional studies to dissect the growth and metabolic control by signal transduction pathways**

**Research Brief:**
In metazoans, nutrient and growth factor availability control cell number, size and metabolic homeostasis. We investigate the specific programs underlying these responses, and their coordination by signal transduction mechanisms.

**Methodologies Used:**
- Mouse models of cancer and metabolic diseases
- Metabolomics
- Genome editing
- Viral vectors
- Signal transduction
- Autophagy flux
- Translation

**Publications**


Barilari M., Bonfils G., Treins C., Koka V., De Villeneuve D., Fagrega S., Pende M. (2017). ZRF1 is a novel S6 kinase substrate that drives the senescence program, EMBO Journal. ()

**Key facts**

**Team**
- Researchers: 14
- Technicians: 8
- Postdoc fellows: 4
- PhD Students: 4

**Translational approaches**
- Patents: 3
- Clinical research grants: 5
- Industry partnerships: 5

**International research links**
- METACARDIS
- Epos
- CMDO (Canada)

**Keywords**
- Nutrition
- Inflammation
- Microbiota
- Obesity
- Fibrosis
- IHC
- Cell culture
- Data mining and integration
- Transcriptomics, (meta)genomics
- animal models

**Biological Resources**
- Peripheral and portal blood
- urine and feces
- DNA, RNA banks (human and bacteria)
- Tissues (adipose tissue depots, liver, intestine)
- PBMC, adipose cells, Immune cells

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**Targeting obesity: From bench to bedside.**

**Research Brief:**
Nutriomics team is focused on the understanding of the patho-physiological mechanisms associated with obesity at its different stages of its natural progression. Since obesity is now recognized as a systemic disorder, the Nutriomics team develops and combines large-scale approaches with innovative bioinformatics and complex systems modeling tools in samples collected from well-phenotyped obese subjects with various metabolic disorders and disease stages. This strategy generates hypotheses on putative new cellular and molecular actors, further investigated by in vitro and ex vivo models and generated knockout mice models. By using these approaches, we identified and proved unexpected capacities of adipose tissue i) to accumulate immune cells, ii) to drive low-grade inflammation and iii) to promote extracellular matrix remodeling and fibrosis, which directly contribute to obesity and its complications. More recently NutriOmic team investigated the importance of environmental changes including nutritional switches on human metabolism and inflammation via modification of the gut microbiota. This last transversal theme uses bioinformatics approaches with multilevel data integration to ensure exploitation of research results towards the identification of signatures associated with obesity and comorbidity stages. Nutriomics is composed of an interactive group of multi-disciplinary PhD, MD-PhD scientists who work in the state-of-the-art facilities.

**Methodologies Used**:
- 2D and 3D Cell cultures, migration, proliferation, differentiation
- Cell biology (western blots, RT-PCR, immunofluorescence, confocal microscopy)
- Immunochemistry, cell sorting FACS
- siRNAs transfection, ChIPs, microarray
- Bioinformatics: predictive analysis, network analysis, data integration, data mining
- Physiology in animal models, genetically modified animals
- Metagenomics, genomics, Genotyping, metabolomics, lipidomics

**Publications**


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Karine Clement
Nutriomique Team
Université de Paris 06
(Université Pierre et Marie Curie)
Inserm U1166
Stéphane Hatem
Paris
In obese subjects, white adipose tissue (WAT) fibrosis represents a maladaptive mechanism contributing to the loss of metabolic fitness and obesity-associated comorbidities. Marcelin et al. demonstrate that elevated PDGFRα+ progenitor subsets with high expression of CD9 promote WAT fibrosis and associate with metabolic deteriorations.

Marcelin et al, Cell Metab 2017
Philippe Lesnik
Integrative biology of cardiovascular and metabolic diseases

Université de Paris 06
(Université Pierre et Marie Curie)
Inserm
Stéphane Hatem
Paris

**Key facts**

**Team**
- Researchers: 8
- Technicians: 3
- Postdoc fellows: 5
- PhD Students: 1

**Translational approaches**
- Patents: 2
- Clinical research grants: 2
- Industry partnerships: 3

**Keywords**
- Lipoproteins
- Lipids
- Lipid sensors/receptors
- Inflammation
- Immunity
- Macrophages
- Metabolic
- Metabolic phenotyping of mouse models
- Reverse cholesterol transport in vivo and in vitro.

**Biological Resources**
- Cohorts of dyslipidemic patients.
- Bank of mRNA from human Monocytes.
- Experimental models of atherosclerosis.
- Genetically-modified mice (tg CD68-hBcl2, tg CD11c-hBcl2, SR-BI flox/flox, ABCG1 flox/flox).

**Research Brief:**

Our research goals are based on the premise that lipid-related inflammation and the associated immune responses are dominant components in atherogenesis. The underlying pathogenesis involves an imbalance of lipid and lipoprotein metabolism and a maladaptive immune response entailing a chronic inflammation of the arterial wall. The validity of this premise is becoming increasingly stronger as basic and clinical data demonstrate disturbed equilibrium of lipid metabolism and immune responses and resolution, shaped by lipoprotein retention, leukocyte trafficking and homeostasis. Our research focus on the clarification of cellular and molecular mechanisms of such lipido-inflammatory and immune responses, with a goal that new diagnostic and therapeutic approaches will emerge from this work. Indeed new reliable biomarkers allowing monitoring of the critical stages of vascular remodeling and thereof of potential complications are urgently required.

Novel molecular mechanisms, translational development and clinical strategies for studying lipid-related inflammation in atherosclerosis and vascular disease represent three major axes of our research program:

Axe 1: To determine how lipids lipoproteins and immune cells crosstalk to influence atherogenesis.
Axe 2: To assess the clinical relevance of novel mechanisms, genes, and biomarkers by studies of human diseases.
Axe 3: To develop novel therapeutic strategies for inflammatory and metabolic disorders and atherosclerosis.

**Methodologies Used:**
- Development of mouse models for cardiometabolic diseases.
- Metabolic phenotyping of mouse models.
- Transcriptomic, Lipidomic, Metabolomic, Metagenomic, Epigenomic, Multivariate analysis.
- Phenotyping and quantification of circulating and tissue leucocytes: flow cytometry/cell sorting.
- Reverse cholesterol transport in vivo and in vitro.

**Publications**


**Expertise in lipoprotein metabolism, atherosclerosis, vascular diseases and mononuclear phagocytes.**
BENEFICIAL OR DETRIMENTAL IMPACT OF LIPID & MONONUCLEAR PHAGOCYTES ON METABOLIC DISORDERS

CHOLESTEROL HOMEOSTASIS IN MONONUCLEAR MACROPHAGES

OVERVIEW OF THE LIPOPROTEIN METABOLISM
Olivier Berteau

ChemSyBio

Université Paris Sud : Paris
11
INRA UMR1319
Stéphane Aymerich
Jouy en Josas

Our team uses biochemical and chemical approaches to solve the mechanism of novel enzymes.

Research Brief:
The ChemSyBio team is investigating novel enzymes catalysing unprecedented post-translational modifications. These enzymes use radical chemistry notably to produces various antibiotics, anti-cancer agents and toxins.

Methodologies Used:
Mass spectrometry, structural biology.

Publications


Novel synthesis of post-translationally modified peptides
Key facts
Team
- Researchers: 9
- Technicians: 3
- Postdoc fellows: 0
- PhD Students: 4
Translational approaches
- Patents: 0
- Clinical research grants: 2
- Industry partnerships: 5
International research links
- Wageningen University
- SCIC, Madrid
- Birmingham University

Keywords
- Food reward
- Satiety
- Glucose homeostasis
- Energy metabolism
- Protein digestion and metabolism
- Metabolic and ingestive behaviour phenotyping
- Molecular biology
- Indirect calorimetry
- Stable isotopes
- Imagery

Biological Resources
- Human volunteers
- Primary cell culture
- Rodent models

Claire Gaudichon
Protein metabolism, energy homeostasis and ingestive behavior
AgroParisTech  Paris-Saclay
INRA UMR0914
Daniel Tomé
Paris

Our team addresses the influence of quality and quantity of protein intake on metabolic, physiological and behavioral responses, using a large panel of approaches (metabolic fluxes by the way of tracers and calorimetry, molecular biology, neurobiology) in rodents and humans.

Research Brief:
Protein intake plays an important role in protein and energy homeostasis. Quantity and quality of protein influence the regulation of protein pathways in different tissues (intestine, liver, muscle, kidney, brain, …) and also interact with glucose and lipid homeostasis, subsequently affecting lean and adipose tissue distribution. Dietary proteins are also involved in different signals interfering with dietary intake, either directly through homeostatic centers or indirectly through food reward. Our team studies the different pathways by which protein intake interacts with caloric intake and with protein and energy metabolism to achieve homeostasis.

In the past years, we provided important integrative knowledge on the adaptive responses to high protein diets that had been proposed as strategies in weigh management. We currently address the consequences of low protein intake on energy homeostasis, given that protein resources are worldwide a main concern for food insecurity.
Moreover, our team is recognized for its strong expertise in the in vivo assessment of protein quality depending on the protein source as well as technological treatments.
Clinical studies are mostly realized in the Research Human Nutrition Center, in Bobigny. Rodent studies are realized in our own animal care facility.

Methodologies Used:
- In vivo exploration of protein digestion and metabolism as well as energy metabolism, using isotopic tracers, gastrointestinal tubes and indirect calorimetry
- Exploration of signaling pathways, especially in intestine, liver and brain, using classical molecular approaches and genetic models
- Phenotyping of ingestive behavior and metabolism using multiscale criteria in rodents
- Development of obesity resistant and prune rodent models
- Exploration of satiety and food reward system using imagery and psychobiological approaches in humans

Publications
Serge Luquet

Central Control of Feeding Behaviour and Energy Expenditure

C2OFFEE

Université de Paris 07
(CNRS UMR 8251)
Jean-Marie Dupret
Paris

**Fully integrated approaches are combined with genetic tools to study the mechanism that link the central nervous system with the regulation of energy balance and peripheral glucose metabolism.**

**Research Brief:**

The core approach of my research group C2OFFEE (http://www.bfa.univ-paris-diderot.fr/spip.php?rubrique81&lang=en) is to leverage the power of modern molecular genetic tools and mouse models in integrated approaches in order to dissect out the role of discrete neural circuit elements in the control of different aspect of energy balance including feeding behavior notably in its rewarding & motivational component together with energy expenditure and nutrient partitioning. A recent achievement was to identify a novel role for a hypothalamic circuitry in AgRP-neurons in the coordination of efferent organ activity and nutrient partitioning, providing a mechanistic link between obesity and obesity-related disorders. In addition we recently demonstrated that when AgRP-neurons ‘activity is compromised through genetic, pharmacologic or dietary intervention (such as diet-induced obesity)-feeding behaviour is no longer dependent on metabolic demands but prominently rely on dopamine-encoded reward and leads to compulsive/comfort feeding. Finally we also highlighted a unique mechanism by which nutritional lipids can directly act on the brain to modulate food reward as a possible mechanism for addictive-like behaviour associated with high fat diet.

**Methodologies Used:**

- Viral-mediated genetic modification of brain nuclei through stereotactic approaches, optogenetic and pharmacogenetic approaches
- Neurons-specific depletion (genetic engineering of Diphtheria receptor specific expression)
- In vivo indwelled chronic perfusion (carotid & jugular vein, intracerebroventricular)
- In vivo analysis of insulin sensitivity (euglycemic hyperinsulinemic clamp, insulin tolerance test)
- Microsurgery (catheter, cannula implant, vagal deafferentation, bariatric surgery in mice)
- In vivo assessment of motivated behaviour and positive reinforcement (conditioned place preference and operant behaviour)
- In vivo assessment of metabolic efficiency and energy balance using integrated indirect calorimetry

**Publications**


**International research links**

- USA
- Germany
- Netherlands

**Translational approaches**

- Patents: 0
- Clinical research grants: 0
- Industry partnerships: 7

**Key facts**

**Team**

- Researchers: 4
- Technicians: 4
- Postdoc fellows: 3
- PhD Students: 1

**Biological Resources**

- Mouse model for conditional inactivation of the N-acylphosphatidylethanolamine phospholipase-D (NAPE-PLD), a key enzyme in the processing of endocannabinoid and N-acyl ethanolamide such as oleylethanolamide (OEA).

- Model for conditional expression of Peroxisome Proliferator-Activated Receptors delta (PPARδ) or a dominant negative form of this receptor

**Keywords**

- Energy balance regulation
- Central nervous system
- Mice model for neuron-specific cell knock out
- Euglycemic hyperinsulinemic clamp study

**Translational approaches**

- Patents: 0
- Clinical research grants: 0
- Industry partnerships: 7

**International research links**

- USA
- Germany
- Netherlands
The core approach of my research group C2OFFEE (http://www.bfa.univ-paris-diderot.fr/spip.php?rubrique81&lang=en) is to leverage the power of modern molecular genetic tools and mouse models in integrated approaches in order to dissect out the role of discrete neural circuit elements in the control of different aspect of energy balance including feeding behavior notably in its rewarding & motivational component together with energy expenditure and nutrient partitioning.
Lionel Bretillon  Catherine Creuzot-Garcher

Eye, Nutrition & Cell Signalling

Université Bourgogne
Franche-Comté  AgroSup Dijon
INRA UMR1324  CNRS UMR6265
Lionel Bretillon
Dijon

Through a translational research approach, our team aims to transform scientific discoveries arising from laboratory on the role of both endogenous and dietary lipids in the retina into clinical applications in the prevention of aging of the retina.

Research Brief:
The demographic forecasts expect the elderly population to increase sharply in the next decades. Since eye diseases are the second most prevalent pathologies after the age of 65 years in Western countries, patients suffering from ocular pathologies are expected to represent a sensitive and growing socio-economic burden. Among those pathologies, age-related macular degeneration (AMD) and glaucoma are the leading cause of visual loss. Aging of the retina is characterized by specific clinical, functional and morphological features. Although lipids are key components of the retina, their roles are not fully defined. Lipids may both promote and prevent aging of the retina. Epidemiological studies have reported that dietary omega 3 fatty acids prevent from the development of AMD. Through a translational research approach, our team aims to transform scientific discoveries arising from laboratory on the role of both endogenous and dietary lipids in the retina into clinical applications in the prevention of aging of the retina. Our projects aim to delineate whether lipids ? namely plasmalogens, cholesterol, and gangliodides ? and lipid metabolism participate in the functioning and dysregulations of the retina. The projects focus on 1) the mechanisms of lipid uptake to the retina, 2) the metabolic pathways that involve lipids as cell mediators in the retina, and 3) the links between pathologies and dysregulations of the lipid metabolism in the retina.

* Methodologies Used:*
- Patient evaluation
- Animal and cell culture experiments
- Electroretinography, funduscopy, angiography, tonometry
- Biological evaluation (qPCR, Western-blotting, flow cytometry)
- Chromatography (thin-layer, gas, high performance liquid), in tandem with mass spectrometry

Publications


Alexandre Benani

Plasticity of brain feeding circuits

AgroSup Dijon Université de Dijon
(Université de Bourgogne)
CNRS INRA
Lionel Brétillon
Dijon

Key facts

Team
• Researchers : 3
• Technicians : 2
• Postdoc fellows : 0
• PhD Students : 2

Translational approaches
• Patents : 0
• Clinical research grants : 0
• Industry partnerships : 0

Keywords
• Obesity
• Food intake
• Neurobiology
• Hypothalamus
• Vulnerability
• Feeding behaviour
• Molecular biology
• Histology
• Stereotaxy
• Tolerance tests

Biological Resources
• AgRP-cre mice
• POMC-cre mice
• RiboTag mice
• St8sia4 KO mice

Research Brief:
The team aims at a better understanding of the neurobiological basis of feeding behaviour.
First, we want to provide details about the structure of neuronal circuits that control food intake.
Second, we want to characterize the morphological plasticity of these networks (i.e. synaptic remodeling, modification of neuro-glial interactions, neurogenesis). We have shown that the structural plasticity of these networks is an essential element in the adaptive control of food intake. Indeed, reduced ability to structural plasticity in these networks could be a risk factor in obesity and related disorders.
Third, we want to characterize the influence of various internal and external factors on the feeding behaviour, such as the metabolic state (effect of overeating), the nutritional history (perinatal imprinting, food experience in adulthood), and pathological context (metabolic diseases, major depression, cachexia).

* Methodologies Used:
Use of standard and transgenic murine models.
Behavioral analysis (food intake, size and frequency of meals, satiety, preferences) and functional investigation (metabolic performance).
Molecular biology (gene regulation, chromatin remodeling, epigenetic).
Histology (neuroanatomy, neuronal tracing, cFos detection, immunohistochemistry).
Targeted intracranial manipulations (stereotactic injections of drugs and viral tools, shRNA-mediated silencing, pharmacogenetic).

Publications


New insight into the mode of action of some metabolic cues

Models and skills

Whole animal physiological studies

Nutritional conditions
- macronutrients, micronutrients, energy expenditure

Feeding behavior
- meal size, meal frequency, interval

Interventional studies for causal relations
- treatment with or without specific nutrients, pharmacological, genetic, etc.
Bruno Vergès

Pathophysiology of dyslipidemia (PADYS)

Université de Dijon
(Université de Bourgogne)
Inserm U1231 CHU
Laurent Lagrost
Dijon

The work of our team is dedicated to the study of the pathophysiology of dyslipidemia in humans, mostly dyslipidemia associated with diabetes and insulin-resistance.

Research Brief:

For more than 10 years, our team has been working on the pathophysiology of dyslipidemia in humans, mostly dyslipidemia associated with diabetes and insulin-resistance. Our team includes scientists and also physicians who are working on both sides (clinic and research). This allows performing many translational studies "from bed to bench". Our main research activities within the field of "pathophysiology of dyslipidemia in humans" are built in 3 axes: 1) dysfunction of lipid metabolism in diabetes and insulin resistance (studied with in vivo human lipoprotein kinetic studies); 2) study of HDL in diabetes and insulin resistance; 3) involvement of the endocannabinoid system (ECS) in diabetes and insulin resistance.

During the 4 previous years we have shown:
- in type 2 diabetes, that hyperinsulinemia is not responsible for increased VLDL secretion and that rbp4 is an independent factor reducing VLDL catabolism
- that both catabolism and production of VLDL1-TG are independent determinants of HDL catabolism in the Metabolic Syndrome
- the importance of peripheral ECS on lipid and glucose metabolism and that ECS activation decreases fatty acid oxidation and increases adipose tissue lipolysis by altering the antilipolytic action of insulin
- that glycation and oxidation of HDL induces the loss of their vasorelaxant effect
- significant reduction in sphingosine-1-phosphate in HDLs from patients with type 1 diabetes or subjects with the Metabolic Syndrome.

Methodologies Used:

In vivo kinetic studies in humans with stable isotopes
Ex vivo on vasorelaxation using rabbit aorta rings
Animal studies
Cell culture
Hepatic tissue explants

Publications


Key facts

Team
- Researchers : 8
- Technicians : 8
- Postdoc fellows : 1
- PhD Students : 6

Translational approaches
- Patents : 0
- Clinical research grants : 0
- Industry partnerships : 3

Keywords
- Eating behavior
- Chylomicrons
- Energy metabolism
- Lipid-binding proteins
- Estrogenic contaminants
- Lipid sensing
- Lipid signalling
- Taste buds
- Small intestine
- Obesity
- Integrative physiology

Biological Resources
- Transgenic mice
- Caco2, immortalized mouse taste bud cells

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Naim Khan

Physiology of Nutrition & Toxicology (NUTox)

Université de Dijon
(Université de Bourgogne)
Inserm U1231 CHU
Laurent Lagrost

Dijon

**Exploration of the molecular and cellular mechanisms responsible for the chemodetection of dietary lipids along the oro-intestinal tract: impacts of estrogenic food contaminants and physiological or pathological consequences on the lipid metabolism, eating behavior and obesity risk**

Research Brief:
The oro-intestinal tract plays a major role in the regulation of energy balance by controlling nutrient bioavailability and eating behavior. Recent data from our team and other investigations support the existence of a specific sensing system responsible for a real-time detection of lipids in ingested foods both in oral cavity and intestinal lumen. We have shown that the plasma membrane receptor CD36 plays a significant role in this detection as a lipid sensor involved in regulation of the spontaneous fat preference, digestive secretions and quality (size and number) of chylomicrons produced during the post-prandial period. Chemoreception of dietary lipids by the oro-intestinal tract appears to be complex since other lipid sensors candidates (e.g. GPR120) have recently been identified in these tissues. Interestingly, estrogenic contaminants of food contact materials might disturb this lipid sensing system by impairing the function of taste buds and small intestine. Our objective is to determine the respective role(s) of these lipid sensors and explore whether dysfunction in this oro-intestinal lipid sensing system leads to physio-pathological states increasing obesity risk and prevalence of associated plethora diseases. A better understanding of these mechanisms might leads to the development of novel therapies.

**Methodologies Used:**
- Metabolic and behavioral phenotyping (indirect calorimetry, Echo MRI, lickometers)
- Micro-surgery in the mouse
- In situ isolated intestinal loop (intestinal lipid absorption)
- Postprandial triglyceridemia, CD36 methylation,
- Organ and cell cultures, genetic polymorphism
- Molecular and cellular biology
- Cell signalling
- Clinical studies in humans

**Publications**


David Dombrowicz

Nuclear receptors, immuno-inflammation and cardiometabolic diseases

Université de Lille
Inserm UMR1011 Institut Pasteur de Lille
Bart Staels
Lille

Research is centered on the regulation, by nuclear receptors, of immune cell contribution to cardiovascular diseases, atherosclerosis and type 2 diabetes.

Research Brief:
Building on our experience in allergic diseases and the immuno-regulatory role of nuclear receptors in asthma and atopic dermatitis, we develop a research on immuno-inflammation in cardio-metabolic diseases, atherosclerosis and type 2 diabetes.

1. We study the regulation by FXR and RORalpha of immune cell functions as well as of the development of atherosclerosis using whole body or cell-specific deletion of these genes in mice and feeding with high fat western diet. Expression of these genes in distinct lymphoid subsets downregulate metabolic inflammation.

2. Using both experimental models and a translational approach, we investigate the link between psoriasis, an inflammatory skin disease, and cardiovascular disease. We demonstrate that high fat diet increases psoriasis severity by altering innate and adaptive immune response through metabolic reprogramming.

3. We characterize the impact of type 2 diabetes on blood and adipose tissue immune cell subpopulations in obese patients with Non Alcoholic SteatoHepatitis and correlate blood phenotype with clinico-biological parameters as well as adipose tissue and liver transcriptome.

*Methodologies Used:
Genetically-modified mice
Pharmacology
Histology and Immunohistochemistry
Flow cytometry
Real-time PCR
Invasive and non invasive plethysmography
Laser capture microdissection

Publications


HFD feeding exacerbated allergic asthma. Obese mice show increased lung ILC and eosinophilia compared to lean mice. Lung ILC2 and ILC3 further increased in HDM-challenged obese mice compared to lean mice, with high IL-33 and IL-17 levels and decreased ILC markers in visceral adipose tissue. ILC depletions followed by T-cell reconstitution, led to a decrease in allergic asthma in obese mice, including TH2 and TH17 infiltration. (Julia et al. Nat. Rev. Immunol. 2015 & Everaere et al. JACI. 2016)

Receptor-induced eosinophil activation in inflammatory, infectious and oncologic diseases

Through release of cytotoxic mediators and cytokines, eosinophils exert a detrimental role in inflammatory diseases but are beneficial in immunity against helminths, mycobacteria and tumors. Cytotoxic and regulatory activities are triggered through the expression of several receptors associated to innate (TLR, TCRgammadelta) or acquired (FcR) immunity. (Decot et al. and Karagiannis et al. J. Immunol. 2005 and 2007; Legrand et al. PlosOne 2009; Driss et al. Blood 2009; Kanda et al. JACI. 2009)

Fractalkine and its receptor in allergic diseases

In lung and skin, antigen-specific Tcell migration is CX3CR1-independent and CX3CR1 is only expressed once T cell reach the tissue. Interaction with CX3CL1, its unique ligand, expressed by epithelial or smooth muscle cells allows T cell survival within the inflamed lung while it regulates retention of Th1 and Th2 cells in skin. A CX3CR1 antagonist blocks survival signal and prevents airway hypereactivity and inflammation (Mionnet et al. Nat. Med. 2010 & Staumont-Salle et al. J. Exp. Med. 2014)
Pierre Déchelotte

Nutrition, inflammation and dysfunction of the brain-gut axis

University of Rouen Normand
Inserm U1073
Pierre Déchelotte
Rouen

Our research focuses on inflammatory bowel diseases and eating disorders performing translational research in close relationship between academic units, clinical departments and industrials to develop rapidly new therapeutics.

Research Brief:
The research of our group, integrated in the IRIB Institute, are mainly dedicated to the study of eating disorders, irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD). These pathologies share common features of intestinal inflammation and dysregulation of the gut-brain axis and offer great opportunities for innovative therapeutic strategies (immunonutrition, neurostimulation). Our projects explore the underlying mechanisms of these diseases from the molecular level to clinical trials. This broad range of research from the bench to the bedside is the result of our research strategy that brings together basic and clinical sciences, in a tight collaboration between laboratory scientists and health professionals. Our dynamic research team has an active research strategy which also drives the translation of research from the laboratory to patenting and creation of biotech company entrepreneurship (TargEdys).

Methodologies Used:
Intestinal models: cell lines, human intestinal explants, experimental colitis
Proteomics: mass spectrometric analysis and in vivo isotopic studies
Body Composition in rodents and humans (DEXA)
Digestive function investigation platform (motility, sensitivity, endoscopy)
Neurostimulation and neuronavigation systems

Publications

Déchelotte Pierre - Pierre.Dechelotte@chu-rouen.fr
Nutrition, inflammation and dysfunction of the Gut-Brain axis
Research teams with secondary association to PMN Institute
Eric Chevet
Protein Homeostasis and Cancer (PROSAC)

Université Rennes 1
Inserm U1242
Eric Chevet
Rennes

Integrated study of Endoplasmic reticulum functions in health and disease.

Research Brief:
Our team focuses on the study of Endoplasmic Reticulum (ER) functions in health and disease. In particular we are interested in better understanding two major molecular machines of the ER, namely the stress signalling machinery and the quality control machinery. The first research axis developed in the laboratory aims at characterizing ER stress signalling actors important for tumour development. Indeed in solid tumours, cells are subjected to major environmental challenges that condition their growth and fate. Under those circumstances, protein folding in the ER is affected and ER stress signalling is activated (the Unfolded Protein Response pathway). Our studies focus mainly on IRE1, the most conserved ER stress signal transducer, in various cancers including hepatocellular carcinoma and glioblastoma. The second research axis developed in the laboratory aims at characterizing novel component of the ER quality control whose expression is regulated upon IRE1 activation. We focus on proteins which specifically participate to the control of misfolded proteins secretion in the liver. Our third research axis focuses on the identification of IRE1 activity modulators through automated screening using the AlphaScreen® technology. These approach provide an integrated framework to better characterize and perturb ER biology in health and disease.

Methodologies Used:
Cell and molecular biology
In vitro and cell-based assays
Automated analases, alphascreen

Publications


Le Reste PJ, Avril T, Quillien V, Morandi X, Chevet E. (2016). Signaling the Unfolded Protein Response in primary brain cancers., Brain Res. (),
Stephen Manon
Mitochondria, Stress and Cell Death
Université de Bordeaux
CNRS UMR5095
Bertrand Daignan-Fornier
Bordeaux

Key facts
Team
• Researchers : 4
• Technicians : 1
• Postdoc fellows : 0
• PhD Students : 2

Translational approaches
• Patents : 0
• Clinical research grants : 0
• Industry partnerships : 0

International research links
• Portugal
• Slovakia
• USA

Keywords
• mitochondria
• apoptosis
• bcl-2 family
• mitophagy
• reconstituted models
• cell biology
• heterologous expression
• protein biochemistry

Biological Resources
• yeast mutants in autophagy-related processes
• yeast mutants in mitochondria-related functions, expressing human proteins
• human cancer cell lines, including KO-lines (HCT-116, HeLa, etc...)
• plasmid constructs for in vitro production of Bcl-2 family members

Our team investigates the central role of mitochondria as both a mediator and a target of degradation processes involved in cellular quality control regulation.

Methodologies Used:
- heterologous expression of human proteins of the Bcl-2 family in model systems
- study of post-translational process of Bcl-2 family members
- role of Bcl-2 family members in survival processes, both apoptosis and non-apoptosis related
- role of mitochondria metabolism, including lipid metabolism, in mitophagy
- molecular mechanisms underlying mitochondria permeabilization during apoptosis

Publications
Deffieu M, Bhatia-Kissova I, Salin B, Klionsky DJ, Pinson B, Manon S, Camougrand N (2013). Increased cytochrome b reduction and mitophagy components are required to trigger nonspecific autophagy following induced mitochondrial dysfunction., J Cell Science. 126(), 415-426


(1) Bax phosphorylation favors a cytosolic localization. (2) The small amount of Bax localized at the mitochondria may create MOMP. (3) When not phosphorylatable, Bax is MOM-localized but is poorly active. (4) In the presence of Bcl-xL, both Bax translocation and retrotranslocation occur, favoring a dynamic regulation of Bax activity. Phosphorylation and dephosphorylation contribute to this regulation. (5) The activity of MOM-localized Bax can be revealed with a BH3-mimetic drug.
Martin Villalba Gonzalez

Lymphocytes differentiation, tolerance and metabolism: basis for immunotherapy

University of Montpellier
INSERM 1183
Christian Jorgensen
montpellier

We have been the first to link tumor cell metabolism to tumor immune escape. We have developed an unique protocol to expand NK cells. We have identified antitumor cells in patients.

Research Brief:
We are interested on understanding how tumor cell metabolism affects tumor immune escape. The mostly glycolytic tumor metabolism generate the activation of intracellular signalling pathways that induces expression on the membrane of ligands for immune receptors. Therefore, it is possible to modulate tumor immune recognition by altering tumor metabolism. We also develop specific protocols to produce large numbers of cytotoxic lymphocytes, in particular natural killer (NK) cells. We use these cells to develop clinical trials.

Methodologies Used:
Lymphocyte expansion
in vivomodels for lymphocyte infiltration
FACs 20 colors

Publications


Nerea Allende-Vega, Ewelina Kryzywinska, Stefania Orecchioni, Nuria Lopez-Royuela, Francesca Reggiani, Giovanna Talarico, Jean-François Rossi, Rodrigo Rossignol, Yosr Hicheri, Guillaume Cartron, Francesco Bertolini and Martin Villalba (2015). The presence of wild type p53 in hematological cancers improves the efficacy of combinational therapy targeting metabolism., Oncotarget. 6(22), 19228

Elena Catalán, Seyma Charni, Juan Ignacio Aguiló, José Antonio Enriquez, Javier Naval, Julián Pardo, Alberto Anel* & Martin Villalba (2015). MHCI modulation due to metabolic changes regulates tumor sensitivity to CTL and NK cells., Oncoimmunology. 4(1),

Tumor metabolism controls MHC-I expression.

Tumor cells choose glycolysis metabolism to generate ATP rather than mitochondrial metabolism even in the presence of oxygen (Warburg effect). The pyruvate generated in the glycolysis is reduced to lactate (fermentation). Surface expression of MHC-I is often reduced in tumor cells to avoid the immune attack. Oxidative phosphorylation (OXPHOS) induces expression of ERK5, which increases MHC-I expression at the transcription level.

Tumor cell metabolism protect them from immune cells

Co-regulation of metabolism and immune function to kill tumor cells.

Metabolic drugs such as DCA and Metformin induce OXPHOS that up-regulate the MHC-I expression, immunomodulators (IMiDs) and IL-2 stimulate the anti-tumor activity of effector immune cells (CTL and NK cells).
Philippe Gérard

Amipem

Université Paris Saclay
INRA UMR1319
Stéphane Aymerich
Jouy-en-Josas

Thanks to the unique tool constituted by the germfree facilities of the MICALIS institute, we developed strategies based on microbiota transfer (from animal models or human patients) to germfree rodents in order to prove the causal role played by the gut microbiota in metabolic and brain diseases.

Methodologies Used:
- gnotobiology (germ-free animal models)
- metagenomics
- transcriptomics

Publications


Gérard P. (2016). Gut microbiota and obesity, Cellular and Molecular Life Sciences. ()
Gladys Mirey
Genotoxicity Signaling
Université Paul Sabatier - Toulouse III
INRA UMR1331
Bernard Salles
Toulouse

Association of DNA damage and DNA repair assays to study genotoxicity mechanisms, particularly after exposure to food contaminants.

Research Brief:
Our team studies the effects of various chemical (pesticides, nanoparticles) or biological (such as bacterial genotoxins) compounds present as contaminants in food, on the integrity of our DNA. We develop in particular cell assays and biotracers to characterize the genotoxicity mechanisms.

* Methodologies Used:
Molecular biology, Cell Biology, Biochemistry, Cell imaging & Cytometry (DNA damage, cell cycle, apoptosis), Genotoxicity assays (comet assay, micronucleus,...).

Publications

Keywords
• DNA repair
• Genotoxicity/DNA damage
• Contaminants
• Metabolism
• Signaling
• Genotoxicity assays
• Cell engineering
• Biotracers/Biosensors
• Repair systems/Biochemistry
• Biomonitoring

Biological Resources
• In vivo/in vitro genotoxic assays
A new mode of action for the Cytolethal Distending Toxin.

We used an association of DNA damage and DNA repair assays to revisit the Cytolethal Distending Toxin mode-of-action and showed the importance of replicative stress to generate DNA double-strand breaks (Fedor et al., Graillot et al., Bezine et al.).

Evaluation of DNA damage for biomonitoring and toxicological studies.

We develop different versions of comet assays in order to detect a large panel of DNA damage on various samples, for either biomonitoring or in vivo toxicological studies (Fedor et al., Lebailly et al., Bezine et al., Bettini et al.).

Real-time observation of micronuclei assay in metabolic-competent cells.

We are using metabolic-competent cells, expressing a chromatin biotracer (Jullien et al.), to study genotoxicity and micronucleus formation.
Yaël Grosjean

Sensory Perception, Interactions between Glia and Neurons

AgroSup Dijon  Université de Bourgogne Dijon
CNRS UMR6265  INRA UMR1324
Lionel Brétillon
Dijon

We try to understand how volatile chemicals are detected and processed into the brain to lead to a specific behavioral response, mainly focusing on glia/Neuron interaction through the study of a family of amino acid transporters.

Research Brief:
Our surrounding environment is bathed in volatile chemicals. Those represent vital information. Our team "Sensory Perception, Glia/Neuron Interactions" explores the molecular and cellular mechanisms allowing the perception of these chemical signals. We aim to respond to the following basic questions:
- How some food odorants are detected and integrated into the brain to lead to a specific meaning?
- How these odorants will stimulate a specific behavioral response? Can they influence social behavior?

* Methodologies Used:
Basic research (molecular genetics, biochemistry, immunohistology, behavior) using drosophila as a model

Publications


Xavier Grosmaitre

Olfactory neuroplasticity and feeding behaviors

Université de Dijon
(Université de Bourgogne)
CNRS UMR6265 INRA UMR1324
Lionel Bretillon
Dijon

We perform electrophysiological recordings of mammalian olfactory sensory neurons in transgenic mice; we analyze the plasticity of these neurons during development, under the influence of the environment and the nutritional status of the animal.

Research Brief:
Our goal is to investigate how the olfactory system evolves during development and neurogenesis as well as under the influence of the odorant environment and internal metabolic signals. We are using rodents as our main experimental model. We are investigating different levels of modulation, from olfactory receptors to central areas of the brain. We develop molecular, cellular, anatomical and physiological techniques as well as behavioral assays.

Main research topics:
1: Functional properties of olfactory receptors and olfactory sensory neurons;
2: Odorant induced plasticity in olfactory neurons: consequences of the odorant environment on the properties of olfactory neurons;
3: Plasticity of the olfactory system induced by homeostatic changes: effects of nutritional status and diet. We evaluate the effects of diet on the physiology of the olfactory system (peripheral and central levels) and olfactory behaviors. We use different types of diet inducing metabolic disorders.
4: Neural structures involved in olfactory, hedonic and feeding behaviors: connections, plasticity and behavioral impact.

We use stereotactic injections of retrograde tracers such as polysynaptic virus (PRV) and monosynaptic cholera toxin (CTb). Double-immunocytochemical characterization of the retrogradely labeled neurons is also performed. The functional significance of the identified circuits is investigated using behavioural paradigms and pharmacogenetics.

Methodologies Used:
Olfactory receptor expression in heterologous systems and functional assays
Electrophysiology (patch-clamp, EOG)
Transgenic mice
Intact epithelium preparation
Connectomics using PRV viruses
Pharmacogenetics
Basic behavioral tests

Publications


Movahedi, K., X. Grosmaitre, and P. Feinstein (2016). Odorant receptors can mediate axonal identity and gene choice via cAMP-independent mechanisms., Open Biology. 6(7), 160019

Tazir, B., M. Khan, P. Mombaerts, and X. Grosmaitre (2016). The extremely broad odorant response profile of mouse olfactory sensory neurons expressing the odorant receptor MOR256-17 includes trace amine-associated receptor ligands., European Journal of Neuroscience. 43(5), 608
Methodological strategy developed in our group

Investigation of olfactory neuroplasticity from olfactory receptors to central areas of the brain and behavior.
**Key facts**

**Team**
- Researchers : 7
- Technicians : 3
- Postdoc fellows : 1
- PhD Students : 3

**Translational approaches**
- Patents : 0
- Clinical research grants : 0
- Industry partnerships : 0

**Keywords**
- membrane biology
- traffic
- lipid transfer
- membrane curvature
- polyunsaturated lipids
- biochemistry
- liposomes
- cell biology
- molecular dynamics

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**Bruno Antonny**

**Dynamics of lipid membranes and protein coats**

Université de Nice - Sophia Antipolis
CNRS UMR7275
Pascal Barbry
Valbonne

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**Molecular approaches**

**Research Brief :**

Various proteins remodel the membranes of organelles involved in intracellular transport. Protein coats deform membranes to promote the budding of vesicles. Golgins, sort of molecular strings, tether vesicles to restrict their diffusion. Lipid transporters adjust the membrane composition. Although very different, most of these mechanisms are controlled by small G proteins of the Arf family and by the physical chemistry of membranes.

We study these mechanisms through molecular, cellular and in silico approaches. With original assays based on fluorescence and light scattering, we follow elementary reactions such as the assembly cycle of protein coats, the tethering of liposomes by a golgin or the transfer of lipids. With fluorescence light microscopy and electron microscopy, we visualize these events in cells and in reconstituted systems. With molecular dynamics, we describe at the atomic level how specific protein motifs sense the chemistry and curvature of lipid membranes.

**Recent findings**

- Intracellular transport of cholesterol through the counter exchange of a phosphoinositide and its hydrolysis.
- Phospholipids with omega 3 acyl chains boost membrane deformation and fission
- Atomic description of the packing of lipids in membranes of various curvature and composition

**Methodologies Used :**

Combination of molecular, cellular and in silico approaches.

Reconstitution experiments with liposomes to study and understand elementary reactions
Molecular dynamics simulations to understand the behavior of lipids in membranes

**Publications**


Endocrinology
Barbara Demeneix
Integration of transcriptional responses induced by thyroid hormones

Museum National d’Histoire Naturelle
CNRS UMR7221
Giovanni Levi
Paris

The lab combines classic endocrinology with cutting edge technology (gene to organism approach to physiology with core facilities for laser microdissection, in vivo gene transfer and neurobiological technique) with a good balance between fundamental research and translational applications.

Research Brief:
Our team has the overarching objective of analysing how thyroid hormone signalling is integrated in whole body physiology during development and ageing. Our research is examining the possibility that it exists a common cellular basis for two apparently distinct areas of thyroid function: metabolism and orchestration of developmental processes. Metamorphosis in fish and anuran amphibians are striking examples of TH-dependent developmental remodelling. Metamorphosis can be seen as a parallel to mammalian postnatal development, with marked TH changes occurring in the maturing nervous system, intestine and bone. Given the links between senescence and tissue renewal capacity and the inverse correlations of TH hormone levels with longevity (see Bowers et al., 2013) we propose the hypothesis that TH availability in tissues controls metabolic responses and self-renewal and regenerative capacity. We have four main research axes:
1. To determine how changes in central, hypothalamic TH availability and action affect metabolism and longevity
2. To discover how cellular transitions in the adult neural stem cell (NSC) niche relate to local changes in TH hormone availability, gene expression, epigenetic signatures and cellular metabolism
3. To identify TH-induced changes determined during development with a potential for reversibility using the regenerating heart as a model
4. To examine how thyroid hormone signalling during development can be modulated by xenobiotics as endocrine disruptors

* Methodologies Used:
- Mouse and Xenopus models, ICV injections (mouse); Non-viral in vivo gene transfer (shRNA, gene overexpression, reporter genes) in mouse brain; germinal transgenesis in Xenopus, crispr/cas9 technology in Xenopus.
- Laser microdissection; In situ hybridisation; immunohistochemistry; hormone assays; gene expression analysis (qRT-PCR); Neurosphere culture, metabolic characterisation.
- Development of fluorescent transgenic reporter Xenopus embryos compatible with high throughput (robotised) readings (pharmaceutical screening and environmental monitoring, exploited by the SME WatchFrog).

Publications
Cell-specific availability of TH is governed by transporters [THT] found at the cell membrane, and deiodinases in the cytoplasm. After release to the circulation by the thyroid gland, TH enter the cells by THTs and undergo deiodination to be activated (by D2 or D1) or inactivated (by D3 or D1). (D1 acts as an inactivating enzyme in the periphery, notably in metabolic tissues like the liver). The biologically active T3 binds to the THRs in the nucleus and activates or represses transcription.

The Xenopus Embryonic Thyroid-disruption Assay (XETA) and its pertinence to human risk assessment

XETA test principle is that the embryonic Xenopus is responsive to thyroid hormone (TH) and thus can be used to detect TH signalling disruption. TH is the same molecule in all vertebrates and many of the main elements of TH signalling are shared between vertebrates (receptors, metabolism, many target genes). Screening in Xenopus has high relevance for human toxicology. Indeed, both Xenopus and humans have key periods critically dependent on TH respectively metamorphosis and the perinatal period.

TH signaling acts as a neurogenic switch by repressing Sox2 in the adult neural stem cell niche:

In the SVZ, TR?1 is expressed in transient-amplifying cells and neuroblasts, whereas Sox2 is expressed in neural stem cells. TR?1 and SOX2 levels are inversely correlated. TR?1 overexpression in SVZ represses Sox2 and induces a DCX+ neuroblast phenotype.
Laurent Sachs
Thyroid hormone receptor function and mechanism of action
Museum Nationale d'Histoire Naturelle
CNRS UMR 7221
Giovanni Levi
Paris

The teams combines classic gene to organism approaches to physiology with core facilities for in vivo gene transfer and whole genome approaches with bioinformatics analysis of high throughput generated data.

Research Brief :
Thyroid hormones (TH) and glucocorticoids (GC) regulate diverse cellular processes from mitosis to apoptosis, from metabolism to growth and development. Perturbation of these two endocrine pathways are among the most common endocrine disorders worldwide with many consequences from early life to the elderly (mental diseases, cardiovascular diseases, metabolism associated disease, cancer, behavior and adverse effect on the quality of life). Prenatal exposure to elevated GC levels, either clinically or through maternal stress or malnutrition, can epigenetically program gene expression in the direction of previously listed diseases. The use of the Xenopus models will provide a powerful tool to understand the epigenome modification activity induced by TH and GC in whole organisms. Xenopus undergoes TH-induced metamorphosis. Interestingly, the metamorphosis and the perinatal period in mammals coincide with a peak of TH and GC. Metamorphosis thus provides a close parallel to the perinatal period. The overall aim is to identify the TH and GC induced regulatory programs operating during this key developmental phase. Such a project needs to be addressed in a physiological context and at the level of the whole genome. Thus, the ability to derive the transcriptome and a whole genome map of transcription factor binding site and their interactions as well as epigenetic modification and the enzymes that control them are crucial for elucidating gene regulatory networks.

Methodologies Used :
Xenopus tropicalis model, Non viral in vivo gene transfer in brain and muscle, Germinal transgenesis in xenopus, Real time qPCR, In vivo Chromatine immunoprecipitation to dissect regulatory mechanisms and epigenetic signatures, Whole genome approach, DNA array, Next generation sequencing, ChIP-Seq, ChIA-PET, MethylCAP-Seq, RNA-Seq, RNA-PET, gPET and Bioinformatics approaches.

Publications
Grimaldi A, Buisine N, Bilesimo P, Sachs LM (2013). High throughput sequencing will metamorphose analysis of thyroid hormone receptor during amphibian development., Current Topics in Developmental Biology. 103(), 277-303
Marc Lombes

Hormone Signaling, Endocrine and Metabolic Pathophysiology

Université de Paris 11
(Université Paris Sud)
CHU Inserm
Marc Lombès
KREMLIN BICETRE

Our project associating basic scientists, biologists and clinicians is entirely devoted to endocrinology and reproduction and is focused on hormone receptor signaling and their implications in human pathophysiology with therapeutic perspectives.

Key facts

**Team**
- Researchers: 18
- Technicians: 5
- Postdoc fellows: 1
- PhD Students: 8

**Translational approaches**
- Patents: 0
- Clinical research grants: 4
- Industry partnerships: 4

**Keywords**
- Steroid hormone signaling
- Endocrinology
- Human reproduction
- Cell Biology
- Transcriptional regulation
- Imaging
- Steroid hormone function

**Biological Resources**
- Transgenic animals
- Cellular model

Research Brief:
The main project of our Unit is devoted to translational research focused on endocrine pathophysiology and hormone signaling. The three partners of Bicêtre campus associating clinicians, biologists and basic scientists are working together to develop a better understanding of molecular and cellular endocrinology.

We pursue studies on the mechanism of action of hormones by analyzing their specific receptors and their molecular partners, their implication in endocrine and metabolic pathophysiology in humans. Using complementary approaches, associating cellular and molecular biology, molecular pharmacology, integrated physiology, genetic analysis, we aim to investigate two interdisciplinary research axes:

- NUClear receptors and transcriptional coregulators dedicated to investigate the expression and the tissue-specific regulation of steroid receptors (mineralocorticoid, glucocorticoid, progesterone, androgens) and of their molecular coregulators, which represent key factors of hormonal responsiveness in target organs (kidney, heart, endometrium, adipose tissues, testis). We are also interested in other nuclear receptors notably xenobiotic receptor and pituitary tumorigenesis as well as adrenocortical carcinoma.
- Reproduction and hormone regulation (including prolactin and growth hormone) of the hypothalamo-pituitary-gonadal axis in the context of ovary and testis dysfunction or hypogonadotrophic hypogonadism associated to puberty and fertility abnormalities.

**Methodologies Used**:
- Cell biology
- Transcriptional and post-transcriptional regulation
- Transgenic animals
- Human pathophysiology
- Hormone signaling

Publications


Our lab is internationally recognized for its expertise on prolactin (all aspects)

Research Brief:

We use translational approaches to identify, understand and target cellular and molecular mechanisms responsible for the progression and/or resistance to treatment of hormone-dependent cancers (breast and prostate cancers).

Aim #1. Determine the identity of castration-tolerant prostate cell(s), decipher their regulation by/downstream of PRLRs signaling, and identify new actionable targets to prevent cancer relapse leading to lethal disease.

Aim #2. Elucidate the vicious circle involving calcium signaling and tissue inflammation in prostate cancer progression, in relationship with nutritional behaviors.

Aim #3. Decipher cell-autonomous IFN-related responses to treatment of breast cancer cells to develop strategies preventing/delaying cancer relapse.

*Methodologies Used:*
- Protein engineering (production/purification of recombinant proteins, mutagenesis)
- Cell bioassays designed for basic studies and pre-clinical studies of therapeutic compounds (proliferation, reporter genes, intracellular signaling, transcriptomic profiling)
- Phenotyping of genetically-modified mouse models, focused on prostate tumors (morphology, tissue anatomy/histology, immunohistochemistry, xenografts, stem cells, gene expression)
- Clinical studies (cohorts, genotyping, immunohistochemistry)

Publications


Structure of the PRL-PRL receptor complex

The crystal structure of PRL bound to its homodimerized receptor was obtained using recombinant proteins produced in the lab. On each receptor, Ile76 (blue) and Ile146 (red) are represented. Mutations of these residues confer ligand-independent receptor signaling activity. These positions correspond to natural SNPs found in the human PRL receptor.

Prostasphere generated from mouse stem cells

Sphere generation in low adherence culture media reflects the stem properties of a cell population. Each stem/progenitor cell gives rise to one sphere in which cells at various stages of differentiation can be visualized using cell-specific phenotypic markers. This figure shows prostaspheres generated after plating a population of epithelial cells dissociated from a mouse prostate. Basal cells, luminal progenitors and mature luminal cells exhibit different colors in immunofluorescence.
Franck Oury

Hormonal regulation of brain development and functions

Paris Descartes
Inserm U1151 CNRS UMR842
Xavier NASSIF
Paris

**Research Brief:**

Hormones are essential factors ensuring proper regulation of our physiological functions by mediating dialogue between organs. Their broad spectrum of actions is not limited to the peripheral organs. Some hormonal factors, such as leptin, insulin, thyroid hormones, steroid hormones reach the central nervous system (CNS) where they modulate the central regulation of whole-body metabolism. Recently, it has been shown that they can also influence more intrinsic functions of the CNS, such as brain development, adult neurogenesis and cognitive functions. Importantly, increasing evidence suggests that changes in their circulating levels may contribute to age-related cognitive decline, as well as to the development of neurodegenerative diseases.

While the functional importance of hormonal factors on brain activities is undeniable, their cellular and molecular mechanisms of action are unclear. Moreover, although the brain expresses receptors for most, if not all, hormonal factors, the role(s) of many hormones in the CNS remain unexplored. Characterizing the influence of hormonal homeostasis during aging may open up new roads for therapeutic intervention to ameliorate age- and disease-related cognitive impairments, and reverse.prevents age-related memory decline.

**Methodologies Used:**

We are currently using an interdisciplinary approach that combines
- mouse genetics
- behavioral/metabolic analyses
- Local brain stereotactic injections
- cellular and molecular methodologies
- lentiviral-based gene downregulation
- Hormonal measurements
- Primary neuronal cells-based assays
- Dendritic morphology and activity
- Collaborative translational studies

**Publications**


**Keywords**

- Memory
- neuronal plasticity
- aging
- Energy balance
- Hormones
- behavioral tests
- stereotactic injections
- lentiviral based gene downregulation
- primary hippocampal neurons
- dendritic analysis
Jean-François Arnal

Estrogen Receptor alpha modulation to prevent atheroma and diabetes

Université de Toulouse 3
(Université Paul Sabatier)
Inserm U1048
Angelo Parini
Toulouse

This in vivo understanding of the beneficial vascular and metabolic actions from the sexual actions will provide molecular rationale to pave the way to selective ER modulators and a better understanding of the mechanisms of action of endocrine disruptors.

Research Brief:

In the last years, we contributed to evidence that, beside the prevention of osteoporosis, targeting estrogen signalling allow to prevent the development or the progression of atherosclerosis and type II diabetes. Thanks to unique transgenic mouse models, we were the first to demonstrate that estrogen receptor ERa, but not ERb is absolutely necessary for the most of the vasculoprotective (including prevention of atheroma) and metabolic (prevention of diabetes type II) actions of E2. The dark side of estrogens is represented by their deleterious long-term action on their two main sexual targets. However, in the absence of associated progestin, the main concern is represented by the deleterious role of estrogen on uterus (endometrial proliferation) and on breast, the proliferative action favoring the risk of cancer.

The full length ERa is composed of 6 domains (from A to F) containing the 2 independent activation functions AF-1 and AF-2. Our team demonstrated recently that ERa AF-1 is not required for the vasculoprotective actions of E2, whereas it is necessary for the proliferative effects of E2 on uterus and on breast cancer cell lines.

Our goal is to further dissect in vivo, for the first time, the respective roles of ERa functions, AF-1 and AF-2 as well as the role of a fraction of ERa localized at the plasma membrane and eliciting «membrane initiated steroid signalling» to open the way to an optimization of ER modulation.

* Methodologies Used:

- Mouse model functional exploration
- Mouse mutants of ERa

Publications


Tissue specific actions of SERMs and selective usage of membrane or nuclear ERα

In this model, whereas E2 activates both membrane and nuclear actions of ERα, E4 (estetrol) is a weak agonist of nuclear activity and an antagonist of the ERα-dependent MISS pathways, and thereby exerts physiological actions in the uterus but not in the endothelium. EDC (estrogen dendrimer conjugate) activates only the membrane effects of ERα and thereby acts on the endothelium, but not on the uterus. The liver appears to be a mixed organ, depending on both membrane and nuclear ERα effects.

Genomic strategies for knockouts or knockins of ERα - I

(A) Schematic representation of the mouse Estrogen Receptor α (Esr1) gene, which encompasses 8 coding exons. The ERα protein is composed of six domains (A to F) comprising a DNA-binding domain (DBD), a ligand-binding domain (LBD) and two activation functions (AF-1 and AF-2).

Genomic strategies for knockouts or knockins of ERα - II

186

(A) Schematic representation of the mouse Estrogen Receptor α (Esr1) gene, which encompasses 8 coding exons. The ERα protein is composed of six domains (A to F) comprising a DNA-binding domain (DBD), a ligand-binding domain (LBD) and two activation functions (AF-1 and AF-2).
Philippe Lefebvre

Molecular analysis of gene regulation in cardiometabolic diseases

Université de Lille 2 (Droit et Sante)
Institut Pasteur Inserm
Bart Staels
Lille

A long term, proven expertise in the field of transcriptional regulation by nuclear receptors in pathology enabling the discovery of novel mechanisms and the design of new screening tools for the pharmaceutical/biotech companies

Research Brief:

Nuclear receptors (NRs) are transcription factors regulated by endocrine, lipidic or environmental (nutrients, drugs, xenobiotics) molecules which control most if not all aspects of biology, including energy homeostasis, inflammation and cellular proliferation. These effects stem mostly from transcriptional events. Indeed, NRs are an assembly platform for distinct macromolecular complexes on DNA, whose activities concur to repress or activate transcription from a limited subset of genes by modifying the chromatin landscape in a tissue-specific manner. The impact of NR-tethered multiprotein complexes on the cellular transcriptome is conditioned by the composition of these macromolecular complexes or interactome. How this interactome varies according to physiopathological events is ill-defined, and our main research area is focusing on these aspects in cardiometabolic diseases. Using PPARgamma, a major regulator of adipocyte differentiation and of insulin sensibility, as well as FXR, the nuclear bile acid receptor, as models systems, we are characterizing the epigenetic origin of interactome variations as well as novel molecular components of this interactome that could bring selective sensitivity to pathological cues. Such a strategy will establish new paradigms to study RN-regulated transcription events which are dysregulated in metabolic diseases and which may lead to the identification of novel, original therapeutic targets.

Methodologies Used:

- Molecular biology: mutagenesis, recombinant proteins, protein-DNA interactions; protein-protein interactions, siRNA and shRNA-mediated gene knockdown
- Transcriptional studies: reporter genes, Q-PCR, microarrays
- Epigenetic regulation: ChIP, ChIP-Seq, RNA-Seq
- Cellular Biology: Immunofluorescence, confocal microscopy, adeno-virus transduction, retroviral and lentiviral transduction
- Animal models: AAV-based hepatocyte transduction

Publications


Dubois, V., Eeckhoute, J., Lefebvre, P. and Staels, B. (2017). Distinct but complementary activities of PPARs in metabolic control, J. Clin. Invest.. (),

Research teams with secondary association to PMN Institute
Key facts

Team
• Researchers : 3
• Technicians : 2
• Postdoc fellows : 1
• PhD Students : 1

Translational approaches
• Patents : 1
• Clinical research grants : 0
• Industry partnerships : 2

International research links
• Brazil - CNRS International Associated Laboratory "Genetics and genomics of children neoplasia" (NEOGENEX)
• USA
• Germany

Keywords
• cancer
• endocrinology
• transcription factors
• regulation of gene expression
• genetics
• mouse models
• molecular biology
• clinical studies
• pharmacology

Biological Resources
• Adrenocortical cell lines with doxycycline-inducible SF-1 overexpression
• Transgenic mice overexpressing SF-1 in steroidogenic tissues
• Access to large Brazilian cohort of carriers of germline R337H TP53 mutation

Enzo Lalli

Mechanisms of gene expression regulation in physiopathology

Université de Nice - Sophia Antipolis
CNRS UMR7275
Pascal Barby
Valbonne

Using an integrated approach including cell biology methods, protein structure analysis, genomics, transgenic animals and clinical studies, we aim to understand the molecular mechanisms of cancerogenesis and to develop novel therapeutic tools.

Research Brief:
We aim to understand the mechanisms of gene expression in cancer, focusing on both transcriptional and post-transcriptional regulations. Particularly, in the field of adrenocortical cancer we have described the critical role of the dosage of transcription factor SF-1 in triggering tumourigenesis, characterized genomic alterations and the patterns of mRNA and miRNA deregulation, identified critically perturbed signalling pathways and demonstrated the efficacy of novel therapeutic agents in the preclinical setting.

Methodologies Used:
- cell culture
- transcriptome analysis
- ChIP-seq
- transgenic mice
- protein expression in bacterial and eukaryotic systems

Publications


SF-1 overexpression triggers adrenocortical tumourigenesis

Nodule developing in the adrenal cortex of a transgenic mouse overexpressing rat Sf-1. Neoplastic cells express gonadal markers (Gata4, AMH) and are probably derived from undifferentiated adrenogonadal precursors.

Subcellular localization of phospho(Ser2448)-mTOR in mitotic adrenocortical cancer cells

The IGF-1R - mTOR pathway has a critical role in regulating proliferation of adrenocortical cancer cells. Drugs inhibiting this pathway significantly inhibit their proliferation. The specific localization of activated (Ser2448-phosphorylated) mTOR in the midbody of telophase mitotic cells suggests a role of this protein in the process of cytokinesis. Green, beta-tubulin; red, phospho(Ser2448)-mTOR; blue, DAPI staining of DNA.

Increased SF-1 dosage in adrenocortical cancer cells induces cytoskeleton remodeling

SF-1 (green) and actin cytoskeleton labeled by phalloidin (red) in H295R-TR SF-1 cells treated with either vehicle or doxycycline (Dox). SF-1 overexpression is heterogeneous in cells treated with Dox. Figure enlargements show filopodia and lamellipodia-ruffles present only in the cell with the highest SF-1 expression level.
Eric Pailhoux
DGP: Gonad Differentiation and its Perturbations

Université Paris Saclay
INRA UMR1198
Corinne Cotinot
Jouy en Josas

The main originality of our team resides in the mammalian models we studied appearing quite divergent from mice according to gonadal differentiation and sexual development.

Research Brief:
The DGP team studied the genes involved in sex determination and sexual development in mammals. One of the aim of the team is to decipher the genetic pathways sustaining the main steps of gonadal differentiation (i.e.: early switch of the gonad toward testicular or ovarian development; germ cell meiosis; ovarian follicles formation; spermatogenesis) in different species of agronomical interest (mainly domestic ruminants and rabbits). Another aim of the team is to understand how these genetic pathways could be influenced by different environmental factors such as endocrine disruptors, diesel particles or maternal nutrition. The team had previously demonstrated that gonad differentiation in farm mammals used genetic pathways that differ from the widely studied mouse mammalian model.

Methodologies Used:
As the BDR unit had a longstanding experience in reproductive biotechnologies, the team develops different strategies of additive transgenesis and, from more recently, of genome editing in domestic mammals such as goats and rabbits. By these technologies we were able to demonstrate the crucial role of the FOXL2 gene in goat ovarian differentiation; role that has been lost in the mouse model.

Key facts
Team
• Researchers : 6
• Technicians : 4
• Postdoc fellows : 0
• PhD Students : 2

Translational approaches
• Patents : 0
• Clinical research grants : 0
• Industry partnerships : 0

Keywords
• Sex reversal
• Gonad differentiation
• Sex determination
• Farm ruminants
• Rabbits
• Molecular biology
• Epigenetic
• Genome editing

Publications


Sex determination process in the goat species: a working model.

In goats, FOXL2 factor appears to repress the male-differentiating pathways, acting directly or not on DMRT1 gene expression. In the goat, DMRT1 may be able to promote SOX9 activation. Moreover, some clues allow the hypothesis that in addition to promoting SOX9 activation, SRY may also be involved in repressing the FOXL2gene.
Pathophysiology of the intestinal epithelium

The team, composed of nine researchers (physiologists, pharmacologists, geneticists) and one clinician gastroenterologist, has a strong expertise on several cellular actors of the intestine: epithelial cells, enteric neurons, immune cells of the lamina propria, intestinal stem cells and fibroblasts.

Research Brief:
We study the mediators released in chronic intestinal diseases with a focus on inflammation, infection, pain-associated pathologies and carcinogenesis. Our ultimate goal is to highlight new therapeutic targets for the treatment of intestinal diseases.

More specifically, we investigate:
- the type of proteases released by inflamed tissues and the pathophysiological effects of these proteases on epithelial barrier function, and in different other cell types involved in the inflammatory response: epithelial cells, leukocytes, mononuclear cells, neurons and fibroblasts
- the mechanisms by which pathogens induce host’s protease release upon infection, and the role of proteases as mediators of host immune response
- the effects of proteases on the transmission of pain message and visceral hypersensitivity symptoms, in the context of irritable bowel syndrome and functional disorders
- the involvement of Protease Activated Receptors (PAR) in carcinogenesis pathways, their crosstalk with integrin signaling in intestinal stem cells
- the effects of the microenvironment of the colon crypts in the transition of the crypts to pre-cancerous and cancerous status, this work involves the study of immune cells, fibroblasts, but also of the enteric nervous system
- the effects of nanoparticles on epithelial barrier function and the induction of carcinogenesis
- the therapeutic potential of protease inhibitors in intestinal pathologies

Methodologies Used:
- Culture of human and murine intestinal organoids
- Primary cultures of sensory neurons (human and murine)
- In vivo models of acute and chronic colitis (DSS, TNBS, CD45RB high, IL10, etc...)
- In vivo models of somatic and visceral pain and electography measures of pain
- In vivo and in vitro gene overexpression and silencing
- Intestinal stem cell isolation
- Ussing chambers
- Protease identification and characterization
- Protease-Activated receptor pharmacology
- mRNA and protein expression studies
- Immunohistochemistry
- In vitro recombinant protein production

Publications


Vergnolle, N (2016). Protease inhibition as new therapeutic strategy for GI diseases, Gut. ()


Nathalie Vergnolle
Pathophysiology of the intestinal epithelium
Université de Toulouse 3
Inserm U1220 INRA UMR 1416
Nathalie Vergnolle
Toulouse

Key facts
Team
- Researchers: 9
- Technicians: 4
- Postdoc fellows: 3
- PhD Students: 4

Translational approaches
- Patents: 2
- Clinical research grants: 1
- Industry partnerships: 4

International research links
- Canada
- USA
- Italy

Keywords
- Proteases
- 3D organoids
- Pain
- Inflammatory Bowel Disease
- Irritable Bowel Syndrome
- Intestinal stem cells
- Inflammation
- Culture of human and murine intestinal organoids
- Primary cultures of sensory neurons (human and murine)
- In vivo models of acute and chronic colitis (DSS, TNBS, CD45RB high, IL10, etc...)
- In vivo models of somatic and visceral pain and electography measures of pain
- In vivo and in vitro gene overexpression and silencing

Biological Resources
- Biobanks: Colonic biopsies from controls, IBS, IBD and colon cancer patients
- Human sensory neurons
- Murine neurons
- In vitro models: Colonic organoids
- Colonic epithelial cell cultures
- Measurements of PAR activation in cell models

Methodologies Used:
- Culture of human and murine intestinal organoids
- Primary cultures of sensory neurons (human and murine)
- Co-culture systems of host epithelial cells and pathogens
- In vivo models of acute and chronic colitis (DSS, TNBS, CD45RB high, IL10, etc...)
- In vivo models of somatic and visceral pain and electography measures of pain
- In vivo and in vitro gene overexpression and silencing
- Intestinal stem cell isolation
- Ussing chambers
- Protease identification and characterization
- Protease-Activated receptor pharmacology
- mRNA and protein expression studies
- Immunohistochemistry
- In vitro recombinant protein production

Publications


Vergnolle, N (2016). Protease inhibition as new therapeutic strategy for GI diseases, Gut. ()


**Axis 1 – Understand the role of proteolytic homeostasis in the gut**

1- Identification of proteases and protease inhibitors present in pathologies  
2- Study of the role of proteolytic actors in intestinal pathologies  
3- Role of proteolytic actors in epithelial cell-neighbored cell interactions 
4- Therapeutic intervention

![Image showing proteases and protease inhibitors in human intestinal tissue](image1)

*In situ zymography of proteases (green, visualizing activity) in human intestinal tissue from healthy controls (CTR) and CRO’s disease (CD) patients. The intensity of color is representative of the intensity of proteolytic activity.*

**Axis 2 – Intestinal Stem Cells**

1- Identification of cellular and molecular events involved in colon tumor initiation 
2- Understanding the impact of (micro)environmental alterations on the crypt cells 
3- Identification of new colorectal cancer biomarkers and therapeutic targets

![Image of intestinal stem cells](image2)

**Axis 3 – Models of intestinal pathologies**

![Image of mouse models of intestinal pathologies](image3)

*Mouse models of IBD, UC, COL and more. (Experimental results)*

![Image of tumor development after treatment](image4)

*Tumor development after treatment and grafting.*
Our team gathers physiologists of the gastro-intestinal tract, basic scientists and clinicians (digestive surgeons, gastroenterologists and nutritionists) to develop basic and transitional researches on gastrointestinal adaptations in response to over- or under-nutrition.

**Research Brief**: We focus on gastro-intestinal adaptations in response to over- or under-nutrition and gut surgeries. We set up unique rat models of bariatric surgeries - vertical sleeve gastrectomy (VSG), Roux-en-Y gastric bypass (RYGB) and one-anastomosed gastric bypass (OAGB) - and Short Bowel Syndrome (jejuno-colon or ileum anastomosis).

Combining experimental research in these models with clinical studies, we identified differences in alimentary glucose absorption and intestinal blood glucose handling after RYGB versus VSG bariatric surgeries. We also characterized the protein malabsorption and oesophagus reflux after the controversial OAGB. Finally, we characterized factors that impact on structural and functional adaptations of the remnant intestinal mucosa and microbiota in humans and rats suffering from SBS. In all those studies we highlighted the plasticity of the epithelial cells constitutive of the gastrointestinal tract.

To decipher the mechanisms of cell remodeling, we now extend our studies to either side of the epithelium: the mucosa layers containing the enteric nervous system (ENS) and immune cells versus the luminal microbiota. We want to determine the functional consequences of intestinal neuro/glio and immune cells changes and how they impact on epithelial cell functions. Finally, the metabolome of intestinal mucosa and microbiota in preclinical models and patients will allow the identification of new biomarkers and/or therapeutic targets to supply or replace surgery.

**Methodologies Used**: In vivo studies, Quantification of gastrointestinal secretions (endocrine, exocrine), Assay of intestinal nutrient transport, Molecular biology and pharmacology, Cell signaling, Clinical studies, Animal models of gastrointestinal weight-loss surgeries and short bowel syndrome, Transgenic mouse models

**Publications**


After RYGB, the jejunal mucosa in the alimentary limb becomes hyperplastic in rats, and we reported this observation in RYGB-operated obese subjects (compared to obese subjects during surgery). These hyperplasia is associated with a metabolic hyperactivity of the alimentary limb and results in increased glucose consumption, which can be visualized by PET / CT Scan analyses in humans. Adapted from Cavin et al. Gastroenterology (2016).

**Cellular and molecular mechanisms involved in the cellular plasticity of gastro-intestinal mucosa**

Both sides of the gastro-intestinal epithelium could contribute to the adaptations in response to surgery readouts since glial and neurons of the enteric nervous system and immune cells communicate with epithelial cells.

**Research strategies**

Our team gathers physiologists of the gastro-intestinal tract, basic scientists and clinicians (digestive surgeons, gastroenterologists and nutritionists) to develop bench-to-bedsides researches.
Intestine: nutrition, barrier and diseases

Université de Paris 06
(Université Pierre et Marie Curie) Université Paris Descartes
Paris 5
Inserm UMRS_1138
Pascal Ferré
Paris

Armelle Leturque

In the context of metabolic disorder epidemics, our central question focuses on the mechanisms through which intestinal cells sense environmental changes and trigger pathways required for tissue adaptation to pathophysiological situations.

Research Brief:
Major functions of intestine are to transfer nutrients to the organism, while maintaining an efficient barrier between external and internal medium, preventing the passage of antigens from bacterial or food origins, while maintaining a tolerance. Intestine, a neglected organ, has gained attention for its roles in metabolic diseases including obesity, insulin resistance, and diabetes via nutrient absorption and sensing, gut cell homeostasis and tissue inflammation.

Gathering complementary expertise in nutrition, metabolic diseases, intestine pathophysiology and epithelial intestinal cell differentiation, we aim 1-to understand how nutrients modify enteroendocrine cell lineage and function and how these changes impact pancreas, intestine and gut microbiota in obesity and diabetes; 2-to analyze the mechanisms involved in intestinal nutrient sensing via absorbing enterocytes and hormone-secreting enteroendocrine cells; 3-to decipher how signals from junctional proteins control the intestinal barrier function and trigger chronic inflammation.

Methodologies Used:
- Bank of jejunal samples from human obese subjects
- Mouse phenotype, glucose homeostasis, food intake, blood parameters
- Cell culture
- Imaging in real time, in tissues sections, confocal and electron microscopy
- Biochemistry, transport assays, protein expression, molecular biology constructs mutations RT-PCR transfections infections RNAI

Publications


Intestine: nutrition, barrier and diseases

Jejunal inflammation in human obesity

Human obesity causes an increase in the jejunal mucosal surface, in both innate and adaptive immune cell densities, characterized by an increased epithelium homing of CD8 alpha beta T cells in the jejunum of obese subjects. T cells from obese subjects impair insulin sensitivity of enterocytes. (Monteiro-Sepulveda et al Cell Met 2015)

Gut nutrient sensing and enteroendocrine cell lineage

Inflammatory digestive diseases: pathophysiology and development of therapeutic targets

Laurent Dubuquoy

Université du Droit et de la Santé Lille 2
Inserm UMR995 CHRU de Lille UMR995
Pierre Desreumaux Lille

Our strength lies in our multidisciplinary composition that promotes interactions between clinicians, scientists and surgeons, and in expertise on various tools ranging from cellular and molecular biology to animal models and histology. Access to patient samples allows for a unique translational approach.

Research Brief:
Our team is interested in the pathophysiology of inflammatory digestive diseases in order to highlight and propose new therapeutic targets for the treatment of these diseases.

A first focus on chronic inflammatory bowel disease (IBD) explores the role of nuclear receptors as well as bacterial flora in the regulation of intestinal homeostasis and is particularly interested in effector mechanisms of immunity as well as the processes involved in post-surgical recurrence.

A second focus on hepatic inflammatory diseases focuses on the pathogenesis of alcoholic hepatitis and the modulation of inflammatory liver damage by the innate immune system, while attempting to model the evolution of these diseases for the purpose to understand the impact of diagnostic methods in therapeutic decision-making.

The strength of our research team lies in its multidisciplinary composition that promotes the interaction between clinicians, scientists and industrials as well as in the expertise on various tools ranging from cellular and molecular biology techniques to animal models through histology and immunohistochemistry. Access to patient samples allows for a unique translational approach.

Methodologies Used:
- Animal models of colitis (TNBS, DSS, HLAB27 Tg...), hepatitis (CCl4, ConA, Ischemia/reperfusion...)
- Cellular models of intestinal epithelium (Caco2, HT29, organoid...), Liver (hepatocyte, progenitor...) and immune cells (PMN, macrophages, lymphocytes...)
- Molecular biology (Q-PCR, plasmids, Transfection, ShRNA...)
- Histology, immunohistochemistry and imaging
- Immunology (FACS, phenotyping...)
- Microbiology (culture, metagenomic...)
- Translational approaches
- Clinical trials

Publications


Translational research toward digestive inflammation

“Bed to bench” approach focused on digestive inflammation

Inflammatory bowel diseases pathogenesis

Better understanding of liver diseases
Research teams with secondary association to PMN Institute
Hervé Blottière

FInE, Functionality of the Intestinal Ecosystem

Université Paris-Saclay
INRA UMR 1319
Stéphane Aymerich
Jouy en Josas

The FInE laboratory applies innovative metagenomics approaches to explore the functionality of the human intestinal microbial communities and study the cross-talk between gut microbiota, food and host cells.

Research Brief:
The FInE team has been the first among international laboratories to design strategies for functional exploration of genes from gut metagenomic catalogue. A platform, named MetaFun, has been created to identify bacterial genes involved in host microbiota interactions by high throughput screening. It is now part of the MetaGenoPolis project, developed in the frame of the French Investment for the Future (2012).

Methodologies Used:
- Metagenomics
- Functional Metagenomics
- MetaProteomics
- Animal models

Publications


Laurent Combettes

Cellular Interactions and Hepatic Pathophysiology

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Laurent Combettes
Orsay

http://www.scicf.u-psud.fr/

Keywords

- regeneration
- Cholangiocyte
- Bile acids
- calcium signaling
- cell culture
- microscopy
- molecular and cellular biology

International recognition for our study of calcium signaling in the liver

Research Brief:

Our main objective is to determine the impact of Ca2+ signaling on the triggering and on the course of hepatocyte proliferation and regeneration, in the wide network of paracrine, endocrine and nervous interactions involved in the regulation of these processes.

We thus propose, to analyze the relationships between Ca2+ mobilizing agonists, hepatocyte calcium signalling and hepatocyte proliferation in the context of liver regeneration and carcinogenesis. Three main axes have been defined:

1. To evaluate the physiological impact of Ca2+ signaling on liver regeneration in rat and mice. We will interfere in vivo with calcium signalling before partial hepatectomy, then we will analyse consequences on regeneration.

2. To analyse the role of Sigma1 receptor, a protein involved in cell proliferation. We will focus on its role during liver regeneration and hepatocarcinogenesis in rats and human.

3. To study paracrine interactions involving calcium-mobilizing agonists, implicated during liver regeneration. We will focus on extracellular ATP, which has been shown to be involved in liver regeneration processes.

Another project of our lab is to studied PFIC2 and syndrome NISCH which are cholestases, due to mutations of BSEP (bile acids canalicular transporter) and claudin 1 (protein of tight junctions), respectively. Our aim is to understand the involved mechanisms, in order to elaborate targeted treatments and take care for the best of the affected children.

Methodologies Used:

- Microscopy (video, confocal, etc...)
- Molecular and cellular biology
- Cell culture

Publications


Our team gathers researchers and clinicians with high-level expertise in toxicology, cell defense and plasticity, metabolism and microenvironment to study the emergence and progression of metabolic and neoplastic hepatogastrointestinal diseases arising in an inflammatory context.

Research Brief:
The liver can be exposed to toxic xenobiotics, nutrient excess and inflammatory mediators released by the gastrointestinal (GI) tract. Although these tissues are able to set up mechanisms of defense and repair, the adaptive responses can be impaired in some individuals, thus favouring the occurrence of diseases such as inflammatory bowel diseases, colorectal cancer, steatohepatitis, fibrosis, cirrhosis and liver cancer. Our team aims to improve the understanding of the mechanisms involved in: i) cell and tissue damage induced by different stressors including infections and sepsis, lipid overload, surgery, hypoxia and xenobiotics, ii) cell defense and tissue repair aiming at limiting stress-induced liver and GI tract injury, iii) the occurrence of different pathological responses that can be secondary to a failure of cell defenses and tissue repair. When appropriate, we also study the impact of obesity and/or NAFLD on the response to stress and on disease progression. These objectives are included in three major research themes that are intertwined, in particular regarding cell defence, tissue repair, inflammation and mitochondrial dysfunction: 1) hepatotoxicity of xenobiotics in normal and fatty liver; 2) response to inflammatory stress and pathophysiological consequences; 3) cell plasticity in liver regeneration, fibrosis and cancer. Our project will provide new paradigms that will help to understand the pathophysiology of several important hepato-gastrointestinal diseases.

* Methodologies Used:
- Animal models: rat and mouse models of liver regeneration, ischemia/reperfusion, hepatocellular carcinoma, (LPK-c-myc) and obesity (genetic and non-genetic)
- Cell models: primary culture of hepatocytes from human, rat or mouse liver. Cell lines (HepaRG, HepG2, Caco2, HT29?), cocultures and 3D cultures, models of steatosis and cholestasis
- Mitochondrial function (oxygraphy, SeaHorse..) and oxidative stress

Publications


Hepatotoxicity of xenobiotics in normal and fatty liver

Numerous xenobiotics including drugs can lead to liver injury. Although hepatotoxicity often involves mitochondrial dysfunction and oxidative stress, several key questions remain regarding these mechanisms. Moreover, hepatotoxicity can be favoured by NAFLD, but the involved mechanisms are poorly understood. Hence, our main objectives are to further characterize the mechanisms of hepatotoxicity and to better understand why some xenobiotics are more deleterious in NAFLD and obesity.

Response to inflammatory stress and pathophysiological consequences

Several diseases (inflammatory bowel diseases, irritable bowel syndrome, sepsis,…) alter the gastrointestinal mucosa. These alterations lead to the translocation of high quantity of bacteria into the portal vein with severe pathophysiological consequences on the liver. The aim of our project is to improve the knowledge of the complex crosstalks between inflammation, hypoxia and cell defense in both gastrointestinal tract and liver in order to design new therapeutic approaches.

Cell plasticity in liver regeneration, fibrosis and cancer

Chronic liver diseases such as alcohol abuse, metabolic syndrome and viral hepatitis are characterized by recurrent bouts of liver damage and chronic inflammation resulting in fibrosis and amplification of the stem/progenitor cells. Our objectives are to study the contribution of liver inflammation to the emergence of pro-tumorigenic microenvironment, to the induction of hepatocyte plasticity and its impact on the stem/progenitor cell proliferation, metabolism and differentiation.
Key facts
Team
• Researchers : 9
• Technicians : 3
• Postdoc fellows : 1
• PhD Students : 3
Translational approaches
• Patents : 3
• Clinical research grants : 2
• Industry partnerships : 2
International research links
• O. Doulembier (University of Mons, Belgium)
• S. Dooley (University of Heidelberg, Germany)
• R. Salem (Northwestern University, USA)

Team
Pascal Loyer  Cédric Coulouarn
TGF-beta signaling, Glutathione homeostasis & innovative Therapies in Cancer (TGTC)

Université Rennes 1
Inserm U1241
Bruno Clément
Rennes

The Team gathers basic researchers and hospital practitioners with a common interest and complementary expertise in primary liver and pancreatic carcinomas to identify new therapeutic orientations for these cancers with specific emphasis on targeted therapy, vectorization and metabolic radiotherapy.

Research Brief:
The hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma and pancreatic ductal adenocarcinoma are aggressive cancers with rising incidence, poor prognosis and limited therapeutic options. Tumor onset and progression are associated with drastic changes in the tumor microenvironment promoting cancer cell survival and proliferation. The objective of the team TGTC is to better understand changes in the tumor microenvironment and their impact on tumor cell fate to provide new therapeutic orientations in cancer. We explore two paradigms, namely the TGFbeta signaling and glutathione (GSH) homeostasis. We aim at better characterizing the contextual determinants that shape the TGFbeta pathway in normal and cancer cells to provide a rationale for efficient targeted therapies using TGFbeta inhibitors. Notably, we explore the role of long non coding RNA as novel effectors and regulators of the TGFbeta pathway in cancer. We also study the contribution of the cystine/glutamate xCT antiporter in the GSH homeostasis in tumor cell fate. At the translational and clinical level, our objectives are to i) identify non-invasive companion biomarkers for targeted therapies and prognosis biomarkers (e.g. exosome content, cytokine production), ii) evaluate new clinical approaches to prevent tumor recurrence (e.g. administration of local anesthetics during surgery) and iii) develop innovative metabolic radiotherapies and synthetic nanovehicles for drug delivery in HCC.

* Methodologies Used:
In vitro models of hepatic and pancreatic cells, establishment of recombinant cell lines, in vivo experiments in rodents, clinical trials, genomic profiling, RNA interference, RT-qPCR, protein expression and catalytic activity analysis, HPLC, phage display, formulation and cell uptake of polymeric nanoparticles, nanotoxicological evaluation, radiolabeling of microspheres.

Publications


Allain C, Angenard G, Clément B, Coulouarn C (2016). Integrative Genomic Analysis Identifies the Core Transcriptional Hallmarks of Human Hepatocellular Carcinoma, Cancer Res. 76(374), 6981


Gene profiling in liver and pancreas cancers is used to characterize genomic alterations and to identify novel therapeutic targets and biomarkers. Our objective is also to unveil new regulators and effectors of the TGFbeta pathways, including transcriptional factors and long non-coding RNA, in order to understand the functional duality of TGFbeta signaling in cancer.

**Tumor microenvironment, Glutathione Homeostasis & Vectorization**

Cancer progression is associated with drastic changes in the tumor microenvironment regulating signaling pathways and metabolic tumor status. We explore the contribution of the cystine/glutamate xCT antiporter in the glutathione biosynthesis and tumor cell fate. We also develop polymeric nanoparticles to target tumor cells and study the influence of the microenvironment on the nanoparticle cell uptake.

**Radiotherapy in Hepatocellular Carcinoma**

Our team is pioneer in the development of metabolic radiotherapy in HCC. The pre-therapeutic evaluation of 99Tc albumin aggregates accumulation led to the concept of personalized dosimetry significantly improving patient survival. We conduct clinical trials for 188Re lipiodol (phase I), 90Y microspheres (multicentric randomized phase II) and neo-adjuvant radioembolization for large tumor down-sizing.
Philippe Gual

Hepatic Complications in obesity

Université de Nice - Sophia Antipolis
Inserm U1065
Patrick Aubinger
Nice

Key facts

Team
• Researchers : 9
• Technicians : 2
• Postdoc fellows : 0
• PhD Students : 5

Translational approaches
• Patents : 0
• Clinical research grants : 1
• Industry partnerships : 1

International research links
• EASD-NAFLD Study group

Keywords
• NAFLD, ALD, NASH
• Mice, Human

Biological Resources
• mouse model of ALD
• mouse model of NAFLD
• Cohorts of alcoholic patients
• Liver and serum banks of alcoholic patients
• Cohort of obese patients
• Liver, adipose tissue and serum banks of obese patients

Study of chronic liver diseases associated with obesity and alcohol: from the diagnosis to the treatment

Research Brief:
The aims of the present team (created in 2008), composed of clinicians and basic scientists, is to better understand the hepatic complications associated with obesity (Non alcoholic fatty liver disease: NAFLD) and, more recently, with chronic alcohol consumption (alcoholic liver disease, ALD). These chronic liver diseases range from steatosis to steatohepatitis (Non Alcoholic or Alcoholic Steatohepatitis, NASH and ASH), fibrosis, cirrhosis and finally hepatocellular carcinoma. NAFLD and ALD are the main causes of cirrhosis and increase the risk of liver-related death and hepatocellular carcinoma. NASH and ALD are also the most common indications for liver transplantation in the United States. Our translational researches mainly focus on 1) the identification of new markers/actors of the progression of NAFLD and ALD. We take advantage of our cohorts of obese (n=1006) and alcoholic patients (n=173); 2) the study of potential players in the progression of NAFLD including the OPN/CD44, endoplasmic reticulum stress and Sirt6 pathways. The impact of targeting these pathways is investigated in mice; and 3) the study of the interaction between alcohol and obesity in the severity of fatty liver disease in mouse and human.

Methodologies Used:
animal models
cellular models
human biopsies
histologic analysis
IHC
Gene and protein expression

Publications


Obesity and regular alcohol use are associated with the development of liver diseases. The prevalence of NAFLD is 25% and up to 40% of patients with severe acute alcoholic hepatitis die within six months. The spectrum of these hepatic abnormalities extends from isolated steatosis to steatohepatitis and steatofibrosis, sometimes leading to cirrhosis and HCC. NAFLD and ALD are two of the three principal causes of cirrhosis and increase the risk of liver-related death and HCC.

Physiopathology of NAFLD AND ALD

Cross talks between the liver, adipose tissue and gut are involved in the pathogenesis of NAFLD and ALD. In obesity, increased adipose tissue inflammation leads to lipolysis and altered adipokines secretion. Obesity and alcohol consumption are associated with dysbiosis and increased gut permeability leading to elevated bacterial products. These factors enhance hepatic inflammation and hepatocyte death which initiate the fibrogenic process and the progression of liver complications.
Uro-Nephrology
Stéphane BURTEY
Endothelial dysfunction and chronic kidney disease

Aix-Marseille Université
Inserm UMR_S 1076
Françoise Dignat-George
Marseille

Our team is the only french team involved in the understanding of the endothelioxicity of the uremic toxins during CKD, focusing on the prothrombotic activation of endothelial cells of indoles through Aryl hydrocarbon receptor stimulation.

Research Brief:
CKD is a public health concern. Understanding the specific pathway involved in the severity of vascular complications during CKD is important to provide new therapy. The identification of the role of AhR in the vessels is important to achieve this goal. The team is real mix of Basic scientists and clinicians involved since many years in a fruitful crosstalk from bedside to bench and now from bench to bedside. This real collaboration is one of our major strength. Uremic toxins are an emerging field in the CKD world. The arrival of AhR pathway in this business confirmed the importance of the concept of endothelio-toxicity in CKD.

1) We identified by a transcriptomic approach the receptor of an important group of uremic toxins involved in the cardiovascular complication of CKD: Aryl hydrocarbon receptor (Kidney international 2013)
2) Tissue factor dependant activation of coagulation in endothelial cells by AhR in response to uremic toxins. We show the acquisition of procoagulant phenotype in vitro (in flow cell culture) and in vivo during CKD.
3) Induction of inflammation in endothelial cells by AhR activation by indolic toxins. AhR is activated in vivo in various tissues (heart, vessels, blood cells) during CKD (JASN 2015)
4) Modulation of the activity of an important drug transporter P-gp by AhR activation, this result allow us to increase the knowledge of how CKD could modify the metabolism of drug normally not excreted by the kidney (JASN in press)

Methodologies Used:
Mouse models, Flow culture, in vivo video microscopy, molecular biology.

Publications


Dou L, Burtey S (2016). The harmful effect of indoxyl sulfate on neovascularization in chronic kidney disease, Kidney Int. 89(3), 532

Key facts
Team
• Researchers : 8
• Technicians : 2
• Postdoc fellows : 0
• PhD Students : 2

Translational approaches
• Patents : 0
• Clinical research grants : 4
• Industry partnerships : 1

International research links
• Belgium
• USA

Keywords
• endothelium
• uremic toxins
• chronic kidney disease
• thrombosis
• Aryl hydrocarbon receptor
• Animals models
• flow culture
• Cellular biology/cytometry
• Molecular biology
• Translational medicine

Biological Resources
• Ahr KO mouse
• EVITHUP cohort
Indoles (indoxy1 sulfate and indole acetic acid) are uremic toxins derived from the tryptophan metabolism in the gut. They activate aryl hydrocarbon receptor in the endothelial cells. AhR activation induces endothelial dysfunction leading to inflammation and procoagulant profil. Endothelial dysfunction, mainly expression of Tissue Factor, could lead to atherothrombosis and explains the increased cardiovascular mortality observed during Chronic kidney disease.
Key facts

Team
• Researchers : 3
• Technicians : 0
• Postdoc fellows : 1
• PhD Students : 0

Translational approaches
• Patents : 0
• Clinical research grants : 0
• Industry partnerships : 0

Keywords
• Hypertension
• Nephrolithiasis
• Chronic Kidney Disease
• Osteoporosis
• Human Studies
• Biomarkers
• Physiological studies
• Immunohistochemistry
• Biochemistry

Dominique Eladari
Pathophysiology of the renal tubule

Université de la Réunion
Inserm UMR1188
Olivier Meilhac
St Denis

Our group propose a unique combination of modern genetic manipulation together with "old but direct" functional techniques to obtain straightforward demonstration of physiological and pathophysiological mechanisms.

Research Brief :
We are located in Reunion Island, a region of France in which the population is very mixed due to the historic immigration of people from different origins, and in which during the last decade dramatic changes in the diet has led to a very impressive increase in the prevalence of obesity, diabetes, hypertension and chronic kidney disease. Therefore, our group focuses on the mechanisms leading to the development of diseases in which renal dysfunction is central. We are particularly interested in the effects of the diet on renal homeostasis, on the progression of chronic kidney disease, or on the onset of essential hypertension.

* Methodologies Used :
Generation of genetically engineered mice & molecular biology
In vivo metabolic studies
System biology
Biochemistry of proteins
Human studies

Publications


Joost Schanstra

Renal fibrosis-mechanisms and detection

Université de Toulouse 3 (Université Paul Sabatier)
Inserm U 1048
Angelo Parini
Toulouse

Development of new concepts of nephroprotection using translational research and state of the art technologies

Research Brief:
Chronic kidney disease (CKD) patient numbers are dramatically rising due to the increased incidence of diabetes and aging reaching today, in 2017, 15% of the general adult population. In contrast to adults in children CKD is mostly due to developmental disease. Individuals, even with early stage CKD, have a significantly increased risk of cardiovascular disease (CVD) complications. Early detection of CKD or prediction of CVD complications and early treatment is key in the clinical management of CKD. We focus our research on the early detection of CKD in both the pediatric and adult population using innovative mostly non-invasive, omics-based, approaches. In parallel we analyze this omics data for the identification of novel targets in CKD using systems medicine and drug repurposing techniques. We believe that such novel approaches will significantly improve the clinical management of individuals, both children and adults, with CKD.

Methodologies Used:
- Animals models of CKD (Unilateral ureteral obstruction, Remnant kidney, Glomerulonephritis, Diabetic nephropathy) and AKI (LPS, Hemorrhagic Shock, rhabdomyolysis).
- Molecular biology (qPCR, ChipSeq).
- Immunohistochemistry (animal and human renal tissue).
- Omics: -transcriptomics, proteomics and metabolomics.
- Bioinformatics.

Publications


Prenatal prediction of post-natal disease:

We have been studying the fetal urinary peptidome using capillary electrophoresis coupled to mass spectrometry to identify peptides that predicted in-utero renal function after birth of fetuses with obstructive nephropathy. This led to the identification of a fetal urinary peptide panel composed of 12 peptides (12PUV) allowing prediction of post-natal renal function with high sensitivity and specificity (For details see: Klein et al., Sci Transl Med. 2013).

Speeding up preclinical research:

A major issue in drug research is that many potential drugs work fine in animal models but do significantly less well in humans (or not work at all). To improve on the validity of preclinical observations we have developed a non-invasive multimolecular humanized readout in mice based on molecular signatures similar in mice and humans. Such read-out will allow to find out at an early stage (eg preclinical) whether a new drug is likely to work in humans as well (Klein et al., Kidney International).

Reproducible urinary metabolome analysis:

We have been working for many years to setup a robust urinary metabolome analysis pipeline in the laboratory which now has fully maturated. We have shown that we can now analyze the metabolite content of the same sample for over 4 (!) years with high reproducibility mainly based on a unique internal normalization procedure. We have used this pipeline to identify urinary metabolite biomarkers of disease (Boizard et al., Sci Rep. 2016).
Christos Chatziantoniou

New Biomarkers and Targets for Therapy of Chronic Kidney Disease

Université de Paris 06
(Université Pierre et Marie Curie)
Inserm UMR 1155
Pierre Ronco
Paris

These studies significantly contribute to a better understanding of the mechanisms involved in the development of renal disease and to provide important clues of how this incurable today pathology can be stopped or even better reversed

Research Brief:
The major objective of our team is to provide a comprehensive approach of mechanisms responsible for renal disease progression and repair. We are using a multi-target strategy to discover mediators of inflammation, apoptosis, initial repair, progression, stabilization or regression of renal lesions. Specific objectives are to:

- Investigate the mechanisms by which Calpains and the Discoidin Domain Receptor 1 act as major mediators of renal inflammation.
- Study the importance of Notch3 to control renal autoregulation and the impact of renal vessel dysfunction in the development of renal failure.
- Examine the role of cell-cell communication in the development of renal disease by focusing on Connexin 43, a protein constituting gap junctions.
- Explore the endogenous regulation of the BMP signalling pathway as opposed to TGFbeta action in mediating Epithelial to Mesenchymal Transition in renal disease.
- Search whether improving Renal Hemodynamics can protect against the progression of renal disease.
- Transfer the knowledge obtained with the experimental models to humans by analyzing the predictive value of the expression of EMT markers detected early, in 3-month protocol biopsies, on the graft function two years after transplantation.

Methodologies Used:
Renal Hemodynamics, BP, RBF, GFR, electrolytes
Transcriptomics, Real Time Q-PCR, siRNA,
Intra-vital microscopy, Renal Morphology, Histology, Immunocytochemistry, Transgenic animals, Experimental nephropathies,
Cell cultures, stable-transient transfections

Publications:


Aurélie Edwards  Pascal Houillier

METABOLISM AND RENAL PHYSIOLOGY

Université de Paris 05
(Université Rene Descartes) Université de Paris 06
(Université Pierre et Marie Curie)
Inserm U1138 CNRS
Pascal Ferré
Paris

Our multidisciplinary team studies the mechanisms by which the kidney regulates ionic transport, adapts to its environment, and controls ionic homeostasis; our investigations span all scales, from genes to the entire organism.

Research Brief:
Our work focuses on the mechanisms of ion transport along the renal tubules, their regulation, and their dysfunction in primary or secondary renal diseases, such as hereditary tubulopathies, hypertension, and disorders of divalent cation homeostasis. We also study the cross-talk between the kidney and other tissues (gut, muscle, bone) in homeostatic regulation. Our studies integrate all scales between genes and the whole organism. The techniques we use are available through our facility for in vivo and ex vivo kidney phenotyping. Our projects have both fundamental goals and clinical objectives (e.g., identification of candidate genes/pathways in hereditary diseases, and development of new drugs). Our connections to clinical departments allow us to perform clinical investigations in patients.

Recent findings include:
* The discovery of a new pathway for sodium secretion in the aldosterone-sensitive distal nephron.
* The demonstration that basolateral ClC-K2 chloride channel may trigger HCO3- secretion by B-intercalated cells in response to alkalosis.
* The finding that nephrotic ascites formation stems from changes in capillary permeability rather than reduced plasma oncotic pressure.
* The demonstration that progesterone is a potassium-sparing hormone in both genders.
* The demonstration that the renal calcium-sensing receptor regulates calcium homeostasis.
* The discovery that MAGED2 mutation causes a new and severe form of antenatal Bartter’s syndrome.

Methodologies Used:
Patch-clamp, voltage-clamp; microdissection and in vitro microperfusion of renal tubules; in vivo analysis of kidney electrolyte balances; SAGE analysis of transcriptomes; mathematical modeling of renal transport.

Publications


Contribution of renal CLC-K2 channels to acid-base balance.


H,K-ATPase type 2 (HKA2) expression in the kidney of mice fed a normal or low K+ diet during one week. Under normal conditions, HKA2 is present in intracellular vesicles of collecting duct (CD) cells (arrows), whereas it is localized at the apical side of cells after K+ depletion.
Djillali Sahali

Renal immunopathology and immunoregulation in transplantation

Université de Paris 12
(Université Paris-Val de Marne)
Inserm UMR 955
Jorge Boczkowski
Crétel

Key facts
Team
• Researchers : 6
• Technicians : 1
• Postdoc fellows : 2
• PhD Students : 4
Translational approaches
• Patents : 3
• Clinical research grants : 2
• Industry partnerships : 1
International research links
• Netherlands, Italy, New Zealand

Keywords
• Signaling
• Lymphocyte
• Podocyte
• Treg
• Pathophysiology
• alloimmunity
• Gene therapy

Biological Resources
• serum library
• Protein library
• RNA library
• DNA library

Molecular pathophysiology of acquired idiopathic nephrotic syndrome

Research Brief :

Our team included two groups of researchers. The first group is interested in the molecular pathophysiology of idiopathic nephrotic syndrome (INS). The mechanisms of immune and podocyte disorders have been studied by transcript profile analyses of lymphocytes and podocytes of patients with INS. In recent years, more attention has been focused on c-mip, which was originally found upregulated in both lymphocytes and podocytes of patients with INS. We generated conditional, global and tissue specific (T lymphocyte and podocyte) mouse c-mip knock down that are underinvestigation. We recently approached the study of molecular mechanisms of nephrotic proteinuria induced by receptor tyrosine kinase inhibitors (RTKI), which target a number of tyrosine kinase receptors. It is crucial to understand the underlying link between RTKI and proteinuria in order to develop a strategy aiming to prevent this complication, allowing to pursue this treatment highly beneficial in most patients.

The second group develops translational research in transplantation with the aim to modulate the immune system during the allogeneic response. The program is focused on two main axes: i) identification of the molecular and cellular mechanisms of the immune system leading to organ rejection or graft-versus-host disease or, alternatively to tolerance; ii) development of strategies of immunomodulation notably relying on CD4+CD25+FOXP3+ natural regulatory T cells (Treg), a central actor of immune tolerance.

Methodologies Used :
Subtractive and differential screening
Cloning and construction of target vectors, sequencing
SiRNA in vivo
Immunohistochemistry and confocal microscopy
Immunohistochemistry
Cell cultures and generation of primary cell lines
Transgenesis and conditional knock out

Publications


Kelhia Sendeyo1, Vincent Audard1, Shao-yu Zhang, Qingfeng Fan, Khedidja Bouachi, Mario Ollero, Catherine Rucker-Martin, Elodie Gouadon, Dominique Desvaux, Franck Briéoux7, Georges Guellaís, Pierre Ronco, Philippe Lang, Andre Pawlik and Djillali Sahali (2013). Upregulation of c-mip is closely related to podocyte dysfunction in membranous nephropathy, Kidney International. 46(5), 997-8
Loredana Saveanu

Modulation of inflammatory response by cell specific endosomes

Université Paris Diderot Paris 7
Inserm U1149
renato Monteiro
Paris

Our hallmark is the identification of insulin responsive aminopeptidase (IRAP) as a regulator of both, innate and adaptive immune response. We are intending to characterize IRAP function in immune and non-immune cells in the inflammatory reactions.

Research Brief:
Although the regulated trafficking of vesicles and their content is essential for a large diversity of cellular processes, the molecular mechanisms involved remain poorly understood. Our results from the last years indicate that the Insulin Responsive Aminopeptidase (IRAP), a type II transmembrane protein, is a dual function factor. Beside its aminopeptidase function, which is involved in antigen processing, IRAP plays an essential role in the trafficking of cell-specific storage endosomes, which is independent of its enzymatic function.

Cell-specific storage endosomes are vesicles that show a slow constitutive recycling, but can translocate rapidly to cell surface under cell-specific stimulation. Despite their broad tissues distribution, storage endosomes were initially studied almost exclusively in adipocytes, where they ensure rapid changes in surface protein composition in response to insulin stimulation. Our results on immune cells show that IRAP storage endosomes regulate the innate immunity by modulation of phagosome maturation, endosomal TLRs signaling and probably cytoskeleton remodeling. Systemic deletion of IRAP in mice generated an aberrant inflammatory response, which culminated with animal death during respiratory infections.

Based on our results obtained mainly in monocyte-derived dendritic cells, our group aims to investigate the role of IRAP storage endosomes in the inflammatory response in both immune cells (monocyte-derived dendritic cells and T cells) and

Methodologies Used:
dendritic cells culture
t cell activation assays
constitutive and ko mouse models
lentiviral expression and knock-down
molecular biology (cloning, qRT-PCRs)
dendritic cells culture
confocal microscopy
TIRF microscopy
recombinant protein expression

Publications


Saveanu L, Lotersztajn S (2016). Focus on "Active vacuolar H+ ATPase and functional cycle of Rab5 are required for the vacuolation defect triggered by PtdIns(3,5)P2 loss under PIKfyve or Vps34 deficiency", Am J Physiol Cell Physiol. 311(3), 363

IRAP describes the cell specific storage endosomes

A. The aminopeptidase IRAP is the hallmark of cell-specific storage endosomes. These vesicles have a constitutive slow recycling and can be rapidly translocated to the cell surface under cell specific stimulation (insulin stimulation in adipocytes or IgE-IC in mast cells). In dendritic cells, IRAP and its endosomal compartment are involved in regulation of both, innate and adaptive immunity.

IRAP endosomes are abundant in alveolar macrophages

Alveolar macrophages were isolated from wt and IRAP deficient (IRAP ko) mice and stained with rabbit anti-IRAP (green) and mouse anti-GM130 (red) antibodies. After respiratory infection with Pseudomonas aeruginosa or influenza, IRAP deficient mice showed an increased mortality, as compared with wt mice (Babdor J, Descamps D et al., Nature Immunology 2017).

IRAP endosomes morphology (immunofluorescent microscopy) in murine dendritic cells.

Bone marrow derived dendritic cells were stained for IRAP (green) and several organelle markers (red). The Q Snare syntaxin 6 is a marker of IRAP endosomes in dendritic cells.
The team has a multidisciplinary and integrative project with three aims:

1) study of the mechanisms regulating neutrophil apoptosis and their phagocytosis by macrophages, which is pivotal for the inflammation resolution and for avoiding autoimmunity
2) elucidation of the mechanisms of neutrophil activation and the role of PR3 in triggering a specific vasculitis. Wegener's granulomatosis
3) identification of target antigens and potential pathogenic role of autoantibodies against endothelial cells and vascular smooth muscle cells in vascular diseases.

* Methodologies Used:

- Molecular biology, cell biology and immunohistochemistry techniques
- Neutrophil isolation, activation and apoptosis measurement by flow cytometry
- Stably transfection of myeloid cell lines, which can differentiate into mature granulocytes allow to perform loss- or gain- of function for functional studies.
- Animal models of inflammation (peritonitis, vasculitis)
- Proteomic analysis two dimension differential in gel electrophoresis (2D-DIGE) Identification of target autoantigens by proteomic combined to immunoblot analysis

Publications


Proteinase 3 is a Phosphatidylycerne-binding Protein That Affects the Production and Function of Microvesicles., Journal of Biological Chemistry. 291(20), 10476-89

Proteinase 3, the autoantigen in vasculitis is a danger signal for the immune system.

During vascular inflammation, neutrophils can express proteinase 3 at the plasma membrane, which activates macrophages inducing the production of inflammatory cytokines. The inflammatory microenvironment favours activation of plasmacytoid dendritic cells which results in an inhibition of the generation of regulatory T cells favoring autoimmunity.

Key role of cytoplasmic PCNA in neutrophil survival

Trimeric structure of PCNA with the nuclear export sequence (NES) in blue at the inner face of the trimer. Immunofluorescence of PCNA (red) and nuclei (blue) in CD34 progenitors (upper panel). At the end of differentiation, PCNA is exported from nucleus to cytosol via its NES. In mature neutrophils, PCNA is exclusively cytosolic and is associated with different protein partners including procaspases to inhibit apoptosis (adapted from Witko-Sarsat et al J Exp Med 2010 and Immunol Reviews 2016).
Osteoarticular system
**Yannick Allanore**

**Translational genetics in systemic sclerosis**

Paris Descartes  
INSERM U1016 UMR 8104

Pierre Olivier Couraud  
Paris

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**Key facts**

Team
- Researchers: 3
- Technicians: 3
- Postdoc fellows: 1
- PhD Students: 4

Translational approaches
- Patents: 1
- Clinical research grants: 10
- Industry partnerships: 8

**Keywords**
- Auto-immunity
- Genetics

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**High translational applications with really from bench to bedside and back**

**Research Brief:**

Immune-mediated diseases comprise a clinically heterogeneous group of diseases affecting about 5% of individuals of European origin. Knowledge of their pathogenic mechanisms has strikingly increased in these recent years taking advantage of the huge progresses made in immunogenetics. Our research offers a unique opportunity both in basic and in translational approaches to continue the deciphering of immunogenic disorders. Our goal is to link inflammation and immune system with human diseases based on a multidisciplinary approach which involves a two-way process going back and forth between the analysis of the genetic data, the dissection of immunological mechanisms and the potential transfer of the findings to the patient management.

**Methodologies Used:**
- Re sequencing
- Genotyping
- Cellular biology
- Molecular biology
- Animal models

**Publications**


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Allanore Yannick - yannick.allanore@inserm.fr - http://www.institutcochin.fr
Ariane Berdal  Sylvie Babajko

Molecular Oral Physiopathology

Université de Paris 07
(Université Denis Diderot)  Université de Paris 05
(Université Paris Descartes)
CHU  Inserm U 1138
Pascal Ferré
Paris

Translational researches are conducted from involved genes to in vitro cell biology, animal models and clinics.

Research Brief :
Our group is dedicated to the oral-facial area, an exemplary composite skeleton where epithelial and neuroectoderm-mesenchymal cells cooperate within a permanently challenged microenvironment. Post-natal physiology harbours specificity (dental cells, bone drug sensitivity and fate related to odontogenic growth and tumours) which determinants are studied in our group. Homogenene patterns were shown to imprint oral cells and impact their post-natal proliferation, differentiation and functions. This was illustrated in transgenic mice: the combinatorial Mxs2, Dlx1, 3, 4, 6 interplay defined the frame of matrix protein expression and thus, regional enamel thickness. Mxs1 and Mxs2 controlled site-specifically osteoblast/osteoclast cross-talks and activity during physiology and healing. Mxs/Dlx transcriptional role is explored on dental and bone genes. An endogenous antisense cis-RNA for Mxs1 was discovered by us. Epigenetic and cell-autonomous mechanisms were evidenced and are presently analysed from sense and antisense promoters to an integrated level. Based on this detailed cell profiles, innovative biomaterials are tested as well some hormonal and toxic factors controlling skeletal morphogenesis. Correspondingly, human rare diseases are studied in our Reference Center. The team is involved in training programs which welcome scientists and health students in oral and mineralised tissue research.

Methodologies Used :
- Molecular in situ studies on mineralized tissues and cells.
- 2D and 3D analysis of the dento-maxillo-facial skeleton.
- Oral and dental genetics - rare diseases
- Experimental surgery and material investigation.
- Molecular and cellular Biology.

Publications


Francis Berenbaum

Metabolic diseases and age-related joint diseases

Université de Paris 06
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Inserm U 938
Bruno Fève
Paris

Our team associates physiologists, cell biologists and clinicians devoted to find new targets and new biomarkers in osteoarthritis, particularly by exploring the role of metabolic diseases, mechanical stress and bone/cartilage/synovial tissue interactions

ResearchBrief:
Our team has been interested for several years in the physiopathology of osteoarthritis in the final objective to discover innovative treatments and novel diagnostic and prognostic biomarkers. For these objectives, we have developed several tools, from cell cultures to human cohort (DIGICOD, a cohort of hand osteoarthritis patients), from preclinical murine models to joint tissue analysis (BioJoint, a biobank of human joint tissues). We focus our projects on the relationship between osteoarthritis and metabolic diseases. Four main projects are currently under investigation:
1 - Role of bone-secreted 14-3-3epsilon protein on cartilage degradation
2 - Role of chondrocyte differentiation on the angiogenesis of the subchondral bone
3 - Role of the parasympathetic system in joint protection
4 - Role of adipose tissues in OA pathophysiology

Methodologies Used:
- Experimental in vivo model of osteoarthritis
- Histological analysis of joint tissues
- Application of mechanical stress on cartilage explants and bone (Flexercell apparatus)
- Cellular analysis (Western-blot, proteolytic activity, ELISA, qRT-PCR, ARN interference, immunocytology)
- Primary culture of articular chondrocytes (mice, human) and costal chondrocytes (mice), of murine osteoblasts (membrane 3D) and of human synoviocytes
- Control of chondrocyte phenotype (hypertrophic differentiation and fibroblastic dedifferentiation)

Publications

Keywords
• inflammation
• adipose tissue
• osteoarthritis
• cartilage
• bone
• synovial tissue
• cell differentiation
• biomarkers
• murine models of osteoarthritis
• human cohort of hand osteoarthritis
• molecular biology
• histology
• cellular biology

Biological Resources
• osteoarthritis mouse model
• BioJoint (a biobank of human joint tissues)
• DIGICOD (a cohort of patients with hand OA)
• Human, mouse, rabbit and rat primary culture (articular chondrocyte, costal chondrocyte, osteoblasts)
Images obtained in the lab

Tools for research

Evaluating 14-3-3ε as a novel prognostic biomarker of OA

Basis for an integrative view of the pathophysiology of osteoarthritis

An integrative view of the pathophysiology of OA
Martine Cohen-Solal

Bone - Cartilage and environment

Université de Paris 07
(Université Denis Diderot)
Inserm U1132
Martine Cohen-Solal
Paris

Key facts

Team
• Researchers : 13
• Technicians : 7
• Postdoc fellows : 5
• PhD Students : 10

Translational approaches
• Patents : 1
• Clinical research grants : 3
• Industry partnerships : 4

International research links
• Europe
• Vietnam

Keywords
• osteoarthritis
• osteoporosis
• cartilage
• Bone
• biobank and patients
• molecular signature
• histology
• bone imaging
• clinical trials

Biological Resources
• Transgenic mice for bone
• Biobank
• Cartilage and bone collection

Physiopathology and Identification of target molecules that regulate bone and cartilage remodeling: from mice to patients

Research Brief:
The research unit has been dedicated to the pathophysiology of bone and cartilage diseases and the expertise have positioned the unit as a leader in the field. Our aim is to characterize the mechanisms that regulate bone and cartilage matrix and to identify the molecular targets that result in the development of osteoporosis and osteoarthritis. The different approaches conducted by the scientists and the clinicians actively involved prompted to the development of tools used from basic to translational research. We have therefore validated biochemical and molecular techniques, cellular and animal models that are then translated in humans through a collection of bone and cartilage tissues as well as human serum and synovial samples.

To identify molecules involved in joint diseases, different projects are under investigation:
- Role of the proteoglycan in cell-cell interactions with bone and cartilage microenvironment.
- Mechanisms of interaction between bone and cartilage to characterize the role of bone cells such as osteoclasts in mechanical-induced osteoarthritis. We focus on the role of Wnt molecules involved in the bone-cartilage crosstalk.
- Characterization of microcrystalline stress on the cartilage and the role of microcrystals in chondrocyte metabolism and apoptosis. This work is translated to humans samples and to a cohort.
- The regulation of chondrocyte function by autocrine and paracrine factors.

Methodologies Used:
- Primary culture of mouse and human bone cells (osteoclasts, osteoblasts, osteocytes), bone resorption and formation assays, pit assays, bone explants.
- Cultures of primary mouse and human chondrocytes and cartilage explants.
- Cell phenotyping (qRT-PCR, Western-blot, proteolytic activity, ELISA, ARN interference, immunocytopology, apoptosis assay, cell imaging)
- Histology analysis (histology, immunohistochemistry, analysis of non decalcified bone)
- Characterisation of systemic bone and subchondral bone (microarchitecture, µCT, bone density)
- In vivo model for murine osteoporosis and osteoarthritis.

Publications


Illustrations of studies on bone

A: Characterisation of microarchitectural changes of bone in young patients with idiopathic osteoporosis. Structural analysis of cortical and trabecular bone is performed by high resolution peripheral quantitative computed tomography and correlated to the genotype (NGS panel)

B: Histomorphometric analysis of cortical and trabecular bone of human and murine undecalcified bone samples

C: Cultures of bone cells (osteoblasts and osteoclasts) and functional tests of bone formation and resorption

D: Evaluation of bone resorption related to inflammation in murine arthritis model (microcomputed tomography).

E: Quantification of bone metastasis by bioluminescence technique and syngenic model of osteosarcoma.

F: Migration test and protein localisation

Illustrations of some studies on cartilage pathology

A: Murin model of joint instability (DMM) that induces a progressive loss of cartilage and osteoarthritis.

B: Analysis of subchondral bone by computed tomography; immunohistochemistry of cartilage; culture of bone and cartilage explants.

C: Microcrystal related joint diseases: characterization of calcifications of meniscus, joint crystals and histology of joints
Céline Colnot

Origins and functions of skeletal stem cells in bone regeneration

Université de Paris 05
(Université René Descartes)
Inserm UMR1163
Stanislas Lyonnet
Paris

We combine expertise in mouse models of bone regeneration, genetically modified mice, primary stem cell culture, cellular and molecular analyses to characterize the role of skeletal stem cells in tissue regeneration and repair.

Research Brief:
The goal of our research is to understand the basic cellular and molecular mechanisms of skeletal regeneration. We aim to define the origins of skeletal stem cells, the factors regulating their recruitment at the bone injury site, and the ontogeny of skeletal stem cells. We showed that the local periosteum (the tissue lining the outer surface of bone) is a key source of skeletal progenitors and that periosteum-muscle interactions are critical for bone repair.

Two main projects:
- Role of periosteum and mesenchymal lineages in skeletal regeneration: (1) characterize the skeletal stem cell populations within adult bone marrow and periosteum, and their embryonic origins, (2) compare the regenerative capacities of skeletal stem cells, (3) assess the molecular regulation of skeletal cell fate decisions during bone repair.
- Role of bone-muscle interactions in musculoskeletal repair: (1) identify the cellular contributions of skeletal and muscle lineages to musculoskeletal repair, (2) determine the role of Bone Morphogenetic Proteins in mediating bone-muscle interactions and (3) identify new factors mediating bone-muscle cross talks.

Our research has implications for cell-based and drug-based therapies in skeletal regeneration and the treatment of various diseases of the skeleton.

Methodologies Used:
- Mouse models of bone regeneration (tibial fracture, cortical defects, segmental defects, bone/muscle grafting, musculoskeletal injuries)
- Renal capsule transplantation
- Genetically modified mice
- Histology, histomorphometry, immunohistochemistry
- Primary skeletal stem cell culture
- Flow cytometry
- Transcriptome analyses

Publications


Origins of skeletal stem cells and recruitment after bone injury

Muscle-bone interactions in musculoskeletal regeneration
Our research offers a unique opportunity in arthritic diseases to link inflammation and immune system based on a multidisciplinary approach which involves a two-way process going back and forth between genetic data, immunological mechanisms and the transfer of the findings to the clinic.

Research Brief :
Three main pillars constitute the organisation of our research program:

- Genomic analysis with diagnostic and therapeutic applications,
- Functional validation of targets,
- Animal models.

Chronic inflammatory diseases result from perturbations of effector cells and soluble mediators of the immune system, and local target tissue abnormalities. The precise mechanisms leading to inflammation in these diseases are incompletely understood and treatments inadequate. The aim of our team is to increase understanding of mechanisms of chronic inflammation. Our goals are: the identification of new genes of susceptibility, and their functional characterization.

These diseases show a strong involvement of the major histocompatibility complex. Because much remains to be learnt on the role of this region in autoimmunity, we are developing specific researches on this topic by focusing on spondylarthrits, autoimmune myasthenia gravis that show a strong association with the MHC and soon on rheumatoid arthritis.

Starting from clinical investigations, and based on genetic and genomic approaches, we use in vitro cellular assays or suitable animal models, as needed. The functional role of dendritic cells and myeloid suppressor cells are analysed. Several targets are already studied. The interactions between scientists and physicians in the team and our large collaborative network contribute importantly to the translation of fundamental research into clinical application.

* Methodologies Used :
Transcriptomics
Genetics
Molecular biology
Cell Biology
Biochemistry

Publications


Spondyloarthritis = complex disorder

Research projects

Integrative Biology of Arthritis

Integrative Biology of Arthritis
Challenge: personnalisé medicine
Laurence Vico
U1059 - SAINBIOSE
Université de Lyon - Saint-Etienne (Université Jean Monnet)
Inserm U1059
Laurence Vico
Saint-Etienne

SAINBIOSE studies the chronic pathologies and astronaut systems for osteo-articular through transversal approaches combining fundamental, technological and clinical research.

Research Brief:
Our missions include the understanding of mechanotransduction at the bone and joint level and the use of mechanical stimuli (intrinsic and extrinsic) to control cellular responses to other stresses in the skeletal environment (hormonal, vascular, and energetic).
We analyze mechanical and metabolic deconditioning occurring in extreme (spaceflight and analogs) and disused (osteoporosis, inactivity, osteoarthritis, rheumatoid arthritis, nutrition disorders) conditions.
The efficacy and feasibility of pharmaceutical or mechanical treatments is evaluated, including analysis of mechanical transfers and numerical models.
We study the regulation between bone cells and their environment, as the components of the extracellular matrix (SIBLING proteins), growth factors and vascularization of bone. Tools are genetically altered mice, in vivo models (marrow ablation, hyper or hypo-gravity, whole-body vibrations, nutritional disorders, and pharmaceutical treatments) and specific techniques for qualitative and quantitative tissue imaging. In vitro 3D models of osteocytogenesis imitating key aspects of the bone environment are developed, including scaffolds optimized for dedicated bioreactors.

Methodologies Used:
- Microgravity experiments (including space flight)
- In vitro and in vivo models of mechanical stimulation (including 2D and 3D cell culture)
- In vivo experimental models on rodents
- Histology, histomorphometry of undecalcified mineralized tissues
- Imaging (photonic microscopy, microtomography)
- Bone vascular imaging and bone blood perfusion estimation in mice

Publications
Florence Apparailly

Genetic and immunopathology of inflammatory osteoarticular diseases

Université Montpellier
Inserm U1183
Christian Jorgensen
Montpellier

The combination of clinicians, geneticists and biologists around the theme of (auto)inflammatory disorders.

Research Brief:
Gathering skills for genetic, functional genomic, molecular and cellular immunology, gene and cell therapy, our team aims at the following objectives:
1- Identify genes associated with chronic inflammatory disorders with rheumatic tropism and study their role in pathophysiological conditions
2- Better characterize distinct sub-populations of monocytes and regulatory T cells and identify pathways controlling their differentiation and function in chronic inflammatory and osteoarticular disorders
3- Propose innovative strategies to restore immune tolerance using tolerogenic myeloid cells or induced Treg cells.

Methodologies Used:
Next generation sequencing - Exome sequencing - miRNome - Transcriptomics - Functional genomics - Multi-parametric flow cytometry - Cell sorting - Human and mouse immuno-monitoring - Experimental models of inflammation (monitoring of clinical, immunological, histopathological and bone architecture parameters) - Isolation and in vitro functional characterization of regulatory T cells, dendritic cells, M1/M2 macrophages and osteoclasts - In vitro and in vivo RNAi - Gain and loss of function studies - Reporter systems for validation of miRNA targets - SeaHorse

Publications


Keywords
- Regulatory T cells
- Arthritis
- (Auto)inflammation
- Genetic
- Monocytes
- Animal models of arthritis
- Multi-parametric flow cytometry
- Molecular biology
- Cell biology
- functional genomic
Focusing on the link between inflammation and bone destruction, our team proposes a novel vision on, not only how immune cells control bone resorption, but also on how bone cells modulate bone hemato-immuno niches as well as systemic immune responses.

**Research Brief:**

- The bone marrow is the site of bone remodeling and differentiation of immune cells, but also a major reservoir of memory lymphocytes. Interactions between bone, immune, and precursor cells are therefore permanent and their deregulation is associated with many pathologies, including chronic inflammatory diseases characterized by bone destruction. Our projects aim to dissect these interactions and to determine how they contribute to maintaining the homeostasis of the bone and immuno-hematological systems.

**Axis 1: Osteoclasts inflammation.**

- Our work revealed that, depending on the context, osteoclasts induce immunosuppressive or inflammatory responses. Our aims are to better understand the origin and the new functions of these different osteoclasts, and to identify markers of the different subsets of osteoclasts in order to be able to target them specifically.

**Axis 2: Memory T cells and their niches.**

- We have characterized the mechanisms by which memory Th17 lymphocytes contribute to inflammatory bone destruction in vivo. Our projects are focused on the analysis of the cellular interactions that allow these memory cells to be maintained in the bone marrow in particular by interacting with bone cells and MSCs. We are also developing new therapeutic approaches to control activation and/or elimination of pathogenic memory lymphocytes.

**Methodologies Used:**
- Primary culture of human and murine bone cells: osteoclasts, osteoblasts, MSCs
- Functional assays to characterize immune cell responses (T cells, dendritic cells, monocytes)
- Flow cytometry for cell phenotyping (immune cells, MSCs, hematopoietic progenitor and stem cells)
- Flow cytometry and cell sorting of osteoclasts
- In vitro generation of CD4+ Th subsets
- Generation of human iPS cells and their derivatives (MSCs, ...)
- Histological analysis on bone and other tissues
- Transcriptomic analysis

**Publications**


Link between inflammation and bone destruction in Crohn’s disease

Bone destruction is a hallmark of inflammation. Gut inflammation generates TNFa-producing Th17 cells that migrate to the bone marrow where they dramatically increase osteoclast differentiation. (i) They produce osteoclastogenic factors (RANKL, TNFa), (ii) they stimulate MSCs to produce RANKL, and (iii) they increase in MSCs the expression of chemokines attracting OCL precursors (monocytes). The resulting osteoclasts have an inflammatory phenotype (i-OCLs) participating to inflammatory responses.

The immune function of osteoclasts

(A) Osteoclasts (OCLs) have different phenotypes according to their environment / origin and are antigen-presenting cells.
(B) In steady state, tolerogenic OCLs (t-OCLs) induce regulatory T cells, that can participate to the immune tolerance to avoid autoimmune reaction against self peptides issued from bone resorption.
(C) In an inflammatory context, inflammatory OCLs (i-OCLs) induce TNFa-producing CD4+ T cells that can participate to autoimmune reactions and link inflammation and bone destruction.

Interaction between memory CD4+ T cells and MSCs in the bone marrow

The bone marrow is a major reservoir for memory T cells. Maintenance of these cells in the bone marrow is controlled by bone marrow cells, in particular mesenchymal stromal cells (MSCs) and osteoclasts (OCLs). Establishment of clones of MSCs derived from induced-pluripotent stem (huiPS) cells represents an original model to study the mechanisms involved in these interactions in human in normal and pathological conditions.
Jean-Claude Scimeca

BIPOA, BioIngénierie et Physiopathologie Ostéo-Articulaire

UCA, Université Côte d'Azur
CNRS UMR7277 Inserm U1091
Stéphane Noselli
Nice

Gathering researchers, clinicians and industrial partners, we aim at developing and transferring into clinic innovative therapeutic solutions for the treatment of musculoskeletal conditions.

Research Brief:
Our project is focused on bone tissue physiopathology and reconstruction in traumatic, tumoral, and aging situations. Within this context, we aim at developing and transferring into clinic innovative therapeutic solutions for the treatment of musculoskeletal conditions.

The main objectives of our experimental work are: (i) to develop calcium phosphate-based new bone substitutes for bone reinforcement and reconstruction; (ii) to design biomaterials incorporating therapeutic compounds targeting bone tumours; (iii) to decipher the molecular mechanisms underlying new bone formation in traumatic and tumoral environments; (iv) to engineer innovative in vitro 3D models of bone-like constructs, as well as in vivo bone cancer models, based on the use of bone substitutes we develop; (v) to use our models to address basic questions about bone cells and cancer cells interactions with each other and with their microenvironment.

In the future, we will continue to use bone substitutes as drug delivery systems to improve bone strengthening and bone reconstruction. We will also investigate strategies involving the combination of these therapeutic agents to enhance their action. Lastly, to identify new therapeutic targets, these bioactive biomaterials will be used to set up in vitro 3D scaffolds allowing us to document the underlying molecular mechanisms governing bone cells and cancer cells interactions within a bone-like microenvironment.

Methodologies Used:
With a view towards building normal or metastatic bone tissue niches, we designed several 2D-3D cell culture models combining calcium phosphate-based biomaterials and either bone or cancer cells. Moreover, we take advantage of both in vitro and in vivo models for the screening of therapeutic compounds that could improve the treatment of bone defects after traumatic or cancer lesions. We are also interested in triggering the host immune response against tumour cells. In this attempt, in vivo cancer models are used to identify therapeutic targets among chemokines and chemokine receptors, which are key partners regulating the interactions among bone, immune system, and cancer cells.

Publications

Bone defect filling in the course of ortho/trauma surgery - Bone reconstruction after tumour resection - Osteoporotic bone strengthening for fracture prevention.
Key facts
Team
• Researchers: 14
• Technicians: 4
• Postdoc fellows: 0
• PhD Students: 3
Translational approaches
• Patents: 1
• Clinical research grants: 0
• Industry partnerships: 4

Keywords
• bone remodeling
• biomaterials
• bone quality
• bone microarchitecture
• bone diseases
• nanocomputed tomography
• FTIR microscopy
• histomorphometry
• Raman microscopy
• microcomputed tomography

Biological Resources
• In vivo animal models
• In vitro models

Daniel Chappard
GEROM: Research Group on Bone Remodeling and bioMaterials
Université d' Angers
ANR LabCom UPRES EA 4658
Daniel Chappard
Angers

The originality of our Unit consists in a large panel of histological techniques applied to the study of bone diseases (metabolic and malignant) and the use of bone biomaterials.

Research Brief:
Bone remodeling allows the constant adaptation of the skeleton to local variations of strains, to hormonal and metabolic changes during all the life. The coordinated action of osteoclasts and osteoblasts in concert influence the properties of the bone mineral matrix (i.e., bone quality) and particularly the microarchitecture of bone tissue. The microarchitecture of trabecular bone can be altered in a variety of metabolic bone diseases such as osteoporosis, primary hyperparathyroidism and also when malignant cells invade the bone marrow (myeloma, lymphomas and metastasis). We have developed a number of techniques to measure bone microarchitecture on human bone biopsies and animal bone specimens. The use of new 2D stereological methods has been proposed using Euclidian and fractal geometry. These techniques allowed us to characterize various types of osteoporosis with different bone microarchitecture (post-menopausal osteoporosis, glucocorticoid-induced or idiopathic male osteoporosis). The use of microcomputed tomography (microCT) was also extensively developed to analyze bone directly in 3D. The importance of microarchitecture was also evidenced in bone biomaterials where it was found to influence the migration of wear debris in prosthetic loosening. The microarchitecture of biomaterials themselves is important to consider and new biomaterials with a 3D architecture mimicking that of bone have been proposed.

Methodologies Used:
• microcomputed tomography (microCT) and nanocomputed tomography
• bone histomorphometry
• videomicroscopy
• animal models of bone diseases
• FTIR and Raman microscopy
• image analysis
• polymer chemistry

Publications
KÜN-DARBOIS J.D., LIBOUBAN H., CHAPPARD D. (2015). Botulinum toxin in masticatory muscles of the adult rat induces bone loss at the condyle and alveolar regions of the mandible associated with a bone proliferation at a muscle enthesis., Bone. 7(), 75-82


MicroCT in a case of bone metastasis in human

Bone biopsy imaged by microcomputed tomography. Note the irregularity of repartition of the trabeculae in the cancellous space (corresponding to focal osteolysis) and the reactive proliferation of woven bone on the periosteal side of the left cortex.

MicroCT analysis of a stack of granules of a bone substitute

MicroCT analysis of a stack of beta-tricalcium phosphate granules used as a bone substitute. Left: original microCT image in 3D, Right: vector analysis of this stack.

Granule of a biomaterial with hyaluronic acid

Hyaluronic acid can facilitate the handling of biomaterials. In addition, it can bind the circulating growth factors.
Research teams with secondary association to PMN Institute
Key facts

Team
• Researchers : 16
• Technicians : 8
• Postdoc fellows : 1
• PhD Students : 8

Translational approaches
• Patents : 3
• Clinical research grants : 0
• Industry partnerships : 5

Keywords
• Bone
• Bioengineering
• Inflammation
• Infection
• Biomaterials
• 3D scaffolds synthesis
• Stem cells
• Bone cells differentiation
• In vivo
• Bacterial biofilms

Our multidisciplinary teams cover the whole spectrum of bone bioengineering coming from biomaterials synthesis through in vivo assessment with a particular emphasis on bone loss-related pathologies.

Research Brief:
The laboratory «Biomaterials and inflammation in bone site» brings together faculty and hospital practitioners (Dental surgeons - Pharmacists - Biologists) and INSERM researcher. The multidisciplinary research project aims to develop new functionalized biomatrices intended for bone filling and to evaluate their regenerative potential during interactions between host cells and biomatrices in inflammatory and/or septic environment.

To achieve these objectives, the laboratory conducted a basic research: 1) to improve the physicochemical potential of biomatrices to optimize their cellularization and their functionalization, 2) to characterize biological processes (inflammation, infection, tissue neoformation...) affecting the regenerative capacities of biomatrices and cellular immunomodulation, 3) to study the pathophysiology of bone in the context of cystic fibrosis.

Our objectives also relate to technological and methodological developments: 1) evaluation of the biocompatibility of new treatments such as superficial nanocrystallization (SMAT process) to reduce the production of articualr prosthesis wear debris; 2) validation of cold plasma sterilization adapted to pre-packaged medical devices.

Methodologies Used:
In vitro: primary human cells and/or bacteria ; Biocompatibility ; Stem cell commitment ; Inflammatory or anti-inflammatory potential ; Degradation (kinetics, particles) ; Anti-bacterial activity ; Bacterial / biofilm adhesion ; Cells / bacteria / biomatrices Interactions
In vivo: Inflammation / infection (mice) ; Air pouch model ; LPS model (IV / IP) ; Infection models Repair / bone regeneration (mice / rat) ; Femoral condyle drill defect model in mice ; Masquelet induced-membrane model in rats

Publications


BIOLOGY AND ENGINEERING OF CARTILAGE

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Bernard Verrier
LYON

Molecular understanding of chondrocyte differentiation, construction and degradation of the cartilage matrix is used to develop multi-factorial approach combining cells, microenvironment, signaling molecules and mechanical conditioning for reconstruction of cartilage in clinical-grade conditions.

Research Brief:
Cartilage is not vascularized and presents poor healing potential. Consequently, traumatic and degenerative lesions of articular cartilage eventually progress to osteoarthritis, a worldwide leading source of disability. Common surgical treatments are not satisfactory since often leading to the production of fibrocartilage, and joint replacement is a short-term therapy because knee prostheses have limited life spans. In this context, cartilage is a good candidate for developing tissue engineering procedures for its repair.

The field of regenerative medicine should be inspired by developmental processes to identify differentiation factors or signaling pathways useful for tissue regeneration. With this view, our group takes advantage of its expertise on chondrocyte differentiation and cartilage development to set up innovative protocols for cartilage repair in collaboration with industrial and medical partners. More recently, we developed new research on regeneration of the pulpodental complex. In brief, our main lines of research are:
- multi-factorial, clinical-grade approach combining cells, 3D matrix, signaling molecules and mechanical conditioning for cartilage reconstruction.
- Identification of epigenetic marks of the osteoarthritic chondrocyte phenotype.
- Advanced characterization of human mesenchymal stem cells after expansion and chondrogenic commitment in hydrogel under serum-free conditions.
- Pathophysiology and regeneration of the pulpodental complex.

Methodologies Used:
- 3D cell cultures of human chondrocytes
- Cell culture in hypoxia
- Tissue engineering in bioreactor
- 3D bioprinting
- pre-clinical animal studies
- Flow cytometry analysis of mesenchymal stem cells isolated from bone marrow, adipose tissue, Wharton jelly and dental pulp

Publications

Bougault C, Pauvier A, Aubert-Foucher E, Mallein-Gerin F. (2009). Investigating conversion of mechanical force into biochemical signaling in three-dimensional chondrocyte cultures, Nat. prot. 4(), 928-938


We develop innovative protocols to engineer cartilage. We explore if mesenchymal stem cells can be used as an alternative to chondrocytes and if physical parameters like mechanical forces or hypoxia can be added in the protocols. We use drug forms of soluble factors and biomaterials that are approved as medical devices.

**Construction of Human Cartilage in Bioreactor**

We have shown that perfusion improves the quality of the cartilage matrix synthesized by human chondrocytes seeded in clinical-grade collagen sponges. The bioreactor is an oscillating perfusion bioreactor which offers the control of several interesting parameters for optimizing the cell culture conditions.

**Cartilage Matrix Reloaded in Hydrogel**

In native articular cartilage, chondrocytes organize in chondrons where cells share their extracellular matrix to form primary tissue units. By using specific combination of soluble factors: a cocktail of FGF-2/Insulin to amplify the chondrocytes then a cocktail of BMP-2/Insulin/T3 to redifferentiate the chondrocytes, it is possible to recapitulate construction of chondrons and cartilage matrix in hydrogel. This cartilage gel can then be implanted by arthroscopy to fill a cartilage defect.