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THE CHAIRMAN'S MESSAGE

Dear Reader,

We are very delighted to present in this annual report the activities carried out during 2006 by the staff of our research centre for health denominated "CRP Santé".

There have been some major changes in the CRP Santé in 2006. On the one hand, the board of administrators has been partly renewed during 2006 and on the other hand, the organisational structure of the centre has been changed in a significant manner. We have now two clearly separated directorates, notably one research directorate with two main fields of activities, namely biomedical clinically oriented research and public health research, and one directorate for administration and finance. These changes have been approved by the Minister for Health and the Minister for Research.

The new structure should help us in the future to better achieve our goals, that means, coordinating and organising quality research in health, medicine and human biology, in improving the public health sector and in contributing towards economic development.

During the last years, the CRP Santé has succeeded in building confidence among its partners by carrying out many high quality research projects. At a scientific level, the CRP Santé is responding to high international quality standards, which was twice confirmed in 2005 and 2006 by its scientific advisory board's evaluation reports. As a matter of fact, the scientific advisory board, composed of high ranking international scientists, pointed out in its reports that many projects are of high quality and are likely to result in new important findings in their respective fields.

At institutional level, the government has confirmed its policy to increase spending for research and innovation up to 1% of GNP. But the policy makers do also require that these additional budgets have to be put into priority research.

Hence we know that there is the need for the CRP Santé to define its priorities and to be selective, to limit the number of areas of research. The mission of the scientific advisory board consists on the one hand of giving an appraisal of the activities in our different laboratories. This is a very important issue because we need to have an efficient external evaluation system using international standards. But on the other hand, it should help us to design a coherent long-term strategy for the CRP Santé's research activities. But by doing this, we will be careful and we will not be too restrictive and compromise well-working research groups.

But it is also important, tremendously important, that we persist in strengthening leadership and governance in our organisation at all levels. So, many measures have already been taken or tightened in order to consolidate the existing achievements. But we do also know that we have to go beyond. A new efficient and flexible framework for decision-making putting the accent on the future is being implemented.

Frank GANSEN

Chairman

THE DIRECTION'S MESSAGE

The year 2006 has been full of challenging situations for CRP-Santé. Nevertheless, despite numerous internal changes and external turbulences impacting on our institution, the core business of CRP-Santé, our research activities, were continued with the greatest success. The excellent performance of CRP-Santé in research has been confirmed for the second time by an independent, international scientific advisory board in September 2006. Scientific excellence of CRP-Santé resulted in an important number of scientific publications in international peer-reviewed high impact journals and in direct contributions to the better understanding of health determinants of the Luxembourg population.

2006 was a year of great opportunities. First of all, a revised mission of CRP-Santé was defined, more precise and closer to our domains of excellence: In line with the major health challenges at European and national levels, CRP-Santé's current mission is to generate knowledge on the pathogenesis, diagnosis and treatment of diseases with large impact on public health, and to perform epidemiological surveillance of these diseases and research on related health determinants in the population. To put it in another way, CRP-Santé will mainly focus on clinically oriented biomedical research and on public health, and will participate to a lesser extend to other fields such as basic research, innovation, education and public debate, domains that will be addressed in collaboration with selected national and international partners. Indeed, with its present orientation, which is unique in our country, CRP-Santé will be a valuable partner for other institutions in the moving national landscape of research, and over the coming months CRP-Santé will be actively looking for new collaborations. As a first step, the scientific collaborations with the Centre Hospitalier de Luxembourg and with the Laboratoire National de Santé, both existing for many years already, have now been clearly defined in formal collaboration contracts.

Since several months, CRP-Santé has a new management composed of a board of three directors, representing public health, biomedical research and administration. This board has succeeded in improving the internal organisation of CRP-Santé. In the future, CRP-Santé will be organised into four research departments grouping together the currently existing large number of research units and independent projects. The four departments, which will rapidly gain critical mass, will be: infections and immunology, oncology, cardiovascular diseases and public health. In addition, better internal working procedures will improve the administration and daily organisation of our institution. As important examples, we can mention the recent approval by the board of administrators of a modern Intellectual Property Policy, or the adherence of CRP-Santé to the European Charter of Researchers. The goal of the management is to transform CRP-Santé, over a short period of time, into a competitive actor in research following international standards. Therefore, however, further improvements in the internal structure of the institution and the management will have to be made.

In order to achieve this vision, the management of CRP-Santé relies on the confidence and positive support of its board of administrators and of the public authorities, mainly the Ministries of Research and Health, and the daily contribution of all its collaborators and partners. CRP-Santé also rapidly needs a commitment from the authorities to build its permanent health research center .The upcoming extension of the laboratory building and the additional office space in the vicinity of the modular building will only transiently improve the situation, but will not be able to cope with the planned development of CRP-Santé in the medium term.

As managers of CRP-Santé, we are confident to have the support of all stakeholders and to be able to lead CRP-Santé to a bright and promising future.



LABORATORY OF PLANT MOLECULAR BIOLOGY



LABORATORY OF PLANT MOLECULAR BIOLOGY

Head of Research Unit: Dr André Steinmetz, D.Sc.

Team members:

Clément Thomas, PhD Céline Hoffmann, PhD (in charge of the Confocal Microscopy Platform) Ning Wang, MD Monika Dieterle, PhD Bernard Grausem, PhD (until April 30, 2006) Cécile Hustin, PhD (since May 1st, 2006) Flora Moreau, Lab Technician

Sabrina Gatti, PhD student, BFR Fellow Jessica Papuga, PhD student, BFR Fellow (since November 1st, 2006)

1_Objectives and focuses

Research expertise of the Unit is on the structural and functional analysis of genes. Present research in that field focuses on a family of actin-binding proteins, the LIM domain proteins, which we have recently identified as novel actin filament stabilizers and bundlers (Thomas et al., 2006; 2007). Current research objectives are to understand the mechanisms controlling the binding of these proteins to actin filaments, as well as to elucidate the roles of LIM proteins in cell differentiation.

Another line of research of the unit is on plant allergen identification and on the development of molecular tools allowing improved tracing of components including plant allergens in food products. The latter aspect is funded by the National Research Fund (FNR) and is carried out in collaboration with three other research laboratories (see below under "national collaborations").

$\mathbf{2}_{-}$ Ongoing projects and main results in 2006

The Plant Actin Cytoskeleton

The actin cytoskeleton is a highly organized and dynamic structure present in all eukaryotic cells where it plays a central role in many processes, including intracellular transport, cell growth, cell migration, signaling, and division. Its assembly from monomeric subunits (G-actin) requires energy, and the resulting filamentous structure is stabilized by a subset of actin binding proteins (ABPs) that bind along the side of the filament. The presence of these proteins also affects the cytoskeletal structure and architecture by mediating the association of actin filaments into cables and bundles and cross-linking these structures into complex networks. ABPs not only regulate the supramolecular organization and function of the actin cytoskeleton but they also control its dynamics via polymerization/depolymerization and severing. Mutations in proteins that affect the stability of actin filaments cause severe diseases in humans and animals and disrupt important cellular functions in plants as well.

Recent work in our and other laboratories have identified plant LIM proteins and the animal Cysteine Rich Proteins (CRPs) as novel F-actin binding proteins. These proteins, which are structurally related and encoded by a small gene family, have a dual, nuclear and cytoplasmic localization. Animal CRPs have been predominantly identified in muscle tissues where they not only play a role in development and differentiation, but are also required for maintaining the architecture of the cells. Understanding the molecular functions of these proteins requires an identification of the target developmental genes on the one hand, and the target cytoplasmic structures on the other hand. It is expected that the plant LIM proteins and the animal CRPs operate via conserved molecular mechanisms.

A comparative analysis of the cellular localization of two GFP-fused tobacco LIM proteins in the tobacco BY2 cell line by confocal microscopy revealed a significant difference in their localization pattern in that one of the proteins (WLIM1) clearly associated with a filamentous cytoplasmic structure. This structure was subsequently identified as the actin cytoskeleton using cytoskeleton-interfering drugs (latrunculin B for actin and oryzalin for microtubules). The second protein (WLIM2) was evenly distributed in the cytoplasm with only occasional weakly

labeled filamentous structures visible. Fluorescence recovery after photobleaching (FRAP) experiments have shown that the interaction of the WLIM1 protein with the actin cytoskeleton is very dynamic. In spite of this dynamic interaction, binding of WLIM1 was found to stabilize the actin cytoskeleton against depolymerization. This stabilization effect appears to be due to the assembly of F-actin filaments into higher order structures such as bundles and cables, as we could demonstrate by co-sedimentation experiments as well as by direct fluorescence microscopy observations. Bundling of actin filaments is triggered essentially by the first of the two structured LIM domains in the protein, while the second domain potentiates the bundling effect. Although the second protein, WLIM2, does not show a clear binding to the actin cytoskeleton in vivo, it binds to and bundles actin filaments in vitro with a dissociation constant similar to that of WLIM1. It is unclear at the moment what causes the discrepancy of the in vivo and in vitro observations for the WLIM2 protein.

Important yet unresolved issues include the elucidation of the molecular mechanism leading to the binding of the WLIM1 protein to actin filaments. Why does WLIM1 bind to actin filaments in vivo and why WLIM2 does not? We have some experimental evidence that F-actin binding of WLIM1 is stress-induced. A most likely mechanism is a stress-induced conformational change of the protein leading to an increased affinity for F-actin. If this is the case, how then can stress induce a conformational change in a protein ? Another important issue is the nuclear role of these proteins in vivo. Several of the plant LIM proteins were found to bind to promoter sequences (e.g. the histone H3 promoter) and may therefore control gene expression. It remains to be seen if these target genes are merely housekeeping genes like the histone genes, or if they include developmental genes involved in the differentiation of specific subsets of cells ? Useful information regarding possible developmental roles of the LIM proteins can be obtained from the study of spatial and temporal expression of the genes by in situ hybridization and real-time PCR. We also expect chromatin-immunoprecipitation (ChIP) and subsequent sequence identification of the isolated DNA fragments to be an appropriate technology to identify candidate genes whose expression is regulated by WLIM protein-binding.

Food Tracing (A. Steinmetz, coordinator of project)

This research project, which is supported by the National Research Fund, aims at developing molecular technologies allowing unambiguous identification and tracing of food components, not only to allow better quality control of food products, but also to identify material coming from allergenic plant species. It requires the identification of stable markers that are specific for each species. The markers we have selected are DNA- and protein-based, and can be detected using simple or multiplex PCR and monoclonal antibodies respectively.

The DNA Approach : Plant LIM protein genes

Tracing of food components via the DNA approach requires not only information on the coding sequence of marker genes used in the study, but also on the structure of the genes. Best specificity is obtained when highly variable sequences in the genes are included. Hence only genes with introns (which are the most variable regions in genes) are appropriate candidates. We have used the LIM protein genes as marker genes in plants since earlier studies had shown that these genes are rather small and contain several short introns whose positions are conserved in unrelated plant species such as sunflower, Arabidopsis and tobacco. Our analysis includes the eight plant species maize, wheat, barley, rice, soybean, peanut, sesame and walnut. Some of these species (e.g. peanut, sesame and walnut) can cause allergies in allergy-prone individuals. The genomic sequences of the marker genes for all above-mentioned plant species except walnut have so far been determined following PCR amplification of genomic DNA using oligonucleotide pairs derived from publicly available cDNA sequences. The analysis of the aligned genomic sequences has revealed that the exonic sequences are rather conserved while the intronic regions differ not only in their sequence, but also in their lengths. For the design of oligonucleotide pairs for specific species identification it was therefore possible to consider not only species-specific sequences, but to identify species also by the length of the fragments produced using a given pair of oligonucleotides. Based on genomic sequence comparisons we designed oligonucleotide pairs that allow unambiguous identification of maize, rice, wheat, barley, soybean, peanut and sesame by producing DNA fragments of defined length for each species.

Using the same approach we have developed, in collaboration with the "Administration des Services Techniques de l'Agriculture" (ASTA), molecular DNA-based probes that allow identification of English ray-grass, Italian ray-grass and Westerwold ray-grass.

The Protein Approach : Plant Lipid Transfer Proteins

The protein approach takes advantage of species-specific differences in the peptide sequence of marker proteins to develop monoclonal antibody-based tests for detection of these proteins, such as allergens, in food products. A major disadvantage of the protein approach over the DNA approach is that a given protein may not be present in all cell types all the time, while DNA is present invariably in all the cells of an organism. Proteins can be present in cells in trace amounts or abundant (e.g. storage proteins in seeds). The more abundant a protein is in edible plant structures or tissues, the easier it can be detected in food products containing the corresponding plant parts. Seeds from a number of plant species (e.g. cereals and legumes) are a common source of proteins and lipids in food products. For seed-based food products the antibodies should be directed against abundant seed proteins. As a consequence this study requires a preliminary identification of abundant proteins in seeds from the various plant species considered in the present study (see DNA approach). Purification and identification of these abundant proteins is easily performed by SDS-PAGE (polyacrylamide gel electrophoresis in the presence of the detergent SDS), followed by staining of the proteins in the gel, and their subsequent partial sequence determination. A family of seed storage proteins comprises Lipid Transfer Proteins (LTPs), some of which have also been identified as potent allergens. Lipid Transfer Proteins are encoded by a large gene family and are highly variable in their sequence ; they all contain four disulphide bridges stabilizing the globular structure of the protein.

Using the above-described approach we have identified seed Lipid Transfer Proteins from maize, wheat, barley, peanut, walnut and soybean. Since their large scale extraction and purification from seed extracts was associated with technological problems, we have tried the expression of one of the peanut proteins in bacteria using a synthetic gene with optimized bacterial codon usage (Genscript, USA). Following cloning into two different bacterial expression vectors, several attempts to express the protein in E. coli failed for yet unidentified reasons. More recently the peanut protein was successfully expressed in the yeast Pichia methanolica. This system will be used now for expression of the other LTPs prior to their use in immunization of mice and hybridoma production for monoclonal antibodies.

3_Publications

• Thomas C, Hoffmann C, Dieterle M, Van Troys M, Ampe C and Steinmetz A (2006). Tobacco WLIM1 Is a Novel F-Actin Binding Protein Involved in Actin Cytoskeleton Remodeling. *Plant Cell* 18, 2194-2206. (Article recommended for reading by the *Faculty of 1000 - Biology*).

• Thomas C, Hoffmann C, Gatti S and Steinmetz A (2007). LIM Proteins: A Novel Class of Actin Cytoskeleton Organizers in Plants. *Plant Signaling and Behavior 2, e1-e2*.

• Lazarescu E, Friedt W, Horn R and Steinmetz A (2006). Expression Analysis of the Sunflower SF21 Gene Family Reveals Multiple Alternative and Organ-Specific Splicing of Transcripts. *Gene* 374, 77-86.

4_Collaborations

Intra-CRP-Santé collaborations

- F. HENTGES (Partner in Food Safety project)
- E. FRIEDERICH (Partner in Food Safety project)

National collaborations

- G. MORIS, Division of Food Control, LNS (Partner in Food Safety project)
- D. RUCKERT, Administration des Services de l'Agriculture (ASTA), Ettelbruck

International collaborations

• **Profs. M. VAN TROYS and C. AMPE**, Department of Health, Ghent University and Medical Protein Research, Flanders Interuniversity Institute for Biotechnology, Ghent, Belgium

• EVOGENE, Rehovot, Israel

• **Dr. W.H. SHEN**, Department of Cell Biology and Plant Development, IBMP Strasbourg, France

• **Dr. M. HEINLEIN**, Department of Virology, Institute of Plant Molecular Biology, CNRS, Strasbourg, France

• Prof. Dr. W. FRIEDT, Institute for Plant Breeding, University of Giessen, Germany

LABORATORY OF IMMUNOGENETICS AND ALLERGOLOGY



LABORATORY OF IMMUNOGENETICS AND ALLERGOLOGY

Head of Research Unit: Dr François Hentges, MD

Team members:

Biomolecular, immune and biochemical characterisation of animal allergens

Christiane Hilger, PhD, senior scientist, project supervisor Cathy Leonard, PhD, project manager Annette Kuehn, PhD, project manager Kyra Swiontek, engineer Paul Felten, technician Caroline Davril, technician Teskrat El-Hassen, student master

Innate immunity at the cellular level

Jacques Zimmer, MD PhD, senior scientist, project supervisor Tatiana Michel, PhD (post-doc) Aurélie Poli, engineer Natacha Ralainirina, doctoral student



Immunolocalisation of Arg r 1 in the salivary gland of Argas reflexus by confocal microscopy

| marker | fario trout | salmon trout | brook trout | salmon | atlantia | pacific | 23 | carp obp | cod | | mackerel | tuna | | herring | redfish |
|--|----------------|-----------------|----------------|--------------|----------|-------------|----|----------|-------------|------|----------|----------------|------------|---------|---------|
| | br | ook tro | out | salmon trout | | | | atl | antic | salr | non | pacific salmon | | | |
| | 1 | 2 3 | 4 | 1 | 2 | З 127 bp | 4 | 1 | 2 28 bp | 3 | 4 | 1 | 2 28 bj | 3 | 4 |
| Top: Detection of Salmonidae fishes by specific PCR. | | | | | | | | | | | | | | | |

Bottom: Brook trout, salmon trout and salmons: Subtyping within Salmonidae by specific PCR.

1_Objectives and focuses

Allergic diseases constitute a major health problem for hundreds of millions of people in developed and increasingly so in developing countries.

It is the strategy of the **Laboratory** of **Immunogenetics** and **Allergology** (LIA) to strengthen its skills in applied research, to develop competitive basic research and to create an optimal environment for improved diagnosis, prevention and treatment of patients with allergic and **immune-mediated** diseases. Research is being performed in close collaboration with the national unit of Immunology and Allergology at the CHL.

In its scientific strategies the LIA aims at performing high quality basic research focussing on the:

- Characterisation at the protein and DNA-level of 3 major animal allergen families: lipocalins, serum albumins and parvalbumins.
- Analysis of the cellular immune response, including cellular regulatory mechanisms to selected allergens belonging to these protein families.
- Understanding some general principals of adaptive and innate immunity through investigation of interactions between natural killer cells and regulatory T cells.
- Analysing interactions between the nervous system and the immune system by studying the effects of several neurotrophins on natural killer cells and regulatory T cells.

Secondly the laboratory applies its research potential at improving patient diagnosis and care. This implies:

- Development of reagents for in vitro detection of patient sensitisation to food and respiratory allergens.
- Development of protein and DNA reagents to detect and trace animal allergens and proteins of animal origin.
- Test in mouse models, modified recombinant allergens susceptible for future treatment of allergic patients.

2_Current projects

Biomolecular, immune and biochemical characterisation of animal allergens.

During the recent years the laboratory has cloned and characterized several proteins belonging to the 3 major animal allergen families: lipocalins, serum albumins, and parvalbumins. Over the next years the lab will extend its biomolecular and cloning activities of allergens, develop the analysis of the regulatory immune response to selected allergens and finish the biochemical characterisation of one further lipocalin allergen.

The projects are:

Further characterisation of guinea pig and rabbit allergens and patent deposition. (project 1a).

Biochemical and pharmacological characterisation of Arg r 1, the major allergen responsible for pigeon tick anaphylaxis (**project 1b**).

Production of genomic and molecular tools for the tracing of parvalbumins of fish, mammalian, or bird origin (**project 2**).

Cloning of new fish allergens belonging to a new protein family and possible patent deposition.

Analysis in an in vitro BALB/c mouse system of the major T cell epitopes recognized by natural and induced T regulatory cells against cat serum albumin, cat Fel d1, and cod parvalbumin. (project 3).

Innate immunity at the cellular level

In this field the LIA laboratory will focus on the study of natural killer cells and natural regulatory T cells (CD4+CD25+ and CD8+CD122+). One major topic will be the investigation of bi-directional interactions between natural killer cells and regulatory T cells. A second major topic will be the analysis of the potential effects of the neurotrophins nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF) and neurturin (NTN) on the phenotypic and functional properties of natural killer cells and regulatory T cells (**project 4**).

3_Scientific results

Allergen characterisation and Cloning

The lipocalin family

The LIA has recently cloned and expressed as recombinant proteins two lipocalin allergens from the guinea-pig and determined the protein sequence of a third lipocalin allergen and also a forth allergen not belonging to the lipocalins. These results have not yet been published, awaiting patent application.

In a collaborative project (Dr. G. Paesen, Oxford) the characterisation of the biochemical properties (histamine binding) and the crystallisation of the lipocalin, Arg r 1 are in progress. A collaborative epidemiological study with several European centres, using the recombinant allergen produced in the LIA is in progress to appreciate the frequency of sensitisation to Arg r1.

Parvalbumins

In the frame of a collaborative FNR project on food safety (SECAL programme) the laboratory works on food and allergen tracing using animal parvalbumins as tracers (protein and DNA level) During the first part of the project the laboratory has cloned the parvalbumin cDNA of 8 major fish species (of whom only 4 were previously known), and expressed them as recombinants. Furthermore the native parvalbumins of these fish species were purified by chromatography. In addition we cloned the α parvalbumins of chicken, turkey,

cattle, pig, horse and mutton. Only the cDNA of chicken was previously known. Furthermore the previously unknown genomic DNA sequence of these parvalbumins was cloned. (project 2). Specific detection of salmonidae subspecies at the DNA level has been performed using appropriate primer pairs.

First monoclonal antibodies which specifically detect carp parvalbumin have been produced.

Immune response to allergens and T regulatory cells

The serum albumin protein family

This project aims at defining T cell epitopes recognized on allergens by natural T regulatory and induced T regulatory cells. Cat serum albumin, overlapping peptides mapping the CSA molecule, mouse serum albumin and a recombinant orthotopic MSA - CSA fragment V fusion protein have been used for the study of the cellular proliferation and cytokine secretion pattern of natural and induced T regulatory cells in the BALB/c mouse upon stimulation by allergen pulsed dendritic cells (*T regs and allergens Project 3*).

Innate immunity at the cellular level

In 2006, we focused our investigations on mouse models (additional work on human cells is planned from 2007 on).

We could show in the field of neurotrophins that the high affinity NGF receptor TrkA is expressed on NK cells and regulatory T cells. In both cases, expression increases after culture in the presence of NGF that thus stimulates the expression of its own receptor, as described for other cell types. In NK cells, TrkA is expressed at a higher density on the CD27^{high} subset. NGF has no influence on the survival of NK cells.

Likewise, the NTN receptor GFR- α 2 is expressed by NK cells, as determined by flow cytometry, RT-PCR and confocal microscopy. Expression is found on resting as well as on interleukin 2activated NK cells. Neither GDNF nor NTN positively influence the survival of NK cells in the absence of interleukin 2. They do furthermore not seem to have a major effect on the cytokine production by activated NK cells. In NTN-KO mice, the repertoire of activating and inhibitory NK cell receptors is not dramatically different from wild-type mice, although a somewhat lower expression (in terms of percentages of positive cells and of expression levels) is evidenced for several of these molecules. Natural cytotoxicity of activated NK cells against the tumour target cell line YAC-1 is higher in NTN-KO than in wild-type mice, suggesting a possible inhibitory effect of NTN on functional NK cell properties. These data will have to be confirmed and extended in future experiments.

4_Publications and presentations in 2006

Original publications directly linked to the program of the lab

• Ralainirina N, Poli A, Michel T, Poos L, Andres E, Hentges F, Zimmer J. Control of natural killer (NK) cell functions by CD4+CD25+ regulatory T cells. J Leukoc Biol. 2007; 81:144-53.

• **Zimmer J, Poli A**, Andres E, Hanau D, Brons NH, **Hentges F**. Reduced cytokine-mediated up-regulation of HLA-DR in TAP-deficient fibroblasts. Immunol Lett. 2006; 107:109-118.



• Zimmer J, Hentges F, Andres E. Natural killer cell deficiencies and severe varicella infection. J Pediatr. 2006; 148:564.

• **Hilger C**, Bessot JC, Hutt N, Grigioni F, De Blay F, Pauli G, **Hentges F**. IgE-mediated anaphylaxis caused by bites of the pigeon tick Argas reflexus: cloning and expression of the major allergen Arg r 1. J Allergy Clin Immunol. 2005; 115(3):617-22.

Original publications in collaboration

• Cvackova M, Kucera P, **Hilger C**. The pigeon tick (Argas reflexus) as a cause of anaphylaxis. Alergie 2/2006.

• Donney C, Kopferschmitt-Kubler M-C, Barderas R, **Hilger C**, Weber M, Vérot A, Pauli G, de Blay F. Occupational rhinitis and asthma due to pig albumin and gammaglobulin. Revue française d'allergologie et d'immunologie clinique 2006;46:31-35

• **Zimmer J, Hentges F,** Andres E. Borderline thrombocytopenia or mild idiopathic thrombocytopenic purpura. PLoS Med. 2006 ;3:365.

Other publications

• Hentges F, Kockhans Bieda MC. Recensement des pollens et des spores atmosphériques. Ministère de la Santé. Station d'Aérobiologie Luxembourg-Ville. Bulletin de l'année 2005.

Oral presentations in conferences and congresses

• **Hentges F**. Food allergy to animal proteins. Postgraduate course for Allergologists and Clinical Immunologists. Grindelwald Switzerland January 20th-22nd 2006.

• Hentges F, Kuehn A. Allergie au Thon. 12^{eme} journée d'actualités en allergologie Strasbourg. March 2006

Poster presentations in conferences and congresses

• **Hilger**, G. Riva , S. Zanotta, G. Guida, J. Kleine-Tebbe, S. Kespohl, G. Pauli, F. de Blay, **F. Hentges**. Diagnosis of allergy to Argas reflexus by detection of IgE antibodies to Arg r1. XXVI th Congress of the European Academy of Allergology and Clinical Immunology, June 2006, Vienna. Poster prize in the poster group: in vitro allergy diagnosis II.

• **A. Kuehn**, P. Felten, C. Hilger, **F. Hentges**. Allergic reaction to tuna fish. IgE sensitisation to a 40 kDa protein distinct from parvalbumin. Poster presentation. XXV Congress of the European Academy of Allergology and Clinical Immunology. Vienna, Austria, 10-14 June 2006.

• C. Leonard, C. Le Pogam, R. Brons, F. Hentges. Proliferation and IFN-secretion pattern of natural CD4+ CD25+ regulatory and or CD4+CD25- cells from naïve or vaccinated BALB/c when stimulated in vitro by dendritic cells pulsed or not pulsed by Fel d1. Poster presentation. XXV Congress of the European Academy of Allergology and Clinical Immunology. Vienna Austria, 10-14 June 2006

• Hentges F, Kockhans-Bieda MC. Trends in medium annual temperature and total annual pollen and fungal spore counts observed in Luxembourg-city during the period from 1991 to 2005. 8th International Congress on Aerobiology, 21-25 August 2006 – Neuchâtel, Switzerland.

• Master 2^{eme} année, Mention Sciences et Santé, Spécialité Physiopathologie Cellulaire et Moléculaire. Université Louis Pasteur Strasbourg I .Analyse du développement séquentiel d'épitopes T et B envers Arg r 1 (allergène majeur d'Argas reflexus) dans la souris BALB/c. El-Hassen Teskrat, june 2006.

5_Collaborations with other CRP-Santé laboratories

The LIA unit has close collaborations in the field of food safety and food allergen characterisation with:

- the laboratory on plant molecular biology of Dr André Steinmetz
- the LMBGM of Dr Evelyne Friederich
- the laboratory on food safety of Gilbert Morris at the LNS

The LIA unit has developed a close collaboration in the field of functional immunology (definition of T cell epitopes on allergens with overlapping peptides)

• with the Immunology Department of Prof. Claude Muller at the LNS

6_International Collaborations/partners

The LIA has ongoing collaborations with the following units and laboratories for clinical, as well as functional studies of allergens.

- Prof. Gabrielle Pauli from the Pneumology department at the University of Strasbourg
- Dr. Guido Paesen from the CEH in Oxford

7_Higher Education and Training

• The LIA unit participates in the master programme "Physiopathologie Cellulaire et Moléculaire" of the University Louis Pasteur of Strasbourg.

• Dr. Jacques Zimmer has qualified at the University Louis Pasteur of Strasbourg to guide research activities (HDR or Habilitation à Diriger des Recherches).

RETROVIROLOGY LABORATORY



RETROVIROLOGY LABORATORY

Head of Research Unit:

Dr Jean-Claude Schmit, MD, PhD

Team Members :

Researchers :

Dr Carole Devaux, PhD, associate head of the laboratory
Dr Sabrina Deroo, PhD, head of research group immuno-virology
Dr François Roman, MD, PhD, head of research group clinical virology
Dr Sylvie Delhalle, PhD, researcher in immuno-virology
Dr Robert Hemmer, MD, researcher in clinical virology
Dr Vic Arendt, MD, collaboration with African countries

Engineers / biologists :

Jean-Yves Servais Daniel Struck Anne-Marie Ternes Aurélie Fischer

Technicians :

Jean-Marc Plesseria Cécile Masquelier Nadia Beaupain Emmanuel Counson

External collaborators : (Fondation Recherche sur le SIDA)

Christine Lambert, technician Terry Baurith, data manager Valérie Etienne, data manager Karin Hawotte, technician Samia Regaia, technician Dr. Thérèse Staub, MD

PhD students :

Franky BAATZ, Université Libre de Bruxelles Cyrille LEJCZAK, Université de Strasbourg

1_Objectives

The Retrovirology Laboratory was founded in 1989 by governmental decision as the national reference laboratory for HIV. Chronic viral infections, especially by HIV and HCV are an evergrowing public health problem in Luxembourg. More than 700 patients infected with HIV have been followed in the Centre Hospitalier of Luxembourg during the past decade. The laboratory is working in close relation with the national department of infectious diseases to provide highly specialized technical support for the clinical follow-up of these patients. The available technologies focus on diagnosis, viral load quantification and pro and rt sequencing for the evaluation of genotypic resistance to current antiretroviral therapy (ART). More specialized assays can also be performed upon request, when specific questions are addressed concerning the disease evolution or the treatment response of a patient. In addition, the behavior of clinical HIV-1 isolates can be documented in vitro, in the presence of antiretroviral drugs, to evaluate the so-called phenotypic resistance. Polymorphisms of HIV-1 coreceptor genes are screened using various technologies (RFLP, sequencing, real-time PCR). This work is largely financed by non-CRP resources.

Besides clinically oriented research programs, the laboratory has developed fundamental and applied research in the field of chronic viral infections (HIV, HBV and HCV). The activities of the laboratory is subdivided into two research units, which interact closely : immuno-virology and clinical virology.

1.1_Immuno-virology research unit

The research topic of the immuno-virology subunit is the role of the humoral immune response in the context of HIV-1 infection. One of the main objectives of the unit is to identify new human antibody fragments directed against HIV-1. Human chemokine receptors, mainly CCR5 and CXCR4, act as HIV coreceptors and play a critical role in the binding and fusion steps of the viral replication cycle. Several new antiretroviral drugs, so-called entry or fusion inhibitors, directed against HIV coreceptors or HIV envelope subunits, have shown potent antiviral activity and are currently evaluated in clinical trials.

We have developed two different approaches to identify peptides that interact with the chemokine receptors: phage displayed peptide libraries and structurally more complex phage libraries. The phage display technology will allow us to study the fundamental aspects of HIV entry into the cell.

1.2_Clinical virology research unit

The clinical virology subunit is an important collaborator within several European HIV networks (EuroSiDA, SPREAD/EuropeHIVResistance, VIRGIL). The main objectives of these studies are to improve knowledge on molecular epidemiology, transmission, prevalence and factors facilitating the emergence and spread of drug resistant HIV-1.

The HCV epidemic is about 4-times larger than infections with HIV and represents a major



public health challenge. Therefore, we extended our database system to HCV but also to HBV cases. HIV and HCV are extremely variable RNA viruses, which has tremendous implications on molecular epidemiology and antiviral treatment. The pathogenic potential of these viruses can be affected by such variability, as it is the case for sensitivity to antiviral drugs.

These past years, bioinformatics and phylogenetics expertise has been implemented in the laboratory to develop complex integrated clinical and virological databases for chronic infectious diseases. The main objectives of the clinical virology unit are (i) to describe the transmission networks and the dynamics of epidemics caused by HIV and HCV in Luxembourg, (ii) to evaluate primary transmitted and secondary resistance to antivirals (HIV-1, HIV-2, HCV, HBV), (iii) to estimate the clinical relevance of viral and host factors involved in HIV-1 entry. In addition, the Retrovirology Laboratory is a partner for developing countries to favour knowledge and technology transfer in such countries.

Ultimately, the main goal of the research programs of the Retrovirology Laboratory is to improve the management of HIV and HCV infections. Our expertise in viral culture, as well as our biosafety P3 level confinement, place us in a leading position to be a partner of biotechnology and pharmaceutical industry for the development of anti-HIV drugs in Luxembourg.

2_Ongoing projects and main results

A_Immuno-virology research unit

Our research is mainly focused on the application of existing phage libraries and the development of innovative "phage display technologies" in the field of HIV to unravel the complex interplay of the viral and host proteins involved in virus entry and to potentially identify new diagnostic and therapeutic lead compounds.

Phage displayed peptide libraries were screened on living cells expressing the chemokine receptor CCR5 used by the virus to enter the host cell. Five phage clones were isolated and are currently analysed for their effect on the receptor. For this purpose, functional assays with the receptor CCR5 are developed in the laboratory (receptor internalization, calcium influx, cAMP production) in collaboration with the laboratory of Dr Galzi in the University of Strasbourg. In parallel, synthetic peptides corresponding to the extracellular loops of CCR5 have been used to screen the peptide libraries. Screening strategies and analysis of the results are ongoing.

We developed in collaboration with a Belgian bioinformatic company, AlgoNomics, an innovative antibody technology based on the display of the third Complementary-Determining Region (HCDR3) of human antibodies. These HCDR3 libraries can be efficiently screened for diagnostic and therapeutic lead compound candidates. We amplified the antibody repertoires of 10 healthy donors and these repertoires were validated by screening against different targets (such as anti-hemagglutinin antibody and lysozyme). We isolated high affinity antibody fragments for the two targets and delivered herewith the proof of concept of the technology.

We are currently engineering HCDR3 repertoires from HIV patients which are not progressing in their infection. These repertoires potentially contain antibody fragments that neutralize the virus and that could be developed as therapeutic lead compounds. To identify such fragments, we started screening strategies with these HIV antibody repertoires on the receptor CCR5. The selected fragments will be analysed in assays to assess their HIV neutralizing activity and will be used to study the interplay of the CCR5 receptor with the virus.

In 2006, we had two presentations at international congresses and three manuscripts are in preparation: one manuscript describing the clones isolated on CCR5 with phage displayed

peptide libraries and two manuscripts related to the validation of the technology developed with Algonomics on two targets (anti-hemagglutinin antibody and lysozyme).

B_Clinical virology research unit

The research activity of the clinical virology unit is focused on (i) the study of the molecular epidemiology and antiviral resistance of chronic viral infections (HIV and HCV), (ii) the clinical implications of viral and host factors involved in HIV-1 entry and HIV-1 entry inhibition and (iii) collaborations with African countries.

The main achievements in the area of molecular epidemiology and antiviral resistance during year 2006 were:

• The optimisation of a robust database system for the collection and exploitation of clinical and virological data on patients infected with HIV-1 and HCV. A new data collection system for the clinical follow-up of patients has been developed in collaboration with the infectiologists of the National Service of Infectious Diseases. An alignment tool has been implemented within the database which is applicable to the full genome of HIV.

- An improvement of our phylogenetic analysis expertise with the utilisation of more complex phylogenetic methods such as maximum likelihood (ML).
- The development of in-house sequencing technologies for the full-length genome of HIV-1 and for the Gag-Pol region of HIV-2.
- The near full-length genome characterization and a detailed phylogenetic analysis of a new HIV-1 unique recombinant form (URF) outbreak in Luxembourg.
- The description of the HIV-2 epidemic in Belgium and Luxembourg, in collaboration with a Belgian AIDS Reference Laboratory (ARL) (Cliniques Universitaires Saint-Luc, Catholic University of Louvain; Prof P Goubau, Phn J Ruelle).
- The characterization of the impact of a rare HIV-1 protease substitution (G48E) on HIV-1 replication capacity in vitro.

The main achievements in the area of HIV-1 entry and HIV-1 entry inhibition were:

- The development of gp41-recombinant virus assay (RVA) for the evaluation of fusion inhibitor sensitivity of HIV-1 clinical isolates in vitro, based on the eGFP reporter system.
- The development of a HIV-1 coreceptor usage assay, based on the recombination of whole HIV-1 envelope inserts into an eGFP-tagged proviral plasmid.

The main achievements in the area of collaboration with African countries were:

• The development of a real-time PCR-based protocol for HIV-1 viral load (VL) determination using dried plasma spots (DPS).

• The evaluation of treatment efficacy using this DPS technology on 150 patients from Mozambique, in collaboration with MSF. Resistance testing has also been realized in case of treatment failure. The same study will be realized in Burkina Faso and Kenya.

• The study of coreceptor polymorphisms on a Long Term Survivor cohort of Rwanda.

During 2006, we had four presentations at international congresses and published two manuscripts in peer-reviewed journals. We developed or intensified collaborations with the Europe-HIVResistance network, the VIRGIL network, the Lerner Research Institute of Cleveland (HIV-1 fitness experiments), the Utrecht Medical Center (gp41 sequencing) and the Belgian AIDS reference laboratories (AIDSARLs). We are in close contact with MSF and the Retrovirology Laboratory of Kigali for African collaborations. Three manuscripts related to HCV epidemiology in Luxembourg, the HIV-1 URF BF outbreak and to the impact of G48E on HIV-1 fitness, respectively are in preparation in our laboratory. Two manuscripts (related to HIV-2 and to patients in Mozambique) are in preparation in the context of our international collaborations.

3_Publications

• Masquelier C, Servais JY, Rusanganwa E, Roman F, Havuga E, Servais J, Tuyizere S, Omes C, Karasi JC, Courteille O, Karita E, Allen S, Schmit JC, Arendt V. A novel 24-base pair deletion in the coding region of CCR5 in an African population. AIDS 2007;21:111-113.

• Roman F, Ammerlaan W, Plesseria JM, Deroo S, Muller CP, Arendt V, Schneider F, Hemmer R, Schmit JC. A new recombinant virus system for the study of HIV-1 entry and its inhibition. Journal Virol Methods 2006;131:99-104.

• Fisher A, Karasi JC, Kibidi D, Omes C, Lambert C, Uwayitu A, Hemmer R, Ende J, Schmit JC, Arendt V. Antiviral efficacy and resistance in patients on antiretroviral therapy in Kigali, Rwanda: the real-life situation in 2002. HIV Med. 2006;7(1):64-6.

• Van de Vijver DA, Wensing AM, Angarano G, Asjo B, Balotta C, Boeri E, Camacho R, Chaix ML, Costagliola D, De Luca A, Derdelinckx I, Grossman Z, Hamouda O, Hatzakis A, Hemmer R, Hoepelman A, Horban A, Korn K, Kucherer C, Leitner T, Loveday C, MacRae E, Maljkovic I, de Mendoza C, Meyer L, Nielsen C, Op de Coul EL, Ormaasen V, Paraskevis D, Perrin L, Puchhammer-Stockl E, Ruiz L, Salminen M, Schmit JC, Schneider F, Schuurman R, Soriano V, Stanczak G, Stanojevic M, Vandamme AM, Van Laethem K, Violin M, Wilbe K, Yerly S, Zazzi M, Boucher CA. The calculated genetic barrier for antiretroviral drug resistance substitutions is largely similar for different HIV-1 subtypes. J Acquir Immune Defic Syndr. 2006;41(3):352-60.

4_Collaborations inside the CRP-Santé

• Laboratoire de Biologie Moléculaire, d'Analyse Génique et de Modélisation (Transcriptomic Core Facility), Dr Evelyne Friedrich and Dr Laurent Vallar: use of the Nanodrop® and the Agilent 2100 bioanalyzer® in several research projects.

• Flow Cytometry Core Facility, Dr René Brons: use of the flow cytometer and cell sorter in several research projects.

RETROVIROLOGY LABORATORY

5_International collaborations

The research projects of the Retrovirology Laboratory have received the support of international collaborations:

- PMEs: Algonomics/Ablynx (Ghent), ABL (Luxembourg)
- University of Strasbourg: UMR 7175, Département Récepteurs et Protéines Membranaires, Pr. J.L. Galzi
- University of Louvain: Département de Virologie, Pr. P. Goubau
- University of Liège: Centre de Référence SIDA, Pr. M. Moutschen.

The Retrovirology Laboratory is participating in several international network/studies:

- Belgian AIDSARLs
- **SPREAD/EuropeHIVResistance (6 EU-FP):** the laboratory was in charge of the database construction and management (17 European countries)
- VIRGIL (6 EU-FP): the laboratory is in charge of the database management (30 European countries)
- **EuroSiDA:** the laboratory is providing samples, sequencing results and information.
- **EuroHIV:** the laboratory is providing statistics on the Luxembourg HIV epidemic.

• INSIGHT (Clinical network financed by NIH): the laboratory gives technical support for clinical trials

• ARTA (African/Dutch collaboration): the laboratory is involved in the development of DBS (Dry Blood Spot)/ DPS techniques for the sequencing of HIV fragments.

IMMUNOLOGY DEPARTMENT LABORATOIRE NATIONAL DE SANTÉ

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IMMUNOLOGY DEPARTMENT LABORATOIRE NATIONAL DE SANTÉ

Head of Research Unit: Prof. Dr Claude P. Muller, M.S.

Team members:

Secretaries:

Ulla Muller Carole Weis

Technical Core Facility:

Fred Fack PhD Wim Ammerlaan Chantal Courtois Nathalie Rodenbour

Virology:

Jacques Kremer PhD Judith Hübschen PhD Mariette Ducatez PhD Christophe Olinger (PhD stud) Julia Kessler (PhD stud) Sebastien de Landtsheer (tech) Emilie Charpentier (tech) Jesse Otegbayo (visiting scientist) Edith Nkwembe (visiting scientist) Christian Tahita (visiting scientist) Anna Vdovichenko (trainee as MS student) Veronique Venard (sabbatical)

Vaccinology (Premavac and Tobavac):

Nathalie Grova, PhD Fabienne Bouche, PhD Stéphanie Willieme (tech) Sophie Farinelle (tech) Tom Bechet (PhD stud) Mario Schellenberger (PhD stud) Mélanie Cassavecchia (trainee and summer student)

Neuropsychoimmunology:

Jonathan Turner, PhD Joana Macedo (PhD stud) Andrea Schote (PhD stud) Laetitia Pelascini (tech) Lynn Folscheid (trainee as MS student) Mélanie Petruzzi (trainee and summer student)

Massspectrometry (MALDI-TOF, Nano-SIMS) and Proteomics:

Fred Fack PhD Patrick Pirrotte (PhD stud) Anja Billing (PhD stud) Dominique Revets (tech)

Peptide and hapten chemistry:

Emmanuel Prodhomme PhD

1_Objectives and focuses

1.1 The Department of Immunology is a department of the Laboratoire National de Santé (LNS). The department was de facto created in 1992. Since then it has gradually expanded its scientific activities with the support of competitive research grants from national (CRP-Santé, Ministère de la Recherche, Ministère de la Santé, Ministère de la Coopération, Fond National de la Recherche, Foundations) and international sources (the European Union DGXII and DGX-III, the World Health Organisation, diagnostic and vaccine industry). By the end of 2001, a contract was signed between the LNS and the CRP-Santé, which serves as a basis for the collaboration between the two institutions. This contract has been updated in 2006.

1.2 Today, the Department of Immunology has grown to a staff of about 30 scientists, engineers, technicians, Ph.D. and M.D students, as well as undergraduate students and support staff. The Department is one of the most productive biomedical research groups in Luxembourg, with about 12-14 peer-reviewed international scientific publications per year, and 50-60 annual contributions to scientific conferences and invited oral presentations per year.

1.3 The Department is affiliated with the Graduate School of Psychobiology of the University of Trier (Chair of Immunology), the Medical Faculty in Homburg/Saar of the University of Saarbrücken and the Graduate School BIOSE of the University of Nancy. Training of Ph.D., M.D. students and laboratory staff of the WHO Network are important activities.

1.4 As a WHO Collaborating Center and WHO European Reference Center for Measles and Rubella the Department has a high profile with WHO and within the WHO Laboratory Network. The Department serves as consultants to International Organisations such as WHO, (Head of the Department member of WHO Steering Committee for Measles) and the European Union 3-7th Framework Programme, scientific institutes and companies.

1.5 The activities range from basic research to contract R&D for the diagnostic and vaccine industry. Major fields of interest are

(i) Immunology and genetic/antigenic diversity of viruses including molecular epidemiology: Public health issues related to infectious diseases in humans (measles virus, rubella virus, hepatitis viruses, influenza virus) and animals (a range of avian viruses including influenza virus). The Dept is responsible for the laboratory surveillance and diagnostic of avian influenza in Luxembourg; (ii) Immunology of vaccines: Development of immune-preventive strategies against infectious agents and against environmental risk factors such as carcinogens; (iii) Immunology and the neuroendocrine system: cytokines in psychoneurological disorders; crosstalk between nuclear receptors and immune receptors

2_Special Highlights 2006 including Avian Influenza Activities

• First seroepidemiological study of avian influenza virus (AIV) in Subsaharan Africa (Owoade et al 2006)

• 02.2006 Rapid intervention (CM Olinger, W Ammerlaan, CP Muller) in Nigeria to establish the first laboratory to diagnose H5N1, 2 weeks after bird flu was first reported from Africa

• 03.2006 Rapid intervention (M. Ducatez) in Burkina Faso to establish the first laboratory to diagnose H5N1, 1 month after bird flu was first reported from Africa

• 04.2006 Interview of CP Muller published by NATURE on the situation of H5N1 in Nigeria (D. Butler. H5N1, from the front lines: Nigeria. CP Muller. Nature 440,726-727, 2006)

• MF Ducatez, CM Olinger, AA Owoade, S De Landsheer, W Ammerlaan, HGM Niesters, ADME Osterhaus, RA Fouchier, CP Muller. Avian flu: multiple introductions of H5N1 in Nigeria. Nature 442, 37, 2006.

• Training in AIV diagnostics of laboratory staff from Nigeria, Burkina Faso, Egypt, Kyrgystan at WHO's + FAO's request

• AIV synergy with other poultry viruses. Collaboration with Y. Guan, H. Chen, University of Hongkong, SAR, China

• Laboratory surveillance of AIV in Luxembourg. Perfect score in EU-wide test panel in all categories

- Head of the Institute served as invited expert for AIV, SARS for:
- Guangxhi Province, China
- EU 7th framework
- FAO (Nigeria)
- Nigerian State Government (Oyo state)
- Kyrgystan
- Coordinator of EUROFLU
- COST B28: BSL3 and BSL4 pathogens (work package coordinator)
- Peer-reviewing of AIV projects and manuscripts

• 11.2006 Appointment of CP Muller as WHO evaluator for the Accreditation Review of the National Reference Laboratory in Bishkek, Kyrgystan

• 05.2006 Appointment of CP Muller as WHO evaluator for the Accreditation Review of the National Reference Laboratory in Skopje, Macedonia

• Jacques Kremer. Surveillance et contrôle de la rougeole: caractérisation du virus et de l'immunité humorale. PhD thesis submitted in July 2006, University of Nancy. Dr. Kremer has published 6 peer-reviewed publications.

• Mariette Ducatez. Comparaison moléculaire des virus de l'Influenza Aviaire, de la Bronchite Infectieuse et de l'Anémie du Poulet en Chine et au Nigéria: L'épidémiologie moléculaire, un atout dans la compréhension de l'évolution des virus aviaires à l'échelle mondiale. PhD thesis submitted in November 2006, University of Nancy. Dr. Ducatez has published 7 peer-reviewed publications, one of which in Nature.

• Mariette Ducatez, Ph.D., spent 1,5 months (February to March 2006) at the Department of Veterinary Medecine, University of Shantou, China to work on avian influenza and

other chicken viruses

• CP Muller, Christophe Olinger, Wim Ammerlaan spent 1 month (14.2.-15.3.06) at the Department of Veterinary Medecine, University of Ibadan, Ibadan, Nigeria to build up the first laboratory for diagnosis of avian influenza in Nigeria

• Mariette Ducatez, Ph.D., spent 2 weeks (March 2006) at the IRSS, Bobo-Dioulasso, Burkina Faso to work on avian viruses and provided a training course for staff from Burkina and Niger

• Mariette Ducatez spent 2 weeks (1.-15.12.06) at the Department of Veterinary Medecine, University of Ibadan, Ibadan, Nigeria to continue the collaboration for diagnosis of avian influenza in Nigeria

3_Current Research projects (for results see publications and highlights)

3.1 Development of a PRE-Vaccine strategy against measles compatible with current immunization schedules to close the window of susceptibility in infants (CRP – Santé CRP/SAN/02/002, 2002-2006)

- Department of Immunology in collaboration with several partners from Europe and US

3.2 Research in microbiology for development II (Ministère de la Coopération, 2004-2008)

- Department of Immunology together with 5 international collaborators

3.3 Collaborative studies of the WHO European Reference Laboratory for Measles and Rubella, Luxembourg (Ministry of Foreign Affairs, 2004-2008)

- Department of Immunology together with WHO and with several international collaborators

3.4 Vaccines against low molecular weight antigens, novel strategies and applications: TOBAVAC (Fonds National de la Recherche, Luxembourg 2002-2008)

- Department of Immunology together with universities of Luxembourg and Metz

3.5 Exploring detection limits of advanced SIMS technology in subcellular biological systems (Fonds National de la Recherche, Luxembourg 2003-2008) - Department of Immunology together with CRP-GL

3.6 Protein processing and profiling by proteomics (2005-2006) - Coordination: Department of Immunology

3.7 Interactions between steroid hormones: hetero- and homodimerisation of their receptors (2005-2006)

- Coordination: Department of Immunology

3.8 Development of new genoproteomic diagnostic tools for the toxicological assessment of endocrine disruptors in food – ENDIF (Fonds National de la Recherche 2006-2008) - Department of Immunology together with 3 partners

3.9 EU COST Action B28: "Array technologies for BLS3 and BLS4 pathogens" (2005-2010)

- In collaboration with 22 European partners, LNS-DI serves as chair of workgroup 2

3.10 "Molecular mechanisms of corticosteroid receptor regulation and activity" (Deutsche Forschungsgemeinschaft 2006-2010)

- In collaboration with 8 partners from the University of Trier and the University of Leiden

4_Collaborations

Laboratoire National de Santé and CRP-Santé and CHL

- F. Schneider, Microbiology Division: 20 joint publications
- R. Wennig, S. Schneider, Toxicology Division: TOBAVAC, PREMAVAC projects; 1 joint publication
- J. Mossong, Epidemiology Unit: 4 joint publications
- F. Hentges, Laboratory of Allergology and Plants: 2 joint projects, peptide synthesis
- E. Friederich, 2 joint FNR projects
- J. Terzis, S. Niclou, U.Rajevic

Other National Collaborations

- Ministry of Research: bourse BFR; peer-reviewing
- Ministry of Health: measles hotline, epidemiological surveillance of measles
- Ministry of Foreign Affairs and Cooperation: molecular epidemiology of infectious diseases in developing countries and New Independent States
- University of Luxembourg (P Seck, F Anton: 1 joint FNR project, P. Heuschling, J. Schiltz: 1 joint publication); teaching
- CRP-GL (HN Migeon, L. Hoffmann): 2 joint FNR projects
- State Veterinary Laboratory: 1 joint publication
- Laboratoire Kutter-Lehners-Hastert (B. Weber): 1 joint project
- Fonds National de la Recherche: programme development
- Clinique Ste Zithe (M. Keipes): 1 joint publication

Collaborations in the Greater-Region (Saar-Lor-Lux, Rheinland-Pfalz, Wallonie): the VIRIM Network

- Interregional Research Price of the 7th Summit of the Prime Ministers of the Greater Region for « VIRIM, a Network of Excellence in Viral Immunology »
- About 30 joint publications

Partners:

- Coordinator: Department of Immunology LU
- Laboratoire de Virologie (A. Le Faou), University of Nancy (since 2000)
- Institute of Virology (N. Muller-Lantzsch), University of Saarland/Homburg (since 2003),
- Service de Génétique Appliquée (A. Bollen), Nivelles, Université Libre de Bruxelles (1994-2000)
- Institut de Biologie Moléculaire des Plantes (A. Steinmetz), Université de Strasbourg (since 1999)
- Institut de Biologie Moléculaire et Cellulaire (J. Hoebeke), Strasbourg (since 2001)
- Laboratoire de virologie fondamentale et d'immunologie virale (C. Sadzot-Delvaux), U.
de Liège (1998-2002)

- Unité de Recherche en Biologie Moléculaire (JJ. Letesson, B. Damien), Facultés Universitaires Notre-Dame de la Paix, Namur (since 2002)
- Lab. de recherche (Ingénierie moléculaire et biochimie pharmacologique, G Kirsch), University of Metz (since 2002)
- Fachbereich Biologie (R.Hakenbeck), University of Kaiserslautern (since 2000)
- Graduate School of Psychobiology (D Hellhammer, H Schächinger, J Meyer, F Anton), University of Trier (since 2001)

The WHO measles and rubella laboratory network

- A. Drishti Robo, Institute of Public Health, Laboratory of Virology, Tirana, Albania
- V. Hutse, Scientific Institute for Public Health, Section Virology Department Microbiology, Brussels, Belgium
- F. Freymuth, Centre Hospitalier Universitaire Clemenceau, Laboratoire de virologie, Caen, France
- Y. Aboudy, Sheba Medical Center, Central Virology Laboratory, Ramat Gan, Israel
- R. Decelis, St Luke's Hospital, Pathology Department, Guardamangia, Malta
- E. Beslagic, Univerzitata u Sarajevu Medicinski Facultat, Sarajevo, Bosnia-Herzegovina
- R. S. Van Binnendijk, National Institute of Public Health and Environment RIVM, Bilthoven, Netherlands
- G. Bosevska, Republic Institute for Health Protection, Skopje, Macedonia
- A. Akcali, Refik Saydam Hygiene Center, Ankara, Turkey
- N.Yilmaz, Refik Saydam Central Institute of Hygiene, Sinhhiye, Turkey
- N. Karadza, Public Health Institute of RS, Microbiology Laboratory, Banja Luka, Bosnia-Herzegovina
- M. Zarvos, Nicosia General Hospital, Central Medical Laboratories, Nicosia, Cyprus
- G. Mulliqi-Osmani, National Institute of Public Health of Kosova, Pristina, Serbia and Montenegro
- T. Kimman, National Institute of Public Health and Environment RIVM, Bilthoven, Netherlands
- N. Chitadze, National for Disease Control and Medical Statistics NCDC, Tbilisi, Georgia
- F. De Ory, Inst de Salud Carlos III, Centro Nacional de Microbiologia, Serv de Microbiología, Madrid, Spain
- J. Nedeljkovic, Institute of Immunology and Virology "TORLAK", Dept of Respiratory Viruses, Belgrade, Serbia
- A. Mentis, Institut Pasteur Hellenique, Athens, Greece
- F. Wild, INSERM, Immunité et Vaccination, Lyon, France
- R. De Swart, Erasmus University, Institute of Virology, Rotterdam, Netherlands
- T. Vilibic-Cavlek, Croatian National Institute of Public Health, Zagreb, Croatia
- H. Rebelo De Andrade, Centro Nacional da Gripe, Instituto Nacional de Saude Dr. Ricardo Jorge, Unidade de Virus Respiratorios e Enterovirus, Lisboa, Portugal
- I. Maïga, Faculté de Médecine et de Pharmacie, Laboratoire de Microbiologie de l'hôpital du point G, Bamako, Mali
- C Bekondi, Virologie, Unité des Rétrovirus et des Hépatites virales, Institut Pasteur, Bangui, Rép Centrafricaine

European and International Collaborations

- G. Kirsch, University of Metz FR (TOBAVAC)
- J. Eichler, V. Wray, Helmholtz-Zentrum für Infektionsforschung, Braunschweig DE

(PREMAVAC)

- V. Yusibov, Fraunhofer-Institute, Philadelphia USA (PREMAVAC)
- A. Madder, C. Bodé, University of Gent BE (PREMAVAC)
- A. Mankertz, Robert-Koch-Institut, Berlin DE (WHO measles network)
- A Osterhaus, R deSwart, R Fouchier, Dept of Virology, University of Rotterdam NL (WHO measles network)
- D. Brown, HPA, London UK (WHO measles network)
- Z. Penzes, University of Prag CZ (MAE)
- F. Lasbennes, University of Strasbourg FR (Nano-SIMS)
- P. Leprince, University of Liège (Proteomics)
- I. Brown, B. Londt, Veterinary Laboratory Agency, Weybridge, UK (avian influenza)
- A. Luppi, Reparto di Virologia e Sierologia Specializzata, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Umbertini", Brescia, IT (avian influenza)
- Dr. V. Palya, Z. Penzes, Ceva-Phylaxia, Veterinary Biologicals Co. Ltd., Budapest, Hungary (poultry viruses)
- V. Venard, SRSMC, University of Nancy, CNRS University of Medicine, Laboratoire de Virologie
- O.C. Meijer Leiden Amsterdam Centre for Drug Research, Leiden, the Netherlands
- D. Brown, Health Protection Agency, London
- Partners in COST B28
- Partners in ELSM Network
- WHO, Geneva
- WHO EURO, Copenhagen
- A. Owoade, Veterinary Institute, Ibadan, Nigeria
- F. Adu, College of Medicine, University of Ibadan, Nigeria
- S. Omilabu, Faculty of Science, Lagos State University, Lagos, Nigeria
- A. Salimonu, O. Arinola, Department of Chemical Pathology, University of Ibadan, Nigeria
- J.O. Olusegun, Eye Foundation, Yaba/Lagos, Nigeria
- J. Otegbayo University of Ibadan, Department of Medicine, Gastroenterology, Liver Unit, Ibadan, Nigeria
- O. Folorunso, National Veterinary Research Institute Vom, Vom, Nigeria
- Dr. Kehinde Adekunle Oyewola, Oakven Multilink Enterprises Ltd, Ibadan, Nigeria
- S. Mairiga, Laboratoire Régional Vétérinaire Zinder, Niger
- Prof. Njayou Mounjohou, Laboratoire de Biochimie Microbienne, Facultés des Sciences, Yaoundé, Cameroon
- P. Okwen Mbah, St. Theresa Catholic Medical Centre, Bamenda, Cameroon
- Prof. Dr. Muyembe Tamfum, National Institute of Biomedical Research, Kinshasa, Congo
- Dr. Nébie Yacouba, Centre Régional de Transfusion Sanguine, Bobo-Dioulasso, Organisation de Coordination et de Coopération pour la Lutte contre les Grandes Endémies – Centre Muraz, Bobo Dioulasso, and District Sanitaire de Dandé, Burkina Faso
- Dr. A. Sow, Laboratoire National d'élevage de Ouagadougou, Ouagadougou, Burkina Faso
- J-B. Ouedraogo, Institut de Recherche en Sciences de la Santé, Bobo Dioulassou, Burkina Faso
- Z. Tarnagda, Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso
- P. Edorh, Laboratory for Ecotoxicology and Environmental Safety, Cotonou, Benin
- I. Gouandjika, Laboratoire des Enterovirus Institut Pasteur de Bangui, Bangui,

République Centrafricaine

- LK. Tuyen, Institute of Hygiene and Epidemiology, Hanoi, Vietnam
- N. Hanh Phuc, The National Institute of Hygiene and Epidemiology, Hanoi, Vietnam
- P. Jutavijittum, Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
- Laurence Bonte, Médecins sans Frontières, Paris, France
- Patrick Hourtane, Médecins sans Frontières, Yola, Nigeria
- H Chen, Y Guan, Joint Influenza Centre, Shantou University Med College and Hong Kong Medical College, China
- E Samoilovich, G Semeiko, M Yermalovich, Research Institute for Epidemiology and Microbiology, Minsk, Belarus
- V. Eremin, N. Lazouskaya, Research Institute for Epidemiology and Microbiology, Minsk, Belarus
- N. Tikhonova, Moscow Research Institute of Epidemiology and Microbiology, Moscow RU
- A. Agafonov, State Research Center of Virology and Biotechnology, VECTOR, Nowosibirsk, RU
- G. Ignatyev, State Research Center of Virology and Biotechnology, VECTOR, Nowosibirsk, RU
- G.Kuroglouglou, Refik Saydam National Hygiene Center, Ankara, Turkey
- K Kasymbekova, Ministry of Health of Kyrgyz Republic, Dept of the State Sanitary, Bishkek, Kyrgystan
- A. Sargaldakova, World Bank
- C. Amaral, Food and Agriculture Organization of the United Nations FAO (TCE)
- V. Yusibov, Fraunhofer Center for Molecular Biotechnology and Vaccinology, Philadelphia, USA

5_Publications

2006 (16 published, 9 in press)

• N Grova, A Valley, JD Turner, A Morel, CP Muller & H Schroeder. Modulation of behavior and NMDA-R 1 gene mRNA expression in adult female mice after sub-acute administration of benzo(a)pyrene. Neurotoxicology. In press.

• MF Ducatez, Z Tarnagda, MC Tahita, A Sow, S De Landtsheer, BZ Londt, IH Brown, ADME Osterhaus, RAM Fouchier, JB Ouedraogo, CP Muller. Genetic characterization of HA1 of HPAI H5N1 viruses from poultry and wild vultures in Burkina Faso. Emerging Infectious Diseases. In press.

• JR Kremer, GH Nguyen, SV Shulga, PH Nguyen, UT Nguyen, NT Tikhonova, CP Muller. Genotyping of recent Measles Virus strains from Russia and Vietnam by nucleotide specific multiplex PCR. J Med Virol. In press.

 MF Ducatez, CM Olinger, AA Owoade, S De Landtsheer, W Ammerlaan, HGM Niesters, ADME Osterhaus, RAM Fouchier, CP Muller. L'étude de l'épidémiologie moléculaire de H5N1 hautement pathogène au Nigéria indique que plusieurs virus distincts y ont été introduits. Epidémiologie et Santé Animale. In press.

• JR Kremer and CP Muller. Measles – Rubeola. Conn's Current Therapy. Invited Book Chapter. In press.



• J Rota, L Lowe, P Rota, W Bellini, S Redd, G Dayan, R van Binnendijk, S Hahné, G Tipples, J Macey, R Espinoza, D Posey, A Plummer, J Bateman, J Gudiño, E Cruz-Ramirez, I Lopez-Martinez, L Anaya-Lopez, T Holy Akwar, S Giffin, V Carrion, AM Bispo, A Vicari, C Tan, B Wolf, K Wytovich, P Borus, F Mbugua, P Chege, J Kombich, C Akoua-Koffi, S Smit, H Bukenya, J Bwogi, FN Baliraine, J Kremer, CP Muller. Identical Genotype B3 Sequences from Measles Patients in 4 Countries, 2005. Emerging Infectious Diseases. In press.

• AOB Oyefolu, AC Nwaeke, KO Akinyemi, Olumuyiwa B. Salu, CP Muller and SA Omilabu. Evaluation of vaccine cold chain system and potency status of measles vaccine administered in Lagos State, Nigeria. African Journal of Clinical and Experimental Microbiology. In press.

• CP. Muller, JR. Kremer, JM. Best, I Dourado, H Triki, S Reef. Reducing global disease burden of measles and rubella: Report of the WHO Steering Committee on research related to measles and rubella vaccines and vaccination, 2005. Vaccine 2006. In press.

• JD Turner, AB Schote, M Keipes and CP Muller. A new transcript splice variant of the human glucocorticoid receptor: Identification and tissue distribution of hGR∆313-338, an alternative exon 2 transactivation domain isoform. Annals NY Acad Scie 2006. In press.

• MF Ducatez, CM Olinger, AA Owoade, S De Landsheer, W Ammerlaan, HGM Niesters, ADME Osterhaus, RA Fouchier, CP Muller. Avian flu: multiple introductions of H5N1 in Nigeria. Nature 442, 37, 2006.

• D. Butler. H5N1, From the front lines: Nigeria, CP Muller. Nature, 440,726-727, 2006.

• MF Ducatez, Y Guan, H Chen, CP Muller, Enhanced laboratory surveillance of group III coronaviruses in life poultry markets in Guangdong province, China, after the SARS outbreak. Proceedings of the 5th International Symposium on Avian Corona- and Pneumovirus, Rauischholzhausen, Germany 132-140, 2006.

• World Health Organization. anonymus. Global distribution of measles and rubella genotypes – update. Wkly Epidemiol Rec 81:469--480, 2006.

• SV Shulga, MA Naumova, TA Mamaeva, AG Gerasimova, NT Tikhonova, PA Rota, WJ Bellini, JR Kremer, CP Muller. The genetic characterization of wild-type measles virus strains, isolated in the Russian Federation in 2003 - 2005. Epidemiology and Vaccino-prophylaxis 5, 25-31, 2006 (in Russian).

• JD Turner, AB Schote, JA Macedo, LP Pelascini, CP Muller. Tissue specific glucocorticoid receptor expression, a role for alternative first exon usage? Biochem Pharmacol 72, 1529-37, 2006.

• AB Schote, JD Turner, J Schiltz, CP Muller. Nuclear receptors in human immune cells: expression and correlations. Molecular Immunology 44, 1436-45, 2006.

• AA Owoade, MF Ducatez, CP Muller. Seroprevalence of Avian Influenza Virus, Infectious Bronchitis Virus, Reovirus, Avian Pneumovirus, Infectious Laryngotracheitis Virus, and Avian Leukosis Virus in Nigerian Poultry. Avian Diseases 50, 222-227, 2006.

• CM Olinger, V Venard, M Njayou, AO Oyefolu, I Maiga, AJ Kemp, SA Omilabu, A Le Faou, CP Muller. Phylogenetic analysis of the precore/core gene of hepatitis B genotypes E and A in West-Africa: New subtypes, mixed infections and recombinations. Journal of General Virology 87, 1163-73, 2006.

• P Pirrotte, JL Guerquin-Kern, JN Audinot, HN Migeon, CP Muller. Potential of NanoSIMS for Life Sciences. In Biophotonics for Life Sciences and Medicine. Fontis Media, pp 77-96, 2006. Invited Book Chapter.

• JR Kremer, F Schneider, CP Muller. Waning of specific antibodies in measles vaccinees and convalescent patients-a longitudinal study. Vaccine 24, 2594-601, 2006.

• AC Frantz, F Fack, CP Muller, TJ Roper. Faecal DNA typing as a tool for investigating territorial behaviour of badgers (Meles meles). Eur J Wildlife Res. 52, 138-141, 2006.

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• MF Ducatez, AA Owoade, JO Abiola, CP Muller. Molecular Epidemiology of Chicken Anemia Virus in Nigeria. Arch Virol 151, 97-111, 2006.

• B Halassy, S Mateljak, FB Bouche, MM Pütz, CP Muller, R Frkanec, L Habjanec, J Tomasic. Immunogenicity of peptides of measles virus origin and influence of adjuvants. Vaccine 7, 34-44, 2006.

LABORATORY OF TOXICOLOGY (ASSOCIATED TO THE NATIONAL HEALTH LABORATORY)



LABORATORY OF TOXICOLOGY (ASSOCIATED TO THE NATIONAL HEALTH LABORATORY)

Head of Research Unit: Professor Robert Wennig, PhD

Team members:

Dr Brice Appenzeller, PhD (research scientist) Dr Marc Schuman, PhD (research scientist) Liliane Martins (PhD student, BFR) Claude Schummer (Master student, PhD student since December 2006) Dr Michel Yegles, PhD (LNS associated research scientist) Dr Serge Schneider, PhD (LNS associated research scientist)

1_Objectives and Focuses

Over the last 10 years, our laboratory has acquired valuable skills in the development of highly sensitive analytical methodologies for the detection and quantification of xenobiotics in classic and more importantly, in alternative matrices, especially hair, sweat and oral fluids. Some of the developed techniques are now routinely used in our laboratory in diagnosis and therapeutic monitoring of patients needing a regular and precise medical treatment (alcohol withdrawal, methadone substitution program, tri-therapeutic treatment of HIV-positive patients, etc.)

Current and future R&D activities:

- Application of scientific advances, derived from internal and external work, to public health problems related to toxicology (drug addiction, chronic alcohol abuse, therapeutic drug monitoring, ...).
- Research on new biomarkers related to chronic intoxication.

• Study of biomolecular dynamics (proteins, endogen molecules...) to identify fundamental mechanisms responsible for pathologies and determine research routes for treatment, and to develop new diagnostic tools.

• Analysis of hair specimens to assess the exposure of human subjects to different kind of organic pollutants.

• Development of analytical methods to detect xenobiotics in sweat, in order to perform monitoring of therapeutic treatment / drugs consumption / exposure to toxics and pollutants in non-invasive way.

From a technical point of view, the laboratory disposes of a large analytical platform including GC-MS (EI, NCI, PCI), LC-MS/MS (ESI, APCI), HPLC-UV and CE-UV.

2_Research Valorisation

Our research efforts are mainly valorised in public health domains such as medical follow-up or diagnosis. Analytical developments and research results are continuously integrated into the routine analytical services of the Toxicology Division of the National Health Laboratory, which offers its services to general practitioners, hospital services and legal services.

Through the organisation (locally) and participation (internationally) at scientific conferences, our research results are not only communicated to directly interested professionals (medical staff, scientists...).

In addition, the laboratory actively participates in higher education by accepting interns and doctoral students, giving lectures and practicals in analytical and organic chemistry.



3_Scientific Results

Publications:

• Appenzeller B.M.R., Agirman R., Neuberg P., Yegles M., Wennig R. Segmental determination of ethyl glucuronide in hair: a pilot study. Forensic Science International. In press

• Appenzeller B.M.R., Schummer C. Boura Rodrigues, Wennig R. Determination of the volume of sweat accumulated in a sweat-patch using sodium and potassium as internal reference. Journal of Chromatography B. In press

• Appenzeller B.M.R., Schuman M., Yegles M., Wennig R. Ehtyl glucoronide determination in white and pigmented hair. Alcohol and Alcoholism. In press

• Frascht M., Schneider S., Schuman M., Wennig R. Formation of scopolamine from Nbutylscopolammonium bromide in cigarettes. Journal of Analytical Toxicology, in press.

• Martins L.F., Yegles M., Chung H., Wennig R., Sensitive, rapid and validated gas chromatography/negative ion chemical ionization-mass spectrometry assay including derivatisation with a novel chiral agent for the enantioselective quantification of amphetaminetype stimulants in hair, Journal of Chromatography B, 2006, 842, 98-105

• Wiens F., Zitzmann A., Lachance M.-A., Yegles M., Pragst F., Wurst F.-M., von Holst D., Guan S.L., Spanagel R. The missing drink: a palm in West Malaysia gets wild mammals to chronically consume alcohol. Submitted to Science.

• Wennig R. and Verstraete A. Drugs and driving. Handbook of analytical separations vol III. Forensic Science, M. Bogusz Ed. Elsevier. Amsterdam. 2006.

Communications:

• Appenzeller B.M.R., Schuman M., Yegles M., Wennig R. Quantification of ethyl glucoronide in white and pigmented hair. SOHT meeting, Vadstena, Sweden. 28-30 May 2006

• L. Martins, M. Yegles, N. Samyn, J. Ramaekers, R. Wennig Segmental hair analysis of MDMA enantiomers by GC/MS-NCI"; Meeting of the Society of Hair Testing (SoHT), Vadstena (Suède), 29 mai 2006.

• Wennig R. Détecter l'indétectable grâce aux progrès des sciences analytiques: défi pour la toxicologie médico-légale moderne. Conférence des CCCTA, Villars, Suisse. 14 Septembre 2006

• Wennig R. Terrorisme et armes CBRN – Aspects toxicologiques. 26^{ème} Journées Nationales de Biologie Clinique. 22-23 septembre 2006

• Wennig R. Tabac, cannabis, alcool. Formation Anti-Tabac / Ministère de la Santé, Centre Hospitalier de Luxembourg. 14 octobre 2006

• Wennig R. From an old therapeutic drug to a potent hallucinogen. Congrès annuel de la Société de Toxicologie. Institut Pasteur, Paris. 23-24 octobre 2006

• Wennig R. Neurotoxicologie: coke, extasy, joint, etc... Séminaire de Neurologie, Centre Hospitalier de Luxembourg. 12 décembre 2006

4_National and International collaborations

• Markers of chronic alcoholism in hair:

- Forensic Medicine Institute, Humbolt University (Prof. Pragst, Berlin)
- Therapeutic Center of Useldange (P. Neuberg, Useldange)
- Institut Universitaire de Médecine Légale, Laboratoire de Toxicologie et de Chimie Forensiques (Dr. M. Augsburger)

Analytical methodology on anti-retroviral drugs:

- Laboratory of Retrovirology (CRP-Sante, Dr J-C Schmit, Dr Vic Arendt)
- LuxDevelopment and ESTHER project in Rwanda (Mme C. Omes)
- Determination of various drugs and benzodiazepines in saliva:
 - Methadone substitution programme (Dr De Winter J-P., Dr Staut W.)

• Detection of amphetamines and congeners in hair:

- Federal Public Service Justice, National Institute of Criminalistics and Criminology (NICC), Brussels, Belgium (Dr Samyn N)

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• Biomolecular dynamics of serum protein:

- Laboratoire d'Immunologie CRP-Santé, Luxembourg (Pr. C. Muller)

• Analysis of organic pollutants in hair:

- Centre de Géochimie de la Surface de Strasbourg, UMR 7517 CNRS – Université Louis Pasteur. (Dr Millet M.)

LABORATORY OF CARDIOVASCULAR RESEARCH



LABORATORY OF CARDIOVASCULAR RESEARCH

Head of Research Unit: Dr Daniel R. Wagner, MD-PhD

Team members:

The laboratory was created at the end of 2003. Starting with three members, the team evolved rapidly and consisted at the end of 2006 of six full-time members:

Didier Rouy, MD-PhD, Associate Director Yvan Devaux, PhD, Scientist Isabelle Ernens, PhD, Scientist Céline Jeanty, Technician Mélanie Vausort, Technician

In 2006, the laboratory welcomed two students, both relying on grants from the Ministère de la Culture, de l'Enseignement Supérieur et de la Recherche:

Huguette Louis, Post-Doctoral fellow Emilie Velot, PhD student

In addition, the laboratory hosted two students from the Master Sciences de la Vie et de la Santé of Nancy's University of Sciences (France) for 3-month periods:

Mohamed Bensattalah Benjamin Haas

1_Objectives and focuses

Congestive heart failure (CHF) has become a disease of epidemic proportion, affecting 3% of the adult population. This will have a major medical and economic impact in Luxembourg in the next few decades. Mortality of CHF is worse than many forms of cancer with a five-year survival of less than 30%. Myocardial infarction (MI) is the leading cause of CHF. Indeed, despite modern reperfusion therapy with thrombolysis or coronary angioplasty, approximately 30% of the patients with MI develop left ventricular remodeling and CHF.

It appears that matrix metalloproteinases (MMPs), a family of collagenolytic enzymes, play an important role in CHF and tissue remodeling after acute MI. Several studies have demonstrated increased expression and activity of TNF- α and MMPs in human and animal hearts during the remodeling process after MI. Most animal models have concluded that MMPs within the inf-arcted tissue are derived from neutrophils.

The nucleoside adenosine is formed in the myocardium through dephosphorylation of AMP when there is an imbalance between tissue oxygen supply and demand which occurs in the setting of acute MI and CHF. Adenosine has been shown in numerous studies to have cardio-protective effects in myocardial ischemia. Whether adenosine has an effect on the expression of MMPs, has not been determined.

The finding that inhibition or targeted deletion of MMP-9 attenuates left ventricular dilatation in the infarcted animal heart led to the proposal to use inhibitors of the MMPs production for patients at risk for the development of heart failure after acute MI.

On the other hand, not all patients with acute MI develop ventricular remodeling and not all patients with ventricular remodeling develop CHF. Therefore, it has become crucial to identify patients at risk for developing left ventricular remodeling and heart failure after acute MI. This has been done with traditional techniques of molecular biology for the risk of developing an acute MI. Microarrays (biochips) have very recently been introduced and allow to study the association between multiple genetic variations (polymorphisms) and the likelihood of developing a certain disease.

As a first step, using microarray technology, we observed that polymorphisms of certain genes (AMPD1, MMP-2, MMP-14, TNF- α) may be associated with a higher risk of coronary artery disease and MI. We have also observed in patients with acute MI that high peripheral levels of MMP-9 are associated with the development of CHF during 2-year follow-up.

Therefore, the objective of the laboratory is to answer the following questions :

- Does adenosine inhibit MMP-9 secretion by neutrophils ?, by monocytes ?
- Which adenosine receptor is involved and what is the signaling pathway involved ?
- Could adenosine analogs be used as therapeutic agents to limit CHF development ?
- Are genetic polymorphisms of the MMP-9 gene responsible of differential CHF development ?

2_Ongoing projects and main results

Several of the above questions have been answered:

• Adenosine inhibits MMP-9 secretion by neutrophils, and this critical finding has been published in one of the top peer-reviewed journals of our specialty (Ernens et al. Circulation Research 2006). The mechanisms responsible for this effect were delineated, as well as the signaling pathway activated and the adenosine receptor involved.

• Interestingly, this effect of adenosine was not found on another population of inflammatory cells, namely monocytes and macrophages, the second wave of cells that are recruited to the infarcted lesion of the heart after neutrophils. Actually, adenosine reproducibly stimulates MMP-9 production by monocytes and macrophages. The mechanisms responsible for this effect are currently being studied in detail. In parallel, we started to prepare a publication on these results which will be submitted in 2007.

• These studies pointed out the need for careful attention at the type of receptor involved and the cell type targeted when suggesting or designing experiments aiming at using the cardioprotective properties of adenosine. However, taken all together, our data reinforce our assumption that adenosine is a promising therapeutic agent to limit CHF development.

• Preliminary experiments aiming at identifying potential genetic polymorphisms of the MMP-9 gene suggest that at least one of them could be correlated to altered ejection fraction following myocardial infarction.

Additional questions have been asked in 2006 and are currently under investigation:

• Since adenosine increases MMP-9 production by monocytes/macrophages and MMP-9 is involved in cell progression and repair after myocardial infarction, can adenosine also modify VEGF expression, another major actor of reendothelialization ?

• The involvement of the A3 type receptor in the cardioprotective role of adenosine is being studied, as it is poorly known. Cell transfection is used to over-express a plasmid encoding the full sequence of the human A3 adenosine receptor. We started experiments in which cells over-expressing the A3 receptor are purified by flow cytometry (collaboration wit the Flow Cytometry Core Facility of the CRP-Santé). Oligonucleotide microarrays and RNA interference will be used to specifically identify genes that are under the control of the A3 receptor when this is activated by adenosine, a collaboration established with the Transcriptomic Core Facility of the CRP-Santé.

• Regarding MMP-9 polymorphisms, we are currently testing whether we could validate the mutation that was identified in a reduced set of patients after myocardial infarction (40 patients) in a larger population (200 additional patients). We also plan to reproduce this mutation in in vitro experiments to test whether it is really associated with an increased MMP-9 activity, presumably due to decreased interaction of MMP-9 with its natural inhibitor TIMP-1.

Finally, a new database for patients with acute myocardial infarction (LUCKY, stands for Luxembourg myocardial infarction registry) is being set up to properly manage the patients samples and to allow future genomic or epidemiologic studies. We are currently testing the functionality of a program developed by the CRP-Santé informatics team and made available through the CRP-Santé intranet. This program will help us to maintain the LUCKY patients database.

3_Valorization

Publications

• Wagner DR, Delagardelle C, Ernens I, Rouy D, Vaillant M, Beissel J. Matrix Metalloproteinase-9 is a Marker of Ventricular Remodeling after Acute Myocardial Infarction. Journal of Cardiac Failure, 12(1): 66-72, 2006.

• Lebrun F, Berchem G, Delagardelle C, Beissel J, Rouy D, Wagner DR. Improvement of exercise-induced cardiac deformation after cell therapy for severe chronic ischemic heart failure. Journal of Cardiac Failure, 12(2): 108-113, 2006.

• Ernens I, Rouy D, Velot E, Devaux Y, Wagner DR. Adenosine inhibits matrix metalloproteinase-9 secretion by neutrophils: implication of A2a receptor and cAMP/PKA/Ca2+ pathway. Circulation Research, 99(6):590-597, 2006.

Patents

- A patent on home monitoring is pending
- A patent on MMP-9 as a risk marker is pending
- A patent on a MMP-9 single nucleotide polymorphism as a prognostic and diagnostic tool for treatment of heart failure is pending.

4_Partnership inside the CRP-Santé

• Dr Evelyne Friedrich, Dr Mikalai Yatskou, Dr Arnaud Muller, Laboratoire de Biologie Moléculaire, d'Analyse Génique et de Modelisation (Transcriptomic Core Facility): collaboration for our projects using oligonucleotide microarrays.

- Dr René Brons, Flow Cytometry Core Facility : use of the flow cytometer and cell sorter in several research projects.
- Marie-Lise Lair, Michel Vaillant: Biostatistiques, Centre for Health Studies

5_National partnership

- Service de cardiologie du Centre Hospitalier de Luxembourg (CHL) : patients samples supply, common set up of the new database.
- Institut National de Chirurgie Cardiaque et de Cardiologie Interventionnelle (INCCI).
- Association pour la Recherche sur les Maladies Cardiovasculaires (ARMCV) : research clinical-oriented programs.

6_International partnership

Medical University, Centre Hospitalier and INSERM U684 (Nancy, France)

- Pr. Dan Longrois : the CRISTAL project
- Pr. Paul-Michel Mertes : the SAHGEN project
- Pr. Faiez Zannad : the CASHEMERE project
- Dr.Patrick Lacolley : the $\alpha 1/\alpha 1$ project.

University of Homburg (Germany)

• Pr. M. Böhm and Dr. I. Kindermann for the home-monitoring program.

LABORATORY OF EXPERIMENTAL HEMATO-CANCEROLOGY



LABORATORY OF EXPERIMENTAL HEMATO-CANCEROLOGY

Head of Research Unit:

Dr Guy Berchem MD

Team members:

Researchers:

Dr Valérie PALISSOT, PhD Dr Nassera AOUALI, PhD Dr Séverine WACK, PhD Dr Etienne MOUSSAY, PhD Dr Marc PAULY, PhD

Technicians:

Manon BOSSELER Sandrine PIERSON Bernadette LENERS Brigitte METZGER Julie JAQUEMIN Nathalie NICOT

Flow cytometry core facility:

NHS BRONS

1_Objectives and focuses

Our main objective is to focus on the study of two haematological diseases, multiple myeloma (MM) and chronic lymphatic leukaemia (CLL). Our approach is based on both patient samples before and after treatment and cultured cell lines for a better understanding of the mechanisms of apoptosis (programmed cell death) and its direct clinical involvement in these two diseases (potential economic downfall).

For this purpose, our lab has also developed a flow-cytometry core facility which now provides an excellent service with different methods to characterize specific markers of the cells (phenotyping, study of differentiation...) and different conditions (calcium flux, mitochondrial depolarization...). We have now acquired a second flow-cytometer which has sorting capacities (FacsAria, Becton Dickinson). This new equipment will be useful in the study of MM and CLL on the bone marrow and blood of patients, respectively. In fact, the phenotyping analysis of bone morrow and blood cells from patients by flow cytometry in more than 10 colors will permit to detect the diseased cells in mixes of different populations of cells. Moreover, with a cell sorter we will be able to select the desired populations of cells for further analysis.

In addition, the microarray project could lead to the development of a prognostic or a predictive test that will be very useful in the decision making phase of chronic lymphocytic leukaemia. A set of genes could be discovered by this method and the set could be later used to predict either outcome or sensitivity to different chemo or immuno therapeutic agents.

Finally, studies in proteomics in our models have been implemented in the laboratory in order to identify proteins differently regulated during the mechanisms of cell death.

Furthermore, we are participating in clinical projects in our field. Through our expertise in cytometry and microarray technologies we can collaborate with these groups and provide them with information necessary for there clinical studies.

2_Main projects

2.1_Histone deacetylase activity on cell death induction in Multiple myeloma (MM)

MM is an incurable hematological disorder characterized by deregulated proliferation of differentiated plasma cells. These malignant plasma cells are predominantly located in the bone marrow. Existing treatments mainly attempt to reduce the malignant cell masses and to overcome the disease-related complications. Whereas initial chemotherapeutic treatment can be successful, drug resistance often develops during disease progression, requiring the use of different drugs.

Histone deacetylase inhibitors represent a novel class of therapeutic agents that regulate gene expression through specific enzymes. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) determine the acetylation state of the histones, the conformational state of chromatin and, consequently, gene expression. Valproic acid (VPA) is already clinically used in the treatment of epilepsy. In 1997, VPA emerged as an anti-neoplastic agent and has been now de-

scribed as an inducer of apoptosis and differentiation in various cancer types and leukaemia.

In the study done in our lab we worked on different multiple myeloma cell lines (RPMI, OMP-2, and U266). Showing a cytotoxic effect on tested cell lines, VPA has a time and dose dependent cytotoxic effect. Moreover VPA induced a slowdown of the cell cycle by diminishing the cell cycle population undergoing G2/S or M phase. In a study performed on several leukemic and B cell precursor cell lines, VPA induced apoptosis mediated by caspase dependent and independent pathways. Flow cytometric analysis of PI and Annexin-V double labelled cells showed the presence of the phenomenon of late apoptosis on the different cell lines even at early time points. This was confirmed by Hoechst staining, showing apoptotic figures. Although ZVAD-FMK treatment, a pan-caspase inhibitor in presence of VPA, inhibits, but not completely abolishes cell death induced by VPA. In conclusion these data indicate that VPA-induced cell death in the tested MM cell lines is not only dependent on caspase activation. We also showed by western blot of caspases 9, 8 and 3 that only procaspase 3 was processed to its active form in all three cell lines. However, no processing of caspases 9 and 8 was observed in any of the tested cell lines.

The link between apoptotic and autophagic cell death, opening the way for a new cell death mechanism, has recently been reviewed. Other studies indicate that both mechanisms can be simultaneously activated within a dying cell. We showed the possibility of autophagic cell death as a potential mechanism provoked by VPA on multiple myeloma cell line with the specific probe monodansylcadaverin (MDC).

A multicentric clinical study is being discussed with Belgian colleagues, aiming at studying the effect of HDAC inhibitors in multiple myeloma patients.

2.2_Analysis of the molecular pathways regulating apoptosis in Chronic Lymphatic Leukemia (CLL)

B-cell chronic lymphocytic leukaemia (B-CLL) is the most common leukaemia in the Western world and is considered to be incurable. It is a clinically heterogeneous disease characterized by the accumulation of CD19/CD5 positive B lymphocytes with significant resistance to cell death and prolonged survival. We are presently investigating the potential relationship between the cell cycle, the expression of cell surface antigens and the sensitivity of B-CLL cells to treatment by several drugs. In addition to classic prognosis markers, we integrated the quantification of CD184 and ZAP-70 to determine the stage of the disease and the Ig mutational status (already published association), respectively.

We currently work on the better understanding of apoptosis in this model too. The cells of different patients before and after treatment and cultured cell lines are studied by flow cytometry, cytotoxicity assays, and microarrays after treatment by chemotherapy (mTOR and HDAC inhibitors, PPAR-antagonists).

Preliminary data showed dose-dependent responses for the different substances tested and the possible combination of drugs is under investigation in order to detect new synergies which could be clinically useful.

The lack of a complete and long-lasting response to chemotherapy is a well-known drawback limiting the clinical potential of chemotherapeutic agents in human cancer treatment. Consequently, we try to determine the reasons why these cells are (or become) resistant in response to chemotherapy. Our study is focusing on two aspects: the wide variety of mechanisms of chemoresistance (MDR), and the dysregulation of apoptosis. Flow-cytometric and micro-array approaches will allow detecting differences in protein and gene expression between sensitive and resistant patient cells. During the year 2005, a bank of patient samples has been constituted for this purpose and the analysis has been already initiated.

2.3_Study of genetic aberrations during cancer in bronchial neoplasia and early stages of oncogenesis

This study is performed in collaboration with the Laboratoire de Recherche en cancérologie pulmonaire, Institut Jules Bordet, Bruxelles, Belgium and the Service de Pneumologie of the Centre Hospitalier in Luxembourg. It concerns patients either with preneoplastic lesions, which will be biopsied and analyzed in Brussels, as well as 4 groups of local patients which will also be biopsied and put into a tumor bank for later analysis. These four groups are: normal nonsmokers, normal smokers, current lung cancer and previous lung or head and neck cancer. After an initial phase of 2 years where the patient samples are collected, the samples will be analyzed using a whole genome Micro-array approach or another technique which might be more appropriate at that time, for this reason, proteins an RNA will be collected and preserved separately. This study is supported by the Fondation Contre le Cancer of Luxembourg.

2.4_Circulating Endothelial Progenitor Cells (EPC) and Remodeling after Acute Myocardial Infarction

A previous study done in collaboration with the Laboratoire de Cardiologie of the CRP-Santé (Dr D. Wagner) concerning the intra coronary injection of autologous progenitor cells prepared in our lab was finished successfully last year and published in early 2006 (ref 4).

A second study in the acute phase after myocardial infarction is in preparation, but meanwhile we plan on starting soon this study on EPCs in acute myocardial infarction. The role of our lab in this study will be to quantify these cells with the help of flow cytometry whereas the logistics of the study will be carried out by the cardiology lab.

Study outline:

Background: Endothelial progenitor cells (EPCs) derived from the bone marrow play an important role in natural repair of the endothelium and are probably involved in neovascularization following bone marrow-derived cellular therapy after acute myocardial infarction. The prognostic value of circulating EPCs on the development of myocardial remodeling following infarction has not been evaluated. Objective of the present study: To determine whether there is a relationship between the number of circulating EPCs at the time of acute myocardial infarction and the development of left ventricular remodeling. Primary endpoints: To determine the time course of circulating EPCs after PCI for ST elevation myocardial infarction (STEMI) < 12 hours. To determine the relationship between circulating EPCs at the time of STEMI and global and regional systolic function at 4 months using echocardiography with strain analysis. Secondary endpoint: To determine the relationship between circulating EPCs and other biomarkers such as C-reactive protein (CRP), brain natriuretic peptide (BNP), matrix metalloproteinase (MMP)-9. Study Design: Patients presenting with STEMI < 12 hours for PCI at the INCCI will all be enrolled in the LUCKY registry (Luxembourg Acute Myocardial Infarction Registry). Patients being followed at the CHL will also be enrolled in the COCONUT registry where they will have additional blood sampling for CD34+ determinations (day 0, day 2, day 5). Number of subjects :> 50 patients

3_Cooperations

Proteomic study of the effect of valproic acid in multiple myeloma. Dr Raes Martine, Laboratoire de biochimie et de biologie cellulaire, Facultés universitaires Notre-Dame de la Paix, Namur, Belgium

Study of the gene expression profile in B chronic lymphocytic leukemia. Dr Evelyne Friederich, Laboratoire de Biologie Moléculaire, d'Analyse Génique et Modélisation. CRP-Santé, Luxembourg

Etude du mécanisme d'apoptose dans la leucémie lymphoïde chronique et le myélome multiple. Pr A. Burny, Laboratoire de Biologie Cellulaire et Moléculaire de l'Institut Jules Bordet (ULB). Bruxelles, Belgium.

Drs JP Sculier, V. Ninane, C. Mascaux, Laboratoire de Recherche en cancérologie pulmonaire, Institut Jules Bordet, Bruxelles, Belgium

Cathepsin D and breast cancer: mechanisms of apoptosis in tumors and sensibilization to chemotherapeutic agents. Dr E. Liaudet-Copman, INSERM U540 Endocrinologie Moléculaire et Cellulaire des Cancers, Université de Montpellier, Montpellier, France

4_Publications

• Lebrun F, Berchem G, Delagardelle C, Beissel J, Rouy D, Wagner DR. Improvement of Exercise-Induced Cardiac Deformation After Cell Therapy for Severe Chronic Ischemic Heart Failure. J Card Fail. 2006 Mar;12(2):108-113

• Chantal Schwartz, Valerie Palissot, Nassera Aouali, Severine Wack, NHC Brons, Bernadette Leners, Manon Bosseler, Guy Berchem. Valproic acid induces non apoptotic cell death mechanisms in Multiple Myeloma cell lines. Int J Oncol., F.I.: 3,0560, 2007, 30, in press

• Beaujouin M, Baghdiguian S, Glondu-Lassis M, Berchem G, Liaudet-Coopman E. Overexpression of both catalytically active and -inactive cathepsin D by cancer cells enhances apoptosis-dependent chemo-sensitivity. Oncogene. 2006 Mar 23;25(13):1967-73.

• Palissot V, Morjani H, Belloc F, Cotteret S, Dufer J, Berchem G. From molecular characteristics to cellular events in apoptosis-resistant HL-60 cells. Int J Oncol. 2005 Mar;26(3):825-34.

• M. Pauly, B. Metzger; M. Bosseler, C. Faber, J. Kayser, V. Palissot, M. Dicato, G. Berchem Improved sensitivity and specificity of K-ras point mutation detection in clinical tumour samples from colorectal cancer patients by PNA-mediated PCR-clamping with a single sensor probe. Submitted

Supplement (International conferences)

• Survey on in vitro cell death induction of multiple myeloma cells upon valproic acid treatment. Schwartz, C., Palissot, V., Brons NHC, Berchem, G. Oral presentation at the Cell Signaling World 2006 Signal Transduction Pathways as therapeutic agents. Luxembourg, January 2006.

• Molecular mechanisms of Bortezomib-induced cytotoxicity in late stage B-chronic lymphocytic leukemia. Palissot V., Schwartz C., Brons NHC, Bosseler M, Leners B., Berchem G. Esh/AACR Conference: Molecular Basis for targeted therapy for Leukemia, Cascaïs, Portugal, February 2006.

• Survey on in vitro cell death induction of multiple myeloma cells upon valproic acid treatment. Schwartz C., Palissot V., Brons NHC, Leners B., Bosseler M., Berchem G. Esh/ AACR Conference: Molecular Basis for targeted therapy for Leukemia, Cascaïs, Portugal, February 2006.

• A novel dimorphism and a new missense mutation in exon 20 of the EGFR gene in patients with advanced colorectal cancer. B. Metzger, M. Pauly, G. Mahon, C. Faber; J. Kayser, M.-A. Dicato and G. Berchem. Poster-Discussion presented at the 8th World congress of gastrointestinal cancer in Barcelona, Spain, 28.6.-1.7.2006, Abstract PD-004 in Annals of Oncology; Suppl 6; 17, vi28, June 2006.

• Improved sensitivity and specificity of K-ras point mutation detection in clinical colorectal malignancy samples harbouring a heterogeneous mix of normal (wild-type) and cancer cells by PNA-mediated PCR-clamping with a single sensor probe. M. Pauly, B. Metzger, M. Bosseler, C. Faber, J. Kayser and M.-A. Dicato. Poster at the 31st ESMO Congress, Istanbul, Turkey, 29.9.-3.10.2006, Abstract will be published in a next supplement of Annals of Oncology.

• Epidermal growth factor receptor (EGFR) gene mutations in exons 18 and 20 in patients with advanced colorectal cancer are more frequent than commonly believed. B. Metzger, M. Pauly, C. Faber, J. Kayser, F. Roman, M. Bosseler and M. Dicato. Poster at the 31st ESMO Congress, Istanbul, Turkey, 29.9.-3.10.2006, Abstract will be published in a next supplement of Annals of Oncology.

LABORATORY FOR NEUROSCIENCE RESEARCH

LABORATORY FOR NEUROSCIENCE RESEARCH

Head of Research Unit:

Priv.-Doz Dr. A.J.A. Terzis, MD, PhD Prof. Rolf Bjerkvig, PhD, co-responsible

Team members:

Simone Niclou P., PhD, senior scientist Uros Rajcevic, DVM, PhD, postdoctoral fellow Claude Danzeisen, MD, doctoral student Vanessa Barthelemy, technician Virginie Baus, technician

1_Objectives and focuses

The main focus of the NorLux laboratory is to find and validate new therapeutic targets and to utilize such targets to develop novel therapeutic strategies for brain cancer. The most malignant brain cancer is Glioblastoma multiforme (GBM), and our research is especially directed towards this disease. By transplantation of human GBM biopsy material into the brain of immunodeficient rats, an animal model was generated that displays a highly invasive phenotype showing stem cell characteristics but no signs of angiogenesis dependent growth (Sakariassen et al. 2006). Through serial passages in the rat brain, the phenotype changed to a less invasive, highly angiogenic phenotype. Using this model system we are currently focusing on the characterization and isolation of the cancer stem cell compartment in human brain tumours. Using this model and high throughput proteomics technologies we are exploring the mechanisms of angiogenesis- independent and -dependent cancer growth. In this context we have also established and characterized an immunodeficient transgenic GFP NOD/SCID mouse model that will allow us to reliably separate the tumour - host compartments.

The overall scientific objectives are to explore newly identified targets and pathways for innovative treatment of brain tumors directed towards the cancer stem cells and tumor vasculature. Then validate these targets on tumour stem cells and blood vessels and create effective and tumour-selective strategies that complement or substitute established therapies.

2_Research Projects in 2006

2.1_Brain Tumour Project

| Title: | New therapeutic principles and biological mechanisms related to brain tumour cell invasion and angiogenesis |
|--------------------|---|
| Acronym: | Brain tumor project |
| Contract number: | SAN/03/004 |
| Financial support: | Ministère de la Culture, de l'Enseignement Supérieur et de la Recherche (MCESR), Luxembourg |

Project Summary

Glioblastoma multiforme (GBM) is the most malignant cancer of the central nervous system, for which no cure is currently available. GBMs are characterized by extensive tumor cell infiltration, cellular atypia, necrosis and new blood vessel formation (angiogenesis). Transplantation of human GBM biopsies into the brain of immunodeficient rats generates a highly invasive tumour showing stem cell characteristics but no signs of angiogenesis (Sakariassen et al. 2006). Socalled cancer stem cells possess the capacity to regenerate a tumor in vivo suggesting that the stem cell represents the cell of tumour origin and is responsible for tumor recurrence. Although some phenotypic markers (as CD133) have been associated with cancer stem cells, there is still an open question if the cancer stem cell in brain tumours represents one or several phenotypes. Our aim is therefore to characterize cancer stem cells from human brain tumour biopsies. Careful phenotyping will allow the isolation of cancer stem cells and identification of novel targets for molecular therapy. We will further explore the possible origin of the cancer stem cell. These cells could arise from malignant transformation of a normal stem cell that has accumulated oncogenic insults over time. Alternatively, they could derive from a transformed, differentiated cell that acquires stem cell properties. These questions will be addressed using our newly-generated GFP-expressing immunodeficient mice as well as a chemically-induced tumor model in rats.

Main Results

The GFP mouse model (GFP-NOD/SCID mouse; Fig.1A) has been characterized in detail in order to confirm its usefulness as a model for tumour biology:

- Demonstration that glioma cell lines injected in these mice are able to reliably generate tumors within 4-5 weeks (Fig.1B).
- Marking tumour cells with a red fluorescent protein (DsRed), tumor cells can be easily recognized and separated from GFP expressing host cells (see Fig.1C).
- Using blood phenotyping and flow-cytometry, we have demonstrated that, as their non-fluorescent counterparts, GFP NOD/SCID mice lack functional immune cells.

A chemically-induced brain tumour model in rats is being used to address the origin of brain tumours:

- Chemically-induced tumour model was successfully set up by transplacental administration of the carcinogen N-ethyl-N-nitrosourea (ENU) in pregnant rats.
- Stem cells and differentiated cells from treated pups have been successfully isolated and cultured.
- Differences in morphology and growth rate between cells from ENU-treated pups and untreated pups were detected.



FIG.1: <u>A</u> Immunodeficient eGFP expressing mouse, photographed under UV light (388nm). <u>B</u> Subcutaneous DsRed expressing human tumour growing in the eGFP expressing mouse (photographed under UV light). <u>C</u> Histological section of the tumour (red) and infiltrated muscular fibers (green). The tumour and host cells can be easily visualized.

2.2_Angiotargeting Project

| Title: | Targeting Tumour-Vascular /Matrix Interactions |
|--------------------|---|
| Acronym: | Angiotargeting project |
| Contract number: | 504743 |
| Financial support: | Integrated Project, EU 6th Framework Programme. Supportive grant for equipment from the MCESR, Luxembourg |

Project Summary

Solid tumour growth depends on a continuous supply of nutrients by new blood vessels that grow into the tumour. This process, termed "tumour angiogenesis" is regulated by a number of complex factors involving both tumour and host cells. The importance of the tumour blood supply has fuelled research into target molecules with anti-angiogenic properties. The animal models developed in the NorLux lab in Bergen generate invasive, non-angiogenic tumor phenotypes as well as tumour phenotypes that heavily rely on angiogenesis (Sakariassen et al. 2006). The molecular mechanisms underlying the change from non-angiogenic to angiogenic phenotype, may reflect an 'angiogenic switch' which has been hypothesized to occur in solid human tumours. This project aims at applying high throughput proteomics technologies to determine in detail the protein expression profiles of the two tumour phenotypes. Brain tumour samples, collected from the two phenotypes, are analyzed by two dimensional in gel fluorescence electrophoresis (2D-DIGE), differentially expressed proteins are isolated and identified by mass spectrometry. The project is also in the process of implementing a gel free proteomics approach based on isotope tags for relative and absolute quantitation and two-dimensional liquid chromatography – quantitative mass spectrometry (iTRAQ 2D LC-qMS). Insight into the differential proteome of the membrane fraction will identify potential biomarkers involved in tumor angiogenesis that may represent novel drug targets to block neovascularization in tumors.

Main Results

- The gel-based separation technique (2D-DIGE) has been successfully implemented in the lab and has generated preliminary data on differentially expressed proteins in human GBM biopsies versus spheroid cultures.
- Mass spectrometry on a MALDI/TOFTOF instrument has been carried out and has lead to the identification of candidate proteins (collaboration with F. Fack, D. Revets, LNS).

• A collaboration has been set up with the Onco-Proteomics Facility of the VU Cancer Center in Amsterdam (C. Jimenez), that will allow us to extend our analysis to gel-free proteomics technologies. This has led to a staff exchange programme (U. Rajcevic), approved by the EU Angiotargeting Consortium, that will be realized at the beginning of 2007.

3_Alginate Bead Project

| Title: | Functional validation of a new therapeutic strategy to prevent neurodegeneration and subsequent cognitive impairments in mouse models of Alzheimer's disease |
|--------------------|--|
| Acronym: | Alginate bead project |
| Contract number: | FNR 06/04/02 |
| Financial support: | Fonds National de la Recherche (FNR), Luxembourg |

Project Summary

Delivery of therapeutic compounds to the brain is a challenge for the treatment of brain diseases, including brain tumours and neurodegenerative diseases. The limited passage of drugs through the blood brain barrier and the short half-life of locally injected therapeutic molecules are major hurdles. Cell-based delivery systems providing continuous delivery of the biologically active compound in situ is a promising strategy for therapeutic applications in the brain. To prevent the immune system from destroying the transplanted cells, the producer cells are encapsulated in an encapsulation device, such as naturally occurring hydrogels (e.g. alginatebased gels). The aim of this project is to optimize the alginate encapsulated cells delivering neuroprotective peptides will be generated and implanted in the brain of murine models of Alzheimer's disease. This will open up new avenues for the use of this novel therapeutic strategy in the treatment of neurodegeneration and brain cancer. This project is carried out in collaboration with the group of Dr. T. Pillot at the INPL in Nancy (Fr).

Main Results

• This project has been submitted to the FNR in April 2006. The application was successfully received by the FNR and financial support was granted in October 2006. The official start date of the project is February 1, 2007.

• Using an electrostatic bead generator we have successfully generated alginate bead encapsulated cells expressing GFP that can be maintained in culture for several weeks (see Fig. 2).



Fig. 2: Electrostatic bead generator for cell encapsulation technology (A). GFP expressing BHK cells encapsulated in alginate beads under phase contrast (B) and in fluorescence (C).

4_Publications in 2006

Publications involving CRP-Santé

• Sakariassen PO, Prestegarden L, Wang J, Skaftnesmo KO, Mahesparan R, Molthoff C, Sminia P, Sundlisaeter E, Misra A, Tysnes BB, Chekenya M, Peters H, Lende G, Kalland KH, Oyan AM, Petersen K, Jonassen I, van der Kogel A, Feuerstein BG, Terzis AJ, Bjerkvig R, Enger PO. Angiogenesis-independent tumor growth mediated by stem-like cancer cells. Proc Natl Acad Sci U S A. 2006 Oct 31;103(44):16466-71.

• Goplen D, Wang J, Enger PO, Tysnes BB, Terzis AJ, Laerum OD, Bjerkvig R. Protein disulfide isomerase expression is related to the invasive properties of malignant glioma. Cancer Res. 2006 Oct 15;66(20):9895-902.

• Terzis AJ, Niclou SP, Rajcevic U, Danzeisen C, Bjerkvig R. Cell therapies for glioblastoma. Expert Opin Biol Ther. 2006 Aug;6(8):739-49. Review.

Other Publications

• Thorsen F, Enger PO, Wang J, Bjerkvig R, Pedersen PH. Human glioblastoma biopsy spheroids xenografted into the nude rat brain show growth inhibition after stereotactic radiosurgery. J Neurooncol. 2006 (Epub ahead of print)

• Boe SO, Haave M, Jul-Larsen A, Grudic A, Bjerkvig R, Lonning PE. Promyelocytic leukemia nuclear bodies are predetermined processing sites for damaged DNA. J Cell Sci. 2006 Jul 25.

• Sundlisaeter E, Wang J, Sakariassen PO, Marie M, Mathisen JR, Karlsen BO, Prestegarden L, Skaftnesmo KO, Bjerkvig R, Enger PO. Primary glioma spheroids maintain tumourogenicity and essential phenotypic traits after cryopreservation. Neuropathol Appl Neurobiol. 2006 Aug;32(4):419-27.

• Thorsen F, Afione S, Huszthy PC, Tysnes BB, Svendsen A, Bjerkvig R, Kotin RM, Lonning PE, Hoover F. Adeno-associated virus (AAV) serotypes 2, 4 and 5 display similar transduction profiles and penetrate solid tumor tissue in models of human glioma. J Gene Med. 2006 Jun 29.

• Werbowetski-Ogilvie TE, Seyed Sadr M, Jabado N, Angers-Loustau A, Agar NY, Wu J, Bjerkvig R, Antel JP, Faury D, Rao Y, Del Maestro RF. Inhibition of medulloblastoma cell invasion by Slit. Oncogene. 2006 Apr 24.

• Brekke C, Lundervold A, Enger PO, Brekken C, Stalsett E, Pedersen TB, Haraldseth O, Kruger PG, Bjerkvig R, Chekenya M. NG2 expression regulates vascular morphology and function in human brain tumours. Neuroimage. 2006 Feb 1;29(3):965-76. Epub 2005 Oct 25.

• Winter FD, Vo T, Stam FJ, Wisman LA, Bar PR, Niclou SP, van Muiswinkel FL, Verhaagen J. The expression of the chemorepellent Semaphorin 3A is selectively induced in terminal Schwann cells of a subset of neuromuscular synapses that display limited anatomical plasticity and enhanced vulnerability in motor neuron disease. Mol Cell Neurosci. 2006 May-Jun;32(1-2):102-17. Epub 2006 May 3.

• Niclou SP, Ehlert EM, Verhaagen J. Chemorepellent axon guidance molecules in spinal cord injury. J Neurotrauma. 2006 Mar-Apr;23(3-4):409-21. Review.

• Majed HH, Chandran S, Niclou SP, Nicholas RS, Wilkins A, Wing MG, Rhodes KE, Spillantini MG, Compston A. A novel role for Sema3A in neuroprotection from injury mediated by activated microglia. J Neurosci. 2006 Feb 8;26(6):1730-8.

5_Collaborations

intra CRP-Santé

- Flowcytometry facility: Rene Brons
- Laboratory for Immunology and Allergology: Dr. J. Zimmer

national

- CRP-Lippmann, Proteomics facility: Dr. Jenny Renaut and Dr. Lucien Hoffmann
- LNS, Mass spectrometry facility: Dr. Fred Fack, Dominique Revets and Dr. Claude Müller

international

- University of Bergen, Norway, Department of Biomedecine: Dr. Per Øyvind Enger and Dr. Frits Thorsen
- University of Bergen, Bergen Center for Computational Science: Dr. Kjell Petersen and Dr. Inge Jonassen
- Vrije Universiteit (VU) Cancer Center, Amsterdam, Netherlands, Onco-Proteomics Facility: Dr. C. Jimenez and Dr. K. Hoekman
- Institut National Polytechnique de Lorraine (INPL), Lipidomix Laboratory, Nancy, France: Dr. T. Pillot and Dr. T. Oster

LABORATORY FOR MOLECULAR BIOLOGY, GENOMICS AND MODELLING

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LABORATORY FOR MOLECULAR BIOLOGY, GENOMICS AND MODELLING

Head of Research Unit:

Dr Evelyne Friederich CRP-Santé/CNRS

Team members:

Microarray facility

Microarray platform

Laurent Vallar, PhD, Molecular biologist André Mehlen, PhD, Molecular biologist, research engineer François Bernardin, Technician

Bioinformatics

Mikalai Yatskou, PhD, mathematician, statistician Arnaud Muller, ingeneer, bio-informatician Jean Muller, PhD student (ULP Strasbourg) Oliver Poch, Co-direction, IBMC, Strasbourg

Molecular cell biology group

Bassam Janji, PhD, molecular cell biologist Guillaume Vetter, post-doc Marie Catillon, engineer Delphine Lentz, technician Sandrine Medvés, PhD student Ziad Altanoury, PhD student
1_Objectives and focuses

Based on our previous achievements and in keeping with the needs of the national research area, the activities of the LBMAGM are interdisciplinary and focus on:

- Basic research aiming at unravelling the molecular mechanisms underlying the progression of epithelial cancer cells towards an invasive state, and more specifically, the role of the actin cytoskeleton in cell morphogenesis and migration.
- High throughput genomics, bioinformatics and modelling. The laboratory runs the national microarray facility (www.microarray.lu, www.bioinformatics.lu) that is accessible to researchers of Luxembourg and the surrounding areas.
- Training of master and PhD students. The LBMAGM is affiliated to the doctoral schools « Vie et Santé », University Louis Pasteur, Strasbourg and « Vie et Santé », University Henri Poincaré, Nancy as well as to the masters Cellular Physio-Pathology , and Bio-informatics, University Louis Pasteur, Strasbourg

2_Research projects

Participation in projects of the CRP-Santé

- 1) Role of the actin cytoskeleton in epithelial cell morphogenesis and migration CRP project 01-01
- **2)** Transcriptomic analysis of cytoskeleton genes associated with tumor progression of epithelium-derived cancers towards an invasive state CRP-Santé (project 03-01)

Cell movement relies on a molecular motor, the actin cytoskeleton, made up by a dynamic meshwork of protein filaments that are closely linked to the plasma membrane. This complex cellular structure not only generates the forces that are required for cell movement but also determines cell shape. The present projects aim at understanding how actin-associated proteins control the assembly of the cytoskeleton in space and time at the level of the cell and how this structure contributes to cell migration, signalling and cancer cell invasion.

Participation in International Research Projects

In partnership with the Curie Institute (coordinator: Dr Cécile Sykes), the University of Ghent and the University of Santa Barbara, California, USA, the LMBGM participates since August 2005 in a research project funded by the « Human Frontiers Science Program Organisation ». This innovative, interdisciplinary project aims at studying the molecular and biophysical bases of actin-dependent movement in a-cellular model systems.

Participation in National Research Programs

• Implementation of a DNA micro-array platform and Novel competences in bio-informatics. Project of the National Research Fund (Biosan FNR/01/04/09). Coordinator: Dr Evelyne FRIEDERICH, CRP-Santé

• Novel protein- and DNA-based methods for tracing of food components. FNR program SECAL. Coordinator: Dr André STEINMETZ, LPMB, CRP-Santé

• Development of new genoproteomic diagnostic tools for the toxicological assessment of endocrine disruptors in food (ENDIF). FNR program SECAL. Coordinator: Lucien HOFF-MANN, CRP-Gabriel-Lippmann.

• Microglial activation: balance between pro-inflammatory secretions and beta-amyloid clearance. FNR program PROVIE. Coordinator: Prof. Paul HEUSCHLING, University of Luxembourg.

3_Major research activities and results in 2006

1_Molecular cell biology unit

A. Role of plastins/fimbrins, actin-crosslinkers, in the control of actin dynamics and actin-based motility.

L-Plastin is a member of a large family of actin filament crosslinkers, including alpha-actinin and dystrophin that stabilise the cortical plasma membrane cytoskeleton. L-plastin harbours two actin filament-binding sites and a regulatory NT-domain. We showed that L-plastin is constitutively phosphorylated on Ser5 in epithelial and mesenchymal cells, conversely to what is observed in hematopoietic cells. Using Ser5 substitution variants and in vitro phosphorylated recombinant proteins, we found that phosphorylation is not absolutely required for binding of Lplastin to F-actin but increased its affinity in vitro and in cells. Importantly, L-plastin endowed epithelial cells with invasive capacity in a collagen assay, dependent on Ser5 phosphorylation but did not affect migration in a 2D space. Our results suggest that constitutively phosphorylated L-plastin might be in a high-activity state in epithelial cells, contributing to the organization of the actin structures important for cell invasion (Janji et al., J. Cell Sci. 2006, collaboration: Jan GETTEMANS, University of Gent, VIB, Belgium).

Transcriptomic analysis of genes implicated in epithelium to mesenchymal transition (EMT), a key step in cancer cell invasion.

To gain a global view on cytoskeleton genes and regulatory circuits involved in EMT we established MCF-7 breast cancer cells expressing sna-1 in an inducible manner. The transcription regulator Sna-1, a homologue of Drosophila Snail, induces EMT in cultured cells by down-regulating cell-cell adhesion proteins. To study the regulatory circuits leading to EMT, we performed, after induction of sna-1 expression, time-course expression profiling experiments with a loop design, using pan genomic and ActiChip microarrays. Analysis of gene expression variations as a function of time at early stages of Sna-1 induction (2-10h) revealed a limited number of genes which exhibited distinct expression kinetics when compared to that of sna-1. Exploration of these data, combined with functional assays, will yield novel information on the cascade of events leading to Sna-1-induced EMT (in collaboration with Olivier Poch, IGBMC, Strasbourg).

B. Research and development activities of the Microarray Center

(www.microarray.lu and www.bioinformatics.lu)

Accessible to academic or industrial users from Luxembourg and surrounding areas, the microarray facility trained scientists from the CRP-Santé and external users and offered services like spotting of DNA or protein probes, running of microarray experiments or mining of array data.

Research and Development

Based on state-of-the-art approaches and methods developed by leading microarray platforms, we established a standardised protocol of statistical procedures (pipeline) dedicated to transcriptomic microarray data analysis. In cooperation with the Curie Institute, Paris, we tested, improved and integrated a novel statistical method for microarray image analysis and automatization of spot quality assessment (MAIA). An environment (BASE) for storage and management of microarray data as well as suitable tools for data mining and gene ontology searches (GoMiner database) were tested and installed. A user-friendly website for free access to local resources, including BASE, CADO4MI, ARPAnno, SRS, EMBOSS and Blast, was developed.

Within research projects of the National Research Fund (SECAL and BIOSAN program), we set up the conditions for protein array-based detection of antigen-antibody interaction and focused on the validation of the procedures for the design and production of function-dedicated oligonucleotide microarrays (ACTIchip). Highly specific 60mer oligonucleotide probes were selected using a program (CADO4MI) we developed in collaboration with Olivier Poch, Strasbourg (PhD thesis Jean Muller, master Céline Thill). Statistical analysis of comparative transcriptomic experiments revealed that ACTIchip performed equally well as other commercial or academic arrays (Affimetrix, Utrecht platform) in terms of specificity, sensitivity and reproducibility (manuscript submitted).

Jean Muller (IGBMC, Strasbourg/LBMAGM, Luxembourg) participated in an interdisciplinary study identifying a gene encoding a chaperonin that is mutated in the Bardet-Biedl syndrome, a disease that is characterized most prominently by a progressive retinal dystrophy, polydactyly, cognitive impairment, and renal dysplasia. This work was recently published in Nature Genetics (Stoezel et al., Nature Genetics 2006).

Valorisation of know-how

Within an EU Integrated project of the PC6 (EVI-Genoret), the microarray facility was asked to develop a function-focused microarray dedicated to the study of retinopathies (in collaboration with Drs Olivier POCH, IGBMC, Strasbourg and Thierry LEVEILLARD INSERM U592, Paris).

In collaboration with the clinicians of the Hospital Centre, the microarray facility contributed to projects aiming at dissecting the molecular mechanisms of complex-treat diseases like cancer (Dr Guy Berchem), cardio-vascular diseases (Dr Daniel Wagner) or allergies (Dr François Hentges).

Teaching and training activities

TRAINING OF DOCTORAL AND MASTER FELLOWS

Jean Muller, PhD obtained a PhD degree of the University Louis Pasteur, Strasbourg in November 2006 (promoters : Drs Olivier Poch and Evelyne Friederich). Since January 2006, he is a post-doctoral fellow at the EMBL Heidelberg.

Céline Thill obtained a master degree diploma in bioinformatics of the University Louis Pasteur, Strasbourg (promoter: Dr Laurent Vallar).

Teaching activities

Practical courses and lectures in biology and molecular cell biology, University of Luxembourg (Evelyne Friederich, Laurent Vallar)



Organisation of scientific meetings

In October 2006, the LBMAGM organized the international Workshop on Bioinformatics and Modelling in Biomedicine: From genes to biological systems, (Luxembourg-City) that was sponsored by the National Research Fund of Luxembourg.

4_National and international collaborations

National collaborations

- Dr André Steinmetz, CRP-Santé
- Dr Guy Berchem, CRP-Santé
- Dr Daniel Wagner, CRP-Santé
- Dr François Hentges, CRP-Santé
- Pierre Plumer, SANTEC, CRP Henri-Tudor
- Dr Lucien Hoffmann, CRP-Gabriel Lippmann
- Prof Paul Heuschling, University of Luxembourg
- Dr Gilbert Moris, Laboratoire National de Santé

International collaborations

- Dr Olivier Poch, IGBMC, Strasbourg, France.
- Dr Cécile Sykes, UMR 168 CNRS/IC, Curie Institut, Paris, France.
- Profs Jan Gettemans and Christophe Ampe, University of Gent, VIB, Belgium
- Dr Rainer Pepperkok, European Molecular Biology Laboratory, Heidelberg, Germany.
- Prof Apanasovitch, Belarus State University, Minsk, Belarus

5_Publications

• Phosphorylation on Serine 5 increases the F-actin-bundling activity of L-plastin and promotes its targeting to sites of actin assembly in cells. Janji, B., Giganti, A., De Corte, V., Catillon, M., Bruyneel, E., Lentz, D., Plastino, J., Gettemans, J., and E. Friederich. 2006. J. Cell Sci. 119:1947-60.

• Stoetzel, C., V. Laurier, E.E. Davis, J. Muller, S. Rix, J.L. Badano, C.C. Leitch, N. Salem, E. Chouery, S. Corbani, N. Jalk, S. Vicaire, P. Sarda, C. Hamel, D. Lacombe, M. Holder, S. Odent, S. Holder, A.S. Brooks, N.H. Elcioglu, E.D. Silva, B. Rossillion, S. Sigaudy, T.J. de Ravel, R.A. Lewis, B. Leheup, A. Verloes, P. Amati-Bonneau, A. Megarbane, O. Poch, D. Bonneau, P.L. Beales, J.L. Mandel, N. Katsanis, and H. Dollfus. 2006. BBS10 encodes a vertebrate-specific chaperonin-like protein and is a major BBS locus. Nat Genet. 38:521-4.

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Contributions to scientific meetings

Posters

Phosphorylation of L-plastin on Serine 5 increases its F-actin-binding activity, promotes its targeting to sites of actin assembly in cells and contributes to cell invasion. Janji, B., Giganti, A., De Corte, V., Catillon, M., Lentz, D., Bruyneel, E., Plastino, J., Gettemans, J., and Friederich, E. 97th Annual Meeting of the American Association for Cancer Research (AACR). April 1-5, 2006. Washington DC. USA.

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Towards a systemic analysis of the cytoskeleton with high throughput bioinformatics methods. The 3rd EMBL Biennial Symposium: From Functional Genomics to Systems Biology. Muller, J., Vallar, L., Stoetzel, C., Muller, A., André Mehlen, A., Vetter, G., Hélène Dollfus, H., Evelyne Friederich, E., and O. Poch. October 2006, Heidelberg, Germany.

Developing mathematical models, algorithms and programming tools for analysis of actinbased motility. Nazarov P., M. Yatskou, Barsukov E.A., Ivashkevich E.V., Golovaty A.A., Apanasovich V.V., Friederich E. The 3rd Wolfgang Pauli International Workshop on "Mathematical Modelling of Actin-Based Motility. November 6-9, 2006, Vienna, Austria.

Talks

Evelyne Friederich

Rôle du cytosquelette d'actine dans la morphogenèse et migration cellulaire. Meeting on "Medical engineering and therapy". May 15-16, Nancy, France.

Laurent Vallar, Evelyne Friederich

Presentation of the national microarray platform and current projects. Workshop on "Bioinformatics and modelling in biomedicine: From genes to biological systems Bioinformatics and modelling in biomedicine: From genes to biological system". October 27, 2006 Luxembourg-City.

Laurent Vallar

Design and evaluation of a thematic oligonucleotide microarray focused on actin cytoskeleton genes. 3rd international meeting on "medical engineering and therapy", May 15-16, Nancy, France.

Jean Muller

Comparative high-throughput genomics to unravel genetic diseases: Application to the Bardet-Biedl Syndrome.Workshop on Bioinformatics and modelling in biomedicine: From genes to biological systems. October 27, 2006, Luxembourg-City.

CENTRE FOR HEALTH STUDIES

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CENTRE FOR HEALTH STUDIES

Head of Research Unit: Marie-Lise Lair-Hillion

Secretaries:

Cathy Fousse-Pires, Christine Gauthier (replacement)

The Centre for Health Studies brings together and coordinates all activities relating to public health, epidemiology and health systems.

The running of the Centre for Health Studies is entrusted to Mrs Marie-Lise Lair, assisted by Dr Sophie Couffignal, head of the Clinical Epidemiology Service and Public Health Service, and by Mr Alain Origer, manager of the Focal Point of the European Monitoring Center for Drugs and Drug Addiction for Luxemburg.

Clinical Epidemiology and Public Health (SECSP):

Dr Sophie Couffignal, Epidemiology Physician, Manager, Dr Alaa Alkerwi, Physician specialised in Public Health, Magali Perquin, PhD Physician in Biological and Medical Engineering, Agnes Columeau, Research Nurse, Michel Vaillant, MSc, Biostatistician Hanene Samouda, Anthropologist Colette Andree, Pharmacist Daniel Theisen, PhD, Physician in Physiotherapy and Rehabilitation Laurence Fond-Harmant, PhD, Sociologist

Systems Analysis Service and Health Services (SASSS):

Gaetane Wibrin, Research Nurse, Christelle Rott, Qualified Biostatistician, Jean-Pierre Cornez, IT

European Monitoring Center for Drugs and Drug Addiction – Focal Point:

Alain Origer, Manager Nathalie Removille, Pharmacist, Scientific Assistant Pascale Straus, Psychologist, Scientific Assistant.

Health Economics:

Laurence Renard, MSc, Health Economist

Health Gateway:

Coralie Dessenne, Information Officer, Data Manager **Patty Mathgen-Geisen**, Editor

Legal Services:

Guillaume Byk, Lawyer

1_Mission of the Centre for Health Studies

The Centre for Health Studies' missions are to:

- carry out research in epidemiology and public health ensuring to assess the Grand Duchy population's health situation and to bring relevant information in order to develop a prevention policy
- collaborate to clinical epidemiology studies carried out by Luxembourg medical establishments' clinicians, by bringing competencies in the management of projects and in biostatistics. It will thus lead to contribute to develop knowledge in health, diagnosis or treatment.
- promote and carry out studies, necessary for the setting up of durable registers-type information systems, on prevailing diseases in the Grand Duchy of Luxembourg. The aim of it is to establish annual or long-term reports allowing a general or more targeted approach reflecting on the development of diseases. It will thus bring information to the decision-making bodies as regards public health to define their policy and strategy.
- carry out research which enables support for public health policies and programmes.
- carry out estimate studies on services or health care provided.
- contribute to inform citizens and to give them a sense of responsibility for the handling of their health.
- contribute to develop a strategy as far as health care is concerned, by promoting innovations both from the manager and the organisation point of view.
- stimulate performance in health care by researching, among others, the most appropriated methods and technologies to Luxembourg health care socio-economic context.

2_Ongoing Projects for 2006

Epidemiology and Public Health Activities

HBSC Survey (Health Behaviour in School-aged Children)

The survey carried out every 4 years by the European Office of the World Health Organisation in 42 countries is a transversal epidemiological study, which targets school-aged children aged 11, 13, 15 and 17, to observe the health behaviour of young people over time, and to identify the influencing factors.

Antenatal Health Monitoring in Luxembourg

Various stages of authentification and data quality control in antenatal monitoring were carried out as a result of the Birth Medical Record ("FIMENA"), before production of the first national report covering the period 2001 to 2003.



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Cardiovascular Health Monitoring

A cross-border monitoring system was designed in 2006 (across Lorraine – Southern Belgium – Luxembourg), with the objective of establishing comparable indicators for cardiovascular health in accordance with a standardised survey method.

Monitoring of the drug phenomenon in the Grand Duchy of Luxembourg

Established by the Council Regulation (EEC) N° 302/93 of February 8, 1993, the EMCDDA overall objective is to supply the Community and its Member states with objective, reliable and comparable information on a European level on the phenomenon of drugs and drug addiction.

Prevalence and spreading of viral hepatitis A, B, C and of HIV in the population of problematic users of illicitly acquired drugs. Early detection, vaccination against HAV and HBV, referral and reduction of risks and damages

Authors included a national cross sectional study carried out on a voluntary and anonymous basis in national prisons, outpatient and inpatient drug treatment centers and facilities. Standardized questionnaires and blood sampling were applied allowing to assess HIV and hepatitis A, B, C prevalence in PDUs on basis of serological evidence.

Prevalence of migraines and headaches in Luxembourg, measurement of the socio-economic impact of the illness: A prevalence study of migraines and headaches among the general public (18 to 65 years old) was carried out and completed by a Family, Social and Economic Impact Assessment. The results are being published.

Study of Obesity and Excess Weight among children and adolescents in Luxembourg: The research compares two models for treatment of obese and overweight children and aims to develop non-invasive, reliable diagnostic methods for childhood obesity and detection of associated complications.

Observational Survey on injuries among young people in sports school in Luxembourg: This study aims to draw up a complete picture of the situation in order to develop prevention policies.

The feasibility study for the establishment of a diabetes register in Luxembourg, together with the pathology and its complications incidence and prevalence study, as well as a study on diabetic patients' life habits and quality: Various information-gathering methodologies for creating a register were tested and compared.

Prospective evaluation of neuropsychological, biological and sub-clinical characteristics at the stage of Mild Cognitive Impairment: A feasability study is currently underway.

Health Report: In 2006, the idea of a health report on the population of Luxembourg was put forward. It will be used as a basis for a first version that will be developed in 2007.

Assessment activities:

External evaluation of the Experimental Action Project for Dependence Insurance: Alternative model for the care management of patients suffering from neuro-dependence.

The objective of the evaluation is to demonstrate whether implenting an innovative and alternative model for the care management of neuro-dependent patients provides added value for these patients as well as for the funds that support them and also to collect all of the information needed with a view to possibly rolling out the project in Luxembourg.

Evaluation of the national PET scan service: The aim of the evaluation is to define the use of the PET scan in the diagnostic and therapeutic chain.



Collaboration Activities with Clinical Research

The Centre for Health Studies brings its skills in Biostatistics to the clinical doctors in the context of the retrospective treatment of data or within the context of clinical research.

- National feasability study of a Luxembourg myocardium infarction register to evaluate reimbursement of medical expenses and future of patients. National Institute of Cardiovascular and Interventional Surgery, Dr Daniel Wagner.
- Randomised clinical test, verified for the evaluation of 3 training methods versus physical control methods for heart weakness.

Cardiology Department, CHL, Dr Charles Delagardelle. Follow-up after 3 months training.

• **Parkinson's disease sleepiness: Retrospective analysis** Neurology Department, CHL, Dr Nico Diederich. Study undertaken in January 2005.

• Nocturnal dysautonomic features in Parkinson's disease. A retrospective polysomnographic study of RR-variability during sleep in PD patients Neurology Department, CHL, Dr Nico Diederich.

• Can non-motor signs be premotor signs in Parkinson's disease? A longitudinal multicenter case-control study.

Neurology Department, CHL, Dr Nico Diederich.

• Before / After evaluation of therapies used on patients suffering from psychiatric disorders with the aid of questionnaires adapted to the illness and also of WHODAS II. Outpatient Clinic, CHL, Dr Pull

Activities within the framework of healthcare funding

Within the framework of hospitals funding, the Centre for Health Studies contributed by carrying out, among others:

- Measurement of care costs required for hospitalised patients in every hospital by using the PRN-methodology, in ambulatory chemotherapy polyclinics,
- The study for the establishment of the workforce endowment pattern in intensive care units according to care costs required for patients, in outpatient units or hospital,
- Projected calculations of medical staff endowment for normal and intensive care hospitalisation units, for the dialysis services, for surgical units.

This work enables to calculate in a provisional way staff endowments appropriated to patients' real needs, in order to use available resources in favour of citizens in the best way. This work is used by the Union des Caisses de Maladie and by hospitals during budget negotiations.

Activities within the framework of health information.

In 2006, the Centre for Health Studies worked on the necessary tools to improve citizens' information in the field of health, notably by:

- The validation of an education frame of reference for Luxembourg's hospitals aiming the patient's education for a well-informed consent, (in collaboration with the Entente des Hôpitaux Luxembourgeois),
- The development of an E-Health in Luxembourg (delegation of Ministry of Health to the Centre for Health Studies for this project)

Activities within the framework of quality improvement and healthcare result.

In order to improve Luxembourg hospitals' health services quality and result, the Centre for Health Studies was the Union des Caisses de Maladie expert to suggest a concept of evolution of the Quality Incitant model used for Luxembourg hospitals funding. This concept introduces EFQM methodology and result indicators.

In 2006, a study commissioned by the Emile Mayrisch Hospital aimed to develop a tool to measure the hospital's performance.

3_Publications in 2006

- Origer, A. (2006). The state of the drug phenomenon in the Grand Duchy of Luxembourg (Annual report on the national network of information on drugs and drug addictions RELIS), CRP-Santé, CES, PF OEDT, Luxembourg
- Final report of the feasability study for setting up a Health Gateway
- (final or intermediate) reports relating to each study carried out in 2006 have been spread to the limited partners: Ministry of Health, Union des Caisses de Maladie. They are the only ones to allow their external publication
- Agnamey, P., Brasseur, P., Eldin de Pecoulas P., Vaillant, M., Olliaro, P. Plasmodium falciparum in vitro susceptibility to antimalarial drugs in Casamance (south-western Senegal) during the first five years of routine use of artesunate-amodiaquine, Antimicrob Agents Chemother. 2006 Apr; 50(4):1531-4
- Wagner, D.R., Delagardelle, C., Ernens, I., Vaillant, M., Beissel, J. Matrix Metalloproteinase-9 is a marker of late onset heart failure following acute myocardial infarction, J Card Fail. 2006 Feb; 12(1):66-72
- Bisdorff, A., Sobocki, P., Cloos, J.M., Andrée, C., Graziano, M.E.Cost of disorders of the brain in Luxembourg, Bulletin Société Médicale des Sciences, 2006; N°3

4_CRP-Health collaborations

Collaborations have been set up between the Centre for Health Studies and the Cardio-Vascular Research Laboratory (Dr Daniel Wagner), the Psychiatry Research Unit (Prof Charles Pull), the haemo-oncology laboratory (Dr Guy Berchem).

5_National-level collaborations

Several national level collaborations are needed to enable the Centre for Health Studies to accomplish its activities.

Numerous studies are carried out in agreement and / or in collaboration with government bodies such as the Ministry for Health and the Health Directorate, the Social Security and National Education Ministries, the Ministerial Department for Sports. Other institutions such as the Union for Health Insurance and the Evaluation and Steering Cell for Dependence Insurance have presented a request to the Centre for Health Studies to carry out evaluation work.

The hospitals (INCCI, Sainte-Thérèse Clinic, Emile Mayrisch Hospital, Centre Hospitalier de Luxembourg), the organisation Entente des Hopitaux and clinical physicians represent a strong hub for partnerships.

Patients' associations, professional associations and representative bodies (AMMD, Collège Médical, Pharmacists, Dieticians...) are involved in numerous studies being carried out by the Centre for Health Studies.

These research projects are carried out in partnership with the University of Luxembourg.

Certain key players in the health sector in Luxembourg are active partners in the projects carried out, such as the Fire Department of Luxembourg Town and the Multi-Sectorial Health Assessment Service.

6_International collaborations

The Centre for Health Studies enjoys the expertise of overseas universities (the University of Montreal, Liège, Bordeaux...) and specialist centres (Operational Research for Health Team, EROS), and assists on projects of organisations having similar or complementary activities (Health Monitoring Centre for the Province of Luxembourg, INSERM in Nancy, Prospective Biology, Swiss Migraine Trust Foundation...). It welcomes Statistics and Public Health students, mainly from Universities in the Greater Region.

LABORATORY OF PSYCHIATRY

LABORATORY OF PSYCHIATRY

Head of Research Unit:

Professor Charles Pull MD, neuro-psychiatry MA, psychology

Team members:

Gloria Aguayo, MD Anne-Marie Schuller, PH.D, neuro-psychologist Nadine Hemmer, clinical psychologist Jessica Schneider, neuro-psychologist Marc Dammé, psychologist Béatrice Strock, psychologue-stagiaire

In collaboration with the psychotherapists of the Psychiatric Day Clinic for Adults of the Centre Hospitalier:

Marie-Claire Pull-Erpelding, PH.D., clinical psychologist Françoise Münster, clinical psychologist Lidiwine Wouters, clinical psychologist Marianne Schomer, clinical psychologist Lizzie Seven, clinical psychologist Paula Pereira, psychiatric nurse Fatima Pezzan, psychiatric nurse

1_Objectives and focuses

The psychiatry unit of the CRP-Santé has focussed on three different aspects

a) Assessment of functioning and disability in mental disorders

Pr Pull is chairman of a task force appointed by the World Health Organization to develop an assessment instrument, the WHO Disability Assessment Schedule II (WHO DAS II), for measuring functioning and disability that is linked to the International Classification of Functioning, Disability and Health (ICF). The WHO-DS-II is a structured clinical interview for exploring 6 domains of functioning and disability in health conditions.

The psychiatry unit of the CRP-Santé has been involved in translating the instrument into French and Luxembourgish, in validating the inter-rater reliability and validity of the instrument for assessing functioning and disability in mental disorders, and is currently involved in a study assessing functioning and disability in anxiety disorders and eating disorders as well as in assessing change in functioning and disability following treatment of these disorders.

b) Neuropsychological Aspects of Aging in Schizophrenia

Impairment in patients with schizophrenia is usually associated with pervasive disturbances in cognitive functioning, in particular in the fields of attention, memory and executive functions. At best, those disturbances remain stable over longer periods of time, elsewhere they keep getting worse over the years.

The psychiatry unit of the CRP-Santé is currently involved in a study supported by a grant from the Fonds National de la Recherche (FNR). It is done in collaboration with the University Louis Pasteur of Strasbourg, the Centre Hospitalier de Luxembourg, and the Centre de Recherche Public Santé. The study intends: 1. to investigate neuropsychological and neurobiological aspects of aging in patients with schizophrenia and 2. to investigate whether there is a specific dementia associated with schizophrenia as a whole or with a subgroup of the disorder.

c) Virtual Reality Exposure Therapy in Panic Disorder with Agoraphobia

Panic disorder with agoraphobia is a frequent and disabling disorder occurring in up to 4% of the general population. Treatment modalities include medication (with selective serotonin reuptake inhibitors or benzodiazepines) and cognitive-behavioral therapy (CBT). A major component of CBT for panic disorder with agoraphobia is progressive and sustained exposure to situations that individuals with the disorder are afraid of. Traditionally, exposure therapy consists in confronting feared stimuli first "in imagination" and then in the real world. Virtual reality exposure therapy or VRET is an exposure "in virtuo" that may be conceptualized as an exposure in between imagination and reality.

The psychiatry unit of the CRP-Santé is currently involved in a study comparing VRET and CBT in panic disorder with agoraphobia. The study is done in collaboration with the Collège de France (Pr Berthoz) and two university hospitals in Lyon (Dr Cottraux) and Paris (Pr Jouvent).

The Laboratory of psychiatry has been audited by Pr Mackel in 2005. In his report, Pr Mackel has recommended that Dr. Pull focus on the project "Virtual Reality and Panic Disorders". According to Pr Mackel, "the basic paradigm is in place, but the next step has to be taken



to get beyond the descriptive stage. The project now needs an empirical base and a more scientific orientation. This will make the results publishable and enhance the visibility of the laboratory. First, the test procedures should be standardized and internally validated. Then, a host of physiological variables should be measured in the virtual surround, quantified and statistically analyzed. This will require the addition of a physiologist or psychobiologist who is familiar with physiological recording and monitoring techniques and statistical analysis. Some (not very expensive) equipment also has to be purchased and it would be very helpful to assign the laboratory a permanent space. Another avenue of expansion of the project is into functional neuroimaging. In sum, there are many ways to enhance the clinical and scientific value of the project".

Pr Mackel's recommendations have been extremely constructive, precise, and helpful. In line with these recommendations, we intend to:

1. create a real unit or laboratory for all future work of the psychiatry unit in the research-field.

2. focus on "virtual reality" and "virtual reality exposure therapy" also known as "cybertherapy" as the main topic of such a unit or laboratory.

3. systematically include the recording of physiological variables in the assessment of participants in future studies.

4. extend the use of virtual reality beyond panic disorder, in particular to other anxiety disorders such as specific phobias, social phobia (social anxiety disorder), test (examination) anxiety and post-traumatic stress disorder, and other mental disorders such as eating disorders.

5. include the assessment and recording of neuropsychological variables in future studies and extend the use of virtual reality to the rehabilitation or remediation of patients presenting with cognitive disorders.

6. continue assessing change in functioning and disability following VRET

Future studies will include measures and monitoring of heart rate, blood pressure, skin conductance, electrocardiogram, electromyogram, electroencephalogram, salivary cortisol, and blood oxygen saturation. We have tested and applied for the purchase of an adequate equipment available from a German company that would allow us to measure and monitor the physiological variables mentioned above. We also intend to integrate measurements from functional MRI into future studies as soon as our neuroradiology department is ready to provide this type of measurement (this should be possible in the next future). Finally, we are in contact with experts in virtual reality concerning the development of all relevant software (virtual environments) required in future projects.

2_Ongoing projects in 2006 and results obtained in 2006

Assessment of functioning and disability in mental disorders

The WHO DAS II is a robust instrument that can be easily administered to measure the impact of health conditions, monitor the effectiveness of interventions and estimate the burden of mental and physical disorders.

With regard to the assessment of functioning and disability in anxiety disorders and eating disorders, and the change in functioning and disability following treatment, more than two hundred patients had been assessed by the end of 2006, using the WHO-DAS-II before and after treatment.

Data collection for follow-up after 3, 6 and 12 months will continue up to June 2007.

Neuropsychological Aspects of Aging in Schizophrenia

By June 30, 2006, 20 patients and 5 controls had been included in the study in Luxembourg and a similar number in Strasbourg. A total of 30 patients and an equal number of controls will be assessed. Assessments will continue in 2006 and 2007.

Virtual Reality Exposure Therapy in Panic Disorder with Agoraphobia

By June 30, 2006, the treatment phase of the trial had been completed. Data from pre- and post-treatment assessments are available for 92 participants, including 32 patients from the Luxembourg centre. On the whole, this is the largest outcome trial comparing CBT to VRET ever completed. Data are currently being analysed for the assessments made pre- and post-treatment. Follow-up assessments (24 and 52 weeks post-treatment) will continue up to June 30, 2007.

3_Publications in 2006

Assessment of functioning and disability in mental disorders

Six papers have been submitted for publication, including

T. Bedirhan Üstün, M.D., Somnath Chatterji, M.D, Jürgen Rehm, Ph.D., Cille Kennedy Ph D, Luis Prieto Ph D, Joanne Epping-Jordan, Shekhar Saxena, M.D., Michael von Korff and Charles Pull, M.D., in collaboration with WHO/NIH Joint Project Collaborators. World Health Organization Disability Assessment Schedule II (WHO DAS II): Development, Psychometric Testing, and Applications.

Neuropsychological Aspects of Aging in Schizophrenia

The methodology of the study has been presented by AM Schuller in 2 oral communications during symposia at international conferences. Data are currently being analysed in Strasbourg and in Luxembourg.

Virtual Reality Exposure Therapy in Panic Disorder with Agoraphobia

The rationale and methodology of the study has been described in several presentations and publications including:

Pull C Current status of Virtual Reality Exposure Therapy in Anxiety Disorders, Current Opinion in Psychiatry, 18(1):7-14, 2005

PulICB Self-help Internet interventions for mental disorders. Current Opinion in Psychiatry, 19(1):50-3, 2006

Pull C. Pharmacologie et thérapie cognitivo-comportementale. In : Guide clinique de thérapie comportementale et cognitive. Sous la direction de O. Fontaine et P. Fontaine. Retz, Paris, 2006.

Pull CB, Cottraux J, Berthoz A, Jouvent R, Zaoui M, Pelissolo A, Pull-Erpelding MC, Wouters L, Aguayo G, Genouilhac V, Duinat A, Znaidi F, De Mey Guillard C, Panagiotaki P, Fanget F, Viaud-Delmon I, Mollard E and Gueyffier F. A Randomized Controlled Study of Virtual Reality

Exposure Therapy and Cognitive-Behaviour Therapy in Panic Disorder with Agoraphobia. In : Abstracts from Cybertherapy 2006, CyberPsychology & Behaviour, 712, 2006.

Pull C. Combined pharmacotherapy and cognitive-behavioural therapy for anxiety disorders. Current Opinion in Psychiatry. 2007 20(1):30-5.

Accepted for publication

Pull C. Illusion de présence et émotions. Le traitement des troubles anxieux par exposition en réalité virtuelle. A paraître dans Progrès en thérapie comportementale et cognitive. La troisième vague : thérapie cognitive et émotions. Sous la direction de J.Cottraux. Masson. Paris.

4_Collaboration within CRP-Santé

• Centre for Health Studies

5_National collaborations

- Fonds National de la Recherche
- CRP Henri Tudor
- University of Luxembourg
- Centre Hospitalier de Luxembourg, Centre Hospitalier Neuro-Psychiatrique

6_International collaborations

- Collège de France University of Strasbourg, University of Lyon
- University of Liège, University of Louvain, University of Bruxelles
- University of Trier, University of Vienna
- World Health Organization

ARTS THERAPIES PROJECT



ARTS THERAPIES PROJECT

Head of project:

Dr Lony Schiltz, Ph D, HDR in clinical psychology

Team members:

Eduardo Crivisqui, Ph D in sociology, senior researcher

Carole Meyer, Ph D in labour psychology, master student in the post graduate curriculum in arts therapies at the UdL, researcher

Laetitia Boyer, DEA in clinical and health psychology, Ph D student in clinical and health psychology at the University of Metz, master student in the post graduate curriculum in arts therapies at the UdL, assistant researcher

Béatrice Denis, DESS in labour psychology, master student in the post graduate curriculum in arts therapies at the UdL, assistant researcher

Mylène Konz, DESS in arts therapy (UdL), assistant researcher

Pascale Fack, professional BA in statistics, research collaborator

Maud Kuhn, master 1 student in psychology at the University of Metz, trainee,

Audrey Ciccarello, master 2 student in psychology at the University of Metz, trainee,

1_General scientific objectives

- Research in clinical psychology and health psychology, with the perspective of combining the two approaches
- Development of appropriate psychotherapeutic interventions using artistic mediations, considering the importance of tertiary prevention
- Thorough investigation of the psychotherapeutic process
- Development of a combined qualitative and quantitative research methodology, suited for the evaluation of psychotherapeutic, psycho-pedagogical and psychosocial interventions
- Investigation of the mathematical basis and possible adaptation of statistical procedures for small samples and data belonging to the nominal and ordinal level of measurement (in collaboration with the SMA of the University of Luxembourg).

2_Current research projects

2.1_Borderline Personality Organization in Adolescents. Diagnostical and Clinical Reflections. Application to Persons Suffering from a Breakdown of their Life Project. (R & D) Head of project : Lony Schiltz

Summary

The aim of the study consists in investigating the links between disastrous life events and the present personality organization, with several subgroups of people suffering from deprivation, exclusion and marginalization. Original research tools will be developed for this project. With the help of multidimensional non parametric statistics, differential personality profiles will be extracted. In a perspective of tertiary prevention we are offering exploratory arts therapeutic sessions to the marginalized persons, with the goal of investigating the indicators of a possible relaunchment of their subjectivation process.

Research results

a) The first part of the study was focussed on the theoretical, historical and conceptual analysis of the phenomena linked to the borderline personality organization and to the summing up of the recent research literature and of personal prior research results with this problematic.

b) The second part consisted in an integrated psychosocial and clinical study of people suffering from exclusion, marginalization and deprivation, i.e. homeless people, long-term unemployed people, refugees and asylum seekers, drug addicts, prisoners.

c) The third part of the study is focussed on the evaluation of the arts therapeutic sessions offered to these persons. The theoretical elaboration of the results of the study will consider especially the recent traumatogenic hypotheses of borderline functioning and psychosis.

National partners

Stemm vun der Strooss, Jugend an Drogenhellef, Foyer Abri-Sud Esch/Alzette, Services sociaux des villes de Dudelange et d'Esch/Alzette, Foyer Ulysse, Centres Pénitentiaires de Givenich et de Schrassig, Naxi, Co-labor, Pro-Actif.

• International partners

Laboratory of Health Psychology of the University Paul Verlaine Metz

Department of Sociology of the University of Kassel

In a study investigating the risk of recidivism with prisoners, the Department of Sociology of the University of Kassel (Germany) is currently testing an original rating scale we have developed for the Rotter test.

2.2_Application of Arts Therapies to some Crucial Problems of our Society. (FNR 02/05/14). Head of project : Lony Schiltz

Summary

The aim of the project consists in studying the cognitive, emotional and motivational disturbances linked to different kinds of developmental or clinical problems (highly gifted underachievers, elderly people confronted to the crisis of third age or suffering from different degrees of dementia, adults psychiatric patients) and in evaluating the efficiency and mode of action of arts therapies in these realms.

The methodological research is focused on the development and clinical validation of new rating scales for projective and expressive tests and of pertinent observational frames for the arts psychotherapeutic sessions and on the investigation of the mathematical foundations and possible adaptation of some multidimensional statistical procedures for small samples and non-metrical data (PLS, CFA, Dynamic Factor Model).

• Research results and perspectives

a) The theoretical and methodological part of the study has been presented in a first report in August 2005. We have given a survey of literature, integrating the data of developmental psychology clinical psychology, health psychology, psychiatry and neurobiology, and personal prior research results. With the help of these analyses we have drawn out the structural, cognitive and behavioral variables to be included in a pretest- posttest design. At the same time the mathematical background of some non parametrical statistical procedures for small samples, respectively non metrical data, has been investigated and the suitability of these procedures for our project has been tested.

b) The second stage of the research project is focused on the application of arts therapies in the different institutions, schools and hospitals participating to the project. The action-research was initiated in March 2006.

c) The third part is focused on the scientific evaluation and thorough investigation of the arts therapeutic process. It was initiated in summer 2006.

d) The fourth part will be focused on the theoretical generalization of our research results.

3_National partners in 2006

CHNP, CHL, Hôpital Kirchberg, Atelier thérapeutique Useldange, Centre Pénitentiaire de Schrassig, Jugend- an Drogenhellef, Foyer Abri-Sud Esch, RBS Seniorenakademie, Maison de retraite Maria Consolatrix (Heisdorf), Foyer St Joseph et Foyer Ste Elisabeth (Bettembourg), EASS, LAML, LTC-Annexe modulaire, ALPEIP.

New partners in 2007 : CHEM, LHCE, AL.

4_International partners

In the realm of clinical and health psychology, there is a collaboration with laboratories of the universities of Metz, Nancy 2, Paris-V, Université de Savoie, Bremen (Germany), Nijmegen (NL), Katowice (PI).

In the realm of mathematics and statistics, there is a collaboration with laboratories of the universities of Kassel (Germany), Lille 2 and Pennsylvania State University (USA).

5_Scientific publications since 2006

Konz M. (2006) L'utilisation des contes de fées et du travail narratif à l'aide d'un bac à sable auprès d'enfants présentant des troubles de la conduite. *Bulletin de la Société des Sciences Médicales du Grand-Duché de Luxembourg. 2 (Numéro spécial) ; 281-295.*

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Schiltz L. (2006) L'être-pour-soi et l'être-pour-autrui. Applications à la recherche en art thérapie. *Revue française de Psychiatrie et de Psychologie médicale X, 94 ; 37-42.*

Schiltz L. (2006) The specificity of music as a stimulus of fantasy. A comparative study of the TAT and the stories written under musical induction. In : Polychroniadou L.(dir) Soma et Psyché. Actes du XVII^e Congrès International de la SIPE. Athènes: Editions PRS. p. 45-62.

Schiltz L. (2006). Grilles d'analyse de contenu basées sur l'approche phénoménologicostructurale. Bulletin de la Société des Sciences Médicales du Grand-Duché de Luxembourg. 2 (Numéro spécial) ; 265-280.

Schiltz L. (2006) Le monde en blanc dans l'anorexie mentale de la préadolescence. Neuropsychiatrie de l'Enfance et de l'Adolescence. 54, 5 ; 297-303. http://dx.doi.org/10.1016/j.neurenf.2006.09.002

Schiltz L. (2006) Reifungskrisen. In : Förstl H. Boehlke E, Heuser M.P. (eds.) Metamorphosen. München : DGPA, ed. GIB. S. 124-130.

Schiltz L. Houbre B. (2006) Analyse multidimensionnelle d'homogénéité sur échantillons restreints : comparaison entre différents sous-groupes d'individus en situation d'exclusion. Bulletin de la Société des Sciences Médicales du Grand Duché de Luxembourg 2, (Numéro spécial) ; 311-325.

Schiltz L. Desor D, Lorang G, Schiltz J. (2006) Elèves surdoués en situation d'échec scolaire. Approche multidisciplinaire de la « résignation apprise » Luxembourg : Saint-Paul. (publication sponsored by the MCESR-CEDIES)

Active participation in international conferences:

| March 2006 | 2 ^e Journée d'études : Procédures statistiques multivariées pour groupes restreints respectivement données de niveau non métrique. Luxembourg | |
|----------------|--|--|
| June 2006 | Congresso Internacional de psicoterapia en la esquizofrenia y otras psycosis. Madrid . | |
| June 2006 | 5 ^e Colloque hongrois de la SIPE. Szentgotthard | |
| June 2006 | 71 st Annual Meeting of the Psychometric Society. HEC Montréal | |
| September 2006 | 18 ^e Congrès International de la SIPE. Belfast. | |
| October 2006 | Jahrestagung der Internationalen Gesellschaft für Kunst, Gestaltung und Therapie. Berlin. | |
| November 2006 | XVII ^{es} Journées Scientifiques de Musicothérapie. Université Paris-V | |
| November 2006 | Journées d'Automne de la SFPE. Paris. | |
| Décember 2006 | Jahrestagung der DGf Kunst u. Psychopathologie d. Ausdrucks. München | |

Teaching and administrative activities at several universities

The head of project has coordinated and directed the **post graduate curriculum in arts therapies,** functioning as a pilot project of the MCESR at the University of Luxemburg and given lectures in clinical psychology, arts therapies and research methodology at the Universities of Metz (Master 2 in Health Psychology and Clinical Psychology) and Paris-V (Master in Humanities and Social Sciences: Arts Therapies).

She belongs, as an associated member, to the Laboratory of Health Psychology of the University Paul Verlaine-Metz and she is co-directing dissertations of the Master 1 and 2 level and PhD theses. She is also co-directing PhD theses at the University de Savoie.

International scientific activities

The head of project has represented Luxemburg in the **Technical Committee: Social Sciences** and Humanities of **COST** and was proposed in 2005 to make the link with the TC of Medicine and Health. She made the report of the COST Strategic Workshop "Food and Health: The Way Forward" for the TC SSH.

In 2006 she has been appointed expert in the Technical Committee : Individuals, Society, Culture and Health.

She has been the national correspondent for the report : " Schulische Begabtenförderung in Europa. Bestandaufnahme und Ausblick" published by the "Bundesministerium für Bildung und Forschung" (Germany).

She has also been appointed expert for the ex-post evaluation of a research project belonging to the programme **"Social cohesion"** by the "Service Fédéral de Programmation de Politique Scientifique" (Belgique).

Organization of seminars and conferences

Organization of two international seminars on research methodology were held in June 2005 and March 2006 at the University of Luxemburg: **Multidimensional Statistical Procedures for Small Samples and Non Metrical Data**, in collaboration with the Department of Applied Mathematics of the University of Luxemburg.

Organization of an international conference "Research strategies for small samples and non metrical data", June $28^{th} - 30^{th}$ 2007, at the University of Luxembourg, in collaboration with the SMA.



MICROBIOLOGY UNIT LABORATOIRE NATIONAL DE SANTÉ



MICROBIOLOGY UNIT LABORATOIRE NATIONAL DE SANTÉ

Head of Research Unit: Dr François Schneider

Head of Projects:

Dr Joël Mossong, PhD

Team members:

Pedro Marques, engineer in research Natacha Koczorowski, technician Akakzia Ovalid, statistician

1_Objectives and focuses

The main activity of Microbiology unit at Laboratoire National de Santé centres around conducting microbiological analyses for hospitals, private practitioners and public health purposes. The unit has particular expertise in mycobacteriology (detection, isolation, antibiogram, typing), characterisation and genotyping of bacteria (e.g. Salmonella, Campylobacter, VTEC, Listeria, MRSA, Legionella,), viral culture and isolation (herpes, CMV, influenza, RSV, adenovirus), inhouse PCR detection (e.g. CMV, Herpes, pertussis, chlamydia) and specialised serology of infectious diseases (e.g. pregnancy-related, hepatitis, HIV, respiratory infections) and non-infectious diseases (tumor markers, allergens, autoimmunity).

The scientific focus of the unit has been applied either to improving the diagnostic and typing capacity of the laboratory or in helping to assist public health policy makers with evidence-based decisions.

The unit disposes of a well-equipped laboratory (P3, real-time PCR, sequencer, pulsed-field gel electrophoresis equipment) and bioinformatics and state-of-the-art software for image, epidemiological and statistical analysis (Bionumerics, Stata).

Personnel: the unit has around 20 members of full-time staff, most of which are laboratory technicians with considerable experience of routine microbiology, 4 PhDs (Joël Mossong, Matthias Opp, Catherine Ragimbeau, Paul Reichert,) and 2 MDs (François Schneider (head of unit) and Jos Even). In 2006 there were 3 members of staff working on CRP-Santé projects.

2_Research activities

Formal research activities in collaboration with the CRP-Santé started in 1999 and include the following projects:

- ESEN 2: European Seroepidemiological network
- Molecular epidemiology of Salmonella infections
- Pilot screening programme of Chlamydia trachomatis infection
- POLYMOD: Mathematical and economic modelling to assist policy makers with assessing the impact of vaccination programmes

ESEN 2

• Project funded by DG Sanco & CRP-Santé (2000-2004), overall project Leader: Health Protection Agency, London

• Aims:

• Standardisation of sero-epidemiological results through the creation, distribution and testing of serum reference panels

o Establishment of comparable large, representative serum banks (N=2679 in Luxembourg)

o Determination of age-specific antibody prevalence against vaccine preventable diseases: measles, rubella, mumps, diphtheria, pertussis, varicella, hepatitis A and B

o Collection of information on vaccination programme histories and past incidence of disease

• Results: presentations for the Local Immunisation Advisory Board: our study demonstrated the impact of the national vaccination programme on population immunity. In particular it identified a high MMR and hepatitis B coverage in the targeted population.

Molecular epidemiology of Salmonella

- Project funded by CRP-Santé, 2003-06
- Technical collaboration: RIVM, Netherlands

• Objective : to subtype the existing Salmonella strain collection using Pulsed Field Gel Electrophoresis (PFGE) method and thus refining epidemiological outbreak detection

- Preliminary results:
 - o 900 strains have been successfully typed

• PFGE is a very useful method to verify and complement serotyping. It has high discrimination for many serotypes (e.g. S. Typhimurium)

• PFGE lacks discrimination for highly clonal types (e.g. S. Enteritidis) and other typing methods are warranted for outbreak investigations

Piloting a screening programme of Chlamydia trachomatis infection

• Funded by CRP-Santé 2004-06

• Collaboration with Planning Familial, Division de la Médecine Scolaire and Service de Santé au Travail- Multisectoriel

Objectives

o to develop and validate a real-time polymerase chain reaction assay for detecting Chlamydia trachomatis in urine

o to estimate the prevalence of Chlamydia trachomatis in secondary schools, family planning, occupational medicine centres

Results

• Prevalences in family planning and occupational medicine are much higher than in secondary schools, and screening should be offered in the former two settings

o prevalence peaks in women around 21-22 years of age and is three times higher than in men

o the inhouse detection PCR is similar in performance to the commercial method

POLYMOD

• to boost policy-making techniques in disease prevention and control (2004-2008)

• DG Research funding 2005-08, Sixth Framework Programme, Contract number: SSP22-CT-2004-502084

- Unit is work package 2 leader. Aims of the work package
 - o Organisation, collection and analysis of social contact surveys

- o Collection and analysis of social and demographic data related to social contact patterns
- o Serological testing of national serum banks for exposure to parvovirus B19 and VZV

• Statistical synthesis of social contact patterns and their relation to sero-epidemiological data

• Results in 2006:

o Large prospectively-collected population-based surveys of epidemiologically relevant social contact patterns were conducted in eight different European countries using a common paper diary approach and covering all age classes

• These data provide novel insights which ought to be taken into account when modelling the spread of infections spread from person by the aerosol or close contact route.

3_Publications

J. Mossong, L. Putz, Z. Shkedy, F. Schneider. *Seroepidemiology of diphtheria and pertussis in Luxembourg in 2000.* Epidemiol Infect. (2006), 134(3): 573-8.

J. Mossong, L. Putz, S. Patiny, F. Schneider. *Seroepidemiology of hepatitis A and hepatitis B virus in Luxembourg.* Epidemiol Infect. (2006), 134(4):808-13.

F de Ory, JM Echevarria, G Kafatos, C Anastassopoulou, N Andrews, J Backhouse, G Berbers, B Bruckova, DI Cohen, H de Melker, I Davidkin, G Gabutti, LM Hesketh, K Johansen, S Jokinen, L Jones, A Linde, E Miller, J Mossong, A Nardone, MC Rota, A Sauerbrei, F Schneider, Z Smetana, A Tischer, A Tsakris, R Vranckx. *European seroepidemiology network 2: standardisation of assays for seroepidemiology of varicella zoster virus.* J Clin Virol. (2006), 36(2):111-8.

J. Mossong, J. Even, P. Huberty-Krau, F. Schneider. *Substantial reduction of human Salmonella Enteritidis infections in Luxembourg in 2005.* Eurosurveillance 2006;11(1): 060119.

WJ Edmunds, G Kafatos, J Wallinga, J Mossong. *Mixing patterns and the spread of close-contact infectious diseases.* Emerg Themes Epidemiol. 2006 Aug 14;3(1):10.

M Schmitz, G Grignard, C Margue, W Dippel, C Capesius, J Mossong, M Nathan, S Giacchi, R Scheiden, N Kieffer. *Complete loss of PTEN expression as a possible early prognostic marker for prostate cancer metastasis.* Int J Cancer. 2006 Dec 12;

V. Arendt, J. Mossong, C. Inwani, B. Farah, I. Robert, A. Waelbrouck, K. Fonck, R. Zachariah. *Primary health centres in urban Kenya as an entry point for early diagnosis of HIV – Infection?* Tropical Doctor (in press).

4_International Collaborations

The unit participates in a number of international surveillance networks

• Enternet: International surveillance network for human gastrointestinal infections (Salmonella, VTEC and Campylobacter)

- o Serotyping and resistance typing, EQA
- o Sharing of data in a European database

• PulseNet Europe (the unit has been certified in spring 2006 to contribute to an international pulsed-field gel electrophoresis typing database of food-borne infections - Salmonella, VTEC, Listeria)

- EWGLINET (Legionella surveillance), EQA, sequence-based typing
- EARSS (MRSA) PFGE and spa typing
- EISS (European Influenza Surveillance Scheme)





FOOD-SAFETY LABORATOIRE NATIONAL DE SANTÉ

Head of Project:

Dr Gilbert Moris

Team members:

Thomas Gaudron, technician Claude Peters LNS, technician
The LNS-food safety team is involved in a FNR-SECAL funded research project which aims at developing novel methods for tracing food components, by employing protein as well as DNA-based methods. The LNS-food-safety-team is involved in the DNA-part, which is dedicated to the cloning and sequencing of LIM-domain protein genes whose structure seem to be suitable for the use as tracer molecules, because the number and position, but not the length and sequence of the introns, are highly conserved between plant species. In this context, the role of the food safety team role has been

1) to develop multiplex-PCR methods for the simultaneous detection of plant species, particularly those linked to allergies or intolerances, based on LIM-DNA sequence informations provided by the team of Dr. Steinmetz at the CRP-santé. For all 5 species for which sequences became available in 2006, PCR systems have been developed. For the simultaneous detection of rice and maize, a robust duplex-PCR system could be set up. For wheat and barley, 2 PCR systems have been developed to distinguish between these two closely related species. A soya PCR was also developed. All PCR systems were tested for adequate specificity and robustness. The aim in 2007 will be to combine soya, wheat and barley PCR's in a multiplex-system together with the rice-maize duplex-PCR already developed and PCR's specific for peanut, sesame and walnut (for which LIM-DNA-sequences will be provided in 2007).

2) To develop multiplex Real-time–PCR methods for the simultaneous detection of genetically modified organisms (GMO). Activities in 2006 were focused on developing and testing of a quadruplex system enabling the simultaneous detection of reference genes for maize and soya (the 2 plant species with the highest number of EU-authorized varieties) together with the most employed regulating elements in GMO's: the 35S promoter of cauliflower mosaic virus and the nopalin synthase terminator of Agrobacterium tumefaciens. A first version of such a quadruplex-system gave satisfying results regarding sensitivity, specificity and robustness. In 2007, efforts will be dedicated to develop other multiplex systems aiming at the simultaneous detection and possibly quantification of EU-authorized GM-maize varieties.

3) A third activity concerned the development of PCR-methods enabling a specific detection of several important fish species, based on DNA-sequences of parvalbumin genes provided by the team of Dr. Hentges of CRP-santé. In the case of carp, brook trout and salmon trout, this aim could be achieved.

Publication of these results has not been considered appropriate in 2006 because of the intermediate character of the results.

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List of partners participating in the financing of the activities of CRP-Santé

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