3ème Colloque « Montpellier Infectious Diseases »

Pôle Rabelais

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Genopolys

Livre de résumés

Session 1 - Infection, transmission and drug design.

Chromosomal DNA replication and segregation in *Leishmania*

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In most biological models, reproduction as identical or similar organisms is based on the extreme accuracy of the mechanisms involved in the even transmission of the genetic material to the two daughter cells. This 'golden rule' does not seem to apply in Leishmania in which asymmetric chromosomal allotments during mitosis are responsible for a unique ploidy organization termed 'mosaic aneuploidy'. To get further insight into this unique feature, we developed an interest in chromosomal replication and segregation which are ill-known in Leishmania. We also studied the 'sister parasite' Trypanosoma brucei, which is diploid and where these key cellular processes are better elucidated. We followed two independent research approaches. First, to determine the physical parameters of the replication process, we analysed DNA replication dynamics in these parasites using DNA molecular combing; this allowed showing particularly large inter-origin distances and high speeds of DNA replication forks. Second, we studied the chromosomal dynamics during mitosis. Using fluorescent in situ hybridization combined with immunofluorescence in T. brucei procyclic forms, we determined the spatiotemporal dynamics of (i) the centromeres of chromosome II and III, and (ii) TbMlp2, the ortholog of a nucleoporin, during the course of the cell cycle. In interphase, the centromeres and TbMlp2 were located at the periphery of the nucleolus. TbMlp2 was then seen progressively migrating from the periphery of the nucleolus to the spindle poles. The position of the centromeres remained unchanged until TbMlp2 had completed migration to the spindle pole; then the centromeres themselves started migrating to the poles. In addition, RNAi knockdown of TbMlp2 lead to aneuploidy. Altogether, these data suggest that, unexpectedly, TbMlp2 may play a key role, as a molecular chaperone and/or transport protein, in the dynamics of chromosomal segregation. In total, both approaches again revealed original features in these divergent eukaryotes as compared to classical models.

Wolbachia interactions with its filarial nematode host: transmission mechanisms and roles of the symbiont.

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Wolbachia are gram-negative, obligate, intracellular bacteria carried by millions of arthropods worldwide. Wolbachia are also symbiotic with filarial nematodes, but exclusively with members of the parasitic Onchocercidae family. Wolbachia are transmitted vertically through the female germline, similar to mitochondria. In nematodes, the Wolbachia-host symbiosis has evolved toward mutualism and bacteria removal interferes with worm development and eventually leads to nematode death.

Filarial nematodes are causative agents of devastating diseases such as elephantiasis and river blindness. These diseases affect ~120 million people in tropical areas. There is currently no drug treatment against adult filarial nematodes. Because Wolbachia is essential to adult worm survival and fertility, Wolbachia is a promising drug target. Using Brugia malayi as a filarial model, a causative agent of elephantiasis, we are focusing on two fundamental aspects of this symbiosis :

- the mechanisms of Wolbachia transmission, from the fertilized egg to the adult tissues.

- the role and contribution of symbionts to the filarial host.

Using cell biology techniques we designed for studying these parasitic worms, we show that Wolbachia asymmetrically segregate during embryogenesis to reach only the hypodermis of the worm, and an ovarian tropism allows the symbionts in the hypodermis to colonize the adult female germline. We will present the defects induced during oogenesis and embryogenesis in the absence of Wolbachia.

Identification of inhibitors of PfCCT, a key enzyme of *Plasmodium falciparum* membrane biosynthesis

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During its life cycle in the human erythrocyte, Plasmodium falciparum, the parasite responsible of malaria, relies on phospholipids to build the membranes necessary for daughter cell development. The parasite membranes are composed of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) which together represent approx. 80% of the total membrane lipids. In P. falciparum, PC and PE are synthesized by the parasite's machinery through the de novo CDP-choline and CDP-ethanolamine (Kennedy) pathways using choline or ethanolamine as precursors. Our studies focus on the two cytidylyltransferases : PfCCT and PfECT. These enzymes catalyze the rate-limiting step of their respective pathway and both contain two cytidylyltransferase domains. Here we focus on the biochemical characterization and the inhibition of PfCCT. Interestingly, both catalytic domains of PfCCT are active while site-directed mutagenesis revealed that only one domain of PfECT is active, suggesting substantial evolutionary differences within this protein family3. Recently, we obtained the 3D crystal structure at 2.4 Å resolution of the C-terminal catalytic domain of PfCCT in complex with its reaction product CDP-choline. By virtual screenings of commercial compounds using docking tools, we identified molecules that competitively inhibit PfCCT activity. We are also developing a second approach for the identification of PfCCT inhibitors by fragment-based drug design. Primary screening of fragment library (230 molecules) has been performed by fluorescence-based thermal shift assay followed by Nuclear Magnetic Resonance Saturation Transfer Difference (NMR STD) as secondary screen to eliminate false positive ligands. Co-crystallization of the protein-fragments complexes will then be used for the optimization process, allowing subsequent rational design of inhibitors of this key enzyme of P. falciparum membrane biosynthesis.

Rationnal Design of small-molecules inhibitors of human Cyclophilins and HCV replication by Structure Based Drug Design.

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The hepatitis C virus (HCV) is the leading cause of chronic hepatitis, of liver cirrhosis and hepatocellular carcinoma. Roughly 170 millions individuals are infected in the whole world and the infection by HCV causes approximately 280.000 deaths per year. The study of the complex of replication made it possible to highlight the crucial role of cellular partners, in particular the cyclophilins1, in the driving process with the synthesis of new viral genomes and inhibition of these enzymes lead to new anti-viral agents. The Cyclophilins are enzymes that have been observed abundantly and ubiquitously in a wide range of tissue types and organisms. They are characterized by the ability to catalyse the cis-trans isomerisation of peptidylprolyl bonds2 (PPIases) which was identified as the rate-limiting step in protein folding. To design news Cyps inhibitors with low molecular mass, we applied a fragment-based screening approach on Cyclophilin D (CypD). We used X-ray crystallography and NMR that are well adapted to identify weak affinity fragments (mM). We solved 14 crystallographic structures of CypD in complex with fragments (2,00 - 0,97Å). Based on the fragments binding modes, we designed and optimized a new Cyps inhibitors family (proline mimetic). Our lead compound have an IC50 of 10 nM on CypD and CypA in vitro and an EC50 of 15 nM for the HCV replication in cellulo. The presentation will show the used of X-ray crystallography for the discovery of news human Cyps and HCV inhibitors.

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[2] Galat, A., Eur J Biochem, **1993**, 216 :689-707.

Investigation of capsid determinants involved in nepovirus transmission: a hybrid structural approach

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Arabis mosaic virus (ArMV) and Grapevine fanleaf virus (GFLV) are two picorna-like viruses from the genus Nepovirus, consisting in a bipartite RNA genome encapsidated into a 30 nm icosahedral viral particle formed by 60 copies of a single capsid protein (CP). They are responsible for a severe degeneration of grapevines that occurs in most vineyards worldwide. Although sharing a high level of sequence identity between their CP, ArMV is transmitted exclusively by the ectoparasitic nematode Xiphinema diversicaudatum whereas GFLV is specifically transmitted by the nematode X. index. The structural determinants involved in the transmission specificity of both viruses map solely to their respective CP. We present here a structural study that allowed us to identify a charged pocket involved in specificity of transmission of Nepovirus by hybrid approach combining X-ray crystallography, cryo-electron microscopy, single particle analysis and molecular dynamics methods.

The Two Human CXCR4 Isoforms Display Different HIV Receptor Activities: Consequences for the Emergence of X4 Strains

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CXCR4 is a chemokine receptor that plays key roles with its specific ligand, CXCL12, in stem cell homing and immune trafficking. It is also used as a coreceptor by some HIV-1 strains (X4 strains), whereas other strains (R5 strains) use an alternative coreceptor, CCR5. X4 strains mainly emerge at late stages of the infection and are linked to disease progression. Two isoforms of this coreceptor have been described in humans, CXCR4-A and CXCR4-B, corresponding to an unspliced and a spliced mRNA, respectively. Here, we show that CXCR4-B, but not CXCR4-A, mediates an efficient HIV-1 X4 entry and productive infection. Yet, the chemotactic activity of CXCL12 on both isoforms was similar. Furthermore, HIV-R5 infection favored CXCR4-B expression over that of CXCR4-A. In vitro infection with an R5 strain increased CXCR4-B : CXCR4-A mRNA ratio in peripheral blood mononuclear cells (PBMC), and this ratio correlated with HIV RNA plasma level in R5-infected individuals. In addition, the presence of the CXCR4-B isoform favored R5 to X4 switch more efficiently than CXCR4-A in vitro. Hence, the predominance of CXCR4-B over CXCR4-A expression in PBMC was linked to the capability of circulating HIV-1 strains to use CXCR4, as determined by genotyping. These data suggest that R5 to X4 switch could be favored by R5 infection-induced overexpression of CXCR4-B. Finally, we achieved a specific siRNA-mediated knockdown of CXCR4-B. This represents a proof of concept for a possible gene therapeutic approach aimed at blocking the HIV coreceptor activity of CXCR4 without knocking down its chemotactic activity.

Session 2 - Infection, immunity and regulation

Identification and characterization of Mabs_4780, a new determinant required for intracellular survival and pathogenicity of *Mycobacterium abscessus*

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Mycobacterium abscessus (Mabs) is an emerging rapid-growing mycobacteria causing severe lung infections, particularly in cystic fibrosis patients. The smooth morphotype displays surface expression of glycopeptidolipids (GPLs) whereas the rough morphotype is characterized by the loss of surface GPL. Rough variants are involved in more severe clinical forms although the underlying physiopathological mechanisms remain obscure. We have recently developed a zebrafish embryo model to decipher the pathogenesis of Mabs and the chronology of the infection process.

Herein, we evaluated the contribution of MABS_4780 in rough Mabs virulence. A mutant was constructed in which MABS_4780 was disrupted by a hygromycin cassette. This strain exhibited a higher susceptibility to thiacetazone, a second-line antitubercular drug, compared to the parental strain and higher sensitivity to detergents, presumably due to alterations of cell wall composition/structure. Consistent with hypothesis, solving the three-dimensional structure of the M. smegmatis orthologue revealed a MaoC-like structure of known dehydratases, potentially involved in cell wall lipid biosynthesis. Since Amoeba may represent the environmental reservoir of Mabs, we also assessed the intracellular fate of the mutant in Acanthamoeba Castellanii. The mutant failed to replicate intracellularly but this growth defect was not due to a general metabolic abnormality since it grew similarly to parental strain in vitro. In addition, unlike the R variant, the mutant strain was extremely attenuated in infected zebrafish embryos and was unable to produce abscesses within the central nervous system and to kill the embryos.

Our findings demonstrate the unanticipated role of MABS_4780 in physiopathology of Mabs infection, emphasizing its potential as an attractive drug target.

Phosphorylation of proteins and bacterial pathogenicity

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The importance of reversible protein phosphorylation to cellular regulation cannot be overstated. In eukaryotic cells, protein kinase/phosphatase signaling pathways regulate a staggering number of cellular processes including cell proliferation, cell death, metabolism , behavior and neurological function, development and pathogen resistance.
While protein phosphorylation as a mode of eukaryotic cell regulation is familiar, many are less familiar with protein kinase/phosphatase signaling networks that function in prokaryotes. Of particular interest, the persistence of bacterial infections in humans and the emergence of antibiotic-resistant strains emphasize the need for novel therapeutic approaches.
In order to sustain treatment of bacterial infections in humans, identification of novel drug targets is pivotal. Thus, a greater understanding of molecular mechanisms underlying bacterial disease pathogenesis is essential for the identification and further development of novel drug targets.
The discovery of eukaryotic-like signaling systems, such as STPKs (Serine/Threonine Protein Kinases) and phosphatases in bacterial pathogens has sparked an interest in understanding their function. This is partly due to the fact that eukaryotic protein kinases are currently the largest group of drug targets, second only to G-protein-coupled receptors. Therefore, studies on the importance of prokaryotic STPKs in human pathogens have gained interest owing to the prospect that these signaling components may be useful in future anti-infective therapies and that a complete understanding of their role is a prerequisite for future evaluation of these enzymes as antimicrobial targets. The increased understanding of their widespread occurrence and the importance of the processes they control emphasize the significance of these eukaryotic-like signaling systems in prokaryotes and especially in pathogens.
Although STPKs and phosphatases regulate important functions in bacterial pathogens, our understanding of the signal transduction mechanism is still in its infancy. The contribution of these signaling enzymes to bacterial growth and pathogenesis is multifaceted as can be expected for any signaling system. In our group, we are exploring the mechanism for how these signaling enzymes mediate diverse functions in a coordinated fashion as it remains to be completely understood.

Host immune response and macrophage behaviour during *Burkholderia cepacia* complex infection in zebrafish embryos

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Chronic respiratory infection in cystic fibrosis patients is characterized by a high level of pro-inflammatory cytokines, leukocyte infiltration, and inflammation in the lungs due to colonization by pathogenic bacteria. Chronic infections caused by bacteria belonging to the Burkholderia cepacia complex (Bcc) can be symptom free, but often cause pulmonary exacerbation with progressive worsening of lung function, sometimes resulting in acute fatal necrotizing pneumonia and sepsis. The reasons for these unpredictable, sudden transitions are not understood.

Using zebrafish embryos, which have an innate immune system very similar to that of humans, we previously found that B. cenocepacia K56-2, belonging to the epidemic ET12 lineage, is highly virulent for zebrafish embryos; it causes a rapidly fatal (2 days) systemic inflammatory infection. In contrast, embryos can control infection with strains such as B. stabilis LMG14294, which cause a persistent infection. Intravenously injected bacteria are rapidly phagocytosed by macrophages, and we found that an intracellular stage is important for fatal infection.

In an attempt to better understand the molecular basis for B. cenocepacia K56-2 or B. stabilis LMG14294 infection outcomes, we performed a global host transcriptome analysis during different stages of both infection types. RNA-seq analysis revealed interesting infection responsive host gene expression patterns. Whereas many host genes were differentially regulated during early (3 hours) as well as later (24 hours) stages of infection caused by B. cenocepacia K56-2, only few genes showed changes in expression level upon persistent infection with B. stabilis LMG14294. In particular, the innate immune response with Toll-like receptor (TLR), NOD-like receptor and apoptosis pathways were strongly activated during acute infection. The “silent” intracellular persistence of B. stabilis coincided with increased expression of genes encoding complement proteins. We will discuss how we are using the zebrafish model to further study the role of the TLR pathway, including the central adaptor protein MyD88 and intracellular stages in the induction of the highly excessive innate inflammatory response.

Role of the transcriptional regulator RegA in establishment of *Brucella suis* persistence in an original in vitro model

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Oxygen deficiency is one of the environmental conditions encountered by Brucella during intramacrophagic replication and chronic infection of the host. At chronic stage of brucellosis, these bacteria can reside in immune structures where anoxic conditions predominate. Our previous studies demonstrated the high metabolic flexibility of Brucella suis with respect to oxygen deprivation. We evidenced the central role of the two-component system RegB/RegA in the coordinated control of oxidative respiration and denitrification respiratory systems, which are crucial for virulence and/or persistence in vivo. More importantly, RegA was found to be essential for B. suis persistence in mice. Recently, we developed an original in vitro model, characterized by progressive oxygen deprivation, which allowed to show that RegA is essential for optimal long-lasting in vitro persistence. To identify RegA-dependent genes and proteins in this model, global transcriptome analysis and whole proteome quantifications were performed by comparison of the wild-type B. suis to a regA mutant strain. These analyses were performed at the time point where anaerobic conditions become established, corresponding to the cessation of wild-type strain multiplication. Genetic validation by quantitative PCR (RT-qPCR) indicated that RegA potentially regulates 12% of the B. suis genes. The down-regulation of genes or proteins involved in cell envelope biogenesis and in cellular division suggests that RegA could be involved in establishment of a non-replicative state. In addition, RegA-dependent repression of an important number of genes involved in energy production may be indicative of a participation of RegA in the slowing-down of central metabolim as it enters into the persistence phase. This was substantiated by the finding that two-thirds of the differentially produced proteins belonging to this functional class were also found repressed, among which isocitrate lyase, the first enzyme of the glyoxylate shunt. Several genes of the virB operon were also found repressed by RegA as was its regulator VjbR. In conclusion, RegA was found to regulate genes that encode proteins of all functional groups. This makes the two-component system RegB/RegA a main regulatory system required for adaptation of B. suis to oxygen depletion, which can contribute to the constraint of bacterial growth, characteristic of chronic infection.

Characterization of RbpA – a master regulator of gene expression from *Mycobacterium tuberculosis*

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RbpA, a RNA polymerase binding transcriptional activator protein from Mycobacterium tuberculosis (Mtb), regulates transcription without binding to the double stranded DNA. RbpA is involved in unwinding of the promoter DNA in transcription complexes containing either the housekeeping sigma factor – sigma A (σA) or the stress-response sigma factor – sigma B (σB). RbpA, predominantly found in actinomycetes, increases the tolerance levels to antibiotics including Rifampicin, most commonly used antibiotic against tuberculosis (the second infection causing highest number of deaths). By using the in-vitro transcription system (IVTS) and electrophoretic mobility shift assays (EMSA), we showed that the action of RbpA is sequence specific, as transcription from the housekeeping sigAP promoter of Mtb requires RbpA for activation, but another housekeeping promoter of B. subtilis, sinP3 doesn't require RbpA for the activation. Furthermore, series of mutations of the nucleotides upstream of the sigAP promoter suppressed the promoter dependency on RbpA. Thus, the fact that RbpA is involved in RNA polymerase - σA and σB mediated transcriptional activation and increased tolerance to rifampicin, corroborates its role in global regulation of the antibiotic-induced stress in Mycobacterium. Hence, a new approach for Mtb, known as Run-off microarray (ROMA), has been elaborated using in-vitro transcription on genomic DNA, for studying the genome-wide regulation of the gene expression by RbpA.

Life Technologies - Technologies CRISPR/TALS (genome editing), nouvelles stratégies de clonage et synthèse de gène

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CRISPRs and TALs are innovative technologies for genome editing. They provide the ability to target, edit and regulate expression at defined sequences within the genome and enable researchers to more accurately study and engineer gene function and develop better cell models. Life Technologies offers a unique flexibility and accessibility to these technologies through its customized solutions.
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Session 3 - Epidemiology, clinical trials and emerging pathogens

Dynamic of the 2011-2012 Hand, Foot and Mouth Disease in Hai Phong city, Vietnam

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Hand, foot and mouth disease (HFMD) is an acute febrile illness in children with a papulovesicular skin rash at the palms or soles of the feet, or both. HFMD is caused by members of Human Enterovirus A family of viruses which include Coxsackievirus A (CVA) and Human Enterovirus 71 (EV71). A large scale HFMD epidemic was reported for the first time between 2011 and 2012 in Northern Vietnam. We focus our attention on the large city-harbour of Hai Phong.

Two main aspects were studied.

The first consisted of determining and understanding the high level of diversity of the clinical signs using the three most solid clinical quantitative parameters; i.e., age, severity score and delay of time of onset to admission from the clinical data set of the 10000 observed cases. A hierarchical classification approach was used to cluster the patients into groups of common symptoms and analyze their distribution over the span of the epidemic. The second consisted in analyzing the spread of HFMD according to time and space. The commune is the smallest subdivision of Hai Phong City. Using the commune for geographical localization and the time distribution of the detected cases, recording was done in Geographic Information System (QGIS). The data were used to create a graph that modelised the outbreak of HFMD. Numerical analyses were investigated to determine some group of communes that presented similarities concerning the spatial dynamic of HFMD.*

HIV patients under suppressive antiretroviral therapy present with various patterns of immune activation: the ACTIVIH study

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Background: HIV infection induces an immune activation fuelled by several causes that may occur in various combinations. These causes are reduced under highly active antiretroviral therapy (HAART), but usually not abolished. In the ACTIVIH study, we analyzed whether immune activation is qualitatively the same for all efficiently treated patients or whether different patterns of immune activation may be identified.
Methods: To this aim, we measured in 120 HIV-positive adults, aviremic under HAART for at least 2 years, 55 cell surface and soluble markers of inflammation, and CD4+ T cell, CD8+ T cell, B cell, NK cell, monocyte, and neutrophil activation. We clustered the dataset using two independent hierarchical clustering analyses: one for variables using the $1-r^2$ (where r is the linear correlation coefficient) as a distance between variables, and one for observations using usual Euclidean distance measured on scaled variables for observations.
Results: The level of many markers of immune activation were increased, but not altogether in a given patient. We identified various subpopulations of patients according to their pattern of immune activation (Figure). Using ANOVA results corrected by False Discovery Rate for multiple testing, more than 90% of variables were on average significantly different for at least one subpopulation of patients with regards to the other ones (p-value < 0.05).
Conclusions: These different patterns of immune activation may be the result of different causes, and may result in different pathogenic consequences. A better understanding of the links between causes, patterns, and consequences of immune activation in virologic responders might pave the way to the identification of markers predictive of specific comorbidities, and to an etiologic and/or symptomatic immunosuppressive therapeutic approach tailored to each subpopulation of patients.

Gender differences in adherence and response to antiretroviral treatment in the Stratall trial in rural disctrict hospitals in Cameroon

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Background: Evidence of gender differences in antiretroviral treatment (ART) outcomes in sub-Saharan Africa is conflicting. Our objective was to assess gender differences in 1) adherence to ART and 2) virologic failure, immune reconstitution, mortality, and disease progression adjusting for adherence.

Methods: Cohort study among 459 ART-naïve patients followed-up 24 months after initiation in 2006-2010 in nine rural district hospitals. Adherence to ART was assessed using 1) a validated tool based on multiple patient self-reports and 2) antiretroviral plasma concentrations. The associations between gender and the outcomes were assessed using multivariate mixed models or accelerated time failure models.

Results: One hundred thirty-five patients (29.4 %) were men. At baseline, men were older, had higher BMI and hemoglobin level, and received more frequently efavirenz than women. Gender was not associated with self-reported adherence (P=0.872, 0.169 and 0.867 for moderate adherence, low adherence and treatment interruption, respectively) or with antiretroviral plasma concentrations (P=0.549 for nevirapine/efavirenz). By contrast, male gender was associated with virologic failure (odds ratio 2.18, 95 %CI 1.31-3.62, P=0.003), lower immunologic reconstitution (coefficient -58.7 at month 24, 95 %CI -100.8;-16.6, P=0.006), and faster progression to death (time ratio [TR] 0.30, 95 %CI 0.12-0.78, P=0.014) and/or to WHO stage 4 event (TR 0.27, 95 %CI 0.09-0.79, P=0.017).

Conclusions: Our study provides important evidence that African men are more vulnerable to ART failure than women and that the male vulnerability extends beyond adherence issues. Additional studies are needed to determine the causes for this vulnerability in order to optimize HIV care. However, personalized adherence support remains crucial.

Chikungunya virus-host interplay at the keratinocyte level

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Transmission of chikungunya virus (CHIKV) to humans is initiated by puncture of the skin by a blood-feeding Aedes mosquito. Despite the growing knowledge accumulated on CHIKV, the interplay between skin cells and CHIKV following inoculation still remains unclear. In this study we questioned the behavior of human keratinocytes, the predominant cell population in the skin, following viral challenge. We report that CHIKV rapidly elicits an innate immune in these cells leading to the enhanced transcription of type I/II and type III interferon genes. Concomitantly, we show that despite viral particles internalization into Rab5-positive endosomes and efficient fusion of virus and cell membranes, keratinocytes poorly replicate CHIKV as attested by absence of nonstructural proteins and genomic RNA synthesis. Accordingly, human keratinocytes behave as an antiviral defense against CHIKV infection rather than as a primary targets for initial replication. This picture significantly differs from that reported for Dengue and West Nile mosquito-borne viruses.

Inflammasome signaling pathways exert antiviral effect against chikungunya virus in human dermal fibroblasts

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Arboviruses represent an emerging threat to human. They are transmitted to vertebrates by the bite of infected arthropods. Early transmission to vertebrates is initiated by skin puncture and deposition of virus in this organ. Events at the bite site remain largely unknown. Here we report that Chikungunya virus (CHIKV), and West Nile virus (WNV) despite belonging to distinct viral families, elicit a common antiviral signature in primary human dermal fibroblasts attesting for the upregulation of interferon signalling pathways and leading to an enhanced expression of IFN-β, interleukines and chemokines. Remarkably, both WNV and CHIKV enhance IL-1β genes expression and induce maturation of caspase-1, indicating the capacity of these pathogens to elicit activation of the inflammasome program in resident skin cells. In this study we also demonstrate that the inflammasome AIM2 sensor is upregulated in infected fibroblasts. Interestingly, AIM2 RNAi silencing interferes with CHIKV- and WNV-induced IL-1β upregulation. Further analysis demonstrated that activation of the inflammasome limits chikungunya virus replication in human dermal fibroblasts. Together, these results indicate that dermal fibroblasts contribute to the pro-inflammatory and anti-viral microenvironment created at the skin level in the early stages of interaction with arboviruses.

Posters

1.

Vif blocks autophagy in HIV-1 infected CD4 T cells

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HIV-1 envelope (Env) triggers autophagy in bystander, non-activated, CD4 T cells which leads to their apoptosis (Espert et al. 2006, JCI), a mechanism now recognized as central to immunodefficiency. In contrast, when CD4 T cells are productively infected, autophagy is repressed (Espert et al. 2009, Plus One ; Zhou et Spector 2008, AIDS), strongly suggesting that one or several viral proteins are involved in the inhibition of autophagy.

Among them Vif (Viral infectivity factor) plays an important role during HIV-1 infection. One of its main known functions is to degrade the antiviral host factor APOBEC3s (Apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3, or A3), and especially Vif binds to A3G and a cellular E3 ubiquitin ligase composed of Elongin B (EloB) and Elongin C (EloC), Cullin 5 (Cul5), Rbx2 and CBF-β to trigger A3G degradation by the ubiquitin proteasome system (UPS). The N-terminus of Vif interacts with A3G and CBF-β while the C-terminal part binds to the different components of the E3 ligase.

Here we demonstrate that Vif neosynthesized in CD4 T cells during a productive infection is responsible, at least in part, for the autophagy blockage. This inhibition requires the C-terminal part of Vif that binds directly to LC3B independently of the presence of A3G. These results reveal an additional important pro-viral function of Vif through inhibition of another innate anti-viral mechanism, autophagy.

2.

Phosphorylation of ParB by Ser/Thr Protein Kinases: Consequences For The Segregation Event In *Mycobacterium tuberculosis*

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Pathogenic bacteria commonly utilize signaling systems to regulate the expression of virulence factors during disease progression and for environmental adaptation. Previously, this was thought to be controlled mainly by two-component systems (TCSs). However, the discovery of eukaryotic-like signaling systems, such as Ser/Thr/Tyr kinases and phosphatases in bacterial pathogens has sparked an interest in understanding their function. In our study, we decided to focus our interest on Mycobacterium tuberculosis (M. tb) cell cycle regulation. Since that bacterial Ser/Thr kinases have been reported to regulate proteins involved in cell division events we focused our interest on the ParA and ParB proteins required for accurate partitioning of replicated chromosomes. ParB is a cytosolic conserved protein that binds specifically to centromere-like DNA sequences parS and interacts with ParA, a weak ATPase required for its proper localization. ParB is thought to be central in the segregation event and would serve as an anchoring platform for the segregation apparatus. Here, we show for the first time that M. tb ParB is phosphorylated by several mycobacterial Ser/Thr protein kinases in vitro. Ten phosphorylation sites were identified by mass spectrometry analysis which were further substituted either by alanine to prevent phosphorylation or aspartate to mimic constitutive phosphorylation. Electrophoretic mobility shift assays revealed a drastic inhibition of ParB DNA-binding activity on centromere-like sequences parS with phosphomimetic ParB compared to wild type. In addition, bacterial two-hybrid experiments showed a loss of ParA-ParB interaction with the phosphomimetic mutant, indicating that phosphorylation is regulating the recruitment of the partitioning complex. Fluorescence microscopy experiments performed in the surrogate Mycobacterium smegmatis ΔparB strain revealed a delocalization of ParB when phosphorylated as the two polar foci observed in the wild-type strain turn into multiple aligned foci distributed along the cell within the phosphomimetic mutant. Therefore, phosphorylated ParB can no longer bind to parS sequences along with ParA, thus free ParB could be recruited by the cytoskeletal machinery to be relocalized in the cytosol and would become available for a future chromosome segregation event.

3.

Role of MgtC in *Mycobacterium* and *Pseudomonas* virulence and its potential inhibition with a natural antagonist.

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MgtC is a virulence factor involved in survival inside macrophages in several intracellular bacterial pathogens, including Mycobacterium tuberculosis. To get further insights into the role of MgtC in mycobacterial virulence, we have analysed the behaviour of a M. marinum mgtC mutant in the Danio rerio infection model using a transgenic reporter zebrafish line that specifically mark neutrophils. This allowed us to uncover a role for MgtC in phagocytosis since the phagocytosis by neutrophils is more important with the mgtC mutant compared to the wild-type. Increased phagocytosis with the mutant strain is also confirmed using J774 macrophage cell line.
MgtC is also present in the extracellular pathogen Pseudomonas aeruginosa and we show that a P. aeruginosa mgtC mutant is attenuated in zebrafish embryos but only in the presence of macrophages. This phenotype correlates ex-vivo with a higher sensitivity of the mutant to the killing by macrophages. MgtC thus provides a singular example of a virulence determinant that promotes subversion of macrophages in both intracellular and extracellular pathogens.
Interestingly, we have previously identified a peptide, MgtR, as a putative natural antagonist of MgtC. Here, we investigate the opportunity of MgtR to antagonize MgtC in Mycobacterium and Pseudomonas species and thus exhibits anti-virulence properties.

4.

Extraction et quantification de l'ADN du Virus de l'Hépatite B sur plateforme PCR ouverte : comparaison des automates Maxwell® 16, Arrow et QIAcube

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Objet de l'étude: La quantification de l'ADN viral dans le sérum par technique PCR en temps réel est nécessaire au diagnostic et au suivi de l'infection par le virus de l'hépatite B (VHB). Des automates d'extraction accessibles en termes de coût et de faibles dimensions ont été mis sur le marché au cours des dernières années. Les performances des automates Maxwell® 16 MDx AS2000 (Promega), Arrow (NorDiag), et QIAcube (Qiagen) ont été comparés.
Méthode : Cinquante échantillons de sérum de charge virale (CV) VHB connue (COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0) et 10 sérums négatifs pour le VHB (AgHBs et anti-HBc négatifs) ont été sélectionnés. L'extraction des acides nucléiques viraux a été réalisée à partir de 200µl de sérum avec des kits d'extraction sur billes magnétiques ou colonnes de silice. Le contrôle interne (CI) hétérologue d'extraction/amplification Eurobioplex DNA QC (Eurobio) a été utilisé afin de s'assurer de la qualité de l'extraction. Après extraction, l'ADN VHB a été quantifié avec la technique Generic HBV PCR kit (Omunis) sur LightCyclerÒ 480 (Roche).
Résultats obtenus: Un défaut d'extraction a été mis en évidence grâce au CI pour 4 sérums (8%) avec l'automate Maxwell. La comparaison entre la PCR ouverte Omunis et le système fermé Roche met en évidence une bonne corrélation des charges virales (p=0.91), et 6 cas de discordance > 1log avec cet automate. Quant à l'automate Arrow, l'extraction a été mise en défaut pour deux échantillons (4%); corrélation (p=0.90), et 13 cas de discordance > 1log. En ce qui concerne l'automate QIAcube, un seul défaut d'extraction (2%) a été mis en évidence ; corrélation (p=0.93), et 8 cas de discordance > 1log.
Conclusion : Les trois automates et kits testés dans l'étude permettent une extraction rapide et performante de l'ADN du VHB. L'utilisation d'un CI apparait indispensable pour réduire les risques de sous quantification liés à l'étape d'extraction. L'appareil Promega est particulièrement compact et simple de maintenance et de mise en œuvre mais il ne dispose pas de lampe UV nécessaire à la décontamination, et a présenté plus de défauts d'extraction que ses concurrents de chez NorDiag et Qiagen

5.

Synthesis and biological evaluation of a new derivative of bevirimat that targets the Gag CA-SP1 cleavage site

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Bevirimat, the first-in-class HIV-1 maturation inhibitor, shows a low in vivo efficacy essentially due to the natural polymorphism of its target, the CA-SP1 junction in Pr55Gag structural precursor. To date its mode of action remains unclear, mainly due to its low hydrosolubility that makes tricky the structural study of its interaction with the CA-SP1 junction. This study was conceived to synthesize new derivatives of Bevirimat by addition of different substituents at the C-28 position with the aim to produce more soluble compounds useful for NMR resolution of complexes formed with the CA-SP1 junction. The final goal is to decipher the mechanism of inhibition of HIV-1 maturation inhibitors in view of their future optimization. The synthesis of new derivatives obtained by substitution of Bevirimat at the C-28 position and their hydrosolubility are discussed. The ability of these molecules to inhibit HIV-1 infection as well as cytotoxicity was evaluated and compared to that of Bevirimat. For the first time an interaction between a Bevirimat-derived compound (16) and the domain CA-SP1-NC was solved by NMR. This interaction was disrupted by the A364V mutation identified in Bevirimat-resistant HIV-1 strains. Highly hydrosoluble molecules retaining Bevirimat activity reported herein therefore represent useful tools to depict the antiviral mechanisms of molecules belonging to the class of maturation inhibitors, a prerequisite for the conception of next-generation Bevirimat-derived compounds generating reduced resistance polymorphisms in the SP1 spacer peptide.

6.

Toxoplasma gondii Vps11, a subunit of the HOPS and CORVET tethering complexes, is essential for the biogenesis of secretory organelles.

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Apicomplexan parasites hold unique secretory organelles (dense granules, rhoptries and micronemes) that play essential functions in host infection. Toxoplasma gondii parasites seem to possess an atypical endosome-like-compartment, which contains an assortment of proteins that appear to be involved in vesicular sorting and trafficking towards secretory organelles. Recent studies highlighted the essential roles of many regulators such as Rab5A and C, sortilin-like receptor and syntaxin-6 in secretory organelles biogenesis. However, little is known about the protein complexes that recruit Rab-GTPases and SNAREs for membrane tethering in Apicomplexa. In mammals and yeast, transport, tethering and fusion of vesicles between early endosomes towards lysosomes and vacuole, respectively, are mediated by CORVET and HOPS complexes, both built on the same Vps-C core. The core consists of the subunits Vps11, Vps16, Vps18 and Vps33 (known to interact with SNAREs). The CORVET complex is defined by additional Vps3, Vps8 and Rab5, while the HOPS complex is defined by extra Vps39, Vps41 and Rab7. Here, we show that a T. gondii Vps11 homologue is essential for the biogenesis or proper subcellular localization of secretory organelles proteins. TgVps11 is a dynamic protein that associates to Golgi-endosomal-related-compartments, vacuole and immature apical secretory organelles. Conditional knock-down of TgVps11 disrupts dense granules, rhoptries and lateral micronemes biogenesis. As a consequence, parasite motility, invasion, egress and intracellular growth steps are affected. Surprisingly, the attachment of the mutant parasites to the host cells increases. Intriguingly, apical micronemes biogenesis seems to be unaffected, suggesting the existence of a specific apical microneme trafficking pathway independent of Vps11. Immuno-purification experiments revealed that TgVps11 is forming a macromolecular complex that remains to be identified. In conclusion, we showed that apicomplexan parasites use canonical regulators of the endolysosome system to accomplish essential parasite-specific roles in the biogenesis of their unique secretory organelles.

7.

Role of the glutamate decarboxylase (GAD)-dependent system of the new *Brucella* species in the resistance to acid stress

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Background. Brucella is the causative agent of brucellosis, the major bacterial zoonosis worldwide. This debilitating infection is transmitted to humans through direct contact with infected tissues, inhalation of airborne bacteria and, mainly, by ingestion of contaminated and unpasteurised dairy products. In the last years, new species and atypical strains of Brucella (as B. microti, B. inopinata and isolates from bullfrog) have been isolated from unusual hosts. These strains are more acid-resistant (AR) than the classical Brucella species (as B. abortus, B. melitensis and B. suis). In several food-borne pathogens such as E. coli, the glutamate decarboxylase (GAD)-dependent system is most efficient for survival under extreme acid stress. Recently, our team has demonstrated that the GAD system of B. microti allows survival of the bacterium at pH 2.5 and contributes to murine infection by oral route. An in silico analysis of genomic sequences reveals the existence of potentially functional GAD systems in the new Brucella species.
Our aim is to confirm the role of the GAD system in the resistance of these strains to acid stress by bacteriological, biochemical and genetic studies.
Present results. In contrast to the classical Brucella species, new and atypical strains of the genus were found GAD-positive, able to export GABA, and also AR (pH 2.5) in the presence of glutamate. The efficient expression of the gad locus of these new strains in a gad mutant of E. coli demonstrates homologies between their systems. A functional GAD system could contribute to improving the adaptability of new species of Brucella in certain natural habitats and/or in the gastrointestinal apparatus of the host.
Perspectives. In this project, we will extend the study of the AR mechanisms by biological validation of comparative transcriptomic data of B. microti versus B. suis at acid and neutral pH.

8.

HIV-1 Env-mediated autophagy degrades selectively the viral transactivator Tat

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Autophagy is a ubiquitous mechanism involved in the lysosomal-mediated degradation of cellular components when engulfed in vacuoles called autophagosomes. Autophagy is also recognized as an important regulator of the innate and adaptive immune responses against numerous pathogens, which have, therefore, developed strategies to block or use the autophagy machinery to their own benefit.
Upon Human Immunodeficiency Virus-1 (HIV-1) infection, viral envelope glycoproteins (Env) induce autophagy-dependent apoptosis of uninfected bystander CD4+ T lymphocytes, a mechanism likely contributing to the loss of CD4+ T cells. In contrast, in productively infected CD4+ T cells, HIV-1 is able to block Env-induced autophagy in order to avoid its antiviral effect. To date, nothing is known about how autophagy restricts HIV-1 infection in CD4+ T lymphocytes. Here we report that autophagy selectively degrades the HIV-1 transactivator Tat, a protein essential for viral transcription and virion production. We demonstrated that this selective autophagy-mediated degradation of Tat relies upon its ubiquitin-independent interaction with the p62/SQSTM1 adaptor. Taken together, our results provide evidence that the anti-HIV effect of autophagy is specifically due to the degradation of the viral transactivator Tat, but this process is rapidly counteracted by the virus to favor its replication and spread.

9.

Functional consequences of UNG2 dysregulation by Vpr during HIV-1 infection

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Vpr is a small accessory protein of HIV -1 that plays a critical role in viral pathogenesis. Known in particular to activate viral gene transcription and to arrest the cell cycle in target cells, Vpr is also capable to interact with many cellular proteins and to interfere with their functions. Among these proteins, Vpr interacts with uracil DNA glycosylase UNG2, an essential enzyme involved in the control of genome uracilation. The interaction of Vpr with UNG2 during the HIV-1 infection leads to a very significant decrease of UNG2 levels, associated with a corresponding loss of uracil removal activity, as recently described by us (Fenard et al. NAR . 2009; Eldin et al. NAR . 2014). This loss of function causes the accumulation of mutagenic uracil bases in the cellular genome. During infection, Vpr is released into the extracellular medium, and concentrations ranging from 0.1 to 100 ng/ml have been detected in the serum of infected patients. This free form of Vpr is able to penetrate through the plasma membrane of many cell types and generate the same effects than those described in cells supporting retroviral replication. The physiopathological consequences of the accumulation of unrepaired DNA lesions in cells that are non permissive for HIV-1 infection, should be carefully scrutinized.

10.

Structural characterization of *Plasmodium falciparum* CCT and fragment-based drug design approach for targeting phospholipid biosynthesis pathway

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Phospholipid synthesis metabolic pathways in Plasmodium falciparum are validated drug targets for new type of antimalarials. In the de novo Kennedy pathway of phosphatidylcholine biosynthesis, the second step catalyzed by CTP:phosphocholine cytidylytransferases (CCT) [2.7.7.15] is rate limiting and appears essential for the rodent parasite survival at its blood stage. We are focused on the structural characterization of this enzyme, the identification of effectors by fragment-based drug design approach (FBDD) and then their optimization to eventually design a lead. We solved the first reported crystal structure of the catalytic domain of the enzyme target (PfCCT) at resolution 2.2 Å and an enzyme-product (CDP-choline) complex structure at resolution 2.4 Å that give detailed images of binding pocket and demonstrate conformational changes between apo- and holo-protein forms at atomic level. The FBDD method uses a library of small molecules (fragments) with molecular weight that does not exceed 300 Da to explore target binding sites. Primary screening of fragment library (230 molecules) has been investigated by fluorescence-based thermal shift assay and Nuclear Magnetic Resonance Saturation Transfer Difference (NMR STD) method is used as a secondary screen to eliminate false positive ligands. This combination of techniques identified so far 4 fragment hits that are currently evaluated for their binding modes and affinities. Co-crystallization of the protein-fragments complexes is carrying out to provide accurate information on the binding modes of the small molecules and topology of interactions will be used to rationally monitor every iterative round of the optimization process allowing subsequent rational design.

11.

Critical role of RON4 for moving junction complex formation and invasion of *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite which belongs to the Apicomplexa phylum, including Plasmodium species which are responsible for paludism. Among this phylum, the invasion mechanism is conserved and involves the formation of a tight interaction between the host cell and the parasite surfaces called Moving Junction (MJ). The MJ contains key parasite components: the surface protein Apical Membrane Antigen 1 (AMA1) and the Rhoptry Neck Protein (RON) complex (RON2/RON4/RON5/RON8). During invasion, the RON complex is secreted into the host cell where RON2 is inserted into the host cell plasma membrane. RON2 interacts directly with AMA1, providing a bridge between the parasite and its host cell. The other members of the RON complex (RON4/5/8) are tethered to RON2 and exposed to the cytosolic face of the host cell membrane, suggesting a role in anchoring the MJ to the host cell cytoskeleton. Here, we present the generation and phenotypic characterization of a conditional RON4 mutant that exhibits a significant invasion defect.

12.

Aedesin: Structure and Antimicrobial Activity against Multidrug Resistant Bacterial Strains

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Multidrug resistance, which is acquired by both Gram-positive and Gram-negative bacteria, causes infections that are associated with significant morbidity and mortality in many clinical settings around the world. Because of the rapidly increasing incidence of pathogens that have become resistant to all or nearly all available antibiotics, there is a need for a new generation of antimicrobials with a broad therapeutic range for specific applications against infections. Aedesin is a cecropin-like anti-microbial peptide that was recently isolated from dengue virus-infected salivary glands of the Aedes aegypti mosquito. In the present study, we have refined the analysis of its structural characteristics and have determined its antimicrobial effects against a large panel of multidrug resistant bacterial strains, directly isolated from infected patients. Based the results from nuclear magnetic resonance spectroscopy analysis, Aedesin has a helix-bend-helix structure typical for a member of the family of a-helix anti-microbial peptides. Aedesin efficiently killed Gram-negative bacterial strains that display the most worrisome resistance mechanisms encountered in the clinic, including resistance to carbapenems, aminoglycosides, cephalosporins, 4th generation fluoroquinolones, folate inhibitors and monobactams. In contrast, Grampositive strains were insensitive to the lytic effects of the peptide. The anti-bacterial activity of Aedesin was found to be saltresistant, indicating that it is active under physiological conditions encountered in body fluids characterized by ionic salt concentrations. In conclusion, because of its strong lytic activity against multidrug resistant Gram-negative bacterial strains displaying all types of clinically relevant resistance mechanisms known today, Aedesin might be an interesting candidate for the development of alternative treatment for infections caused by these types of bacteria

13.

Cryo CLEM: a new technique to investigate infected cells

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In order to visualize and gain structural information of viruses directly into cells, we have recently developed the cryo correlative light and electron microscopy method (Cryo-CLEM). Briefly, this technique allows us to observe by fluorescence a viral event in a frozen cell and then visualising this region of interest by cryo-transmission electron microscopy. Therefore, combined with cryo-electron tomography, we can access to viral structures at cellular level.
Unfortunately, this methodology is restricted to viral events located at the vicinity of the plasma membrane. Indeed, the more we go into the cells, the thicker it is and the harder we can see. So this approach is perfectly adapted to structural investigations of assembling or disassembling viral steps.
We have first established cells growth conditions directly onto electron microscopy grids and defined freezing conditions (conditions of blot, quantities of cells left after blot, thickness of the ice...). The next step was to work with infected cells by viruses. Once again, we needed to set up experimental conditions (quantities of viruses, infection time...) in order to observe events of interest. As these events are extremely rare, it is like looking for a needle in a haystack. To solve this problem, which is the aim of cryo-CLEM, regions of interest are localized by light microscopy in using fluorescent proteins.
Thanks to the use of fiducial fluorophore of 100 nm diameter scattered on the grid, which can be observed both in light and electron microscopy, we can then accurately view the region of interest in cryo-electron microscopy.
To illustrate this new methodological approach, we present here results we obtained on visualising of Chikungunya replication complexes.

14.

Role of the autophagy protein TgATG8 in the development and survival of the human parasite *Toxoplasma gondii*

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Autophagy is a self-degradative process which is conserved in most Eukaryotes. It involves a double membrane compartment called the autophagosome to sequester and degrade intracellular components. Toxoplasma gondii is an obligate intracellular protozoan parasite that can cause congenital toxoplasmosis and a severe pathology in immuno-compromised individuals. This parasite possesses an apparently reduced autophagic machinery but is nevertheless able to generate autophagosomes upon stress conditions. The protein TgATG8 occupies a central position in this process and can be conjugated to the autophagosomal membranes. Surprisingly, this protein also localizes to the apicoplast, a plastid which hosts essential metabolic pathways. With the aim of elucidating the function of this protein, we have generated a TgATG8 conditional mutant that is severely affected in cell-growth and in the homeostasis of the apicoplast. The precise role of TgATG8 at this organelle is presently unknown, so to identify the TgATG8 proteins sub-network, we have performed immuno-precipitation experiments on GFP-TgATG8 parasites extracts, followed by proteomic analysis. It will hopefully help us deciphering the dual functions of TgATG8: i) in canonical autophagy, by identifying the interactions of TgATG8 with the rest of the autophagic machinery ii) the non-canonical role at the apicoplast, by identifying organelle-specific partners.

15.

Substrate specificity of an antibiotic resistance conferring enzyme: aminoglycoside 2''-phosphotransferase Iva

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The aminoglycoside O-phosphotransferases constitute a large class of bacterial enzymes that is widely distributed among pathogens and confers a high level of resistance to several clinically used aminoglycoside antibiotics. APH(2")-IVa is a prominent member of this class and its steady state kinetics with several aminoglycosides have been studied extensively by the use of a coupled enzyme system but there is little information on its reaction pathway, in particular the initial steps. Such information can only be obtained by transient kinetics.
We present a study in which the steady state parameters (kcat and Km) with several members of the group of aminoglycosides were obtained by determining the product ADP directly by HPLC, a method that allows transient kinetic studies. We confirmed its substrate specificity for aminoglycosides that contain a 4,6-disubstituted 2-deoxystreptamine core and its inability to phosphorylate 4,5-disubstituted ones.
We determined the dissociation constants (Kd) together with the thermodynamic parameters for the binding of several aminoglycosides by isothermal titration calorimetry. The binding of the aminoglycosides tested, even those that are not substrates, is energetically favored.
To predict the important interactions of the different antibiotics with the enzyme, a study was carried out by molecular docking. These results are discussed in the context of crystal structures of APH(2")-IVa.aminoglycoside complexes.

16.

The proviral and antiviral properties of DHX9 are manipulated by the Chikungunya virus

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The Chikungunya virus (CHIKV) relies on the host cellular RNA binding proteins to promote its replication. Previous proteomic studies have revealed interactions of these macromolecules with the viral nonstructural proteins (nsPs) forming the replication complex of this alphavirus. One of these cellular proteins is the RNA Helicase A, also called DHX9, known as a cofactor assisting replication of various viruses and as a sensor of double-stranded RNA capable to activate cellular innate responses. In this study, we show that DHX9 is depleted in CHIKV-infected cells while a fraction of this protein is sequestered in nsP2/nsP3-positive foci. Together with immunoprecipitation experiments showing a direct interaction of DHX9 with the nsPs, these data indicate that DHX9 is sequestered in replication complexes (RCs). By using shRNAs targeting DHX9, we pinpoint a decrease in subgenomic promoter expression suggesting that DHX9 recruitment into the replication complexes assists viral replication. This proviral effect is abolished by ectopic expression of a DHX9 mutant lacking the RNA helicase activity. Thus, CHIKV has evolved to conscript DHX9 into the replication complex to improve the replication of the viral genome, and to degrade this helicase, probably to prevent the activation of the innate cellular responses.

17.

Role of the E.coli RNA polymerase sigma region 3.2 in resistance to lipiarmycin

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RNA polymerase is an essential enzyme of transcription and a target for fidaxomicin (Fdx): a macrocyclic antibiotic produced by Dactylosporangium aurantiacum. Fdx was approved for clinical use in 2011 under the name of Dificid® for treatment of Clostridium difficile. Our project aimed at understanding the mechanism of resistance and tolerance to Fdx. Previously we showed that the RNA polymerase sigma70 subunit region 3.2, that is highly variable between the alternative sigma factors, may be implicated in resistance/tolerance to Fdx. Indeed, the deletion of the residues sigma70 residues 513-519 in the region 3.2 makes RNAP more sensitive to Fdx. Furthermore, the RNAP bearing stress-response sigma38 displayed higher sensitivity to Fdx that may be caused by the difference in the region 3.2 sequence. We hypothesized that sigma region 3.2 contributes to the formation of Fdx binding site or allosterically affects Fdx action by interacting with the RNAP clamp domain. In order to define which amino acids are responsible for resistance/tolerance to Fdx we constructed several mutant sigma's bearing the substitutions in the region 3.2 and performed analysis ofthe mutant sigma factors using in vitro transcription and EMSA assays. Our results suggest that antibiotics targeting RNAP function as a growth phase-specific transcriptional regulators.

18.

HIV-1 NC and ESCRT-component Tsg101 interplay prevents HIV from turning into a DNA-containing virus

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HIV-1 agent of the AIDS pandemic is a RNA virus reverse transcribing its RNA genome (gRNA) into DNA shortly post entry into cells. Within cells, retroviral assembly requires thousands of structural Gag proteins and two copies of gRNA as well as cellular factors to converge to the plasma membrane in a finely regulated timeline. In this process, the nucleocapsid domain of Gag (GagNC) ensures gRNA selection and packaging into virions. Then, budding and virus release require the recruitment of cellular ESCRT machinery. Interestingly, mutating GagNC results into the release of DNA-containing viruses by promoting reverse transcription prior to virus release, through an unknown mechanism. Therefore, we explored the biogenesis of these DNA-containing particles, combining live-cell total internal-reflection fluorescent microscopy, electron microscopy, trans-complementation assays and virus biochemical characterization. Our study reveals that DNA virus production is the consequence of budding defects with Gag aggregation at the plasma membrane and deficient recruitment of Tsg101, a key component of the ESCRT-I machinery. Indeed, targeting Tsg101 to virus assembly sites restores budding and decreases DNA levels in cells and particles in favor to RNA packaging. Altogether, our results highlight the role of GagNC in the spatiotemporal control of reverse transcription, via an ESCRT-I-dependent mechanism.

19.

MLV requires Tap/Nxf1-dependent pathway to export its unspliced RNA to the cytoplasm and to express its spliced RNAs

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Eukaryotic cells have evolved stringent proofreading mechanisms to ensure that intron-containing-mRNAs do not leave the nucleus. However, all retroviruses must bypass this checkpoint. Retroviral primary polycistronic transcript (Full-Length) must reach the cytoplasm to be either translated or packaged as genomic RNA in progeny viruses.
Murine leukemia virus (MLV) is a prototype of simple retroviruses with only two splicing events generating partially and fully -spliced RNAs. MLV splicing is a well-regulated process that directly influences viral leukemogenic properties in mice. Several cis-elements were identified in the FL RNA that regulate its cytoplasmic accumulation. However, their connection with an export mode is yet unknown. Our goal was to identify the cellular pathway used to export the MLV FL RNA into the cytoplasm of the host cells.
Host mRNAs generally rely on Tap factor to exit the nucleus, designing Tap as candidate for MLV RNA export. To examine whether Tap is recruited by MLV, we inactivated Tap with a dominant-negative mutant or a RNA competitor. Their effects on the expression, rate, and localization of MLV RNA were studied at different time points by Western blotting, RT-qPCR, fluorescence microscopy, and RNA-ChIP assays. Taken together, our results showed for the first time that MLV required the Tap-dependent pathway for its spliced and unspliced RNA expression and to export its FL RNA.
By contrast to Human Immunodeficient Virus type-1 (HIV-1), MLV recruits the same pathway for the cytoplasmic expression of its spliced and unspliced RNAs. Thus, MLV replication relies to coordinated splicing/export processes. The Tap-dependent nuclear export addressed the FL RNA to translation. Whether the pool of FL RNA to be packaged is also exported by Tap remains a crucial issue that awaits investigation.

20.

Persistence of *Mycobacterium abscessus* in foamy macrophages: deciphering the role of enzymes involved in the accumulation of lipids in the form of intracytoplasmic lipid inclusions (ILI).

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As is the case with Mycobacterium tuberculosis, the emerging pathogen M. abscessus can persist during latent infection in organized granulomatous lesions during . Foamy macrophages (filled with lipid bodies - LB) lining the necrotic centers of granulomatous lesions is the primary intracellular niche for persistent mycobacteria. We recently developed a model system where macrophages infected with M. avium were induced to become foamy by lipoprotein-(VLDL) treatment. Using this system, we clearly showed how LB and intracytoplasmic lipid inclusions (ILI) were formed in the macrophages and the mycobacteria, respectively. Importantly, we observed that the presence of large ILI resulted in an arrest of mycobacterial division, phenotypic of mycobacteria that persist for extended periods of time. However, it remains to be determined whether M. abscessus will behave similarly in foamy macrophages and which are the key actors involved in this process. Triacylglycerol (TAG), the most abundant neutral lipid found in ILI is produced through the action of TAG synthase (Tgs) which is likely crucial to ILI formation. The M. abscessus genome contains 7 tgs genes, but it is unknown which of these are important to ILI formation in foamy macrophages. This study is aimed to describe the morphological characteristics and growth dynamics of the M. abscessus smooth (S) and rough (R) variants in our lipoprotein-(VLDL)-induced foamy macrophage model as well as to delineate the function of Tgs in ILI formation. Our preliminary observations are that the M. abscessus S and R strains neither accumulate ILI when cultured under standard in vitro growth conditions nor when infecting macrophages not treated with VLDL. This supportsour hypothesis that extensive ILI formation will only occur while infecting foamy macrophages. In addition, tgs1 and tgs2 deletion mutants and tgs1 and tgs2 over-expressing strains have been generated successfully. Changes in ILI morphology and growth of these mutants in VLDL-treated foamy macrophages are currently being investigated and will provide insight into the molecular mechanisms by which M. abscessus produces the neutral lipids necessary for ILI formation. This may provide novel insight into how M. abscessus establishes and maintains a latent infection.

21.

New allosteric inhibitor of a bacterial enzyme confering resistance to aminoglycoside phosphotransferase(3')-IIIa

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Bacterial resistance often leads to treatment failure due to inappropriate antibiotic therapy, which may have serious consequences, especially in critically ill patients. Resistance to aminoglycosides is mainly due to the expression of enzymes which modify these antibiotics. One important mechanism of aminoglycoside modification is ATP/GTP-dependent *O*-phosphorylation that is catalyzed by a large group of aminoglycoside phosphotransferases (APHs). These enzymes confer antibiotic resistance to many pathogenic.

The aim of this study is to prevent this resistance by designing specific inhibitors against APHs. Here, we focus on the search of APHs allosteric inhibitors that will bind to small cavities of the protein and thereby block the enzyme function by perturbing its dynamics.

Potentially interesting APH cavities were identified by comparing their volume in different protein conformations produced by normal mode analysis. A cavity belonging to a large groove of variable volume, splitting the protein into two parts, was chosen as a first target. By molecular docking, we screened large libraries of commercially available compounds (Zinc database) after applying orally drug-like filtering criteria (Lipinski's rules of five). Seventeen of the highest ranked compounds were tested by *in vitro* steady state kinetic experiments in order to evaluate their inhibition potential. One compound was shown to be a specific inhibitor of APH and the determination of the inhibition mode revealed an allosteric inhibition mechanism. The co-crystallization of APH-inhibitor complexes is in progress with the aim of confirming the inhibition mechanism determined kinetically, as well as the *in silico* prediction of inhibitor-protein interactions. Two other strategies were used to confirm the inhibitor binding site: research of inhibitor analogs and construction of a mutant containing a single amino acid substitution (R211A) in the target APH cavity. This study gives new insights into the inhibition mechanism of APHs by such allosteric compounds, and already provides the basis for future development of combined therapies (antibiotic with APH inhibitor) which may reverse the antibiotic resistance in a clinical context.

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