Systèmes de sécrétion de type IV et virulence bactérienne

Rapport Hcéres

agence d'évaluation de la recherche
et de l'enseignement supérieur

Section des Unités de recherche

AERES report on the research unit

Virulence bactérienne et maladies infectieuses

From the

University Montpellier I

INSERM

Mai 2010

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INSERM

Le Président
de l'AERES

Jean-François Dhainaut

Section des unités
de recherche

Le Directeur

Pierre Glorieux

Mai 2010

Research Unit

Name of the research unit: Virulence bactérienne et maladies infectieuses

Requested label: UMR_S INSERM

N° in the case of renewal:

Name of the director: M. David O'CALLAGHAN

Members of the review committee

Chairperson:

Ms. Florence Niedergang, Université Paris 5

Other committee members

M. Christoph DEHIO, Biozentrum der Universität, Basel, Suisse

M. Camille LOCHT, Institut Pasteur de Lille

M. Jean-Louis HERRMANN, Université de Versailles-Saint Ouen

M. Jean-Jacques LETESSON, Université de Namur, Belgique

M. Vladimir PELICIC, Imperial College of London, Royaume-Unis

Committee members nomminated by staff evaluation committees (CNU, CoNRS, INSERM and INRA CSS....)

M. Thierry NAAS, member of INSERM CSS

Observers

AERES scientific advisor:

Ms. Claire POYART

University or School representatives:

Research Organization representatives:

Ms. Christine TUFFEREAU, INSERM

Report

1 • Introduction

- Date and execution of the visit:

The visit took place on January 20th (whole day). Time was devoted to presentation of results and projects, as well as private discussions with the director, the staff researchers, the students and post docs, and the technicians. The entire scientific presentation was given in English by the director of the research unit, the other scientific staff participated in the discussion following the presentation.

- History and geographical localization of the research unit, and brief presentation of its field and scientific activities:

The group in its current form was created in January 2007. It is now composed of two basic research groups and a clinical research group. Three fundamental research themes (Brucella, role of MgtC and Burkholderia) and one clinical (virulence of MDR bacteria) have been developed. One of the basic research themes (MgtC) will not be continued, because the principal investigator has left the group.

- Management team:

The director is a full time scientific researcher. The entire team is involved in the decision making process. There is no secretary, which is recognized by all members of the team as a major problem.

- Staff members

	Past	Future
N1: Number of researchers with teaching duties (Form 2.1 of the application file)	3	4
N2: Number of full time researchers from research organizations (Form 2.3 of the application file)	2	4
N3: Number of other researchers including postdoctoral fellows (Form 2.2 and 2.4 of the application file)	5	2
N4: Number of engineers, technicians and administrative staff with a tenured position (Form 2.5 of the application file)	2	1
N5: Number of other engineers, technicians and administrative staff (Form 2.6 of the application file)	0	1
N6: Number of Ph.D. students (Form 2.7 of the application file)	4	1
N7: Number of staff members with a HDR or a similar grade	6	6

2 • Overall appreciation on the research unit

- Overall opinion

This unit is focused on type IV secretion systems in pathogenic bacteria and has good relations with clinical studies. In addition to its core activity on *Brucella* type IV secretion systems, the unit recently developed additional experimental models (*C. elegans* and zebrafish) to address questions on bacterial virulence using *Burkholderia* as a new model. The zebrafish model has recently been published (Infect. Immun.) and should lead to interesting results. In addition, the *Brucella* work will be broadened to more global genomic studies. The two staff researchers who are in charge of sub-projects in addition to the director are solid, highly motivated and experienced. However, because of the relatively small size of the unit, it will be very important to prioritize the projects that appeared too numerous. Also, the link between the basic research and the clinical studies are not obvious, as the clinical research is not on *Brucella* or *Burkholderia*, but is centered around a number of other pathogens, including *Staphylococcus aureus* and *Escherichia coli* (UPEC). The unit suffers from a lack of secretary and the unique permanent technician will retire during the next quadrennial.

- Strengths and opportunities

Excellent spirit;

Very good visibility in the domain of Brucella research;

Excellent networking and international collaborations allowing the team to develop ambitious programs (screening, sequencing, …) as collaborations;

Excellent ability to raise funds through competitive grant application;

Excellent insertion into the local translational network;

Very good attractiveness for postdoctoral fellows and young CR1 researchers;

Very good connections with the hospital

Very bright and highly motivated young staff researchers

- Weaknesses and threats

It is not clear how the (too) numerous projects will be prioritized given the reduced number of persons potentially involved;

While it is clear how the clinical studies benefit from the developments of the basic research (in terms of read out assays, C. elegans, zebrafish models, etc), it is much less apparent how the basic research is translated into clinics;

Less than optimal expertise and equipments in cell biology and biochemistry;

Lack of technician and secretary, high administrative burden for researchers.

- Recommendations to the head of the research unit

The efforts should be more focused toward the more promising projects such as type IV secretion systems and effectors, the use of the zebrafish model for the study of Burkholderia as this project appeared as a good niche for the team, also to use this model for type IV secretion read-out. In contrast, projects in the field of innate immunity might be too complicated and competitive.

- Data on the work produced :

(cf. http://www.aeres-evaluation.fr/IMG/pdf/Criteres_Identification_Ensgts-Chercheurs.pdf)

A1: Number of permanent researchers with or without teaching duties (recorded in N1 and N2) who are active in research	7
A2: Number of other researchers (recorded in N3, N4 and N5) who are active in research	3
A3: Ratio of members who are active in research among permanent researchers [(A1)/(N1 + N2)]	1
A4: Number of HDR granted during the past 4 years	3
A5: Number of PhD granted during the past 4 years A6: Any other relevant item in the field	3

3 • Specific comments on the research unit

- Appreciation on the results

This team is internationally recognized in the field of *Brucella* microbiology and has good achievements in clinical studies.

Project 1 (*Brucella*): This is historically the major research theme, for which the group has an important international visibility after discovering the T4SS in this species. T4SS are complex multi-protein machineries. The main goal is to gain detailed understanding of the role of the T4SS in *Brucella* virulence, which is an important zoonotic disease.

One of the components of T4SS, VirB8, has been studied in detail, leading to good (Infect Immun 2005 and 2006 and J Bacteriol 2009) and very good publications (PNAS 2006), some of which were obtained through collaborations. A yeast two hybrid screen has been performed to identify several host proteins interacting with T4SS pilus components (CD98 and Gal 1). Two PhD theses were completed on this subject. The role of Gal 1 has been studied further and 2 papers are now in preparation.

Project 2 (*Salmonella* MgtC): this project was developed independently by the leader of an Avenir group to study the role of the MgtC virulence factor, conserved in numerous intracellular bacterial pathogens. This led to the discovery of a new post-transcriptional regulation of MgtC by a short peptide MgtR. Although the field of *Salmonella* is very competitive, this was an extremely successful research theme and has led to 3 good (J. Bact 2005, Infect Immun 2005, Microb Pathog 2008) and 3 very good publications (Mol Microbiol 2007, EMBO J. 2008) with the leader of the Avenir group as senior author . One student defended his PhD thesis on this work. He also obtained the EraNet Pathogenomics Prize. Because the leader of the Avenir group left the team in 2008, the project will not be continued.

Project 3 (*Burkholderia cepacia*): this new project was started because this pathogen has a T4SS that may play an important role in virulence. It is an opportunistic pathogen of the lungs in cystic fibrosis patients. *Burkholderia* are difficult to study because of the absence of good models and the difficulty to use genetic approaches with the bacteria due to its high resistance to antibiotics. The zebrafish model has been established in the laboratory to visualize intracellular life of bacteria and a first article had just come out at the time of the visit (Infect Immun 2010). The model appears promising and paves the way for many exciting projects.

Project 4 (*clinical studies*): This is developed with the hospital and generates the majority of the publications of the group. Some were published in good journals (Diabetes care 2007 and 2008, IF7.8), many in lower IF journals and some even in French (J Clin Microbiol 2005 and 2007, Diabetologia 2008, Diabetes Metab 2008, PlosOne 2008 and 2009, Clin Microbiol Infect 2005 and 2008). There are two main distinct projects. In the first one, study of the microbiology of diabetic foot ulcer showed that the bacterial populations differ between early and late stages infections, which was used to develop a PCR test to determine ulcer grades. This led to efficient cost reductions in the local hospital and will now be proposed to other hospitals in France.

In the second one, a novel population of multi drug resistant *E. coli* strains involved in urinary tract infections has been identified. Their virulence in the *C. elegans* model has been tested and shown to be reduced. In addition, the antibacterial effect of cranberry juice was assessed in collaboration with a company.

- Appreciation on the impact, the attractiveness of the research unit and of the quality of its links with international, national and local partners

The team has very good visibility in the Brucella field, the team leader has been frequently invited to participate in international Brucella meetings or to give seminars. The clinicians are very active in attending national clinical microbiology meetings and workshops and they will participate in the organization of a meeting in Montpellier in 2010.

One student obtained the EraNet Pathogenomics Prize for the best PhD thesis in the field of Pathogenomics.

Although the team is not located in one of the most scientifically active French cities, it manages to be very attractive for students and post docs (5 post docs in total, including 4 foreigners). In addition, the group was recently strengthened by the arrival of 2 experienced staff researchers (CR1) and one Professor (PU).

The director is extremely efficient in networking, at the national and international levels. The ability of the team to raise funds is excellent (European programs EraNet Pathogenomics and RNAi-NET, ANR MIE, PHRC) and there are ample funds for all the projects.

- Appreciation on the strategy, governance and life of the research unit

The organization of the team in three sub groups (Brucella, Burkholderia, Clinical studies) with many connections is appropriate. The small size of the team and frequent meetings appear to generate a great spirit of collaboration that overcomes the heterogeneity of the topics. The zebrafish project is a promising model that should be further developed and used for different aspects. The clinical studies are quite heterogeneous but are supported by funding and collaborations with industry. However, while it is clear how basic research- with the developed animal assays (C. elegans and zebrafish)- is useful for the clinical studies, it is not obvious how the clinics might benefit the basic research.

After the recruitment of a CR1 INSERM researcher in December 2007, who developed the zebrafish project, the group has been further strengthened by the recent arrival of another CR1 INSERM who will concentrate on the RNAi screen on Brucella and also participate in clinical studies. The project will also benefit from the arrival of University Professor.

Several team members are involved in teaching (1 PUPH, 1 MCUPH, 1PR1, 1 CR1 Inserm).

There is a very strong support and commitment form the local university and hospital authorities.

- Appreciation on the project

Project 1 (Brucella) :

In general the group will shift more towards genomics and cellular microbiology by studying cell signaling during invasion by Brucella. This appears as a necessity in the field of Brucella, because of the difficulties in the identification of Type IV secretion system effectors.

An ANR grant has been obtained to pursue the characterization of the role of the host protein CD98 in Brucella pathogenesis, in collaboration with another Inserm team. It is unclear how exactly the group will contribute to this project and what experiments will be performed in the laboratory. One senior post-doc will focus on the sub-project.

The Btp1 and Btp2 putative effector proteins of the T4SS will be characterized further using a well-designed strategy. It is planned that other potential effectors with different eukaryotic domains will be characterized shortly, which appears somewhat optimistic. Moreover, if the topic evolves towards the field of innate immunity, it might be very competitive and at high risk.

A genome-wide RNAi screen will be performed to identify the host factors required for Brucella infection, in collaboration with the Max Planck Institute for Infection Biology in Berlin. The analysis and exploitation of the potential hits will be performed by the CR1 staff researcher who recently joined the group and has expertise in this

type of work. To maximize the impact of this interesting project, the team will need to develop more expertise in cell biology and invest in the appropriate equipment.

It is less likely that the two other projects, that are at a very early stage, will be feasible in the near future considering the limited manpower in the group and should therefore be treated as low priority.

Identifying host specificity determinants using the many Brucella genomes obtained at the Board Institute, which is undoubtedly a very useful resource for the community, is risky. Indeed, it might be difficult to identify the gene(s) that may be responsible for a particular virulence phenotype, especially on a larger scale and the approach cannot take multigenic effects as well as various types of regulations into account. A good aspect is that it is neither time- nor manpower- consuming, as it is performed outside the laboratory. The team leader will only supervise this project, which might provide the community with large amount of information. Similarly, the possibility to look for small non-coding RNAs (part of a new EraNetPathogenomics grant application) is a very popular research theme, but it may be difficult to carry out in a competitive way by the team, because of the limited manpower in the group.

Project 2 (Burkholderia):

The Burkholderia project will become one of the important focuses of the research in the group, which appears a logical evolution after the establishment of the zebrafish animal model. It is planned that the fish will first be used to dissect the intracellular life stages of Burkholderia survival and replication within the host and then to screen a bank of mutants and/or use a microarray approach to identify bacterial genes that are important for the different stages of intracellular survival. Notably, the role of the T4SS in virulence will be further investigated. This is likely to be a highly profitable project. The use of transgenic fish to identify the host proteins important for infection and/ore the use of the model to screen for inhibitory molecules, although very interesting as well, look more like projects for the longer-term. Similarly the possibility to study host immune response is interesting but may again be far from the current expertise in the group. The model will also be used to test clinical isolates.

In conclusion, this project is interesting and may lead to important publications. However, the relevance of the model for cystic fibrosis research and how the fish model compares with in vitro lung epithelial cftr-/- cells remains to be seen.

Project 3 (clinical studies):

The collaboration with the Broad Institute will be extended to the clinical project to sequence the microbiome in samples from patients with diabetic foot ulcers (metagenomic approach). This is a very ambitious initiative, which should lead to a better link between the basic and clinical research themes in the group.

Furthermore, it is planned (i) that the previous PCR assay to distinguish between colonization and infection will be further confirmed by the study of more samples obtained on a national basis, (ii) to compare S. aureus strains from foot ulcers with strains isolated from nasal swabs to see whether they are identical or different, and (iii) to further use of the fish and worm models to test the role of bacterial cooperation in virulence. It was felt that point (ii) was confirmatory and therefore appeared to be less interesting (it is already known that a “healthy” carriage may lead to infection of the individual, who becomes contaminated with his or her own strain when the skin-mucosal barrier is breached).

For the second project on multi-drug resistant E. coli strains giving UTI, the fish and worm models will be used to test the impact of multi-drug resistance acquisition on virulence. This project seems less competitive, as there are several identified competitors in France and abroad.

Note de l'unité	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A+	A	A

Administration Droit Economie Gestion Médecine Odontologie Pharmacie Sports

Université Montpellier 1

Montpellier, le 14 avril 2010

Le Président
Ph.A/NG
Départ N° 214

Monsieur Pierre GLORIEUX
Directeur de la section des unités
de recherche
Agence d'Evaluation de la Recherche et de
l'Enseignement Supérieur (AERES)
20, rue Vivienne
75002 PARIS

Monsieur le Directeur,

Je vous adresse mes remerciements pour la qualité du rapport d'évaluation fourni à l'issue de la visite du comité d'expertise concernant l'unité de recherche **«Systèmes de sécrétion de type IV et virulence bactérienne»**

Vous trouverez ci-joint les réponses du Directeur de l'unité auxquelles le Vice Président du Conseil Scientifique et moi-même n'avons aucune remarque particulière à rajouter.

Je vous prie d'agréer, Monsieur le Directeur, l'expression de ma considération distinguée.

Philippe AUGE

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Université Montpellier 1

Instituts thématiques

Inserm

Institut national
de la santé et de la recherche médicale

14th April 2010

We thank the AERES committee for their positive and constructive suggestions that will help us focus our research efforts more efficiently. Here we will give information that will reply to and complement some of the comments made by the committee. The committee clearly highlighted the strong points of our laboratory however, we believe that the excellent working conditions that we enjoy (not seen by the committee as they did not visit the laboratory) should also be noted.

- It is not clear how the (too) numerous projects will be prioritized given the reduced number of persons potentially involved

We agree that we have presented a very ambitious project considering the size of the group. The project presented was for the next 4 years with perspectives for the following 4 years. With hindsight, the way that the *Brucella* projects were presented in the director's presentation, starting with the broader post genomics based projects and finishing with the more detailed projects that are the built on our recent exciting results, may have given the wrong impression of our priorities. We agree that the genomics based projects are long term which will open new paths for the end of the new quadrennial and the following one. To our minds, it is clear that we must prioritize the analysis of our candidate T4SS effectors, the cell signalling during bacterial infection (a project that has ANR funding) and the siRNA screen. Other aspects will be addressed as the manpower and funding becomes available.
We do not understand why the committee wrote that we have 'reduced numbers of persons'; the group has recently been strengthened by the arrival of a fourth tenured scientist and a university Professor.

- While it is clear how the clinical studies benefit from the developments of the basic research (in terms of read out assays, C. elegans, zebrafish models, etc), it is much less apparent how the basic research is translated into clinics

It is clear that the clinical applications of the *Brucella* project in France are limited, however it should be remembered that brucellosis is one of the most important zoonotic diseases in the world, and is a serious public health problem in the countries in which it is endemic. We also see this as a fascinating opportunity to understand the interplay between a pathogen and its host. *Burkholderia cepacia* complex (Bcc), however, is a serious health problem for CF patients. Fortunately (for the patients) Bcc is not yet a problem in the CF patients in our region; however we have established close collaborations with clinicians in Toulouse and in Prague. We will be using the zebrafish model to study virulence of clinical isolates and to identify bacterial and host factors that could lead to the development of prognostic tools (as we have done with the diabetic foot ulcers). We have also established collaboration which will allow us to use a HTS platform to identify anti-infectious molecules in Bcc infected zebrafish embryos. We are also discussing with a local start-up company that plans to develop the model to identify novel antimicrobials for other CF pathogens.

- Less than optimal expertise and equipments in cell biology and biochemistry

- To maximize the impact of this interesting project, the team will need to develop more expertise in cell biology and invest in the appropriate equipment.

Over recent years, the new discipline of Cellular Microbiology has become a key aspect in the study of host-pathogen interactions. Studying such interactions requires entering this research area. Over the last 3 years, we have made an important effort to develop our skills in cell biology but agree that this effort must continue. This will be done by both introducing and developing the techniques in the laboratory and through collaborations with experts in the field. The CR who has recently joined the lab has experience in cell biology and we will try to recruit other skilled personnel in the required areas.

We do not understand the comment concerning equipment; we have very good tissue culture facilities which are now being extended into a second culture room. We have a good inverted fluorescence microscope and a recently acquired fluorescence microscope for zebrafish. Both are equipped with sensitive CCD cameras and will soon be moved into a new dedicated room. We also have access to a FACS in the building. For confocal and electron microscopy we use the RIO imaging platform and the CRIC in Montpellier.

We have no ambition to become specialists in biochemistry. We are equipped for the more simple aspects such as SDS PAGE, simple protein purifications and protein-protein interactions. We will continue to use national and international collaborations for more detailed biochemical analysis; for example we have recently established collaboration with the CEA in Marcoule who are world experts in the use of mass spectrometry to analyze the bacterial proteome.

- Lack of technician and secretary, high administrative burden for researchers.

We agree completely with this comment, at present the administrative activities normally carried out by a secretary are performed by the director, a CR1, an IE and a post doc. This is all time lost for their research projects.

- The Btp1 and Btp2 putative effector proteins of the T4SS will be characterized further using a well-designed strategy. It is planned that other potential effectors with different eukaryotic domains will be characterized shortly, which appears somewhat optimistic. Moreover, if the topic evolves towards the field of innate immunity, it might be very competitive and at high risk.

We are already studying one additional candidate effector, and have identified several very promising candidates. New reporter cells to study protein translocation are now being constructed, and this will allow a fast screen of putative interesting candidates without loosing much time.

- In contrast, projects in the field of innate immunity might be too complicated and competitive

As mentioned above, the study of host-pathogen interactions implies an understanding of host factors. We do not plan to enter the complex and competitive field of innate immunity, but the understanding of how the bacterial TIR domain proteins function will require some studies in this area.

- An ANR grant has been obtained to pursue the characterization of the role of the host protein CD98 in Brucella pathogenesis, in collaboration with another INSERM team. It is unclear how exactly the group will contribute to this project and what experiments will be performed in the laboratory.

We do not understand this comment; the team leader wrote and is coordinating this project, and at least half of the proposed work will be performed in the coordinators' laboratory. A full time post doc is working on the project in our lab.

- In conclusion, the zebra fish project is interesting and may lead to important publications. However, the relevance of the model for cystic fibrosis research and how the fish model compares with in vitro lung epithelial cftr-/- cells remains to be seen

At present there is no perfect model to study infections in CF. The zebrafish is not the perfect model to study all aspects of infection in CF, however we do find that it provides possibilities that other models do not. We have been able to follow infection in real time and provide the first clear evidence that Bcc can multiply within macrophages. The model will allow us to study these intracellular stages in real time in vivo and identify the bacterial factors required for this key aspect of virulence. We agree that validation of such identified bacterial and host factors in other models is essential.

- The use of transgenic fish to identify the host proteins important for infection and/ore the use of the model to screen for inhibitory molecules, although very interesting as well, look more like projects for the longer-term. Similarly the possibility to study host immune response is interesting but may again be far from the current expertise in the group

We have already successfully used transgenic reporter fish to study the role of host immune cells in infection, and will continue these promising experiments. Identification of important host factors will give a much clearer focus to future research, and we believe that it is important to initiate these experiments rapidly. The genetic tractability of the zebrafish (using morpholinos for short term knock down) and ease of transcriptome analysis makes it the ideal system. We do not have the intention to study the immune response in detail but, here again, host and bacterial factors play an equally important role in infection, meaning that some analysis of the host is essential.
Screening for inhibitory molecules is planned in the longer term as collaboration.

- It was felt that comparing *S. aureus* strains from foot ulcers with strains isolated from nasal swabs to see whether they are identical or different was confirmatory and therefore appeared to be less interesting (it is already known that a "healthy" carriage may lead to infection of the individual, who becomes contaminated with his or her own strain when the skin-mucosal barrier is breached).

We do not agree, while it is a common assumption that this is the case, there is little documented evidence. A recent paper from the CHU de St Etienne (Eur J Clin Microbiol Infect Dis (2010) 29:373–382) highlights this.

- For the second project on multi-drug resistant E. coli strains giving UTI, the fish and worm models will be used to test the impact of multi-drug resistance acquisition on virulence. This project seems less competitive, as there are several identified competitors in France and abroad.

We agree that this is a competitive field; our work here is restricted to collaborations initiated by the two leading French laboratories working on enterobacterial resistance.

Dr David O'Callaghan

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