VPB - Virulence bactérienne précoce : fonctions cellulaires impliquées et contrôle dans l'infection aiguë et subaiguë

Rapport Hcéres

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

Research Units Department

AERES report on unit:

Early bacterial virulence: cellular functions and control of acute and sub-acute infections (EBV)

Under the supervision of the following institutions and research bodies:

University of Strasbourg

February 2012

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

Research Units Department

President of AERES

Didier Houssin

Research Units Department

Department Head

Pierre Glaudes

Unit

Name of unit:	Early bacterial virulence: cellular functions and control of acute and sub-acute infections
Acronym of unit:	EBV
Label requested:	Equipe d'Accueil
Present no.:	EA-4438
Name of Director (2009-2012):	Mr Bertrand LUDES
Name of project leader (2013-2017):	Mr Gilles PRÉVOST

Members of the committee of experts

Chair:	Mr Eric OSWALD, Toulouse
Experts:	Mr Alain BONNIN, Dijon
	Ms Dominique BUZONI-GATEL, Nouzilly
	Mr Bernhart RYFFEL, Orléans
	Mr Dirk SCHLÜTER, Magdeburg, Germany
	Mr Michel SIMONET, Lille (CNU representative)
	Mr Marco VIGNUZZI, Paris

Representatives present during the visit

Scientific Delegate representing AERES:

Mr Joost VAN MEERWIJK

Representative(s) of the unit's supervising institutions and bodies:

Mr Eric WESTHOF, University of Strasbourg

Report

1 • Introduction

Date and conduct of visit:

The site-visit of the complete AERES committee took place on Wednesday February 1st 2012 (8:30 to 12 am) at Forum de la Faculté de Médecine, rue Kirschleger, 67000 Strasbourg. The visit started with a presentation by the head of the research unit followed by presentations by the leaders of the three projects of the unit. In three parallel meetings, members of the committee then met with PhD students and postdocs, engineers and administrative assistants, and with researchers of the two units visited that day. The morning session ended with a meeting with the unit's director and finally a closed-door meeting of the committee. In the afternoon, the committee met with the representatives of the Strasbourg University.

History and geographical location of the unit, and overall description of its field and activities:

The research unit entitled "Early bacterial virulence: cellular functions and control of acute and sub-acute infections" (EBV) results from the split of the multi-thematic EA-4438 unit and the subsequent association of two bacteriology research teams. The EBV unit focuses on the pathophysiology of acute and sub-acute infections caused by two pathogenic bacteria: *Staphylococcus aureus* and *Borrelia burgdorferi sl.* Approaches include epidemio-clinical investigations, genomic and proteomic identification of virulence factors, analysis of the cellular impact of virulence factors on target cells and assessment of new therapies.

Management team:

EBV is directed by an associate professor of the medical faculty, who heads also the research team on *S. aureus*. The second team on *Borrelia* is headed both by an associate professor and a professor of the medical faculty who is also director of the hospital bacteriology laboratory and in charge of the National Reference Center of *Borrelia*, two structures that directly interact with the EBV research unit.

Unit workforce:

Workforce	Number on 06/30/2011*	Number on 01/01/2013*	2013-2017 Number of producers**
N1: Professors or assistant professors	8	10	8
N2: EPST or EPIC researchers	1 for 1.5 year	1	0
N3: Other professors and researchers*)*	-	5	1
N4: Engineers, technicians and administrative staff *****on a permanent position	5	5	
N5: Engineers, technicians and administrative staff ***** on a non-permanent position	2 (1 for 1 year)		
N6: Postdoctoral students having spent at least 12 months in the unit*)*	1		
N7: Doctoral students	5		
N8: PhD defended	8		
N9: Number of Habilitations to Direct Research (HDR) defended	1 (Dec 2011)		
N10: People habilitated to direct research or similar	6	9	
TOTAL N1 to N7	**22 (2 for 1 year)**	**21**	**9**

* **If** different, indicate corresponding FTEs in brackets.

** Number of producers in the 2008-2011 period who will be present in 2013-2017.
Definition and downloading of criteria:
http://www.aeres-evaluation.fr/Evaluation/Evaluation-des-unites-de-recherche/Principes-d-evaluation.

2 • Assessment of the unit

Overall opinion on the unit:

One of the rationales for the creation of this unit is the opportunity to gather the medical bacteriologists of the faculty of medecine of Strasbourg. This unit is relatively small but previous attempts to have a larger biomedical unit have been unsuccessful so far. There is no strong scientific interaction between the two research groups of EBV but it's a young unit and one can be confident that the director of the unit will be able to foster such interactions. It should be also stressed that discussions with the Dean of the Faculty of Medicine and the Vice-President of the University Scientific Council have shown that EBV is strongly supported by local authorities.

Strengths and opportunities:

The committee appreciates the integration in the research unit of clinicians that participate to the training of PhD students. On the other hand, the planned recruitment of a CNRS staff scientist will help to stabilize the unit. One of the members of the unit heads the National Reference Centre for *Borrelia* which will be of great help for the unit's research activities. *In vivo* experimental approaches will greatly benefit from the new animal facilities dedicated to infectious microbiology. In partnership with a private company developing diagnostic products, the unit develops several opportunities of technology transfer (e.g. anti-enterotoxin antibodies). Finally, it has developed good national and international scientific collaborations.

Weaknesses and risks:

The national and international competition on *Staphylococcus aureus* and *Borrelia burgdorferi* is very strong and thus poses a potential threat. The committee also notes that support funding is not high enough for fundamental research and needs to be reinforced. Finally, the relatively low number of PhD students and post docs of the unit hinders its research activity.

Recommendations:

The committee strongly recommends substantially improving the scientific and technological collaboration between the two research groups. This and other measures would also improve the unit's attractiveness for PhD students and post docs. The committee felt that too descriptive investigations should be avoided and that mechanistic approaches should be favored. Development of a local federation of biomedical research units is also strongly encouraged.

3 • Detailed assessments

Assessment of scientific quality and production:

The *Staphylococcus* team has good publication record. This research was reported in journals with a mean impact factor of 4.1 (range, 1.7 to 10.6), notably one of them was published in PNAS. Beside basic research, the group also developed investigations with clinicians to establish relationships between bacterial virulence factors (i.e.toxins) and staphylococci-induced pathologies (published in PLoS One and Journal of Hospital Infection), and also an antibody-based typing method for staphylococcal enterotoxins (European patent) which is a valuable tool for food industry and human medicine. The Lyme Borreliosis team has also good publication record. The group has published several papers on clinical and experimental aspects of borreliosis (2007-2011: 10 papers with 4 papers IF > 6). Since 2012, the group is the Lyme Disease Reference Center, which will further support its research.

Assessment of the unit's integration into its environment:

This research unit gathers the medical bacteriologists of the faculty of medecine of Strasbourg and is therefore fully integrated into its medical, scientific and academic environment. For instance, the director of EBV is the co-manager of the master degree on Cellular and molecular pathophysiology. The Lyme Borreliosis transmitted by ticks is also an important regional health concern and therefore any scientific contribution to better understand transmission and pathogenicity may have an economic and health impact. The Borrelia team has therefore obtained support by the region Alsace.

Assessment of the research unit's reputation and drawing power:

The *Staphylococcus* group has a long-term, internationally-recognized expertise on toxinology (as substantiating evidence, the group leader [h-index, 32] organized, in 2009, the 14th European Workshop on Bacterial Protein Toxins), in particular on staphylococcal exotoxins which are essential for *Staphylococcus aureus* pathogenesis. The two PIs of the Lyme team participate and are regularly invited to international meetings and have good international collaborations. Furthermore, the team participates in different national and international networks: Eucalb-European commitee against Lyme Borreliosis; REID: Réseau des Interactions durables: maladies à tique; CNEV: centre national d'expertise sur les vecteurs; Consortium International on mites and tick genome sequencing.

Assessment of the unit's governance and life:

The organization of the research unit is appropriate. Regular laboratory meetings are organized and the implementation of guest seminars, regular postgraduate seminar and journal club is planned . However, the scientific and technological collaboration between the two research groups should be improved.

Assessment of the strategy and 5-year project:

The project of the *Staphylococcus* group is a deepening investigation, through molecular and cell biology, biochemistry and cell imaging approaches of leucotoxin activity, and the mode of action of leucotoxin inhibitors and their activities in vivo using experimental models of staphylococcal infection. The group has scientific and technological expertise to develop its project. However, funding is problematic as financial support decreased during the past four years, notably compromising the renewal of obsolete equipments required for research development.

The *Borrelia* group focused on the role of tick saliva, the local and systemic inflammatory/immune response, local proliferation and spread of the spirochetes. The proposed investigation is sound and relevant. The contribution of the team is of good quality and may contribute to the clinical management of the prevalent Lyme disease in this Eastern part of France. Furthermore, one of the leading PIs has planned a sabbatical in San Diego to get more molecuar training for the project. However the international competition is definitively hard and the team could take benefit of the already established teams working on insect saliva and subsequent consequences upon a transmitted infectious disease.

Assessment of the unit's involvement in training:

The different members of EBV are involved in several master degrees and lectures. As teachers at the school of medicine and pharmacy, the different permanent members of the unit are involved in the Pharm D degree and Medical D degree. The director of EBV is the co-manager of the master degree on Cellular and molecular pathophysiology (35 to 40 M2 students every year). He also organizes the Master 2 examination committees. At the research unit level, regular laboratory meetings are organized to allow PhD and master students to present their research progress.

4 ● Project-by-project analysis

Project 1: Cell biology and inhibition of staphylococcal virulence factors

Name of project leader: Mr Gilles PRÉVOST

Workforce

Workforce in Full-time Equivalents	06/30/2011	01/01/2013
FTE for professors or assistant professors	5x0.5=2.5	6x0.5=3.0
FTE for EPST and EPIC researchers	1 till May 2009 = 0.35	1
FTE for engineers, technicians and administrative staff on a permanent position	1 + 0.25 (2011)	2
FTE for engineers, technicians and administrative staff on a non-permanent position	1+0.25 (12 mo)	
FTE for postdocs having spent at least 12 months in the unity	0.28 (13.5mo)	
FTE for doctoral students	4.27 (205mo)	
TOTAL	**9.9**	**6.0**

• Detailed assessments

The group has a long term, internationally-recognized expertise on toxins in particular on staphylococcal exotoxins which are essential for *Staphylococcus aureus* pathogenesis. It investigated the structure and mode of action of bicomponent toxins termed leucotoxins, which form pores in cell membranes. Notably, the group focused on interactions of the two toxin components with each other and with the cell membrane, on dynamics of pore formation and inhibition of the latter by specific antibodies and calixarenes.

The project, in continuity with past, is almost exclusively a deepening investigation, through molecular and cell biology, biochemistry and cell imaging approaches of (i) leucotoxin activity at aiming to define membrane protein ligands and target cells as well as influence of Ca2+ channels on cell signaling and inflammation, and (ii) the mode of action of leucotoxin inhibitors and their activities *in vivo* using experimental models of staphylococcal infection. Such a focusing research confers competitiveness to the group. Undoubtedly, the group has scientific and technological expertise to develop its project. However, funding is problematic as financial support decreased during the past four years, notably compromising the renewal of obsolete equipments required for research development. Another weakness of the group is its difficulty to recruit young researchers (including postdocs) who should revitalize it: seeking financial resources from French and European agencies should partly correct human resource deficit. The group should also increase its international visibility (its presence at international meetings must be strengthened and international collaborations should be reinforced): this should contribute to attractivity for students and postdocs from abroad**.**

Conclusion:

Staphylococcus is the most common Gram-positive bacterium involved in a broad range of pathologies in humans and, surprisingly, very few French research groups study bacterial virulence factors. The Strasburger group, headed by a recognized expert (strongly involved in educational activities elsewhere), is one of them, and its high-quality, basic research contributed to a better knowledge in the above-mentioned field, with potential medical valorization. A better international visibilility would facilitate its financial support and, as a consequence, development of its original on going research.

Project 2: Cellular and molecular biology of tick-borne infections

Name of project leader: Ms Natalie BOULANGER - Mr Benoit JAULHAC

Workforce

Workforce in Full-time Equivalents	06/30/2011	01/01/2013
FTE for professors or assistant professors	3x0.5=1.5	1.5
FTE for EPST and EPIC researchers	-	-
FTE for engineers, technicians and administrative staff on a permanent position	2.8	2.8
FTE for engineers, technicians and administrative staff on a non-permanent position	-	
FTE for postdocs having spent at least 12 months in the unity	-	
FTE for doctoral students	2 (100mo)	
TOTAL	**6.3**	**4.3**

- Detailed assessments

The group combines clinical and experimental murine studies to obtain new insights into the pathogenesis and epidemiology of borreliosis. Since this disorder is of great medical importance in France, especially in Alsace, their work is highly clinically relevant. In the following 5 years, the group will focus on the interaction of *Borrelia* with the skin, in particular keratinocytes, and the modulation of this interaction by tick saliva.

A major finding of this small unit was that vector (tick) saliva downregulates the cutaneous inflammatory response induced by invading Borrelia, which may support survival and spread of *Borrelia* in its new host. To further study this important aspect, the group has set up in vitro models with human keratinocytes, mast cells and fibroblasts as well as a murine model of Borreliosis, which both are straightforward to further characterise (i) the effect of the vector saliva on the local and systemic inflammatory/immune response and (ii) influence of tick saliva on spread of different *Borrelia* species in the skin. These proposed investigations are sound and relevant. The approach is original and the team is aware of the strong international comptetition.

In the past, the team has obtained support by the region Alsace and DGA (Direction Générale de L'armement) for two of the PhD thesis. Furthermore, the team is well integrated in different national and international networks (Eucalb-European commitee against Lyme Borreliosis; REID: Réseau des Interactions durables: maladies à tique; CNEV: centre national d'expertise sur les vecteurs; Consortium International on mites and tick genome sequencing). The proposed project will be performed in collaboration with national and international colleagues, which will further contribute to the international visibility of the group. The latter is also documented by invitations of the project leader as speaker to international conferences.

Conclusion:

The *Borrelia* group has a good scientific productivity and links clinical and experimental research. The project for the following 5 years is focussed, solid and well planned. Currently, the group is relatively small and it will be important to enlarge the group with PhDs and Postdocs to further increase productivity, to augment the scientific atmosphere, the international visibility, and competitiveness.

5 • Grading

Once the visits for the 2011-2012 evaluation campaign had been completed, the chairpersons of the expert committees, who met per disciplinary group, proceeded to attribute a score to the research units in their group (and, when necessary, for these units' in-house teams).

This score (A+, A, B, C) concerned each of the four criteria defined by the AERES and was given along with an overall assessment.

With respect to this score, the research unit concerned by this report (and, when necessary, its in-house teams) received the overall assessment and the following grades:

Overall assessment of the unit "Early Bacterial Virulence: cellular functions and control of acute and sub-acute infections EBV":

Unité dont la production, l'organisation et l'animation sont très bonnes. Le rayonnement et le projet sont bons mais pourraient être améliorés.

Grading table:

C1 Scientific quality and production.	**C2** Reputation and drawing power, integration into the environment.	**C3** Laboratory life and governance.	**C4** Strategy and scientific project.
A	B	A	B

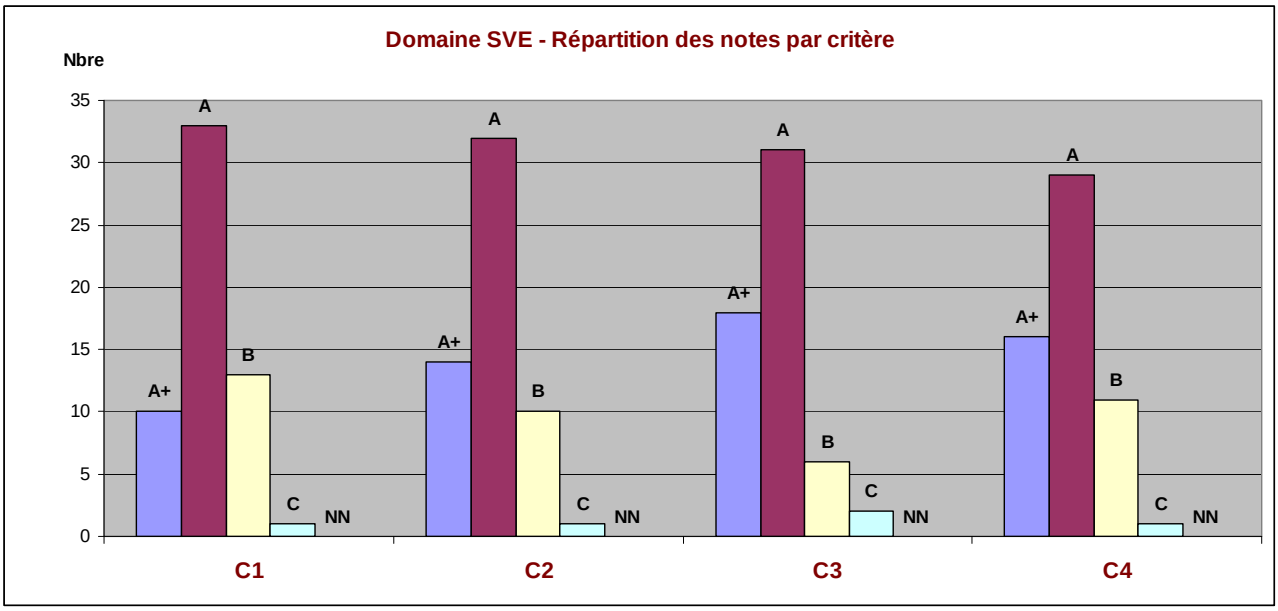
6 • Statistics per field

Notes

Critères	C1	C2	C3	C4
	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Gouvernance et vie du laboratoire	Stratégie et projet scientifique
A+	10	14	18	16
A	33	32	31	29
B	13	10	6	11
C	1	1	2	1
Non noté	-	-	-	-

Pourcentages

Critères	C1	C2	C3	C4
	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Gouvernance et vie du laboratoire	Stratégie et projet scientifique
A+	18%	25%	32%	28%
A	58%	56%	54%	51%
B	23%	18%	11%	19%
C	2%	2%	4%	2%
Non noté	-	-	-	-



7 • Observations générales des tutelles

UNIVERSITÉ DE STRASBOURG

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Directeur de la Section des Unités de recherche
Agence d'évaluation de la recherche et de l'enseignement supérieur (AERES)
20 rue Vivienne
75002 PARIS

Alain BERETZ
Président

Strasbourg, le 25 avril 2012

Objet : Rapport d'évaluation du projet d'EA « Virulence bactérienne précoce : fonctions cellulaires et contrôle de l'infection aigüe et subaigüe » (réf. S2PUR130004511-RT)
Réf. : AB/EW/N° 2012-198

Affaire suivie par
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Direction de la recherche

Cher collègue,

Je vous remercie pour l'évaluation du projet d'équipe d'accueil « Virulence bactérienne précoce : fonctions cellulaires et contrôle de l'infection aigüe et subaigüe » porté par Monsieur Gilles Prévost.

Vous trouverez ci-joint les réponses du porteur de projet concernant les erreurs factuelles et les remarques et appréciations du comité d'experts.

Je tiens à préciser que l'Université avait déjà prévu de constituer une fédération universitaire de médecine translationnelle regroupant les unités de recherche de biomédecine et de dentaire, anticipant ainsi les recommandations du comité d'experts.

Je vous prie d'agréer, Cher Collègue, l'expression de mes sentiments distingués.

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P.J. :

- **Une première partie corrigeant les erreurs factuelles**
- **Une seconde partie comprenant les observations de portée générale**

Research Project:

Early bacterial virulence: cellular functions and control of acute and sub-acute infections (EBV) – Dir: G. Prévost

Université de Strasbourg

Comments of the AERES report dated February 2012, receive March 23rd 2012

Despite initially constituted of three research groups the project now gathers two groups with the same initial personnel that are called:

- Cell biology and inhibition of staphylococcal virulence factors, PIs **G. Prévost**, F. Jehl (assistant Professors)
- Cellular and molecular biology of tick-borne infections, PIs **N. Boulanger** (assistant Professor), B. Jaulhac (PU-PH)

This proposal was the result of the fruitful dialogue between all members with the collaboration of the responsible of the scientific department from University. It also took into account the double concern to fit indications from the faculty of medicine dealing with the attractiveness of clinician colleagues and flowing in the developing tasks contributing to a scientific Federation for Translational Medicine in Strasbourg. This project reflects in fact a continued approach that consisted in the recruitment of colleagues that were considered to get interest in our fields of interest and will continue to take an active part in the next period (Pr T. Bourcier, Dr P. Riegel, Dr T. Lavigne; but also Pr Y. Hansmann, Pr D. Gaucher, Drs D. Levêque, N. Lefebvre, Dr E. Jover (CNRS-CR1).

We thank the AERES committee for his detailed analysis of the project and the report.

One major point has to be corrected: Dr N. Boulanger is assistant Professor who will be in charge of the *Borrelia* project, while Pr B. Jaulhac is in charge of the National Reference Center fully attributed 2012.

AERES Committee pointed out several topics:

- **Financial support**: in order to begin the next period in more favorable conditions, several applications were deposited this year by the different groups (2 ANR, DGA, VLM, Alsace Région…) and we are confident that a sell of one intellectual property will be achieved this year with a manufacturer involved in diagnostic. Beside of these quite usual applications, we are also willing to prepare bi- or multi-lateral applications for funding with foreign collaborators, e.g. collaborators of the Rhine Valley. Despite both the two earlier groups were regularly sustained by local funding (University, Alsace Region) that contributed to both fundamental research, PhD grants, building new animal facility, we recognized that other funding has mainly allowed to buy an FPLC, maintenance and consumables of specific programs. We hope that the new initiatives will strengthen our potential and will let possibility to buy some equipments

- **Publications and attractiveness**: Even some were operated for past, much effort is made to get more publications in journals with IF ranking from 5 to 11-12, in a volume of more than 80 international publications. The two groups totalize 10 publications of IF > 6.0 from 2007 to date, but it would complete by 4 others this year. We also are motivated to propose consistent reviews to high ranked dedicated journals. It is right that rare funding impacts our participation to much international congresses, despite participations to "Gordon Conference on Spirochetes", "Pores 2012" and American Society for Microbiology congresses. Creation of a website and better funding would positively contribute to our visibility and possibility to recruit young researchers. For 2011 and 2012, these are 6 PhD theses that will be defensed; but among them Drs Maher Saleh and Sophie Lefèvre will stay in the team through hospital carriers, and we hope to maintain Wardi Moussaoui in a 9-12 month post-doctoral position. Otherwise, the politic of the unit will be, if possible, to sustain one application at least for a post-doctoral fellow. For the next year, we also hope to welcome 4-5 PhD students, three in the Borrelia's project, two of them are already sure.

- **Scientific activities**: both the two projects are interested with clinical studies that involve new clinical facts, new diagnostic approach/recommendation, pertinent new technologies, but they mainly do not affect the recurrent unit budgets. The latter studies have vocation to prepare future fundamental investigations, and may be published in good journals as mentioned by the committee. However, the budgets are mainly devoted to fundamental research that consists for:

- The *Staphylococcus* project aims to decipher the chronology of the various leucotoxins interactions with target cells, translocation and kinetics of calcium signals triggered in target cells. We wish to compare these elements with cell inflammatory response and examine the possibilities from different target cells that can give insights in further applications. In that sense, we will interact with the *Borrelia* group for their expertise with keratinocytes and fibroblasts culture and cell response.

  The other facet is to define interactions of leucotoxins with receptors and also with specific antibodies and other inhibitors. The understanding of the molecular interactions may contribute to development of new effective drugs, while the efficacy of actual ones is ought to be considered. In these objectives, new collaborations are establishing.

- The *Borrelia* project is aware that at this point the work has been mainly descriptive. To analyze the molecular mechanisms of the transmission and being still competitive, three aspects will be developed:

  - Skin microbiome and TLRs with Pr R. Gallo (San Diego)

  - Host vascular permeability and development of the disease with Dr L. Bockenstedt (Yale university) and with Dr T. Moriarty (Toronto University) to analyze this process by confocal intravital microscopy

  - Role of resident skin mast cells with Dr. S. Mécheri (Pasteur institute, Paris).

  - *P6- last paragraph*: It is not clear to which group working on insect saliva, the AERES committee refers to. If it is the group at the IBMC working on the interaction of mosquito-*Plasmodium*, their area of interest concerns the innate immunity of the vector to the *Plasmodium*, while we work on the innate immunity of the vertebrate host to

Borrelia and tick saliva. We do not exclude a future collaboration with this group, in case we develop a more specific project on the vector.

- N. Boulanger is part of a national network on arthropod saliva and is part of "Vector saliva network" headed by the NIH (National Institute of Health, Bethesda, USA).

Therefore, this project mainly deals with understanding mechanism of molecular processes in the focused objectives.

Interactions between the two groups

The further meetings between members from the whole initial project allowed delineate several common interest areas:

- Influence of bacterial flora according to moisture on tick bite localization
- Influence of staphylococcal product interacting with TLR-6 on Lyme disease development
- Influence of *Borrelia* on cell calcium levels, involvement of major calcium pathways
- Establishing a cell model derived from neuroborreliosis
- Confocal microscopy, multiplex titrations,

where the two projects would produce common work and can gain interactions.

Strasbourg, 02.04.2012

G. Prévost