Aussois (France), 23-27 mai 2009

La réponse immunitaire des insectes en action : des mécanismes fondamentaux de la défense de l'hôte à la résistance aux infections dans le milieu naturel

Insect immunity in action: from fundamental mechanisms of host defense to resistance against infections in nature

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Rapport sur la Conférence

Conference Report

RESUME DU RAPPORT

Conférence Jacques Monod intitulée :

La réponse immunitaire des insectes en action : des mécanismes fondamentaux de la défense de l'hôte à la résistance aux infections dans le milieu Naturel

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Conférence s'est tenue à Aussois du 23 au 27 mai 2009 et a réuni les meilleurs spécialistes de l'immunité des insectes dans l'esprit de la première Conférence Jacques Monod à Roscoff (2006) portant sur cette thématique. Cette Conférence a permis de faire le point sur l'avancée des travaux dans des domaines variés allant de la recherche la plus fondamentale en immunité innée à des aspects plus appliqués illustrés par les exposés présentés par Jay Evans sur l'immunité des abeilles ou un représentant de l'industrie (John Dorsch).

Aux 29 conférenciers invités se sont ajoutés 26 orateurs qui ont exposé leurs travaux dans des présentations plus courtes. Nous avons aussi convié cette année les Pr. Paul Schulze-Lefert et Thomas Böhm qui ont présenté respectivement leurs vues sur l'immunité innée des plantes et sur l'immunologie des vertébrés. Les sessions ont couvert les réponses immunitaires locales (essentiellement au niveau de l'épithélium intestinal) et systémiques, les réponses cellulaires, les interactions hôte-pathogène/parasite/parasitoïde-symbiontes, l'immunologie des insectes vecteurs de parasites ainsi que plusieurs aspects de l'immunité antivirale. Des présentations ont aussi porté sur d'autres invertébrés, essentiellement les crustacés et le ver nématode *Caenorhabditis elegans*. Les travaux exposés ont couvert plusieurs types d'approches, de l'étude moléculaire fine des voies de transduction du signal et des phénomènes de reconnaissance entre hôte et microbes à l'étude de l'évolution du système immunitaire inné en passant par le comportement des insectes sociaux vis-à-vis de leurs congénères infectés.

En conclusion, cette Conférence a offert un panorama des recherches de pointe dans le vaste domaine de l'immunité des insectes avec souvent la présentation de données non encore publiées. Un rapport plus détaillé rédigé en anglais est aussi disponible. Cette Conférence a aussi fait l'objet d'un *Meeting Report* dans la revue *Cell Host&Microbe* (Welchman *et al.,* 2009).

Welchman, D.P., Aksoy, S., Jiggins, F., and Lemaitre, B. (2009). Insect immunity: from pattern recognition to symbiont-mediated host defense. Cell Host Microbe 6, 107-114.

CONFERENCE REPORT

Final report from the Jacques-Monod Conference entitled:

Insect immunity in action: from fundamental mechanisms of host defense to resistance against infections in nature

Aussois, May 23-27, 2009

General description of the meeting:

During the past three decades insects have been successfully used to study innate immune reactions and many of these studies paved the way to novel discoveries in vertebrates. Insect immunity had been the focus of a previous Conference Jacques Monod, which took place in Roscoff Bretagne in 2006 and was a major success. For this year's meeting in Aussois, we invited additional world-leading researchers and two representatives from the field of plant- and vertebrate immunology (Paul Schulze-Lefert and Thomas Böhm). The meeting in Aussois provided a comprehensive and comparative view of innate immunity, often in an infection context and addressed practical implications of insect immunology (see for example the talks by Jay Evans on honey bee immunity and the presentation of John Dorsch, an industry representative). Emphasis was placed upon the key areas including:

- **Immunity against viruses:** Antiviral Immunity session
- **Cellular immunity:** Cellular Immunity session
- **Peripheral immunity:** Systemic and Local Responses I and II sessions
- **Comparative immunology:** Ecological Immunity, Priming sessions and Genomics – other invertebrate models session
- **Vector insect immunity:** Insects as vectors of human diseases session
- **Host-pathogen interactions:** Intestinal immunity session

as well as other sessions

In addition to the 29 invited speakers we selected an additional 26 talks from applicants who had described cutting edge research in their Abstract. One of the important aspects of the Aussois meeting was the fact that all participants work on topics related to innate immunity. This ensured lively discussions both during and outside sessions. As anticipated, this format of conference worked more effectively than larger meetings such as the *Drosophila* conferences (American and European *Drosophila* Research Conference) or conferences on more specialized topics (Gordon Research Conference, Keystone and Cold Spring Harbor Meetings). The ambition of this new conference was to convene an assembly of scientists working on fields of innate immunity that are as diverse as ecological immunity or molecular signal transduction. We could not achieve a comprehensive discussion of immune priming as two of the major protagonists of this concept (Schmuecker, Schulenburg) were unfortunately unable to attend the meeting due to relocation of their laboratories.

The individual presentations included the following:

Opening session

Paul SCHULZE-LEFERT (Köln, Germany) gave a keynote lecture on plant immunity and gave a comprehensive overview of the different levels of plant immune defenses. He left the participants with the impression that the fields of plant and invertebrate immunity are moving closer together. Examples for this include the convergent evolution of glucan sensing in plants and insects and the use of endogenous signals in the regulation of immune responses. **Ronald VAN RIJ** (Nijmegen, The Netherlands) **s**howed that antiviral immunity in Drosophila requires systemic RNAi spread via uptake of dsRNA by non-infected cells. **Ingrid FAYE** (Stockholm, Sweden) shared with us her memories of Hans Boman, one of the pioneers in insect immunity who had passed away in December 2008.

Session - Systemic and local responses I

Julien ROYET (*Marseille, France)* using a cellular depletion strategy presented data showing the contribution of blood cells to immune responses. In addition he presented evidence that mitochondria might be playing a role in the *Drosophila* immune response. **Ulrich THEOPOLD** (*Stockholm, Sweden*) using a knock-down approach presented evidence that the hemolymph clot has an immune function in preventing systemic spread of bacteria and subsequent sepsis. Further supporting the clot's immune function, **Shun-Ichiro KAWABATA** (*Fukuoka, Japan*) identified a lipopolysaccharide-responsive homologue of C3 convertase in horseshoe crab which contributes to opsonization of both Gram(+) and Gram(-) bacteria. **Vincent LECLERC** (*Strasbourg, France*) discussed the evidence for the contribution of endogenous danger signals to insect immunity (see also above the talk by Schulze-Lefert). **Marie-Odile FAUVARQUE** (*Grenoble, France*) identified Ubiquitin Specific Proteases as negative regulator acting on *Drosophila* immune signals via the *imd* pathway and its downstream targets (compare also the results by Neal Silverman below). **Ylva ENGSTRÖM** (*Stockholm, Sweden*) showed how combinatorial use of transcription factors including novel members of the POU family affects inducible and tissue-specific epithelial expression of *Drosophila* immune genes and presented data on a *Candida* infection model.

Session - Cellular immunity: haematopoiesis, phagocytosis

Dan HULTMARK *(Umeå, Sweden)* described a recently isolated Nora virus and how this virus can be present in high titres with only limited apparent consequences on life traits. **Michelle CROZATIER** *(Toulouse, France)* summarized her work on larval haematopesis and on the effects of Latran, a non-signalling Domeless receptor homologue, which acts as a negative regulator of JAK/STAT signalling. By antagonizing Domeless function in hemocytes, Latran appears to promote lamellocyte differentiation. **Shoichiro KURATA** *(Sendai, Japan)* reported on the function of PGRP-LE during induction of autophagy, a form of cellular demise which helps to evade intracellular infections, for instance by *Listeria monocytogenes.*

Session - Cellular immunity: haematopoiesis, phagocytosis (continued)

Róbert MÁRKUS *(Szeged, Hungary*) and coworkers identified subepithelial sessile hemocytes as a novel source of lamellocytes that are recruited upon parasitoid infections. **William WOOD** *(Bath, United Kingdom)* presented time-laps confocal microscopy and evidence that the *Photorhabdus* virulence factor Mcf1 leads to immobilization of hemocytes in a RAC-dependent manner. Using

Salmonella infections, **Naokaki SHINZAWA** *(Obihiro, Japan*) showed that sequestration of bacteria within the phagosome confers infection tolerance properties to *Drosophila*. This process is dependent on p38 MAP kinase and has been dubbed phagocytic encapsulation. **Petros LIGOXYGAKIS** *(Oxford, United Kingdom)* brought our understanding of bacterial recognition a step further to the *in vivo* situation by showing the masking effect of teichoic acid on recognition of Gram(+) bacteria by PGRP-SA and by showing that different PGRP receptor complexes are required for the recognition of different bacteria and subsequent systemic activation of the Toll pathway.

Session - Viral immunity - Ecology, parasitic wasps and associated viruses

By resequencing immune genes in various *Drosophila* populations **Frank JIGGINS** *(Edinburgh, United Kingdom)* identified a small subset of genes, which show adaptive evolution. In contrast to vertebrates, little evidence was found for the maintenance of polymorphisms or adaptive evolution of antimicrobial peptides. **Darren OBBARD** *(Edinburgh, United Kingdom)* presented comparative genome data from a systematic search for naturally occurring *Drosophila* viruses. Systematic searches for virulence factors employed by parasitoid wasps specific of *Drosophila* allowed **Marylène POIRIE** *(Sophia Antipolis, France)*to identify serpins and proteins acting on Rac GTPases as well as additional factors. Comparing specialist and generalist wasps revealed striking differences in the usage of virulence factors. **Beatrix LANZREIN** *(Bern, Switzerland)* gave a comprehensive overview of her lifetime work on the different levels of virulence strategies that are used by parasitoid wasps. Adding to our understanding of the fine specificity of host-pathogen interactions **Ioannis ELEFTHERIANOS** *(Strasbourg, France)* documented a protective effect of an ATP-sensitive potassium channel in the heart for survival to infections with Flock House Virus but not *Drosophila* C virus. **Shubha Govind** *(New York, USA),* presented multilevel studies of two parasitoid species and her initiative to organize whole genome data into a common format and database.

Session - Insect as vectors of human disease

During recent years, a number of proteins have been implicated in the mosquito response towards malaria causing *Plasmodium* species. Work generated by **Georges CHRISTOPHIDES** *(London, United Kingdom)* and **Elena LEVASHINA** *(Strasbourg, France)* place the thioester-containing protein Tep1 and two leucine-rich repeat proteins of *Anopheles gambiae* into a complement-like system required for specific parasite killing. The LRR proteins prevent binding of Tep1 to self tissues and thus likely control the specificity of action of Tep1 by targeting it to the parasite surface. Studies reported by **Serap AKSOY** *(New Haven, USA*) suggest a role for obligate symbionts *(Wigglesworthia)* in the tsetse fly. When *Wigglesworthia* is removed by antibiotic treatment, the flies become highly susceptible to infection by trypanosomes. PRGP-LB plays a role in sustaining the symbiosis and preventing immune activation elicited by the presence of the symbiont. A possible consequence is that PGRPs also prevents infection with trypanosomes. **Alexander RAIKHEL** *(Riverside, USA)* described the different levels of regulation of melanization responses in the mosquito *Aedes aegypti* both at the transcriptional and post-translational level. In addition in the latter case, regulation differs between hemolymph and other tissues. **Sirley DAFFRE** *(Sao Paolo, Brazil)* has identified a novel tick antimicrobial peptide called Microplusin which has copper-chelating properties suggesting a possible mechanism for its activity which may involve depletion of copper from microorganisms. Finally in this session **Georges DIMOPOULOS** *(Baltimore, USA)* described another level of protection against *Plasmodium* infection, which involves the exponential growth of the mosquito midgut microbiota after a blood meal, which elicits a strong induction of a local epithelial immune response that is detrimental to the parasite while controling bacterial proliferation. Data from **G. Christophides** indicate that PGRP-LC likely mediates this response.

G. Dimopoulos also reported on the relative contribution of the negative regulators Cactus, Caspar (NF-kappaB pathways) and PIAS (JAK-STAT pathway) and data from other infection models such as Dengue virus and *Brugia malayi.*

Session - Systemic and local responses II

Neal SILVERMAN *(Worcester, USA)* provided further insight into the regulation of the *imd* pathway, the importance of caspase-mediated cleavage of IMD and subsequent K63linked poly-ubiquitination through Diap2 and ubiquitin-conjugation enzymes. He also discussed the role of phosphorylation of Relish, which he finds to be dispensable for its cleavage but required for the recruitment of RNA polymerase II to target promoters. **Alain ROUSSEL** *(Orléans, France)* presented the N-terminal structure of GNBP3 which comprises an immunoglobulin fold in which the ß-glucan binding site is masked by a loop that appears to be displaced only by long chain but not short chains ß(1,3) glucans thus ensuring specificity of binding to long polymeric sugar chains. **Susanna VALANNE** *(Tampere, Finland)* and coworkers identified novel regulators of the Toll pathway, in particular an evolutionarily conserved regulator, which appears to also act in zebrafish and human cells. **David GUBB** *(Derio, Spain)* identified the Drosophila Low Density Lipoprotein receptor homologue LpR1 as the potential receptor that mediates endocytosis and degradation of serpins in the Garland cells and pericardial athrocytes. **Cyril GUEYDAN** (*Gosselies, Belgium*) described a novel level of regulation of the expression of antimicrobial peptides, which involves the stability of the corresponding RNA. The stability is mediated by AU-rich elements in the 3' UTR and by deadenylation of the RNA.

Session - Ecological immunity, priming

Karyn JOHNSON *(St Lucia, Australia)* **Luis TEIXEIRA** *(Oeiras, Portugal)* had independently shown that *Drosophila* strains infected by *Wolbachia* are better protected against a number of RNA viruses. Texeira showed that in the case of the Flock House Virus this is due to tolerance rather than resistance and Johnson presented data indicating different levels of protection in different *Drosophila-Wolbachia* combinations. **Yixin YE** (*Brisbane, Australia*) and coworkers had applied a selection scheme involving *Pseudomonas aeruginosa* to isolate more resistant flies which showed changes in immune induction pathways but also pronounced differences in expression of genes involved with cellular immunity. The evolutionary costs of these changes were revealed in a number of life history traits. **Fabrice VAVRE** (*Villeurbanne, France*) has studied *Wolbachia* in obligate association with a wasp (*Asobara*), whereby removal of *Wolbachia* leads to sterility. Transcriptome and histological analyses indicate that the parasite manipulates host physiology in particular at the level of iron metabolism and programmed cell death. **Sylvia CREMER** (*Regensburg, Germany*) presented an overview of the different levels at which social behaviour in insect colonies can prevent or limit the spread of infections and provided evidence for a social vaccination where individuals are better protected against microbes their colonies have been previously exposed to, as compared to "novel" microbes. **David SCHNEIDER** (*Stanford, USA*) using a mutant in a protease that is required for phenoloxidase activation described how this mutation had very different consequences depending on the infectious agent used. In opposite outcomes, microbes were either eliminated (resistance) or not eliminated but were tolerated by the host organism (tolerance). Yet others induced a combination of both phenotypes. **Valerie SMITH** (*St Andrews, United Kingdom*) gave a comprehensive overview of crustacean immunity much of which appears to rely on pre-produced effectors and less on their induction.

Session - Vertebrates versus invertebrates

Thomas BÖHM *(Freiburg, Germany)* gave an overview of the design principles of adaptive immune systems indicating how non-anticipatory mechanisms create diversity within immune recognition repertoires. Some of his points were followed up in the subsequent general discussion on immune priming (see also general comments). **Douglas BROOKS** *(Adelaide, Australia*) presented evidence that killing and phagosome maturation are delayed upon infection with *Helicobacter pylori* as compared to *E.coli*, which may explain part of *H. pylori's* infectivity irrespective of virulence factors. **Tetyana SHANDALA** *(Adelaide, Australia)* reported secretion defects in *Drosophila* mutants lacking 14-3-3ε, which affected the release of antimicrobial peptides from the fat body and leads to immune defects.

Session - Intestinal Immunity

Won-Jae LEE (*Seoul, Korea*) described the complex regulation of Duox proteins in the Drosophila gut, which is required to both control gut homeostasis and to combat infection by pathogenic microbes. Both p38 and a phospholipase C pathway are involved in this process. **Dominique FERRANDON** *(Strasbourg, France)* reported results from a genome-wide RNAi screen using orally applied *Serratia marcescens* to identify genes affecting survival to this intestinal infection. More than 800 genes were identified and along the way the importance of the JAK/STAT pathway for epithelial homeostasis through the control of the proliferation of intestinal stem cells was shown although in this infection model an excess of proliferation appears to be counterproductive. **Nicolas BUCHON** *(Lausanne, Switzerland)* and colleagues had applied *Erwinia carotovora* in a similar intestinal infection model but in their case the renewal pathway protected the flies from the detrimental effects of the infection with this normally nonpathogenic bacterium. The JAK/STAT pathway is also required for the activation of hemocytes in an exciting tumor model presented by **José Carlos PASTOR-PAREJA** *(New Haven, USA),* whereby disruption of the basal lamina, an event also occurring in wounds, was shown to activate hemocytes and to a partial control of tumor growth. **François LEULIER** *(Gif-sur-Yvette, France)* showed that Pirk (also known as PIMS or Rudra) is a negative regulator of the *imd* pathway that participates in setting up the threshold of pathway activation at a level sufficiently high so as to preserve the tolerance to commensal gut bacteria. Using a lepidopteran model, **David HECKEL** *(Jena, Germany)* showed how the lepidopteran midgut modulates an immune response upon feeding on bacteria. A strong maternal and weaker paternal transmission of the induced immune status was observed.

Session - Genomics-other invertebrate models

Jay EVANS *(Beltsville, USA)* described approaches to use knowledge of insect immune systems to control diseases affecting honeybees caused by known agents such as the American foulbrood bacterium and the less characterized Colony Collapse Disorder. **Bok Luel LEE** *(Busan, Korea)* and colleagues have purified the proteins involved in the activation and molecular regulation of the *Tenebrio* Toll signaling pathway which may allow them to develop detection kits for minute quantities of peptidoglycan and/ or glucans. **Kenji KUROKAWA** *(Busan, Korea)* focused on one particular aspect of this cascade namely the recognition of peptidoglycan which requires the cooperation of PGRP-SA and GNBP1 to activate *Tenebrio* Spätzle. In the final presentation of the day, **John DORSCH** *(Research Triangle, USA)* presented approaches used in the industry to control pest insects and proposed to include strategies that target the insect immune system.

The session on the final day was dedicated to the nematode *C.elegans* as a model to study innate immunity. **Jonathan HODGKIN** *(Oxford, United Kingdom)* described whole genome screens for

genes that affect a specific swelling reaction that is induced by many microbes upon infection of nematodes. Lysozymes, C-type lectins and antimicrobial peptides were identified and some validated using RNAi. **Jonathan EWBANK** *(Marseille, France*) summarized his work on the regulation of antimicrobial activity in *C.elegans,* which involves p38 protein kinase C and G proteins signaling and thus a combination between generic stress and pathogen-specific responses.

Concluding remarks

In general the meeting showed that many researchers in the field are analyzing innate immune responses in a more integrative manner which takes into account the specific infection model, the organism studied and the interconnection between different effector pathways and general host physiology. This is an exciting trend, which has led to many, often unexpected points of convergence with vertebrate and plant immunology and general implications for our understanding of innate immunity.

Many participants including invited and selected speakers as well as students commented that they very much enjoyed the meeting and gained a lot from it. Several participants expressed their satisfaction with the poster sessions, which were all well attended and led to lively discussions. Also the selection of talks was received very well. One slight problem this year was that a couple of conferences on related fields where held in the same period while for example there was none last year, which was our initial intended date for this meeting. Although we were able to attract sufficient applications it may be wise to avoid such overlap in the future. The local organization was excellent, with special thanks to Dominique Lidoreau, who helped at all stages of the organization of the meeting and insured that it ran smoothly.

Perspective

The participants felt there is a strong need for organizing another meeting on invertebrate immunity in 2012. They elected on the last day of the Conference Dr. George Christophides (Imperial College London) to act as a vice-president and Dr. Dominique Ferrandon (CNRS, Strasbourg) as president for the next conference. A feedback from the participant evaluation forms will help ensure an adequate organization and further scientific excellence of this meeting. Of note, a meeting report has been published in the Cell group journal Cell, Host& Microbe, the top journal in the field of host-pathogen interactions.