Pathogens under scrutiny in the South of France

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While the nature of the pathogens and the diseases they cause may be incredibly diverse, the mechanisms and pathways these organisms exploit to survive and usurp the cellular machinery to their own benefit are in many instances very similar. With that in mind, Pascale Cossart, Robert Menard and Felix Rey invited world experts on a variety of human pathogens to come to La Colle sur Loup, share their latest discoveries and learn from each other. The result was the EMBO workshop ‘Emerging Themes in Infection Biology’. More than 100 participants were graced with a rich, top-quality scientific programme and also with clear skies that day and night (!) helped to keep the spirits high throughout the meeting.

Françoise Barre-Sinoussi (Institut Pasteur, Paris) and Rino Rappuoli (Novartis Vaccines and Diagnostics, Siena) started the meeting with two memorable keynote lectures. Françoise naturally focussed on human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) and the insights that studies on human HIV controllers and primate models that do not develop simian immunodeficiency virus (SIV) disease have brought to our understanding of HIV pathogenesis and protection from disease. Despite the clear success in the therapeutic control of HIV infection, an effective vaccine is not yet within reach and after Rino Rappuoli’s talk the audience was certainly convinced of the power of effective vaccines. His lecture was punctuated by successful vaccine examples such as the recent results obtained with eradication of meningococcal C and B meningitis in the UK and New Zealand, respectively, but focussed mostly on the future of vaccine research. Now that many childhood infections can be prevented by vaccination, Rino emphasized that it is time to refocus efforts towards developing vaccines against agents that infect the elderly and the poor as well as emerging infections.

In this meeting report we will follow the pathogen’s encounters with the host, how they travel, ingeniously invade and target key cellular processes and how the host responds to its aggressors.

Reaching the ideal replication site

Different pathogens have adapted to survive and proliferate in specific environments. Reaching these niches is sometimes a challenging feat as pathogens have to travel long distances across organs, cross several cellular barriers, invade cells and travel inside the cell itself. The talk by Robert Ménard (Pasteur Institute, Paris) followed one of these journeys. Plasmodium, the etiologic agent of malaria enters the body through mosquito bites. It travels the blood stream and reaches the liver where it divides, forms merozoites, infects other
liver cells and finally enter the host’s bloodstream to invade erythrocytes and initiate disease. Using intravital imaging, Robert’s lab tracked the parasite from its injection site to the liver and came across several surprises. First, a large number of parasites persist in the skin, multiply and even form merozoites. Nevertheless, a proportion of parasites enters the circulation and reaches the liver. A second surprise was that the exit step from the liver sinusoids occurs by endothelial cell traversal, a process that does not appear to require Kupffer cells as was generally thought. Infection and invasion of the host cell is another difficult task for intracellular pathogens. Arriving in proximity with host cells, pathogens can exploit the specific morphology of host cells to enter them. Ari Helenius (ETH, Zurich) described how Vaccinia viruses use cellular extensions known as filopodia to reach the cellular body before entering host cells. Exposed phosphatidyserine groups in the Vaccinia virus viral membrane mimic apoptotic bodies and are used by the virus to enter the host cell by macrophagoctosis in an epidermal growth factor receptor (EGFR)-dependent manner. Bacterial pathogens on the other hand have at their disposal an arsenal of secretion systems to invade cells such as the needle-like type III secretion system (T3SS). Salmonella enterica, for example is an intracellular pathogen that replicates within host-cell vacuoles and delivers virulence factors (effector proteins) through a T3SS. The process involves assembling the needle and shutting the effector proteins across two bacterial membranes and the host vacuolar membrane in a highly coordinated sequential set of events. David Holden (Imperial College, London) showed recent data from his laboratory that address one aspect of this spectacular coordination. As the T3SS is assembled and crosses the vacuolar membrane, the intravacuolar Salmonella can sense the neutral cytosolic pH, trigger degradation of a regulatory complex and finally allow effector translocation. Legionella pneumophila is another intracellular pathogen that thrives inside macrophages in a vacuole that evades fusion with lysosomes. To control endosomal traffic and guarantee its intravacuolar survival Legionella secretes an unexpectedly high number of effectors in the host cytoplasm through a type IV secretion system. Craig Roy (Yale University, New Haven) identified and functionally characterized a family of such effectors displaying an ankyrin repeat homology domain. One of these, AnX fragments the Golgi and affects several endocytic Rab proteins possibly interfering with the endocytic pathway in this manner. Parasites have also developed competent cellular strategies to enter their hosts. John Boothroyd (Stanford University) and Isabelle Tardieux (Institut Cochin, Paris) both discussed Toxoplasma gondii invasion and the role of rhoptries and their components in this process. Isabelle showed how, toxofilin, one such component secreted into the host cell can mimic cellular cofillin by locally increasing actin flow and turn-over to disassemble the cortical actin meshwork at the site of invasion and facilitate vacuole folding. John focussed on different rhoptry components that enable the formation of a so-called moving junction that allows the parasite to slide into host cells in an intracellular vacuole. He highlighted the importance of ROP16, a polymorphic kinase that mimics cellular Jak2 and is injected into the host cell to activate STAT6 and alter the host immune response.

Further hurdles have to be overcome inside the cells. Matt Welch (University of California, Berkeley) described how Rickettsia express an actin modulator, Sca2 that acts as a cellular mimic of formins that binds profilin to assemble the long parallel actin filaments that Rickettsiae use for their intracellular movement. This molecular mimicry leads not only to the movement of the bacterium in the host cell cytoplasm but it also allows bacteria to move to neighboring cells. Herpes viruses are another striking example as they travel from the epithelium to the nervous system. Lynn Enquist (Princeton University) described how, once inside neurons, pseudorabies virus take advantage of the microtubule network to travel large distances from the cell body to the tip of the axon and back. Lynn also discussed a viral strain, Bartha that is impaired in its anterograde movement. Surprisingly, it can instead ‘jump’ to the axon of a neighbourong neuroun and then move in a retrograde fashion to the cell body.

After efficient replication and the full exploitation of the local resources pathogens may need to move to a different site inside the same host or to another host. Guillaume Dumenil (Paris Cardiovascular Research Center) explored this understudied step of the infection process during Neisseria meningitidis infections. Guillaume showed that after proliferating on the epithelial cell surface in the throat, Neisseria trigger a cascade of events involving post-translational modifications of its type IV pili leading to the detachment of individual bacteria from the microcolonies. This detachment step allows the propagation of bacteria to new host but also the dissemination of bacteria across the epithelium, a prerequisite for invasive infections.

From the experimental point of view, observation of some of these events requires real-time imaging techniques that are now a requirement in the field. Spinning disc confocal microscopy and two-photon microscopy are necessary to efficiently describe how pathogens travel across tissues. At the cellular level, observation of single cells reveals a wealth of information but novel assays are needed to describe specific steps. For instance, Jost Enninja (Institut Pasteur, Paris) reported the design of a fluorescence resonance energy transfer (FRET)-based assay to visualize the exit of single Shigella flexneri from the intracellular vacuole following its internalization. In another elegant analysis, Jost took advantage of molecular beacons to ‘image’ gene expression in real time in single cells. This revealed a temporal link between the induction of interleukin 8 (IL-8) with specific steps of the invasion process.

Pathogens hijack and target key cellular processes

In an attempt to maximize the impact of the limited number of proteins coded by the small genomes of the majority of the organisms that were discussed at the meeting, these pathogens have developed strategies to take advantage of the cell machinery for their own benefit or to
target it as broadly as possible with their limited resources. One example common to many pathogens is exploiting regulators of post-translational modifications such as phosphorylation, ubiquitination or SUMOylation. These modifications can affect the stability, localization or activity of the proteins that are targeted and pathogen effector molecules can use several of these components for different purposes. Indeed, Ari Helenius (ETH, Zurich) presented data showing that VACCINIA virus proteins are highly ubiquitinated and that the virus depends on a cluster of proteasome and ubiquitin related genes for disassembling its core and for its replication. Ari’s approach consists of high throughput cellular small interfering (siRNA) screens to identify what he calls the infectome, i.e. the cellular proteins and pathways involved in the entry of the viruses studied. Jorge Galan (Yale University) also reported on Salmonella typhimurium and the ubiquitination of its type III effector phospho-nositide phosphatase SopB. SopB is ubiquitinated by the cellular machinery and its localization and functions depend on this ubiquitination. Eric Oswald (Universite de Toulouse) showed that also the cycle inhibiting factors (Cifs), produced by the enteropathogenic Escherichia coli (EPEC) usurps another ubiquitin ligase complex. Cifs are known to stabilize the cell cycle inhibitors p21 and p27, hence inducing cell cycle arrest. Eric showed that EPEC Cifs induces accumulation of Nedd8-conjugated cullins and binds components of the Cullin-Ring E3 ligase complex. By modulating the ligase activity of this complex, Cifs could target proteins involved in processes such as control of the cell cycle progression, but also dynamic of actin cytoskeleton and host immune response. Like some of the Salmonella effector proteins discussed by Jorge Galan, IpaH9.8 is another bacterial effector with E3 ligase activity. Chihiro Sasakawa (University of Tokyo) reported that this Shigella E3 ligase promotes aberrant polyubiquitylation of NEMO and tempers the NF-kappaB-mediated inflammatory response to bacterial infection. Clearly, the ubiquitin and proteasome machinery are part of the infectome of many pathogens. David Ribet (Institute Pasteur, Paris), from Pascale Cossart’s laboratory gave yet another example whereby the pathogen appears to be dampening the host response by affecting SUMOylation. Small Ubiquitin-like Modifier (SUMO) are ubiquitin-like proteins that can also be covalently linked to protein substrates. David showed that Listeria monocytogenes infection, and in particular the expression of its virulent pore-forming listeriolysin O (LLO), induces degradation of the SUMO E2 ligase Ubc9 both in cell culture and in infected mice. The result is a decrease in the levels of cellular SUMO-conjugated proteins, which is likely to be beneficial for the bacteria as SUMO overexpression leads to decreased growth of intracellular Listeria.

Among other broad regulatory molecules that pathogens elegantly use are small ribonucleic acids (RNAs). The ‘never-ending tale of antiviral RNA silencing’ in plant pathogens was the topic of the talk of Olivier Voinnet (IBMP, Strasbourg). Olivier detailed a fascinating story of the host–pathogen arms race focusing on the RNA interference (RNAi) pathway. Although RNAi is thought to act as a host defense mechanism, Olivier described how plant viruses produce counter–defense proteins that suppress RNAi pathways, and the host in turn produces resistance proteins that can counteract the pathogen’s counter–defenses. Bryan Cullen (Duke University) focused on human viruses and micro-RNAs coded not only by the host genome but also by the virus itself. Bryan detailed the role of the oncogenic Kaposi’s sarcoma-associated herpesvirus (KSHV) miR-K1 that appears to directly down-regulate p21 and overcome the cell cycle blocking activities of p53. This in turn will promote proliferation, hence increasing the carcinogenic potential of the virus. KSHV miR-K11 is another interesting example of miRNA-based interactions between viruses and humans. miR-K11 is an ortholog of the cellular miR-155 and consequently can affect expression of the same miR-155 targets. While in this case, the relevance of this regulation for viral infectivity, pathology or immune response is not yet clear the fact that Epstein–Barr viruses (EBV) also affect the same miRNA155—

this time by promoting its expression in B-cells and possibly affecting the NF-kappaB pathway stabilizing latent persistence— indicates an important function for this molecule in the context of infections. The apicomplexan T. gondii also expresses several small RNAs, including heterochromatic siRNA typical of plants and metazoan like miRNAs that are likely to act as translational regulators in this system. Mohamed Ali Hakimi (Universtité Joseph Fourier, Grenoble) has been studying the small RNAome of this parasite and showed how varied it is and how dynamic its expression is. The fact that expression levels of several parasite miRNAs differ greatly in freshly egressed or intracellular growing parasites indicates that they too are important regulators of Toxoplasma’s life cycle.

The host cell response

In infection to fight the host fights back at every stage of the process. The first line of defense against pathogens is the presence of cellular barriers such as epithelia but pathogens use these barriers as docking sites to invade the host. In the case of HIV, it has been known that semen prevents the adhesion of the virus to epithelial cells. Sebastien Amigorena (Curie Institute, Paris) reported that the soluble protein Clusterin is the active component in semen that blocks HIV capture. Clusterin is involved in several cellular processes such as apoptosis and cellular adhesion. Although this protein is expressed ubiquitously, its glycosylation state is key for the effect and specific for semen. Despite this early blockage, the virus often succeeds in invading host cells where it faces further barriers. The tripartite motif 5 (TRIM5) is a host E3 ubiquitin ligase which plays an important role in restricting HIV-1 infections in several primates. TRIM5 directly binds to the HIV-1 capsid in the cytoplasm and prevents reverse transcription of the virus by mechanisms that remain unclear. Studying downstream signaling cascades and transcription regulation Jeremy Luban suggested that TRIM5 acts as a pattern-recognition receptor to detect HIV and trigger an intracellular antiviral response.
Mounting an efficient acquired immune response requires the coordination of several different cell types such as dendritic cells, B cells and T cells. One key challenge is understanding how rare populations of cells, for example antigen specific T cells and Ag bearing dendritic cells encounter each other in infected animals. Using intravital two-photon microscopy, Ron Germain demonstrated that T cells move along a fibroblastic reticular cell (FRC) network in the lymph nodes. This movement favours T-cell contacts with dendritic cells that are sitting on the same network and deposit chemoattractants on the fibres. In his talk, Ron also presented intravital movies of (BCG)-induced liver granulomas. Of particular interest was the correlation observed between antigen-induced arrest of effector T-cell migration and interferon (IFN)-gamma production, consistent with the immune system focusing effector cytokine production locally where and when it is needed.

Both innate and acquired immune responses need to be tempered before their activation causes unnecessary damage. Arturo Zychlinsky (Max Planck Institute for Infection Biology, Berlin) provided a striking example of this necessity by describing the role of neutrophils and neutrophil extracellular traps (NETs) in systemic lupus erythematosus (SLE). NETs are extracellular structures composed of deoxyribonucleic acid (DNA), histones and neutrophil proteins that capture and kill pathogens. Serum DNase1 is essential for disassembly of NETs after an infection and Arturo reported that a considerable number of SLE patients’ sera do not degrade NETs efficiently. These patients either lack an active DNAse I or the NETs produced are resistant to DNase I activity because of the large amounts of autoantibodies accumulated in these structures. The net result (so to speak) is the accumulation of NETs and the inflammatory syndrome that results.

A workshop on infection biology would not be complete without mentioning the well-established role of several infections in cancer development. Harald zur Hausen (DKFZ, Heidelberg) dedicated most of his career to proving the involvement of high-risk human papilloma viruses in cervical cancer and his closing keynote lecture highlighted many other instances where an association between a particular infection and cancer exists or is to be expected. zur Hausen put forward the hypothesis that an infectious agent may be associated with the higher incidence of colorectal, lung and breast cancer in red meat consumers.

The field of infection biology is replete with incredible examples of strategically planned and well-executed pathogen interventions. The host response is not always effective and indeed the outcome of an infection is often deleterious to the host. However, the quality of the science, the nature of the discoveries and the imaging and technical advances presented at this meeting made it clear that we are well on the way to knowing our enemies and ourselves. Sun Tzu would have liked to live in these exciting times…

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