The Global Health Institute (GHI), at Ecole Polytechnique Federale de Lausanne, plays an instrumental role in understanding various aspects of infectious diseases. The Life Science Symposium 2012, titled “Global Health Meets Infection Biology”, created a platform for excellent interaction among the top infection biologists across the globe. Infectious diseases which claim 18 million human lives each year and account for half of the deaths in the developing world is still the least understood. Prof. Stewart Cole (GHI, EPFL), gave a brief overview of the status of various infectious diseases in the world and shared some very interesting facts. The health budget can range from as low as $11/year in Eritrea to as high as $8262/year in Luxemburg. Zoonotic diseases are on constant rise with 2.5 billion cases and 2.7 million deaths per year. Keynote speaker Dr Rino Rappuoli from Novartis Vaccines and Diagnostics talked about “Vaccines and Global Health”. As pointed by Dr Rappuoli, the major events in the history can be linked to the infectious diseases. Siena, one of richest cities in Italy, suffered from terrible attack of plague, leading to “Black Death”, in 1348, which led to an unfinished cathedral. To combat the infectious diseases, vaccines came into picture. Twenty-first century vaccines still work on “inactivation and injection”. In the 21st century, there will be problems of aging society, emerging infection, and poverty. The biggest challenge will be to combat poverty and emerging infectious diseases. Dr Rappuoli emphasized new thinking which will lead to successful vaccine development. There were four sessions that dealt with various aspects of infectious diseases.

In the first session on “Global health”, Prof. Barry Bloom, a stalwart in the area of tuberculosis biology from Harvard School of Public Health, emphasized that “TB or not TB” is no longer the question. The success rate of current treatment is 84%. The highest burden of TB is in India, China, Indonesia, Nigeria, and South Africa. Further, the complications that continually threaten us are different forms of TB. Prof. Bloom further pointed out that mouse should be totally stopped as a model system when it comes to research with Mycobacterium tuberculosis. Prof. Myron Cohen, from University of North Carolina, talked about the prevention of transmission of HIV-1. Prof. Cohen pointed out that the enemy in this whole game is the “variant”. The mantra is “Tnt”, test and treat. Treat enough to the end of the pandemic of HIV was the message given by Prof. Cohen. Dr Christopher Dye, from WHO, touched upon a very vital aspect of infectious disease, i.e., “infectious diseases and changing lifestyle in developing countries”. Dr Dye pointed out that diabetes is a risk factor for TB. The barrier is thinning and the metabolic disorders can play an important role in infectious diseases. Dr Steven Sinkins, from the University of Oxford, talked about control of mosquito borne diseases and its relation to Wolbachia.

Session 2, titled “Evolutionary genomics of pathogens and their hosts”, dealt with bacterial genomic revolution, presented by Dr Mark Achtmann from University College Cork. Dr Achtmann talked about how bacterial evolution doesn’t follow Darwinian law! Dr Carmen Buchreiser, from Institut Pasteur, talked about intercellular parasitism of Legionella. Prof. Jean-Laurent Casanova, from Rockefeller University, discussed the genetic theory of infectious diseases. Prof. Casanova pointed out that primary lesion is the host genetic lesion. Prof. Beatrice Hahn, from the University of Pennsylvania, shared some of the very interesting facts about the great ape reservoirs of HIV and malaria.

Session 3, titled “Mechanism of infection”, threw light on the ways by which pathogen attacks the host. Dr Guillaume Dumenil from Inserm gave a presentation on how Neisseria meningitidis colonize the vascular endothelial cells. Prof. Denise Monack from Stanford School of Medicine spoke about how chronic Salmonella carriage is controlled by host regulator of fatty acid metabolism. Further, Prof. Natalie Strynadka from the University of British Columbia gave an insight into the importance of crystal structures into understanding the protein transport across bacterial membrane. Prof. Feng Shao from the National Institute of Biological Science talked about detailed dissection of the bacterial virulence factors and its interaction with macrophage.

Session 4, named “Innovative interventions”, was a very interesting session that talked about “out of the box” ideas.
This session saw pioneers like Prof. Antonio Lanzavecchia from Institute for Research in Biomedicine, Prof. Bali Pulendran from Emory Vaccine Center, Lutz Gissmann from the German Cancer Research Center, and Lennart Hammarstrom from Karolinska Institut. This symposium created very strong waves among the participants because of the wonderful short talks by the various researchers and the posters by the vibrant group of graduate students. Prof. John McKinney, from GHI, EPFL, who is an expert in research on *Mycobacterium tuberculosis*, played a very vital role in posing excellent questions and food for thought. The success of this meeting also goes to the administrators, Cécile Scherer Hayward and Lamy Suzanne, along with many others who made sure that almost everything was perfect! Such symposia bring together the researchers across the globe and form a very strong platform for further avenues in infectious biology.

### Vaccines and Global Health

**Rino Rappuoli**  
Novartis Vaccines and Diagnostics; Siena, Italy

During the 20th century, vaccines have eliminated most childhood diseases with the major exceptions of the diseases caused by meningococcus and respiratory syncytial virus (RSV). What is the role of vaccination in the 21st century? The first target will be to develop vaccines for meningococcal meningitis, which is perhaps the last disease that in a few hours can attack and kill healthy children and young people, and RSV, which affects virtually every single child in the first few months of life. Fortunately, thanks to several revolutionary technologies developed during the past 30 y, including conjugation, genomics, and new adjuvants, we are in the final stages to conquer meningococcal meningitis and new approaches are being tested for RSV. The second and perhaps most important target of vaccination in the 21st century will be to take care of the global health problems of this century. These include taking care of the aging population, with new vaccines targeting the diseases typical of the elderly with an aging immune system, to control emerging antibiotic resistance, to preventing cancer, taking care of the diseases present only in countries affected by poverty, and taking care of emerging diseases such as pandemic influenza. Overall, vaccines in the 21st century will have an increased safety, and will be used as an insurance to ensure health across all ages, for the entire life.

**Prevention of HIV-1 Infection**

**Myron S. Cohen**  
Institute of Global Health and Infectious Diseases; The University of North Carolina at Chapel Hill; Chapel Hill, NC USA

HIV-1 is transmitted by blood and blood products, from mother to baby, and through mucosal exposure to infected genital secretions. The magnitude of the HIV pandemic is governed by the per person contact transmission efficiency, the duration of infectiousness and the number of people exposed. The efficiency of transmission of HIV depends on the infectiousness of the index case and the susceptibility of those exposed. Infectiousness is dictated by the concentration of HIV-1 in relevant fluids (regardless of route of transmission) and the viral genotype and phenotype. Susceptibility (and resistance) to infection depends on the integrity of skin or mucosal surfaces, hereditary resistance, acquired immunity and any local factors that enhance or reduce transmission. People newly infected with HIV-1 (i.e., acute infection) and those with STD co-infections excrete such a large concentration of virus as to be “hyperinfectious”. The actual transmission of HIV likely occurs in the first few hours after exposure. The probability of transmission may be as low as 1/10000 episodes of intercourse or 1/10 sexual exposures when anal intercourse is practiced. The transmission of HIV is generally limited to one or a small number of founder variants which themselves may be “hyperinfectious”. Synergistic behavioral and biologic HIV prevention strategies have been developed. Safer sex includes limiting the number of sexual partners, use of male latex condoms, and structural interventions to reduce exposure. Biological interventions include: treatment of inflammatory cofactors, voluntary male circumcision, and use of antiviral agents either for infected people (who can be rendered much less contagious) or as pre- or post-exposure prophylaxis. There is increasing ecologic evidence that broader use of antiviral treatment is reducing incidence of HIV. However, maximal benefit of HIV “treatment for prevention” will likely require a program of universal “test and treat”, where many more infected patients are identified, linked to care, and treated very early in disease and for life. This strategy is resource-intensive and demands that therapy evolve toward a cure, so that HIV-infected people can eventually reduce or stop...
Hyperpathogenism: Viral Parasites of Human Pathogens and Their Influence on Disease

Catherine Ronet, Mary-Anne Hartley, Patrik Castiglioni, Matteo Rossi, Onur Eren, Steeve Beverley and Nicolas Fasel

Because viral replication depends on the vigor of its host, many viruses have evolved incentives of fitness to pay their keep. When the viral host is a human pathogen, these fitness factors can surface as virulence: creating a Russian doll of pathogenesis where parasites within parasites complicate the disease process. We dub this process “hyperpathogenism” and have exposed its importance as major clinical consideration in metastatic leishmaniasis. Here, our lab has recently shown that a virus within metastatic leishmania parasites (Leishmania RNA virus: LRV) can act as an independently immunogenic entity, where its RNA-based nucleic acid acted as a potent innate immunogen, triggering a destructive hyper-inflammatory cascade through Toll-like receptor 3 recognition. Interestingly, our preliminary data show this LRV-specific immune response can be rendered protective if primed before the infection as a vaccine, raising the importance of cross talk between the innate responses to the various components of this nested infection. By appreciating the microbial-virus as a backseat driver of human disease, we may be able to better formulate appropriate clinical intervention or even exploit its presence for having potential as novel molecular target for therapeutic and prophylactic intervention.

Infectious Diseases and Changing Lifestyles in Developing Countries

Christopher Dye
World Health Organization; Geneva, Switzerland

Low body mass and diabetes have been treated as distinct risk factors for tuberculosis (TB) although they are linked components of the nutritional profile of populations. While diabetes enhances the risk of TB, a greater body mass index (BMI) is protective, and yet diabetes is more frequent among people who are overweight. To add to the complexity at population level, BMI distribution, diabetes prevalence, and TB incidence vary by age and sex and differ between rural and urban areas. In particular, TB incidence changes with age both directly (because the prevalence of infection and the risk of progression from infection to active TB are age-dependent) and indirectly (through its effects on BMI and diabetes as risk factors). Population aging is expected to affect TB incidence through these direct and indirect routes, and the same is true of urbanization. TB offers just a glimpse at the web of interactions that exist in developing countries as they undergo demographic and epidemiological transitions. Will infectious disease control programs be helped or hindered as the prevalence of non-infectious diseases increases with better nutrition in growing, aging, urbanizing populations?

Wolbachia and the Control of Mosquito-Borne Diseases

Steven Sinkins

The maternally inherited bacterial endosymbiont Wolbachia pipiens is capable of rapid spread into insect populations using cytoplasmic incompatibility (CI), the imposition of crossing sterilities that give infected females a reproductive advantage. Recent studies have also demonstrated that the presence of certain strains of Wolbachia in mosquitoes can inhibit the development/dissemination of various important human pathogens. In the invasive mosquito Aedes albopictus, we have created a transfection with the wMel strain of Wolbachia from Drosophila, and shown that it can block the transmission of dengue and other arboviruses. Bidirectional CI is generated with wild-type populations, which provides a mechanism for introducing this strain to high frequency/fixation in natural populations of this species in a stable and geographically limited manner. Mosquito immune gene transcription was not elevated by the transinfection, suggesting that strong Wolbachia-mediated viral inhibition can operate independently of the innate immune pathways. We have created somatic infections of Wolbachia in Anopheles gambiae and observed both immune gene upregulation and inhibition of the development of Plasmodium parasites; prospects for the use of Wolbachia in malaria control will also be discussed.

Comparative Genomics of Drug Resistance in African Trypanosomes

Fabrice Graf, Philipp Ludin, Christina Kunz-Renggli, Harry P. de Koning and Pascal Mäser

We are investigating the molecular mechanisms of drug resistance in African trypanosomes, the causative agents of human sleeping sickness. Two bloodstream-form T. b. rhodesiense lines
have been selected in vitro over the course of two years for resistance against the clinical drugs melarsoprol and pentamidine, respectively. Both lines exhibit cross-resistance to either drug, as well as to other diaminidines and adenosine analogs. We applied next generation sequencing to find the mutations causing drug resistance. Comparative transcriptomics and genomics revealed the complete absence of the gene \( TbAT1 \) in the melarsoprol-selected line and a non-synonymous point mutation in \( TbAT1 \) of the pentamidine-selected line. \( TbAT1 \) encodes an adenosine transporter that also mediates cellular uptake of melarsoprol and pentamidine. However, genetic knockout of \( TbAT1 \) in wild-type trypanosomes produced a smaller drug resistance phenotype than exhibited by the two selected lines. Thus additional mutations must be involved. Interestingly, both resistant \( T. b. rhodesiense \) lines have lost the aquaglyceroporin gene \( TbAQP2 \). Human AQP9 was shown to transport arsenite. Our findings suggest that \( T. brucei \) AQP2 transports organic arsenicals and plays a major role in the long-known phenomenon of cross-resistance between melarsoprol and pentamidine in trypanosomes.

**Population Genetics and Genomics of Genetically Monomorphic Bacterial Pathogens**

Mark Achtman

University College Cork; Cork, Ireland

Despite the dramatic genetic diversity associated with most bacterial taxa, some show only limited diversity, and have been called genetically monomorphic. These include multiple deadly pathogens which have been assigned species designations, including *Bacillus anthracis* (anthrax), *Mycobacterium leprae* and *Yersinia pestis* (plague). However, each of these represents a recently evolved clade of a species with more diversity, and multiple genetically monomorphic clades exist within many species, e.g., serovar Typhi (typhoid) within *Salmonella enterica* or epidemic *Vibrio cholerae* (cholera). A general feature of many (but not all) genetically monomorphic pathogens is that they show clonal patterns of descent, where homologous recombination and horizontal gene transfer are rare. This makes them ideal for comparative genomics based on single nucleotide polymorphisms that can be revealed by short-read genomic sequencing. In some cases, the mutation rate is sufficiently high that real-time microevolution over decades has been observed, e.g., clades of *Staphylococcus aureus*. In other cases with a slower mutation rate, the most recent common ancestor first evolved a few millennia ago, e.g., *Y. pestis*. Phylogeographic correlations with genetic lineages are increasingly being used to reconstruct the historical patterns of spread of these bacteria, as will be illustrated with recent reconstructions of the history of plague.

**Intracellular Parasitism, the Driving Force of Evolution of Legionella, the agent of Legionnaire Disease**

Carmen Buchrieser

Institut Pasteur, Paris, France

*Legionella pneumophila* is the etiological agent of Legionnaire disease and Pontiac fever, the latter being a less acute disease. It is a gram-negative bacterium present in environmental and artificial water environments that replicates in protozoan hosts and is also found in biofilms. When aerosolized *Legionella* are inhaled, they are able to colonize the respiratory tract, invade alveolar macrophages and replicate intracellularly, thereby causing the disease. In the environment, replication within protozoa is essential for the survival of the bacterium. Analysis of the genome sequence of *L. pneumophila* revealed the presence of an unexpected high number and variety of eukaryotic-like proteins, predicted to be involved in the exploitation of the host cellular cycle by mimicking specific eukaryotic functions. Among those are proteins containing F-Box domains, U-Box domains, SET domains, Ankyrin repeat proteins, or a sphingosin-1-phosphate lyase. Eukaryotic-like proteins have also been identified in other bacterial pathogens, however a comparative analysis shows that *L. pneumophila* ranks as one of the pathogens that encodes the most and the widest variety of eukaryotic-like proteins and/or proteins with eukaryotic domains. Recent published results and our ongoing work show that many of these proteins are implicated in modulating host cell functions during the intracellular life of *L. pneumophila*. A systematic phylogenetic analysis indicates that lateral gene transfer from eukaryotic hosts, such as protozoa, has contributed to the necessary adaptation processes with respect to the evolution of virulence and the emergence of a human pathogen, *L. pneumophila*, from the environment.

**PE_PGRS Genetic Diversity in Mycobacterium tuberculosis Is Not Related to Immune Escape**

Mireia Coscollá,1,2 Richard Copin,3 Salome Seiffert,1,2 Stephanie Birrer,1,2 Daphne Davis,3 Joel Ernst3 and Sebastien Gagneux1,2

1Swiss Tropical and Public Health Institute; Basel, Switzerland; 2University of Basel; Basel, Switzerland; 3New York University School of Medicine; New York, NY USA

Recent evidence has shown that human T cell epitopes in human-associated *Mycobacterium tuberculosis* complex (MTBC) are evolutionarily hyperconserved and under strong purifying selection. However, putative antigenic properties have been attributed to PE_PGRS gene family, in part because of their high genetic diversity compared with the otherwise highly conserved MTBC genome. The objective of this study was to explore whether
human T cell recognition is driving the increased genetic diversity in PE_PGRS genes of MTBC.

We first sequenced the open reading frames encoding 25 PE_PGRS and 2 PE_Unique domain proteins in 94 phylogeographically diverse clinical isolates of MTBC. We then analyzed the 64 annotated PE_PGRS proteins in the H37Rv reference genome for the presence of putative CD4+ and CD8+ T cell epitopes using bioinformatic analyses. Finally, we combined these epitope predictions with our sequence diversity data. We found that individual PE_PGRS genes differed widely in their genetic diversity and in the direction and extent of selective pressures acting on them, suggesting many of these genes have distinct and non-redundant functions. We found that the large majority of predicted CD4+ and CD8+ T cell epitopes were confined to the conserved PE domain, and that despite being genetically diverse, the PGRS domains harboured hardly any predicted T cell epitopes. Taken together, these results weigh against the view that PE_PGRS proteins are a source of variation of T cell antigens in MTBC, and reinforce the observation by Comas et al. that MTBC epitopes are in fact hyperconserved.

Role of the Group B Antigen of Streptococcus agalactiae: A Peptidoglycan-Anchored Polysaccharide Involved in Cell Wall Biogenesis

Shaynoor Dramsi,1 Elise Caliot,1 Marie-Pierre Chapot-Chartier,2 Pascal Courtin,2 Saulius Kulakauskas,2 Christine Péchoux,3 Patrick Trieu-Cuot1 and Michel-Yves Mistou2

1Unité des Bactéries Pathogènes à Gram Positif; Institut Pasteur; Paris, France; 2CnrS ERL; Paris, France and INRA; UMR1319; MICALIS; Jouy-en-Josas, France; 3INRA; Plate-forme MIMA2; Jouy-en-Josas, France

Streptococcus agalactiae (group B streptococcus, GBS) is a leading cause of infections in neonates and an emerging pathogen in adults. The Lancefield Group B carbohydrate (GBC) is a peptidoglycan-anchored antigen that defines this species as a group B streptococcus. Despite earlier immunological and biochemical characterization, the molecular basis and the function of this abundant glycopolymer have never been addressed experimentally. Here, we inactivated the gene gbcO encoding a putative UDP-N-acetylgalactosamine-1-phosphate:lipid phosphate transferase thought to catalyze the first step of GBC synthesis. Indeed, the gbcO mutant was unable to synthesize the GBC polymer, and displayed an important growth defect in vitro. Electron microscopy study of the GBC-depleted strain of S. agalactiae revealed a series of growth-related abnormalities: random placement of septa, defective cell division and separation processes, and aberrant cell morphology. Furthermore, vancomycin labeling and peptidoglycan structure analysis demonstrated that, in the absence of GBC, cells failed to initiate normal PG synthesis and cannot complete polymerization of the murein sacculus. Finally, the subcellular localization of the PG hydrolase PcsB, which has a critical role in cell division of streptococci, was altered in the gbcO mutant. Collectively, these findings show that GBC is an essential component of the cell wall of S. agalactiae whose function is reminiscent of that of conventional wall teichoic acids found in Staphylococcus aureus or Bacillus subtilis. Furthermore, our findings raise the possibility that GBC-like molecules play a major role in the growth of most if not all β-hemolytic streptococci.

Toward a Genetic Theory of Infectious Diseases

Jean-Laurent Casanova

The Rockefeller University; New York, NY USA

The hypothesis that inborn errors of immunity underlie infectious diseases is gaining experimental support. However, the apparent modes of inheritance of predisposition or resistance differ considerably between diseases and between studies. A coherent genetic architecture of infectious diseases is lacking. We suggest here that life-threatening infectious diseases in childhood, occurring in the course of primary infection, result mostly from individually rare but collectively diverse single-gene variations of variable clinical penetrance, whereas the genetic component of predisposition to secondary or reactivation infections in adults is more complex. This model is consistent with (1) the high incidence of most infectious diseases in early childhood, followed by a steady decline, (2) theoretical modeling of the impact of monogenic or polygenic predisposition on the incidence distribution of infectious diseases before reproductive age, (3) available molecular evidence from both monogenic and complex genetics of infectious diseases in children and adults, (4) current knowledge of immunity to primary and secondary or latent infections, (5) the state of the art in the clinical genetics of non-infectious pediatric and adult diseases, and (6) evolutionary data for the genes underlying single-gene and complex disease risk. With the recent advent of new-generation deep resequencing, this model of single-gene variations underlying severe pediatric infectious diseases is experimentally testable.

Great Ape Reservoirs of AIDS and Malaria

Beatrice Hahn

University of Pennsylvania; Philadelphia, PA USA

It is now well established that HIV-1, the cause of the global AIDS epidemic, is of chimpanzee origin, while Plasmodium falciparum, the cause of malignant malaria, is derived from a gorilla parasite. Thus, two of the most widespread and virulent diseases of modern man appear to have originated in our closest relatives, the African apes. This talk will compare and contrast the ape origins of AIDS and malaria, and discuss important parallels that could provide insight into the prospects of future zoonoses.
Vascular Colonization by Neisseria meningitidis

Guillaume Duménil
INSERM U.970; Equipe Avenir/Equipe 9; Centre de Recherche Cardiovasculaire; Paris, France

Bacterial infection of human vasculature can lead to unregulated systemic activation of coagulation and innate immunity and rapidly becomes life threatening. Neisseria meningitidis is a vascular pathogen that causes fatal sepsis and meningitis. Post-mortem histological analysis of tissues from individuals infected with N. meningitidis show large bacterial aggregates in close association with the vascular wall of small vessels. The ability of this bacterium to colonize blood vessel endothelium is likely to impact its capacity to both multiply in the blood stream and reach the brain. Focusing on the bacterial–host interactions recent work describing sequential steps in N. meningitidis vascular colonization will be described.

Chronic Salmonella Carriage Is Controlled by a Host Regulator of Fatty Acid Metabolism

Denise Monack
Stanford School of Medicine; Stanford, CA USA

The global burden of disease caused by intracellular pathogens remains one of the largest challenges facing the international biomedical community. Millions of human cases of salmonellosis are reported worldwide every year, resulting in huge economic costs to society and thousands of deaths. Salmonella Typhi is a human-specific bacterial pathogen that causes the disease typhoid fever. Typhoid fever affects more than 20 million people per year and causes greater than 220 000 deaths annually. A significant percentage (1–6%) of typhoid patients become chronic carriers of S. Typhi. Chronically infected hosts transmit disease to naïve individuals and are critical reservoirs for disease and thus pose significant public-health problems. From the bacterial perspective, persistent infection is essential for microbial survival in nature. However, very little is known about the molecular mechanisms involved in chronic Salmonella infections of mammalian hosts. Thus, investigating the chronic carrier state in salmonellosis should provide insight into bacterial survival strategies. We have shown previously in a mouse typhoid model that S. Typhimurium persists in macrophages within systemic tissues of chronically infected mice. However, very little is known about the physiological state of the macrophages that harbor intracellular Salmonella in chronic carriers. In the studies described here, we show during a long-term systemic S. Typhimurium infection in mice that there is an initial pro-inflammatory/Th1 cytokine response followed by an anti-inflammatory/Th2 cytokine response. S. Typhimurium preferentially associates with anti-inflammatory/M2 macrophages at later stages of infection, indicating that these cells might represent a unique niche for its long-term intracellular survival. In addition, we show that PPARγ, a transcriptional factor that plays a role in sustaining fatty acid metabolism in M2 macrophages, is upregulated in Salmonella-infected macrophages. In the absence of PPARγ, S. Typhimurium is unable to replicate in macrophages and we link this to availability of glucose. Importantly, PPARγ-deficient mice are not chronically infected with S. Typhimurium. Altogether, this work describes the molecular mechanisms underlying a bacterial pathogen’s dependence on macrophage metabolism for chronic carriage.

Single and United! Essential Virulence Strategy of Salmonella to Evade the Host Defense

Dipshikha Chakravortty1 and Sandeepa M. Eswarappa2
1Department of Microbiology and Cell Biology and Center for Infectious Disease; Indian Institute of Science; Bangalore, India; 2Cleveland Clinic; Cleveland, OH USA

Salmonellosis! Gives a shiver. Each industry, be it food, dairy or poultry, dreads this organism. Salmonella belongs to group of gram-negative bacteria with 2300 serovars. CDC has placed this organism in bioterrorism category II as it has a great potential to cause economic damage along with high morbidity and mortality. Salmonella has a capability to cause wide range of infection from localized gastroenteritis to systemic typhoid fever. Salmonella requires a specific niche for its successful life inside the host. This niche is the Salmonella-containing vacuole (SCV). Being an intracellular pathogen, Salmonella has evolved strategies to counteract intracellular microbicidal agents like reactive oxygen species, reactive nitrogen species and antimicrobial peptides. However, it is not clear how exactly Salmonella escapes from lysosomal degradation. Here, we have addressed this matter by investigating whether the infected host cell has sufficient quantity of lysosomes to target growing number of Salmonella. Immunofluorescence microscopy and transmission electron microscopy demonstrated that infected host cell contains many Salmonella-containing vacuoles (SCVs) having single bacterium per vacuole. This was due to the division of SCV along with the bacterial division which increases the number of SCVs per host cell that are to be targeted by lysosomes. Flow cytometry demonstrated that Salmonella decreases the quantity of acidic lysosomes inside the host cell. These events potentially result in a condition where an infected cell is left with very few lysosomes to target the overwhelming number of SCVs. This condition favors Salmonella as there are not enough lysosomes to counteract them. Overall, our results demonstrate that on one hand SCV undergoes division, increasing the number of vacuoles to be targeted by lysosomes and other microbicidal agents, and on the other hand, Salmonella decreases the number of acidic lysosomes in the infected host cells.
Salmonella Typhimurium (S. Tm) cooperatively express costly virulence factors (VF) to elicit inflammation, a “public good” that aids the pathogen to outgrow the intestinal microbiota. By definition, cooperation is prone to be sapped by the invasion of defectors, i.e., mutants which never cooperate but profit from the “public good”. How S. Tm deals with defectors during infection was, until now, unclear. Among VF, the type three secretion system 1 (T1) is essential to trigger inflammation. Interestingly, from the same genome, S. Tm stochastically forms T1 expressing (T1+) and T1 non-expressing (T1-) cells. Switching from one state to the other occurs by a process called bistable gene expression. Thus only a part of the S. Tm population undergoes the cost of virulence expression. We therefore hypothesized that bistable T1 expression could stabilize cooperation by generating a subpopulation (T1-) excluding defectors. This was studied by computational modeling, within-host evolution experiments, genome re-sequencing, and mutant analysis. Results observed in vivo demonstrated that hilE, a negative regulator limiting T1 expression to a small subpopulation of the S. Tm cells, was of key importance. Disrupting hilE, i.e., increasing T1+ cells proportion, accelerated defector invasion to such an extent that inflammation subsided and the S. Tm population declined. This study shows for the first time how a fine-tuned bistable switch in gene expression allows a division of labor stabilizing cooperation-based virulence: T1+ cells produce the costly “public good”, while T1- cells compete against defectors.

Biochemical Dissection of Bacterial Virulence and Macrophage Innate Immunity

Feng Shao

National Institute of Biological Sciences; Beijing, China

Gram-negative bacterial pathogens use a conserved type III secretion system to inject virulent effector proteins into host cells. These effectors often use fine and sophisticated strategies to manipulate or block host signal transduction pathway, particularly those related to innate immunity, thereby promoting bacterial survival and infection. I will present our work on the mechanisms of three important bacterial effector families, each of which defines a novel enzymatic posttranslational modification, as well as the functional role of these modifications in bacterial infection. Specifically, the OspF effector from Shigella flexneri employs an unprecedented MAPK phospho-threonine lyase activity to shut down host MAPK signaling pathways and block IL-8 production during infection. The CHBP/Cif family of type III effectors from Burkholderia pseudomallei and certain strains of enteropathogenic E. coli (EPEC) function as glutamine deamidase to modify host ubiquitin and ubiquitin-like protein NEDD8, thereby inactivating the ubiquitination pathway and disrupting many important host cellular processes that are regulated by ubiquitin modification. The NleE effector from EPEC and Salmonella Typhimurium carries out a novel cysteine methylation modification on the key ubiquitin-chain sensory proteins in host NFκB signaling pathway, leading to block of the NFκB signaling during EPEC infection. On the host side, the newly proposed inflammasome complex is believed to play important roles in macrophage innate immune defense against bacterial infection. I will also discuss our recent identification and characterization of the NAIP family of NOD-like proteins (NLRs) that indeed serves as inflammasome receptors for directly sensing bacterial flagellin and also the type III secretion apparatus, which plays an important role in restricting various bacterial infections.

Structure-Based Analysis of Protein Transport Across Bacterial Membranes

Natalie Strynadka

Department of Biochemistry and Molecular Biology; University of British Columbia; Vancouver, BC Canada

Bacteria have evolved several dedicated and sophisticated assemblies to transport proteins across their biological membranes. Recent advances in our understanding of the molecular details governing the specific actions of these protein secretion systems has come from an integrated approach of X-ray crystallography, NMR, mass spectroscopy, electron microscopy, and in vitro reconstitution/in vivo phenotypic analysis. Highlights of recent advances will be presented with an emphasis on that of the type III secretion system, the so-called bacterial “injectisome” encoded exclusively by pathogenic gram-negative strains including Salmonella Typhimurium, Yersinia pestis, Pseudomonas aeruginosa, and enteropathogenic Escherichia coli. A structure-based and genetic piecing together of the type III secretion system indicates that more than two dozen proteins assemble into a large needle shaped complex spanning the inner and outer bacterial membranes as well as that of the infected host cell, providing a direct conduit for the transport of essential bacterial virulence effectors from bacterial to host cytosol. A molecular understanding of the type III systems being garnered from these studies provides the foundation for the development of new classes of vaccines and antimicrobials to combat these pathogens in the clinic and community.
**Infection and Cancer**

Lutz Gissmann  
German Cancer Research Center (DKFZ); Heidelberg, Germany

The current estimation of the cancer cases that are related to infectious events varies between 18% and 20% and there is evidence that this number is still growing. Besides *Helicobacter pylori* and some parasites there are only viruses for which a causative relation to malignant diseases has been demonstrated. Establishing such a link depends on the epidemiologic profile of the disease and the putative causal infectious agent as well as its biologic properties in experimental cell culture and in vivo models. Often one of the classical Koch postulates (isolation of the putative agent from the affected tissue) cannot be fulfilled and, in some instances, not even traces of the microbe (i.e., its nucleic acid) can consistently be found in the tumor. The reason might be that cancer is the late and rare consequence of a chronic infection where replication of the infectious agent does no longer take place and gene functions are not required for maintenance of the tumor growth. Therefore a combination of observational and experimental strategies has to be applied to collect sufficient evidence and to initiate attempts for preventive measures that target the infectious agent such as development of vaccines. Examples of classical and innovative approaches will be presented.

**Antibody-Mediated Treatment of Infections Using Genetically Modified Bacteria and Plants**

Lennart Hammarström  
Department of Laboratory Medicine; Karolinska Institutet at KUS Huddinge; Stockholm, Sweden

Replacement with immunoglobulins is a standard form of prophylaxis for antibody deficient patients. Gammaglobulin therapy is also given to selected patients with a variety of infections. Two forms of treatment have been given as intravenous infusions but this has been gradually been replaced by home treatment with gammaglobulin given by the subcutaneous route. As some patients still suffer from infections at mucosal sites, we have supplemented their therapy with topical application of antibodies or antibody fragments given as eye drops, nose drops or as a drinking solution. Novel methods for the production of these antibodies are currently being developed, including transformed lactic acid bacteria and transgenic plants. In the former case, antibody fragments (scFv, VHH) are being secreted by transformed lac bacteria, selected for tropism at the respective site, allowing local, in vivo production of the desired antibodies at various mucosal surfaces (including the oral cavity, the stomach, the intestines, and the vaginal tract). The latest applications of this technology, includes the use of bacteria producing two antibody specificities in order to reduce the appearance of escape mutants. Recently, we have extended our work to encompass production of selected VHH fragments in transgenic plants (rice), where production of endogenous storage proteins has been suppressed, allowing high yield production. The unique thermostable properties of VHH fragments allow boiling of the rice, subsequently using the rice water for therapy in a mouse model of rotavirus infection.

**Force Volume and Stiffness Tomography**

**Investigation of Bacterial Membranes**

Giovanni Longo,1 Laura Marques Rio,2 Andrej Trampus,3 Alain Bizzini,1 Giovanni Dietler1 and Sandor Kasas1

1Laboratory of Physics of Living Matter; Ecole Polytechnique Fédérale de Lausanne (EPFL); Lausanne, Switzerland; 2Institute of Microbiology; Faculty of Biology and Medicine; University of Lausanne and University Hospital Lausanne (CHUV); Lausanne, Switzerland; 3Infectious Diseases Service; Department of Internal Medicine; University Hospital Lausanne (CHUV); Lausanne, Switzerland

During the past years, the importance of high-resolution analysis of the microbial cell surface has been increasingly recognized. A thorough investigation of the surface modifications induced by different environmental conditions can prove to be essential for the rapid diagnosis and treatment of infections. In particular, any kind of study leading to a better comprehension of the effects of antibiotics on bacteria and to identify the most effective antimicrobial treatment could significantly decrease overall morbidity and mortality.

Several instruments are available to observe at high-resolution specific properties of specimens. Specifically, AFM, due to its versatility and simplicity, is among the most widely used instruments to perform mechanical and adhesive characterizations of living biological systems in liquid environment. Indeed, AFM can routinely image single bacteria at a nanometric scale, in physiological environment and can study the mechanical properties of its membrane.

Such analyses coupled with high resolution investigation of morphological properties of the cell surfaces, are increasingly used to characterize the state of single cells: it has been demonstrated that some of their properties can reflect their physiological state and that they can be clearly altered in some pathological conditions or in specific environmental conditions. It seems reasonable to extrapolate these observations to bacteria.

In this work we present an analysis of the surface mechanical properties of two different strains, *Escherichia coli* and *Staphylococcus aureus*, exposed to different environmental conditions. We will show images collected by performing several force curves on bacterial surfaces in order to reconstruct a map of the membrane stiffness. We will show the variation of the bacterial membrane stiffness when exposed to different media such as antibiotics. These measurements will allow defining how the surface stiffness can change between dormant or active states and what is the effect of the antibiotic on the bacterial structure.
The existence of phenotypic heterogeneity within isogenic cell populations and promoting survival in fluctuating environments has been extensively demonstrated. Quantitative analysis of individual cells as a function of time allows a more accurate delineation of the cell-to-cell variations compared with population-averaged measurements. Our studies focus on real-time single-cell analysis of Mycobacterium tuberculosis (Mtb), in order to elucidate its phenotypic diversity, specifically looking at the growth dynamics during infection. We postulate that phenotypic heterogeneity may promote the long-term survival of Mtb within the microenvironments of the lung.

In several bacterial species it has been observed that the number of ribosomes per cell positively correlates with the growth rate of the cell. Therefore, we decided to explore the utility of rRNA output as a correlate of the growth rate at the single cell level and to estimate the variation in mycobacterial growth state at different levels of environmental complexity namely, axenic cultures and host environments. Toward this, we constructed fluorescent Mtb reporter strains expressing short-lived GFP variants under the control of the 16S ribosomal promoter (P_16S). We used microfluidic tools in conjunction with snapshot and time-lapse fluorescence microscopy to measure in real-time the variation in growth rates, and fluorescence of individual cells.

Our preliminary results indicate that our P_16S::gfp reporter strain faithfully reflected mycobacterial growth dynamics at the single-cell level, displaying a positive correlation between the green fluorescence (rRNA output) and growth rate. We also observed a considerable amount of phenotypic heterogeneity with respect to growth rate and rRNA output when Mtb was subjected to different stresses such as nutrient starvation, antibiopic exposure, residence inside macrophages or in vivo host tissue environment. Interestingly, the levels of green fluorescence (rRNA output) also appeared to be a good predictor of the ability of the cells to resume growth after exposure to stress.

Memory T and B lymphocytes and long lived plasma cells represent a repository of the antigenic experience of an individual. By analyzing the specificity and function of such cells we can gain insights into the human immune response, understand host-pathogen interaction and identify correlates of protection and pathology. I will describe three methods that we have used to interrogate T, B, and plasma cell repertoires and I will focus on two main aspects. The first deals with the identification of subsets of effector and memory CD4+ T cells and their role in immune surveillance and protection against different classes of pathogens. The second deals with the dissection of the human antibody response to viruses and with the identification of human monoclonal antibodies with potent and broadly neutralizing activity.

**Systems Vaccinology: Enabling Rational Vaccine Design with Systems Biology**

Bali Pulendran

Despite their great success, we understand little about how effective vaccines stimulate protective immune responses. Two recent developments promise to yield such understanding: the appreciation of the crucial role of the innate immune system in sensing microbial agents and tuning immune responses, and advances in systems biology. In this presentation, I will discuss how these developments are yielding insights into the mechanism of some of the most successful vaccines ever developed. Furthermore, such developments promise to address a major challenge in vaccinology: that the efficacy of a vaccine can only be ascertained retrospectively, upon infection. The identification of molecular signatures induced rapidly after vaccination, which correlate with and predict the later development of protective immune responses, would represent a strategy to prospectively determine vaccine efficacy. Such a strategy would be particularly useful when evaluating the efficacy or immunogenicity of untested vaccines, or in identifying individuals with sub-optimal responses among high risk populations, such as infants or the elderly. We have recently used a systems biology approach to identify early gene signatures that correlate with, and predict the later immune responses in humans vaccinated with the live attenuated yellow fever vaccine YF-17D, or with the influenza vaccines. I will review these studies, and discuss their broader implications for vaccinology.