A temporal and spatial framework for vaccine design

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Control over *when* and *where* vaccines deliver antigen and adjuvant signals profoundly shapes the magnitude, quality, and durability of protective immunity. This seminar will highlight two complementary approaches - temporal dosing optimization and mucosal boosting - that together can inform the design of next-generation vaccines.

Firstly, “extended prime” immunizations that prolong initial antigen exposure enhance humoral immune responses to a variety of subunit vaccines. We show that a simplified two-dose extended prime regimen, timed to synchronize with the germinal center (GC) reaction, can amplify humoral responses. Computational modeling of the GC response suggested that this is mediated by antigen delivered in the second dose being captured as immune complexes in follicles, an effect that can be amplified by prolonged antigen exposure in the second dose administration, predictions we verified experimentally.

Next, we introduce Bioactive Enhanced Adjuvant Chemokine Oligonucleotide Nanoparticles (BEACON) as a mucosal adjuvant for vaccination against herpes simplex virus (HSV). Following systemic priming, mucosal boosting with BEACON and HSV glycoproteins increased vaginal $CD8^+$ tissue-resident memory T cells and mucosal IgG and IgA responses, while inducing minimal vaginal inflammation. This strategy conferred superior protection when compared with intramuscular boosting, reducing disease severity and suppressing viral load in vaginal mucosa and dorsal root ganglia.

Together, these findings illustrate how temporal and spatial modulation of vaccination can amplify systemic and mucosal immunity, providing a blueprint for vaccines against infectious diseases and cancer.

Dynamic membrane remodeling by a self-organizing bacterial kinase–phosphatase duo

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The ability to self-organize and form dynamic structures is a fundamental property of living organisms. Although there are theoretical models describing how self-organization arises, only a few biochemical systems have been rigorously characterized. Here we report a self-organizing system consisting of a bacterial phosphoinositide kinase and its opposing phosphatase that exhibit spatiotemporal patterns, including traveling waves, to remodel host cellular membranes. The *Legionella* effector MavQ, a phosphatidylinositol (PI) 3-kinase, is targeted to the host cell's endoplasmic reticulum (ER). MavQ and the *Legionella* PI 3-phosphatase SidP, even in the absence of other bacterial components, drive rapid PI 3-phosphate turnover on the ER and spontaneously form traveling waves that spread along ER subdomains and induce vesicle/tubule budding. Evidence from in vitro reconstitution strongly suggests that a Turing-like reaction–diffusion mechanism accounts for the behavior of the MavQ/SidP system. Our results not only exemplify the importance of self-organizing behaviors that result from chemically interacting kinases and phosphatases in complex cellular behaviors but also reveal a mechanism that intracellular bacterial pathogens use to remodel host cellular membranes for survival.

Viral genetic factors influencing mosquito transmission and mouse pathogenicity: contrasting African and Asian Zika Virus lineages

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Zika virus is a mosquito-borne flavivirus primarily transmitted among humans by *Aedes aegypti*. Over the past two decades, it has caused significant outbreaks associated with birth defects and neurological disorders. Phylogenetically, Zika virus is divided into two lineages: African and Asian, each exhibiting distinct biological properties. African lineage Zika virus strains are more transmissible by *Aedes aegypti* and more pathogenic to mice than their Asian counterparts, however the genetic determinants underlying these differences are unknown. In this study, we addressed this question by comparing recent African and Asian strains by engineering a panel of chimeric viruses, in which segments of the parental genomes were swapped. We infected mosquitoes and mice with these chimeric viruses to investigate the effects of specific viral genes. Our findings revealed that the structural genes from the African strain enhanced viral internalization, while the non-structural genes improved the production of infectious particles in mosquito cells. When we compared the chimeric viruses in mosquitoes *in vivo* via oral exposure, we found that transmission prevalence (the proportion of mosquitoes with a systemic infection whose saliva contained infectious virus) was most significantly influenced by the structural genes, irrespective of the mosquito genetic background. Additionally, we developed a stochastic model of *in vivo* viral dynamics in mosquitoes that mirrors the observed patterns, suggesting that the primary difference in transmission prevalence between the African and Asian strains lies in their ability to traverse mosquito salivary glands. We further infected *Ifnar1* knockout mice with the chimeric viruses through subcutaneous injection and found that the non-structural genes from the African strains were associated with an increased clinical score, while the structural genes from the same strains led to severe viremia.
Overall, our findings suggest that the phenotypic differences the two lineages are influenced by multiple genetic factors rather than a single mutation. This underscores the complexity of viral adaptation, which is often constrained by epistatic interactions and lineage-specific adaptive landscapes.

Innate immune response to *Vibrio cholerae* infection

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Vibrio cholerae colonizes the human small intestine and causes an acute, severe diarrhea that kills ~100,000 people every year. Young children are especially susceptible to death by cholera. Despite the importance of innate immunity in preventing *V. cholerae* infection, how the host's innate immune system responds to *V. cholerae* remained largely unclear.

We have analyzed the transcriptome profiles of epithelial cells and immune cells at the single-cell level in an infant mouse *V. cholerae* infection model. *V. cholerae* infection increased a subset of enterocytes specialized for defense responses, which express antimicrobial proteins and fibrinogens. In CD45+ immune cells, type 3 innate lymphoid cells (ILC3) dominantly respond to the infection. ILC3s express interleukin-22 (IL22) in response to infection, which mediates the increase in the defense subset of enterocytes.

Administration of IL22-Fc fusion protein protects infant mice from *V. cholerae* colonization, diarrhea, and death. Mechanistically, 1. Antimicrobial protein Reg3β, secreted by enterocytes, directly kills *V. cholerae*, 2. IL22 promotes stem cell differentiation into mucus-secreting goblet cells. 3. Mucus protein (Muc2) impedes *V. cholerae* from colonization on intestinal epithelium. These results highlight the crucial role of the innate immune response, mucus barrier, and point to host-directed approaches for cholera therapeutics.

Capturing bacteria-host interactions at high resolution during pathogenesis

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Bacterial secretion systems are molecular complexes that directly translocate substrates into host cells and extracellular environments. Type III secretion systems (T3SS) include two evolutionally related nanomachines that promote pathogenesis: the injectisome, a needle-like appendage that injects virulence proteins into hosts, and the flagellar motor, required for bacterial motility. Here, cryo-electron tomography (cryo-ET) combined with biological approaches is used to capture bacterial nanomachines *in situ*, revealing how the cytoplasmic complex of the injectisome enables protein translocation and how the flagellar system generates high torque for migration in the human stomach.

In *Shigella flexneri*, the injectisome is a model T3SS that allows the pathogen to invade human enterocytes. The cytoplasmic complex of this system is essential for both formation of the needle and the translocation of virulence proteins. Here, a combination of biological approaches with cryo-electron tomography (cryo-ET) revealed the intact structure of the injectisome and solved how its cytoplasmic complex is organized within the native environment. Moreover, *in situ* structural analysis demonstrated that assembly of the cytoplasmic complex induces conformational changes of the gate through which proteins must pass to reach the extracellular region.

In *Helicobacter pylori*, a distinct flagellar system enables colonization of the highly viscous mucus layer, contributing to stomach cancer. *In situ* structural analysis of the flagellar motor revealed the complete periplasmic spoke-ring network consisting of a distal spoke-ring, which recruits the stator unit that generates rotational power for motor function, and a proximal spoke-ring, which stabilizes rotation of the MS-ring that transmits rotational power through the rod and hook to the filament. Notably, genetics and cryo-ET approaches also showed that components of the cage around the motor are homologous to those of type IV pilus machinery in other Gram-negative bacteria. These findings demonstrate how essential structures contribute to a fully functional motor in a high-priority pathogen. In addition, high-resolution structures of sheathed flagellar filaments determined using *in situ* single particle cryo-electron microscopy (cryo-EM) revealed post-translational modifications that enable smooth filament rotation.

Overall, these studies illustrate how the crucial synthesis of cutting-edge cryo-EM and biological techniques accelerates the discovery of structural and functional insights into nanomachines at the bacteria-host interface. Importantly, this versatile approach also applies to studies of macromolecules and cellular processes in diverse prokaryotic and eukaryotic cells.