Co-Evolving Host-Pathogen Molecular Pairings Determine Host Range

Some pathogen- and parasite-host pairs are locked in reiterative cat-and-mouse chases, with molecular mimicry sometimes propelling the dynamics

Shannon Weiman

Molecular interactions between microbial virulence factors and host defense proteins dictate host range, disease pathology, and, possibly, evolutionary trajectories of both hosts and pathogens, according to several researchers who spoke during the plenary session, "Intricacies of Host-Microbe Co-evolution," at the 2013 ASM General Meeting, held in Denver last May. Many bacterial and viral pathogens, as well as several parasites, they say, depend on specialized proteins to thwart host immune responses by mimicking host ligands.

This molecular-level relationship subjects host-pathogen interfaces to a reiterative cat-and-mouse chase, putting constant selective pressure on these interacting gene sets, one from the pathogen and the other from its host. Compatible interfaces between microbial factors and host immune effectors determine the cell, tissue, and species specificity of each pathogen, influencing the overall course of disease within the host.

Viruses and Their Hosts: Mutual Evasions, Perpetual Pursuit

Virus-host interactions drive a constant cycle of mutual evasion and pursuit, rapidly introducing changes into key proteins from both sources that interact with one another, says plenary session participant Nels Elde of the University of Utah in Salt Lake City, who describes this process as a "molecular arms race." He uses evidence of positive selection in these proteins, surfaces, and specific amino acids to track determinants of host range and mechanisms of rapid evolution in the host and in double-stranded DNA viruses, including poxviruses.

When exposed to such viruses, the host innate immune system quickly attempts to shut down viral replication. One such mechanism that defends against double-stranded DNA viruses depends on protein kinase R (PKR). PKR first senses double-stranded RNA, an intermediate in viral replication, in the cytoplasm of infected cells. Then, it phosphorylates the eukaryotic translation initiation factor 2α (eIF2α), a host protein that is critical for initiating protein synthesis, thereby inactivating it and blocking the production of viral particles.

"The crucial function of PKR in innate immunity is reflected by the evolution of numerous factors from various viruses that disable PKR to promote viral production, including a poxvirus-encoded mimic of eIF2α, called K3L," says Elde. This mimic of the host eIF2α protein makes it particularly difficult for host PKR to develop resistance to the viral K3L protein because it must maintain interactions with its own phosphorylation partner.

Elde analyzes PKR sequences from primates, which provide a record of PKR evolution, to bet-

SUMMARY

- **Molecular interactions between microbial virulence factors and host immune effectors dictate host range, disease pathology, and, possibly, evolutionary trajectories for both species.**
- **Double-stranded DNA poxviruses are engaged in a molecular arms race with hosts, with viral proteins mimicking and subverting key proteins of the innate immune response.**
- ***Staphylococcus aureus* similarly interferes with innate immune mechanisms through proteins that mimic host ligands, including the virulence factor Panton-Valentine leukocidin and other leukotoxins.**
- **Virulence factors with plentiful genetic and structural variation enable *Toxoplasma gondii* to evade immune responses across a broad range of host species.**

ter understand host mechanisms for evading viral mimics. "One branch in Old World monkeys was calculated to have undergone one of the most intense episodes of positive selection reported for any primate gene," he says. Across the primate lineage, base-pair substitutions resulted in changes to 17% of the amino acid sequence, altering interactions with viral K3L and other inhibitors while maintaining essential interactions with eIF2α. Indeed, despite their sequence divergence, all primate orthologs maintain their ability to phosphorylate eIF2α, yet show marked differences in binding to vaccinia K3L. "Rapid evolution of primate PKR did not seem to alter eIF2α recognition significantly, but resulted in considerable differences in susceptibility to K3L," says Elde. "In particular, we find in the hominoid lineage that human, chimp, gorilla, and orangutan PKR orthologs are 1,000-fold more resistant than gibbon PKR to K3L."

To map regions and point mutations that confer resistance, Elde makes chimeric proteins by interchanging various domains of PKR from one host species with those of another. He focuses on three regions that exhibit positive selection—the dsRNA-binding domain, the spacer region, and the kinase domain, including three key residues in the kinase domain that directly contact eIF2α. These residues "are among the fastest-evolving residues in PKR, suggesting that selective pressure to evade mimics of eIF2α may have driven changes in these residues," he says. Moreover, these domains have an extensive history of direct binding by viral factors.

Elde traced resistance to the αE and αG helices of the kinase domain. Two of the three residues in question, S 492 and F 489 in the αG helix, prove essential to resistance of human PKR. An additional residue in the αE helix, L 394, which is positioned away from the K3L interface, is also essential for resistance in human and orangutan PKR. "Helix αE masks αG in terms of K3L resistance," he says. "Only when helix αE is susceptible does the configuration of αG matter." These multiple interacting surfaces enable the host protein to discriminate against rapidly evolving viral mimics.

This versatility puts evolutionary pressure on viral K3L to maintain interactions with PKR, particularly when switching between hosts with different PKR interfaces. Elde explores mechanisms of rapid evolution in DNA viruses, which have much lower point mutation rates than RNA viruses, in experimental evolution studies. He finds that successively passaging vaccinia virus in HeLa cells leads to rapid increases in viral fitness, which can be traced to increased K3L gene copy number. "Frequency of these duplications may be on par with or exceed the frequency of nucleotide substitutions," he says.

Extra copies of K3L result in higher levels of this protein, which saturates PKR. Additional copies also set the stage for accumulating mutations. "K3L expansion facilitated the appearance of (a beneficial) variant by providing additional targets for mutation," Elde says. Viruses that acquired beneficial mutations soon reduce their K3L gene copy number, retaining the beneficial mutation while doing away with the original copies. This "gene accordion" mechanism allows for rapid evolution of virulence factors in DNA viruses despite low rates of point mutation.

S. aureus also Can Undermine Innate Immune Responses

The gram-positive bacterial pathogen *Staphylococcus aureus* similarly interferes with innate immune mechanisms through proteins that mimic host ligands, according to Victor Torres of New York University in New York, N.Y., and his collaborators. Leukotoxins are staphylococcal virulence factors that lyse immune cells by forming pores in their plasma membranes. Multiple leukotoxins exist, possibly as a product of gene duplication events, each with a unique tropism for cell type and host species.

Torres set out to identify the molecular determinants of this specificity. "LukAB targets phagocytes such as polymorphonuclear cells (PMNs, or neutrophils), which are an integral part of the host innate immune response to *S. aureus*," says Torres. He finds that its molecular target is a receptor with several aliases, including CD11b/CD18, integrin αM/β2, complement receptor 3, and macrophage-1 antigen (Mac-1).

This receptor is found on the full range of cells that LukAB targets, including PMNs, macrophages, monocytes, and dendritic cells. In binding Mac-1, *S. aureus* selectively destroys innate immune cells that would otherwise eradicate this pathogen. In fact, Mac-1 is a target of several other bacterial pathogens, including *Bordetella pertussis*, *Bacillus anthracis*, pneumococcus, and gonococcus.

More specifically, LukAB binds within the I-domain of Mac-1, mimicking the binding of host ligands to this same region, says Torres. This interaction accounts for *S. aureus* species specificity, he says. The gorilla I-domain amino acid sequence is "most similar to human," with a 98.6% identity, followed by rabbit at 79.1%, and then mouse at 78.1%. "These data correlate with the tropism of LukAB toward PMNs from each species," he says.

This analysis suggests a strategy for treating *S. aureus* infections in humans—namely, with antibodies that block the I-domain binding site, according to Torres. In vitro, such antibodies protect human neutrophils against LukAB lysis.

Meanwhile, another virulence factor from this pathogen, Panton-Valentine leukocidin (PVL), binds to the C5aR and C5L2 chemokine receptors, says Torres, citing findings reported by Jos van Strijp at the University Medical Center Utrecht in the Netherlands and his collaborators. PVL mimics natural ligand C5a, competing for the same binding site and potently inhibiting C5a mediated-activation of neutrophils. Conversely, C5a antagonizes PVL and protects human neutrophils against PVL-mediated cytotoxicity, suggesting a possible treatment strategy.

Meanwhile, yet another virulence factor, leukotoxin ED (LukED), targets the chemokine receptor CCR5, which also serves as a coreceptor for several other pathogens, including human immunodeficiency virus (HIV) and poxviruses as well as *Toxoplasma gondii* parasites. When LukED binds to CCR5, mimicking host ligands, it inhibits CCR5 signaling. Targeting host myeloid cells and T lymphocytes, it depletes macrophage, dendritic cells, Th1 and Th17 effector cells, and memory progenitor cells.

"We predict that virulent clinical strains producing large amounts of LukED use the toxin to eliminate antigen-presenting cells as well as *S. aureus*-specific CCR5+ Th1 and Th17 cells, which protect against infection," says Torres. Consistent with these findings, the antiviral drug maraviroc, a CCR5 antagonist developed to treat HIV, blocks LukED and protects against its toxicity. Moreover, individuals who are resistant to HIV through deletion of the CCR5 gene are also resistant to *S. aureus*. These findings suggest that this antiviral drug or others that target this receptor might provide an alternative approach for treating *S. aureus* infection, he points out.

Toxoplasma gondii: Adept at Modulating Immune Defenses across Many Host Species

The intracellular parasite *Toxoplasma gondii* is another microbial master when it comes to disrupting the host immune response, modifying inflammatory pathways to maintain a chronic infection, according to Jeroen Saeij of the Massachusetts Institute of Technology in Cambridge. "The worldwide distribution of *Toxoplasma* and its ability to chronically infect multiple animal species, including birds and mammals, raises the question of how this unicellular organism manages to control immune responses of such different species," he says. He and his colleagues identified one mechanism for undermining immunity in mice, a host that shares a long evolutionary history with *Toxoplasma* species, that may apply more broadly to other hosts as well.

T. gondii resides intracellularly, protected within vacuoles in host cells. The host disrupts these vacuoles, killing the parasites held within, using immunity-related GTPases (IRGs), which bind to the vacuole surface and disassemble the membrane. IRGs are plentiful, varied, and essential for surviving *Toxoplasma* infection in mice, which carry 23 *Irg* genes, according to Saeij.

T. gondii counteracts the actions of IRGs with the virulence factor rhoptry protein kinase 18 (ROP18). Differences in ROP18 alleles partially explain differences in virulence from one strain of parasite to another. This kinase is highly expressed in types I and II strains, but not in avirulent type III strains, where an insertion in the promoter prevents expression. Extensive ROP18 sequence divergence suggests it is engaged in an evolutionary cat-and-mouse chase with host IRGs, Saeij says.

However, ROP18 does not directly interact with IRGs. Pseudokinase ROP5 acts as an adapter, and its interactions with IRGs determine host susceptibility, Saeij continues. Like vaccinia virus K3L, the ROP5 gene duplicated and diversified. Copy number and sequence vary by strain, imparting different virulence characteristics and accounting, in part, for the broad host range of *T. gondii*.

"Both the [host] IRGs and the ROP5 locus have expanded, perhaps due to an evolutionary arms race whereby new host IRG genes required new ROP5 genes so *Toxoplasma* could continue to evade IFN-mediated killing," says Saeij. This hypothesis is supported by phylogenetic analysis

of IRG sequences, which are highly divergent and polymorphic in mice and other hosts, as well as of ROP5, particularly in the domain that binds IRGs.

"Tandem copies of different alleles of ROP5 might also affect the ability of the parasite to adapt to hosts other than mice," Saeij continues. Thus, variations among ROP5 and ROP18 alleles, and their combinations, may allow for interaction with divergent host IRGs, possibly explaining virulence differences among strains, as well as differential effects exerted in different hosts. "It has been argued that different *Toxoplasma* strains and their effectors have coevolved with different hosts in different niches," he says. For example, rats, whose IRG sequences diverge widely from those of mice, are far more resistant to *T. gondii* than mice.

Divergent hosts present *T. gondii* with a dizzying array of immune epitopes and mechanisms. Thanks to virulence factors with promiscuous binding capacities, gene duplication and divergence, and reassortment of alleles, *T. gondii* proves adept at navigating diverse host environments, and is able to chronically infect a broad range of host species.

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