Immune System Variations Affect Responses to Pathogens

Polymorphisms in Toll-like receptor signaling pathways may explain varied responses to pathogens and differences in vaccine efficacy

Shannon Weiman

Rare Toll-like receptor (TLR) deficiencies are widely recognized risk factors for infections by specific pathogens. Additionally, researchers are finding that more subtle variations in innate immune signaling pathways also contribute to susceptibility to such pathogens as well as to how individuals respond to antimicrobial drug treatments and vaccines. Recent developments could lead to novel, genotype-specific drugs to modulate host immune responses as a way of treating infections among such individuals, according to several researchers, who presented findings during the 2013 Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) held in Denver last September.

Variations in Signaling Affect Susceptibility to Pathogens

Polymorphisms predispose specific individuals to various infectious diseases or increase their susceptibility to particular pathogens, including tuberculosis (TB), leprosy, Legionnaires’ disease, and Helicobacter pylori, according to Thomas Hawn of the University of Washington-Seattle. A hallmark of his research is delving beyond well-known TLR deficiencies, while unmasking polymorphisms in downstream effectors and signal modulators that underlie variability in susceptibility throughout the population. He shared some of his recent findings during the 2013 ICAAC symposium “Genomic Analysis Provides New Perspective in Infectious Disease.”

TB ranges from being asymptomatic among 90% of those infected with Mycobacterium tuberculosis to causing severe pulmonary or meningeal infections. TLRs 1, 2, 6, and 9 respond to this pathogen. However, those responses may vary, depending on polymorphisms in Toll-interacting protein (TOLLIP) and how it responds to signaling molecules, according to Hawn. These variations, in turn, influence both the host immune response and disease outcome, he finds.

Other researchers report that TOLLIP is a negative regulator of TLR signaling, based on experiments with rodents. According to Hawn, in humans TOLLIP is a negative regulator of TLR2 and 4 signaling. “TOLLIP has an anti-inflammatory effect on TLR signaling in humans, and TOLLIP deficiency is associated with an increased risk of TB,” he says. He and his collaborators also identified a single nucleotide polymorphism (SNP) with the same phenotype. This SNP, designated rs5743899, decreases expression of TOLLIP, leading to increased TLR2-dependent pro-inflammatory IL-6 and decreased anti-inflammatory IL-10 in response to M. tuberculosis. The SNP also is associated with greater-than-average susceptibility to TB in a Vietnamese population. In patients, excessive TB-mediated inflammatory responses prove detrimental and are treated typically with anti-inflammatory glucocorticoids.

Another SNP, rs3750920, has the opposite ef-
fect, increasing TOLLIP and protecting individuals against TB, according to Hawn. In this case however, cytokine responses are unchanged, suggesting distinct mechanisms, he says. “TOLLIP plays an important role in the pathogenesis of tuberculosis by regulating both pro- and anti-inflammatory pathways. Modulating TOLLIP activity and function may lead to important breakthroughs in tuberculosis vaccine design as well as immune drug development.” This is the first reported instance of TOLLIP polymorphisms being associated with an infectious disease, he adds.

*Staphylococcus aureus* is another bacterial pathogen with diverse manifestations, ranging from asymptomatic colonization to life-threatening infection,” according to Vance Fowler of Duke University in Durham, N.C., who spoke during the symposium “*Staphylococcus aureus* and Hypervirulence: Mechanisms and Clinical Implications.” In experiments with inbred mice with differential susceptibilities to this pathogen, he and his collaborators identified a series of genes involved in immune responses to infections.

One such gene, *tnfaip8*, is downregulated in A/J mice, which are susceptible to *S. aureus*, according to Fowler. Macrophage cells from such mice have an aberrant inflammatory cytokine response, he says. For example, down regulation of this gene increases production of GM-CSF, a Th1-type cytokine that leads to severe inflammation, but decreases interleukin (IL)-1β. “IL-1β is a key inflammatory cytokine in orchestrating host defense against *S. aureus*, with mice deficient in this cytokine exhibiting markedly increased susceptibility to [that pathogen],” he says.

### Changes in Host Genes Can Lead to Different Symptoms during Infections

Polymorphisms in immune signaling pathways can predispose individuals to manifest specific symptoms when infected by particular pathogens—providing a new angle for understanding infectious diseases and perhaps for identifying who is more apt to develop specific symptoms from that pathogen and maybe even better ways of preventing and treating specific infections.

“It is not known at present which host genetic, environmental, and bacterial virulence factors influence the different clinical presentations of TB,” says Hawn. However, he and his collaborators find that a SNP in the Toll-Interleukin 1 Receptor Domain Containing Adaptor Protein (TIRAP), a protein that mediates signaling by TLRs 1, 2, 4, and 6, is more strongly associated with meningitis than with pulmonary symptoms among individuals infected by *M. tuberculosis*.

“TIRAP SNPs could alter the initial host containment of bacilli in monocytes or macrophages and the early bacteremia that results in seeding of the meninges,” Hawn says. “These results . . . suggest that the Toll-like receptor pathway influences susceptibility to meningeal and pulmonary TB by different immune mechanisms. Because TIRAP plays a central role in the innate immune recognition of a wide variety of pathogens, we speculate that TIRAP polymorphisms will be associated with susceptibility to a wide spectrum of human infections.”

### Host Changes Affecting Responses to Three Different Infectious Agents

Host and pathogen genetics affect susceptibility to specific strains of *M. tuberculosis*, according to Hawn. “Different genotypes of *M. tuberculosis* induce different patterns of host immune response,” he says. Host susceptibility thus depends on which immune responses are elicited. A particular SNP may predispose an individual to infection by some strains harboring virulence factors that enable more invasive infection, versus other less virulent strains, thereby influencing disease manifestation.

For example, a polymorphism in TLR-2 increases susceptibility to meningeal TB by making individuals more susceptible to infection with the Beijing strain of *M. tuberculosis*, says Hawn. This strain, which causes disseminated disease, is prevalent in Asia but now is causing multidrug-resistant outbreaks of TB in the United States (US). “This is the first time a relationship between bacterial and host genotype has been observed in TB, although it has previously been observed with other pathogens,” he says. “Future vaccine candidates may need to be evaluated against a range of *M. tuberculosis* genotypes and host ethnicities if they are to prove globally effective, particularly against disseminated disease.”

Infections with *Plasmodium falciparum* lead to malaria but also can manifest more narrowly as meningitis, according to Johanna Daily of Albert Einstein College of Medicine in Bronx, N.Y., who spoke during the same symposium. Why do some
“Cytokine biomarkers may provide a new paradigm for... stratifying patient risk for morbidity,” he says. “Variations in immune responses could affect vaccine efficacy.”

By affecting immune responses to antigens, polymorphisms that influence susceptibility to pathogens may also affect vaccine efficacy, according to Hawn of the University of Washington. Individual differences in TLR signaling thus might help to explain variable responses associated with the widely used but far from satisfactory Bacille-Calmette-Guerin (BCG) TB vaccine that dates back to 1921, he says. “Host genetic factors may be a primary reason for BCG’s variable and inadequate efficacy.”

Several common variants in both the TLR1 and TLR6 genes that influence susceptibility to TB infection also alter T cell responses to BCG vaccination, according to Hawn. Hyporesponsive alleles promote a Th1-type response, increasing immunostimulatory interferon (IFN)-γ and/or IL-2 production, while decreasing Th2-type cytokines IL-6 and IL-10. The mechanism likely involves altered interactions with TLR2, which forms a heterodimer with either TLR1 or TLR6 and mediates immune activation. TLR2 signaling in antigen presenting cells such as monocytes, macrophages, and dendritic cells promotes Th2-type T-cell responses in mice, while TLR2 inhibition promotes Th1-type response. “To our knowledge, this is the first description of polymorphisms in innate pathway genes that affect the adaptive response to in vivo vaccination against a bacterial pathogen in humans,” he says.
How these polymorphisms might affect BCG-induced immunity is under investigation, Hawn continues. However, these results might explain highly variable IFN responses in BCG-vaccinated infants. “Our data suggest that these previous observations may be partially attributable to variation in TLR1 or TLR6 and that host genetics are an important variable influencing the immune response to BCG vaccination,” he says.

These findings carry important implications for clinical evaluations of vaccines. Genetic differences among individuals could confound results when, for example, IFN-mediated inflammation at the site of antigen exposure is used as a positive indicator. If “genetic background of patients influences the results of immunodiagnostic tests, alternative assays may need to be developed that are not dependent on host genetic variation,” Hawn says. “Genotypes may need to be considered when analyzing efficacy data.” Segmenting the population by genotype may provide a fairer way of evaluating vaccines, ushering in vaccines that are administered on a genotype-specific basis, with low-response individuals perhaps receiving higher doses. These findings could also “guide novel adjuvant vaccine strategies, and hold potential for rational selection of adjuvants,” he adds.

**Genotype-Specific Treatments**

Reaching a fuller understanding of how genetic susceptibilities vary among individuals could lead researchers to develop treatments that augment host immune responses against pathogens. Such treatments would not be subject to the forces that lead pathogens to acquire resistance to antibiotics, according to Hawn. “Supplementing anti-TB therapy with host modulators may lead to shorter treatment times, a reduction in lung damage caused by the disease, and a lower risk of relapse or reinfection,” he says.

Some immunomodulatory treatments are already being administered to TB patients, such as anti-inflammatory glucocorticoids that minimize immunopathology. However, this treatment might be more effective if it were administered on a genotype-specific basis, Hawn contends. “TB is not only a disease of failed immunity,” he says. “People succumb for the opposite reasons of an inadequate or excessive immune response.” However, identifying the underlying pathology in each patient is difficult and thus is often overlooked, leading to a one-size-fits-all treatment strategy that has a high failure rate.

Measuring a particular polymorphism in the inflammatory signaling molecule LTA4H could help to remedy this situation, Hawn continues, citing research done in collaboration with Lalita Ramakrishnan of the University of Washington-Seattle, David Tobin of Duke University, and Guy Thwaites, Sarah Dunstan, and Jeremy Farrar of Oxford University Clinical Research Unit in Saigon, Vietnam. LTA4H helps to activate macrophage cells that control inflammatory responses to TB. “The ability of macrophages to contain mycobacterial infection is critical throughout infection, and this ability is dependent on finely tuned LTA4H levels,” he says. If LTA4H is too low, macrophage cells fail to control the infection. However, if LTA4H is too high, excessive macrophage activation causes immunopathology. A specific promoter polymorphism in LTA4H appears to be responsible for overactivating macrophage cells, leading to intracerebral inflammation in cases of meningeal TB, he says. Only individuals with this genotype appear to benefit from dexamethasone treatment, he adds. “We speculate that the observed modest beneficial effects of these adjunctive therapies in the general patient pool may in fact represent their substantial benefit in the LTA4H-high genotype, as we have found for TB meningitis.”

A simple genotyping assay could rapidly and inexpensively identify those individuals who are likely to benefit from glucocorticoid treatment, while sparing others the expense and serious side effects of such inappropriate treatments, Hawn says. Whether anti-inflammatory treatment is simply ineffective in patients with low LTA4H activity, or exacerbates disease severity by further damping inflammatory response is under investigation. Either way, patients with low LTA4H activity would more likely benefit from treatments aimed to boost inflammatory responses, not damp them. “If people succumb to TB for two fundamentally different reasons, then it is imperative to design therapeutic strategies that reflect this dichotomy,” he says. “The ability to tailor therapies to these divergent inflammatory states and specific LTA4H genotypes could improve patient outcomes.”

Shannon Weiman is a freelance writer in San Francisco, Calif.