Genetic Analysis of Host Responses in Sepsis

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Introduction
The contest between nature and nurture is everywhere apparent in biology, and nowhere more so than in the realm of infectious disease research. On the one hand, what could be more environmental than an infection? Microbes are environmental by their very nature, invading the body from without. Surely they encode their virulence within their own genomes, and just as surely they are able to “outrace” the ponderous host in an evolutionary contest by virtue of their short generation times and their enormous proliferative capacities. On the other hand, differences in host susceptibility are equally obvious. Some mammalian species offer suitable models for specific human infections; others simply do not support infection at all. Moreover, some humans seem impervious to infection with a given agent, while others are infected very easily. Are differences in host susceptibility inherited, or do they merely reflect vagaries in the infectious properties of microbes?

During much of the past century, the microbe itself stood at the heart of microbial pathogenesis. Little thought was devoted to the host per se, though it was granted that differences in susceptibility to certain infections did exist between individuals, and between different ethnic groups. During the past 20 years, extraordinary strides in our grasp of mammalian genetics have made the host side of the equation far more approachable. A restricted collection of genes now presents itself as the likely repository for genetic differences that foreshadow susceptibility to infectious disease. The Toll-like receptors, of which 10 are presently known to exist in humans, offer an excellent example of this genetic reductionism, in that they embody the afferent component of the innate immune system, and strongly influence the containment of an infection from its earliest stages. The Toll-like receptors were identified as the culmination of a long and relentless inquiry into the yet-unsolved clinical problem of sepsis.

Though it might seem difficult to measure the net heritability of infectious disease resistance, the tools with which to make such a measurement have been in existence for a long time, and such a “coarse genetic” appraisal is a necessary antecedent to fine genetic analyses that implicate an individual locus, or ultimately, individual mutations. In 1998, Sorensen et al. [1] performed a retrospective adoption study in order to determine the heritability of premature death of diverse causes. Examining 960 adoptees who had been raised apart from their biologic parents since shortly after birth, these workers were able to show that the premature death of a biologic parent was strongly associated with premature death among the adoptees. When mortality from specific causes was examined, no cause of death was found to be as heritable as death attributed to infectious disease. Given that a biologic parent had died before the age of 50 years as a result of infection, the offspring of this parent, though raised in a separate environment, were 5.6-fold more likely to die of infection than controls, at any age across a six-decade range. It has become fashionable to consider that most phenotypes are heritable, and susceptibility to infectious disease is particularly so.

What are the genes that determine infectious disease susceptibility (or conversely, resistance)? Are some loci specific for one disease or another, or is the effect always a general one? Data derived from chance mutations in humans and in animals have begun to yield answers to these questions. To the largest degree, it has been the study of “sepsis” that has proved most productive. Although most clinicians recognize sepsis when they see it, there is much debate over how it is best defined. While bacteremia is common in sepsis, it is not necessary for the diagnosis of sepsis. Moreover, bacteremia may exist in the absence of the severe pathologic derangements that sepsis implies. The causa sine qua non of sepsis is infection. The infection may or may not involve the intravascular compartment. But it is clear that bloodborne factors, produced partly by the pathogen and partly by the host, lead to disturbances of coagulation, metabolism, and ultimately, vasomotor collapse.

The attempt to understand the pathophysiology of sepsis led first to the concept that microbial pathogens elaborate toxins that evoke the septic syndrome. An important advance occurred much later with the realization that these toxins work their effects by inducing the release of the host factors normally intended to contain infection, but also potentially injurious if produced in excess. The
link between molecules that originate in microbes and the induction of the host response has been provided by genetic studies that have revealed the sensing mechanism of host innate immune cells. A great deal of progress has also been made in elucidating the signaling pathways that are triggered by host receptors. These biochemical events lie at the very heart of sepsis, and without them, sepsis would be a process very different from the one that we know it to be.

On the Microbial Side: Molecules That Trigger the Septic Syndrome

With the late 19th century recognition that microbes are primarily responsible for infectious diseases, efforts were almost immediately directed toward the identification of toxins, produced by bacteria, that might account for the lethal properties of a systemic infection. At this stage, there was little understanding of interplay between the host and pathogen per se, and no inkling of the importance of endogenous mediators of sepsis. Although a number of early workers had identified and even partially purified microbial toxins in the “premicrobial” era, it was Richard Pfeiffer, a student of Robert Koch, who identified and studied “endotoxin,” a substance produced by *Vibrio cholerae*, which could induce fever and shock in experimental animals. Other microbial inducers were also identified, each with its own story. Among these were the zymosan of yeast, the peptidoglycan of both gram-positive and gram-negative bacteria, the lipoteichoic acid of gram-positive organisms, and the lipoarabinomannan of mycobacteria. But in many respects, endotoxin, ultimately known as lipopolysaccharide (LPS), took center stage because of its extreme toxicity, its abundance, and its evident relevance to the septic syndrome, in that LPS alone could recreate most of the problems that were observed in the course of an authentic bacterial infection [2]. For decades, however, there was little understanding of how LPS could exert its toxic effects. Several advances were required to achieve this understanding, which was then swiftly generalized to encompass other microbial inducers as well. The central discoveries (Fig. 1) are recounted below.

The lipopolysaccharide response is minimally dependent on a single gene product

In 1965 it was observed that mice of the C3H/HeJ strain were highly resistant to most, and perhaps all, of the biologic effects of LPS [3]. This resistance was ultimately ascribed to a genetic defect that was mapped to mouse chromosome 4 [4]. An allelic mutation was later identified in mice of unrelated C57Bl/10ScCr strain [5]. At this point, many of the earliest hypotheses concerning endotoxicity, which revolved around assumptions of nonspecific effects on biologic membranes, immediately became untenable. It could be guessed that LPS must exert its effect through a single biochemical pathway, ultimately delimited by a single protein. The availability of an LPS-resistant animal model opened the way for inquiry into the cellular basis of endotoxicity. In due course, C3H/HeJ mice were shown to be abnormally susceptible to infection by many gram-negative (but not gram-positive) bacteria [6]. This finding supported the view that LPS recognition is important as a host defense mechanism.

The toxicity of lipopolysaccharide is conferred by cells of hematopoietic origin

In 1980, Michalek et al. [7] showed that cells of hematopoietic origin were essential for mediation of the lethal effect of LPS. Using adoptive transfer studies in which LPS resistant animals served as donors or recipients, these authors determined that the strain origin of the lymphoreticular system foretold endotoxin sensitivity in radiation chimeras. Hence, only a minority cell population seem to be required for LPS to exert its lethal effect. Evidence from many sources suggested that these cells were probably macrophages [8].

Tumor necrosis factor mimics lipopolysaccharide effects and is required for endotoxicity

In 1985, it was recognized that at least one of the lethal endogenous mediators of endotoxicity was tumor necrosis factor (TNF). Beutler et al. [9] demonstrated that passive immunization against TNF would significantly weaken the lethal effect of LPS. Moreover, in many separate studies, it was shown that TNF alone could cause systemic inflammation and shock similar to that evoked by LPS [10]. Hence, while other cytokines also contribute to the LPS response, TNF is of major importance. The complexity of LPS-induced reactions was daunting, in the sense that hundreds of endogenous genes are activated by LPS, some encoding insoluble mediators, and others structural proteins. TNF provided a convenient and sensitive endpoint to follow in analyzing responses to LPS, particularly as it was induced within minutes following contact between LPS and LPS-responsive mononuclear cells [11].

CD14 binds lipopolysaccharide and is required for the lipopolysaccharide response

In 1990 Ulevitch and his colleagues discovered the first biologically relevant receptor for LPS on the surface of host mononuclear cells [12]. This was CD14, a glycosylphosphatidylinositol-tethered protein rather abundantly represented on the surface of host mononuclear cells, and also present in a soluble form in plasma. Cells lacking CD14 were insensitive to LPS, but their sensitivity was restored by transfection-induced expression of this protein. Moreover, mice lacking CD14 were found to be approximately 1000-fold more resistant to LPS than mice with a normal copy of the CD14 gene [13]. The Ulevitch group also showed that LPS was engaged by a plasma protein called LBP (lipopolysaccharide-binding protein), which acted to transfer the microbial ligand to cell-bound CD14 [14]. Hence, the chain of events initiated by LPS began to take shape.