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COMMENTARY

Genetic Screening for Susceptibility to Infection in the NICU Setting

Commentary on the article by Ahrens *et al.* on page 652

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"An ounce of prevention is worth a pound of cure." So goes the old adage that is particularly relevant for the practice of infectious disease. Manipulating the acquired immune system via immunization has been extremely successful in preventing infection. When specific immunization is not possible, the use of prophylactic antimicrobial agents is considered. Prophylaxis with antimicrobial agents can be effective (1), but is associated with two important disadvantages: the potential for drug toxicity (2) and the selection of resistant microorganisms (3).

During the past several decades, substantial progress has been made in understanding the molecular basis of innate immunity. The innate immune system is activated by recognition of molecular patterns unique to microbes (4). Recognition of microbes can be initiated by soluble components of plasma, including mannose binding lectin (MBL), which binds to surface components of microorganisms and serves to both opsonize the microbial particle and trigger the complement system (5) (Fig. 1). Recognition of foreign particles by soluble plasma components is often followed by receptor-based recognition on monocytes and other host cells. In particular, the important role of the toll-like receptors (TLRs) in recognition of microbes and other danger signals is increasingly appreciated (6). TLRs contain an extracellular leucine-rich repeat motif and a cytoplasmic TIR domain. In several instances, including the recognition of Gram-negative bacterial lipopolysaccharides, the TLRs act in synergy with the CD14 coreceptor (7). Activation of TLRs results in the subsequent activation of kinase-based cytosolic signaling cascades, culminating in the translocation of NF-κB to the nucleus.

In addition to recognition by surface TLRs, microbial constituents, such as peptidoglycan-derived muramyl dipeptide, can activate cells directly via cytosolic proteins of the nucleotide-binding oligomerization domain (NOD) family that contain a leucine-rich repeat region homologous to that of the TLRs coupled with a nucleotide binding site (8). As with the TLR pathway, the NOD-based pathway culminates in translocation of NF-κB to the nucleus and subsequent synthesis and release of multiple genes encoding proinflammatory proteins, including cytokines.

Among the cytokines released early in the innate immune response is IL-6, a pleiotropic cytokine produced by most tissues in response to infection or trauma (9, 10). IL-6 regulates multiple inflammatory responses, including the acute-phase synthesis of an array of hepatic products, induction of fever, and stimulation of hematopoiesis. In addition IL-6 induces B-cell terminal differentiation and activation of both T cells and thymocytes. Of note, plasma IL-6 concentrations rise in human newborns early during bacterial sepsis (11).

For many years the bulk of research on the role of inflammatory mediators in sepsis was conducted with the underlying assumption that the overexuberant release of cytokines was a central and common pathway by which sepsis progressed to septic shock and death. With our increasing fund of knowledge, a more nuanced view of the innate immune response has developed. The overall purpose of the innate immune response is to initiate acute host defense mechanisms, including recruitment of leukocytes to sites of infection and release of antiinfective molecules into the systemic circulation. In addition, it is increasingly recognized that the innate immune system, by facilitating the activation of antigen-presenting cells, initiates and instructs the acquired immune response (12). Consistent with this view, alleles of *TLR4* and *CD14* that encode proteins with reduced function have been associated with an increased risk of invasive bacterial infection in adults (13, 14). Thus, there is now both a theoretic and empiric basis for believing that inadequate innate immune responses leave the host susceptible to microbial infection. Moreover, a new paradigm of insufficient immune response or "immune paralysis" has now been suggested as a frequent contributor to the progression of sepsis to septic shock and death (15).

Our increased understanding of innate immunity has raised interest in leveraging the innate immune system in the prevention and treatment of infections. As early diagnosis and therapeutic intervention increase the likelihood of a favorable outcome, there is a great desire to identify early markers of infection (11). Efficient diagnosis of infection is particularly relevant in neonatology. Newborns are at an increased risk of invasive microbial infection, and this risk is particularly high in premature neonates of very low birth weight (VLBW). In such newborns the risk of sepsis can be as high as 10 to 20% (16).

The article by Ahrens and colleagues (17) is an early step toward assessing whether the application of molecular biologic

tools to define host innate immune genes may help predict which patients are at greatest risk of sepsis. In a prospective, multicenter study, the authors studied 372 VLBW newborn infants. Using PCR and restriction fragment-length polymorphism analysis of DNA extracted from buccal swabs of newborns, the authors focused on variant alleles of *MBL*, *CD14*, *TLR4*, and *NOD2* predicted to confer diminished innate immune responses and that have been shown in prior studies to correlate with increased risk of infection in adults (13, 14, 18, 19), as well as an IL-6 allele (IL6-174G) that apparently regulates IL-6 production in neonates but not adults (20). Correlations were made between the presence of blood culture–proven sepsis and the presence of the variant innate immune gene alleles.

Using multivariate logistic regression analysis, the authors endeavor to rule out confounding factors (*e.g.* exclude effects of gestational age, sex, and antecedent glucocorticoid administration) and demonstrate that the homozygous IL6-174G allele was predictive of the development of sepsis (odds ratio, 1.9; $p = 0.039$). There was also a strong trend toward association of the *NOD2*–3020insC allele with culture-proven sepsis (odds ratio, 3.2; $p = 0.052$). The sepsis rate was modestly higher for patients with the CD14-159T allele, but this trend did not achieve significance ($p = 0.18$). Within the limits of the study size, the *TLR4* allele and *MBL* B/C/D alleles analyzed did not appear to substantially alter risk of neonatal sepsis.

Of note, the increased rate of sepsis noted in carriers of homozygous IL-6 mutation was largely related to increased rate of Gram-positive bacteremia. Two of the centers participating in the study used routine prophylaxis against Gram-positive infection in all VLBW neonates with i.v. catheters, using the glycopeptide antibiotic teicoplanin (similar to vancomycin). Remarkably, the higher rate of culture-proven sepsis in carriers of the homozygous IL6-174G allele was related to the high rate of Gram-positive bacterial infection in those VLBW infants who did not receive teicoplanin prophylaxis. This correlation suggests that screening for IL6-174G allele could be the basis for selective prophylaxis of high-risk neonates.

The results of this intriguing study raise a number of questions. More work is needed to better understand the effect of the −174G IL-6 allele on plasma IL-6 protein concentrations during infection. Moreover, the mechanism by which the IL-6 allele apparently confers selective susceptibility to Gram-positive bacteria is worthy of study. A confirmatory study with a larger sample size would help resolve whether the *NOD2*-3020insC allele is associated with a distinct subset of infections, as suggested in the Ahrens study [see Table 3 in article by Ahrens *et al.* (17)]. The high carrier frequency for the innate immune alleles studied [8 to 40%, (17)] would suggest that despite the fact that these alleles appear to be associated with decreased innate immune responses, there is a strong selective pressure for their maintenance. By analogy to the maintenance of sickle cell Hb alleles in selected populations as a result of the advantage conferred against malaria (21), it is possible that an attenuated innate immune response confers a host advantage in certain infections (or other disease processes) but not others. Owing to the wide variation of polymorphism frequency among populations, the authors excluded from analysis the 16 infants of African and Far Eastern descent. Similar studies should be performed to evaluate whether the observed associations hold true for these populations as well. Finally the issue of cost-effectiveness of any such genetic screening with respect to morbidity and mortality outcomes is important.

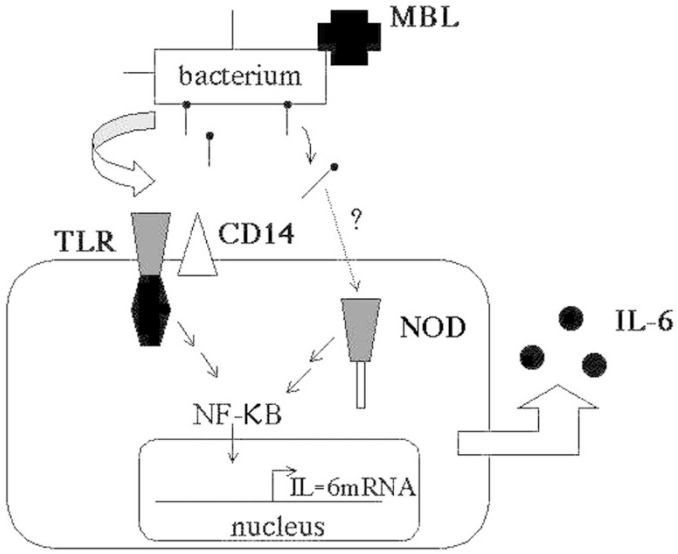

Figure 1. Activation of the innate immune system by bacteria.This basic schema highlights the roles of the proteins encoded by the genes studied in the article by Ahrens *et al.* (17). Bacteria and bacterial surface components (generically symbolized by lines with small circles at their base) are recognized by soluble plasma proteins such as MBL that enhance delivery of bacteria to phagocytes via distinct receptors (not shown). In addition, receptor-based innate immune recognition of bacterial surface components occurs via TLRs, often in conjunction with CD14. Alternatively, bacterial components can translocate to the host cell cytosol by an incompletely defined process (?) and then be recognized by the cytosolic NOD proteins that contain a leucine-rich repeat region with homology to that in the extracellular domain of TLRs. Both TLR and NOD activation activate signaling cascades that culminate in the release of NF-κB from the cytosol to the nucleus, thereby activating the transcription of inflammatory genes, such as that encoding IL-6. Release of IL-6 into the systemic circulation results in pleiotropic effects, including activation of hepatic synthesis of acute-phase proteins.

The promising results of Ahrens and coworkers, including the potential applicability of basing antibacterial prophylaxis on such genetic analyses, will need to be reproduced and verified with multiple larger study populations before any general clinical application. Much more remains to be learned about the expression and function of the innate immune system at birth and during early development (22). Despite these qualifications, the completion of the human genome project coupled with rapid advances in the manipulation and analysis of nucleic acids will likely result in an increasing array of techniques by which physicians more accurately predict the risk of disease and tailor prophylactic therapy accordingly.

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