Polymorphisms of the Toll-Like Receptors and Human Disease

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The Toll-like receptor (TLR) family regulates both innate and adaptive immune responses. Given its broad effect on immunity, the function of TLRs in various human diseases has been investigated largely by comparing the incidence of disease among persons with different polymorphisms in the genes that participate in TLR signaling. These studies demonstrate that TLR function affects several diseases, including sepsis, immunodeficiencies, atherosclerosis, and asthma. These findings have resulted in new opportunities to study the pathogenesis of disease, identify subpopulations at greater risk of disease, and, potentially, identify novel therapeutic approaches.

Toll-like receptors (TLRs) recognize conserved molecular patterns on specific classes of pathogens and initiate a series of signaling events that leads to the expression of proinflammatory genes. TLR function might, therefore, affect the incidence or progression of inflammation-related human diseases, such as asthma, atherosclerosis, and rheumatoid arthritis. As more genetic polymorphisms in TLRs are identified and as our knowledge of TLR signaling improves, our understanding of the relationship between TLRs and pathogenesis of disease will also improve and likely will provide a rational basis for developing novel therapies to treat these important diseases.

TLR function is better understood in mice than in humans, largely because humans lacking specific TLRs have not yet been identified. Although there are many similarities between murine and human TLRs, important differences also exist, particularly with regard to the specificity and expression of TLRs in humans and mice. For example, constitutive expression of mouse tlr2 in peripheral blood leukocytes is low but inducible in many cell types, including T cells. In contrast, human TLR2 is constitutively expressed by many cell types but not T cells [1, 2]. Mice and humans also differ with regard to the regulation of expression of TLR genes. For example, lipopolysaccharide (LPS) specifically up-regulates tlr3 expression through the induction of IFN-β in mouse cells, but the up-regulation of TLR3 by IFN-β is blocked by LPS in humans [3]. These findings suggest that mice and humans may respond differently to simultaneous infection with viruses and bacteria, a common occurrence in clinical settings.

With regard to ligand binding, the lipid A analogue, lipid IVa, is a potent antagonist of LPS signaling in human cells but acts as an LPS mimetic in mouse cells [4]. In this case, the species specificity resides within the TLR4 accessory protein, myeloid differentiation protein–2, rather than TLR4 itself. Another TLR4 ligand, taxol, is a plant-derived anticancer agent that mimics the action of LPS in mice but not humans [5]. In addition, penta-acylated LPS stimulates murine, but not human, cells [6]. It is likely that there are other as-yet-unidentified differences between murine and human TLR gene expression, ligand binding, or signaling pathways that could lead to divergent innate or adaptive immune responses. Finally, progression of human disease is complicated by multiple genetic differences and environmental factors that are not pertinent to well-controlled experiments with inbred strains of mice. Thus, although in vitro and in vivo experiments can provide the fundamental rationale for specific pathophysio-
icical studies in humans, the role of TLRs in regulating human adaptive responses, including the disease setting, can be achieved only through studies of human patients.

Our knowledge of TLR function in human disease is based primarily on analyses of persons with polymorphisms in TLR genes. The first identified TLR polymorphism encodes an Asp299Gly amino acid substitution in TLR4 [7]. This polymorphism is associated with a decreased signaling response to LPS in vitro and decreased airway response to inhaled bacterial LPS [7]. However, the effect of the Asp299Gly polymorphism on LPS-induced TLR4 signaling ex vivo is controversial [8, 9]. Here, we discuss recently identified associations of TLR polymorphisms with various human diseases and how these associations can improve our understanding of disease pathologies and treatments.

Sepsis is a syndrome associated with bacterial infection that causes >100,000 deaths each year in the United States. The clinical features of sepsis are caused, in part, by the release of proinflammatory bacterial products, including LPS, into the bloodstream. Determining associations between pathophysiological responses to these toxins and specific genetic polymorphisms might eventually allow persons who are most at risk for sepsis to be identified.

More than half of the cases of sepsis are caused by gram-negative bacteria [10]. Because TLR4 is required for innate immune responses to LPS, several groups have investigated possible associations between the Asp299Gly polymorphism and sepsis. Two of these studies demonstrated that this polymorphism increases the risk of gram-negative infections [11, 12], and another study linked this polymorphism to an increased incidence of systemic inflammatory response syndrome [13]. In addition, meningococcal infections have been associated with rare, heterozygous missense TLR4 mutations [14], although not with the Asp299Gly polymorphism [14, 15]. It is not currently known whether these rare TLR4 polymorphisms have a greater impact on in vivo TLR4 function than does the more common Asp299Gly polymorphism. However, it is likely that the impact of the Asp299Gly polymorphism on sepsis is restricted to gram-negative infections, because the Asp299Gly polymorphism does not affect polymicrobial sepsis [16]. With regard to other pathogens, an association between the Asp299Gly polymorphism of TLR4 and severe respiratory syncytial virus–induced bronchiolitis was recently reported [17]. Respiratory syncytial virus is a known ligand for TLR4 [18, 19]. Taken together, these data suggest that human TLR4 has a critical role in the innate immune response to gram-negative bacteria and respiratory syncytial virus, although the cellular and molecular events affected by the TLR4 polymorphisms in pathogenesis and adaptive responses to infection have not yet been identified. Human trials evaluating the role of TLR4 antagonists in the treatment and/or prevention of clinical sepsis [20] may be particularly informative.

TLR2 recognizes lipoproteins from the cell wall of several bacteria, including Borrelia burgdorferi, Treponema pallidum, and Mycobacterium fermentas. Cells of persons with an Arg753Gln polymorphism in TLR2 are less responsive to bacterial peptides derived from B. burgdorferi and T. pallidum. Moreover, this polymorphism may also predispose persons to staphylococcal infections [21] or tuberculosis [22]. Another polymorphism of TLR2 (Arg677Trp) affecting the intracellular region of the protein blunts activation of nuclear factor–κB by Mycobacterium leprae and Mycobacterium tuberculosis [23] and appears to enhance susceptibility to leprosy [24, 25] and tuberculosis [26] in humans.

TLR5 signals in response to bacterial flagellin [18, 19]. In humans, a common stop codon polymorphism of TLR5 (392STOP) results in decreased flagellin signaling and is associated with enhanced susceptibility to Legionella pneumophila, a flagellated bacterium [27]. It will be interesting to learn whether polymorphisms in TLR11 exist in the human population and whether they affect urogenital infections, because this recently identified molecule appears to control uropathogenic bacterial infection in mice [28].

Immunosuppression is a frequent complication of sepsis. In view of the requirement for TLR4 in the biological response to gram-negative LPS, it is likely that this receptor is involved in the development of immunosuppression. Several possible mechanisms leading to immunosuppression might involve TLR4. First, the apoptosis and impaired function of B and T cells seen in sepsis likely results, at least in part, from TLR4 signaling, either through direct induction of apoptosis or by the production of secondary mediators of apoptosis, such as glucocorticoids [29–31]. Second, cells previously exposed to LPS have a decreased capacity to respond to a second challenge. Although the mechanism responsible for tolerance to LPS is poorly understood, it likely involves TLR4. Decreased TLR4 signaling after exposure to LPS would likely affect both innate recognition of bacteria and priming of adaptive immune responses and contribute to immunosuppression in patients with sepsis.

Several recent findings suggest that polymorphisms in the human TLR4 gene are associated with the development and progression of atherosclerosis. For example, the Asp299Gly polymorphism is associated with a reduced risk for carotid artery atherosclerosis [32], acute coronary events [33, 34], and an improved response to statin therapy [34]. In keeping with this observation, persons with the Asp299Gly polymorphism also have lower levels of circulating proinflammatory cytokines, such as IL-6, fibrinogen, and soluble vascular cell adhesion molecule–1 [32]. One interpretation of these data is that the protection against vascular inflammation afforded by the Asp299Gly polymorphism is greater than the detrimental im-
pact of more frequent infections seen in these persons. It will be important to identify additional molecules participating in TLR4-mediated atherogenesis and to determine whether polymorphisms in these genes are also associated with protection or susceptibility to this disease.

As more genotypic information for patients becomes available, it will be possible to identify persons within a target population who are most likely to benefit from specific drugs or are most at risk of experiencing adverse effects. It is, therefore, interesting that persons with the Asp299Gly polymorphism may have significantly more benefit from treatment with pravastatin than will those without this polymorphism [34]. Findings such as these could allow tailoring of doses of drugs, such as the statins, to specific persons on the basis of their genotype, as well as contribute to our overall knowledge of disease mechanisms and progression.

The association between TLR4 function and atherosclerosis is consistent with findings showing that TLR4 mRNA and protein are more abundant in plaques in atherosclerotic lesions than in unaffected vessels [35, 36]. Among the cellular components of atherosclerotic plaques are several TLR4-expressing cells, including macrophages, endothelial cells, smooth muscle cells, T cells, and dendritic cells. It is not yet clear whether the primary action of TLR4 in atherogenesis resides within one or more of these cell types, although a function for TLR4 expression in dendritic cell-mediated progression of lesions is suggested by the finding that murine dendritic cells loaded with a heart-specific peptide can induce myocarditis in a TLR4-dependent fashion [37]. Limitations inherent in human studies do not permit direct experimental analyses of cellular requirements in atherosclerosis, but it is likely that both innate and adaptive immune mechanisms contribute to the generation and progression of atherosclerotic lesions.

Given the importance of LPS in airway disease, several groups have studied the effects of the Asp299Gly TLR4 polymorphism on asthma. The rationale for these studies is based on 2 separate, and somewhat contradictory, lines of reasoning. First, exposure to LPS increases the severity of asthma. In persons sensitive to house dust mite allergen, the severity of their asthma correlated more closely with levels of LPS than with those of the allergen itself [38]. Subjects with allergic asthma are also more sensitive to the bronchoconstrictive effects of inhaled endotoxin [39] than are persons without asthma. LPS can clearly exacerbate asthma, probably by increasing the extent of airway inflammation.

Alternatively, exposure to LPS and other TLR ligands in early childhood may, paradoxically, decrease the incidence of asthma later in life [40, 41]. This latter observation is part of a large body of data documenting an inverse relationship between atopic asthma and exposure to pathogens or their products during childhood (reviewed in [42]). It has been suggested that this protection against asthma is related to an increase in the number or activity of regulatory T cells, whose function is to down-regulate immune responses. Regardless of the mechanism, it is clear that LPS can either exacerbate or diminish the severity of asthma, depending on the timing and dose of the exposure to LPS and whether the disease or its exacerbations result primarily from LPS or allergens.

Given the opposing effects of LPS, it is perhaps not surprising that some studies have not demonstrated that the Asp299Gly polymorphism has an impact on the overall incidence of asthma [43]. However, asthma specifically associated with LPS in house dust revealed that persons with the Asp299Gly polymorphism had a decreased risk of bronchoreactivity [44], and the systemic inflammatory response to inhaled LPS was significantly diminished among persons with the common TLR4 Asp299Gly polymorphism [45]. Another study found that asthmatic persons with the Asp299Gly polymorphism have an increased severity of atopy [46]. These findings are consistent with the known ability of LPS to exacerbate existing asthma and to decrease atopy, respectively, and suggest that the Asp299Gly polymorphism is predictive of airway and atopic responses in a specific subset of the population. Interestingly, a study among children of European farmers found that a promoter polymorphism of TLR2 (−16934 A→T) was associated with a lower risk of developing asthma [42], presumably because of a protective effect of TLR2 expression. Moreover, a promoter polymorphism for TLR9, a receptor for prokaryotic DNA [18, 19], appears to be related to the development of asthma in Europeans but not African Americans [47].

Several possible mechanisms involving TLR4 might account for its effect in asthma and atopy. First, although signaling through TLR4 is usually associated with production of Th1 cytokines by dendritic cells, it can also induce IL-10 production [48]. Thus, persons with the Asp299Gly polymorphism may have decreased production of either IL-10 or Th1 cytokines. Alternatively, the Asp299Gly polymorphism may affect the function of regulatory T cells, which not only express TLR4 but are activated by LPS [49]. Thus, the increased severity of atopy in asthmatic persons carrying the Asp299Gly polymorphism could result from decreased activity of allergen-specific regulatory T cells.

Interestingly, in animal models, cytosine-phosphodiester-guanine DNA, in the form of synthetic oligonucleotides, has a unique capacity to serve as an adjuvant for anti-DNA induction and can lead to antibody responses to mammalian DNA, even under conditions in which complete Freund’s adjuvant is ineffective [50, 51]. Other TLR ligands may also promote autoantibody production, although the mechanism may differ from that of cytosine-phosphodiester-guanine DNA. Thus, in experimental models, repetitive stimulation with LPS leads to anti-DNA production [52, 53]. This response may reflect both polyclonal immune cell activation and specific stimulation by...
DNA self-antigen that is released by cells as a result of downstream effects of LPS on the induction of apoptosis [54]. In this context, it is interesting that the TLR4 Asp299Gly polymorphism has recently been found to be protective in the development (but not the severity) of rheumatoid arthritis [55] and both Crohn disease and ulcerative colitis [56, 57]. In view of these findings, the role of infection in the origin of collagen vascular diseases has long been considered, although the exact role of the TLR system remains a subject of future investigation.

Thus, as these considerations indicate, stimulation via the TLR system is likely to have an important impact on human disease. The ability to modulate this response with specific agonists and antagonists is likely to affect to the pathogenesis of many diseases. For example, enhancing adaptive immune response will prove useful in development of vaccines, whereas reducing these responses should benefit cardiovascular or arthritis patients. In addition, an improved understanding of how polymorphisms in TLR genes affect disease progression will permit more accurate assessments of risk for these diseases, so that therapies can be specifically tailored to individuals within defined risk categories.

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