The Role of Toll-Like Receptors in Herpes Simplex Infection in Neonates

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Toll-like receptors (TLRs)—and their associated signal-transducing proteins—on the surface of cells have been demonstrated to account for most, if not all, of the events associated with bacterial sepsis. Using human cells expressing different TLRs, we demonstrated that the interaction between TLR2 and herpes simplex virus (HSV)–1–2 leads to the production of cytokines. Using peripheral-blood mononuclear cells, we tested the ability of cells from people of different age groups to make cytokines in response to HSV. An examination of the host responses of neonates to HSV indicates that, rather than producing less interleukin-6 and interleukin-8 in response to HSV than adults do, neonates produce more of these cytokines than adults do. This may explain the sepsis syndrome that is seen with HSV (and other virus infections) in neonates.

Herpes simplex virus (HSV)–1 causes lifelong infection and periodic disease in the majority of the world’s human population [1, 2]. Herpesviruses (including HSV, varicella-zoster virus, and cytomegalovirus) usually cause only limited disease in adults and older children. In neonates (most often in those <1 week of age), however, they may result in a sepsislike picture that, although rare, can be devastating and is characterized by fever, jaundice, hepatosplenomegaly, and the development of disseminated intravascular coagulation seen in serious TORCH (toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex) infections [1, 2].

The reasons for the disparity between the picture seen in neonates and that seen in adults are not apparent and have been attributed to several putative deficiencies in the innate immune response to HSV in neonates [1, 2]. Although this devastating early-disseminated disease is most often acquired in the birth canal, acquisition through contact with others with active HSV lesions may also occur. The individual’s risk of disseminated disease may correlate with the dose and the duration of the exposure, but the similarity in the presentations of diseases caused by entirely different organisms suggests that the pathogenesis of the disease is determined not by the source of infection but, rather, by the response of the host [1, 2]. The production of cytokines in response to infection has been shown to occur through the interaction between the infectious agents and pattern-recognition proteins on the surface of the cells of the innate immune system. In work published elsewhere, toll-like receptors (TLRs) have been identified as playing a role in the human response to bacteria—and, recently, to viruses [3]. Studies of HSV–1-induced secretion of cytokine have demonstrated that HSV-1 activates murine macrophages through TLR2 [4] and signals TLR9 in murine natural interferon-producing cells [5]. Moreover, the presence or absence of TLR2 is critical to the survival of mice in an in vivo model of HSV-1 infection [4]. Ironically, the presence of TLR2—and, thereby, a more robust innate immune response to HSV—makes it more likely that neonatal mice will succumb to an infectious challenge [4].

In our previous study, we established that TLR2 plays a role in the response to HSV-1, but we did not address the role that it might play in the response to HSV-2 [4]. Although HSV disease may involve destructive lesions of both the liver and the brain, many of its features suggest a sepsislike picture that could be related to the ability of these viruses to stimulate TLRs. To examine the effect that HSV infection has on the transcription of host proteins (particularly cytokines) and to compare the innate immune responses to HSV-1 and HSV-2, we first investigated the abilities of HSV-1 and HSV-2 to induce a cytokine response from human peripheral-blood mononuclear cells (PBMCs) (figure 1A). Challenge with either HSV-1 or HSV-2 activated the secretion of cytokines (interleukin [IL]–6 and IL-8) from human PBMCs in a dose-dependent manner (figure 1A). Both HSV-1 and HSV-2 activated nuclear factor κB (NF-κB) in TLR2-transfected human embryonic kidney (HEK) 293 cells, but neither virus activated NF-κB in either the TLR4-expressing HEK cells or the control HEK cells (figure 1B). In these experiments, virus was inactivated by UV light prior to cell challenge, indicating that virus replication was not necessary either for activation of NF-κB or for stimulation of secretion of...
cytokine. Experiments performed with knockout mice revealed an absolute requirement for TLR2 before HSV-1 [4] and HSV-2 (data not shown) would induce a cytokine response.

Thus, normal adult leukocytes mount a robust innate immune response to both HSV-1 challenge and HSV-2 challenge as measured by their production of inflammatory cytokines. The relative magnitude of in vitro responses observed for HSV-1 compared with those for HSV-2 may not correlate well with clinical outcomes because these studies used inactivated virus and did not account for any additional tissue destruction that may be caused by the viruses themselves. The high multiplicities of infection necessary to see these responses suggest that a threshold level of virus could be necessary to trigger the TLR response.

There is evidence that neonates have higher levels of secretion of cytokine in response to certain microbial pathogens than adults do [6]. To determine whether these observations apply to responses to HSV-1, we compared the cytokine responses of neonatal and adult cells. In a cohort of healthy neonates and adults, we quantified, using whole-blood assays, the secretion of cytokine (IL-6 and IL-8) in response to HSV-1 challenge. In whole-blood assays (figure 2), the per-cell levels of secretion of cytokine are lower than those in cultures of isolated PBMCs (figure 1). However, both polymorphonuclear and mononuclear cells are represented in whole blood. Moreover, whole-blood cultures use autologous serum, rather than exogenous fetal calf serum, as the source of soluble accessory proteins such as CD14. Therefore, whole-blood assays more accurately reflect the functional capacity of the donor to respond to a microbial challenge. After encountering a clinical case of a neonate with disseminated HSV-1 disease who had elevated levels of cytokines in serum, we decided to examine the cytokine responses of adults and neonates to HSV-1. Analysis of the IL-6 response revealed that cord-blood cells from neonates produced significantly higher levels of IL-6 in response to stimulation with HSV than did adult blood cells (figure 2A). Similarly, neonatal blood cells secreted higher levels of IL-8 than did adult blood cells (figure 2B).

As in adults, in neonates the clinical picture of sepsis is associated with the production of inflammatory cytokines, particularly IL-6 [7]. The clinical characteristics of disseminated HSV-1 disease in neonates include hemodynamic instability and laboratory abnormalities that suggest that the clinical picture is the result of cytokine production by the host.

Why is the disease seen in neonates so different from that seen in older children or adults? The host responses of neonates are deficient in many ways. Defects both in production, migration, and complement levels of polymorphonuclear leukocytes and in interferon production have been documented [8, 9]. In addition, macrophages of neonatal mice have less antiviral activity than macrophages of adult mice do [10]. Thus, it would be anticipated that neonates would have higher levels
of virus than adults would. However, the symptoms of most infectious diseases (e.g., fever, vascular instability, thrombocytopenia) are thought to be caused not by the bacterial or viral invaders themselves but by the host response to antigens on these microbes.

The data presented here, documenting an exuberant cytokine response in neonates to HSV-1, may provide a possible explanation for the unique clinical presentation of herpesviruses in neonates compared with that in adults. In studies with yeast and bacterial products, we (data not shown) and others have found that, rather than being weaker than that in adults, the response in neonates to certain antigens, particularly those responses involving TLR2, may be even stronger [6, 11].

The clinical constellation of findings that typify disseminated neonatal herpesvirus infections includes fever, tachycardia, hemodynamic instability, and laboratory abnormalities (including leukocytosis and thrombocytopenia). These clinical and laboratory findings are commonly associated with production of inflammatory cytokines. That toxoplasmosis [12], cytomegalovirus [13], and HSV are all TLR2 ligands suggests that the common clinical features of TORCH diseases [14, 15] may relate to the common interaction between these pathogens and TLR2. Whether rubella also interacts with TLR2 is an issue that requires further investigation. The development of therapies that bind and block TLR proteins on the surface of cells might be considered in the treatment of these diseases.

References