A Paradigm Shift: Vaccine-Induced Antibodies as an Immune Correlate of Protection Against Herpes Simplex Virus Type 1 Genital Herpes

Sita Awasthi and Harvey M. Friedman
Infectious Diseases Division, Perelman School of Medicine, University of Pennsylvania, Philadelphia

(See the major article by Belshe et al on pages 828–36.)

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More than a half-billion people worldwide are infected with genital herpes [1]. During primary infection, herpes simplex virus (HSV) establishes latency in lumbarosacral dorsal root ganglia. The natural history of genital herpes is characterized by frequent episodes of recurrences that may be symptomatic or asymptomatic and that transmit virus to sexual partners or from mother to infant during labor and delivery [2–4]. Genital herpes increases the risk of human immunodeficiency virus (HIV) acquisition and transmission by 3- to 4-fold, which underscores the importance of a vaccine to prevent genital herpes [5].

The Herpevac Trial for Women was a large, multicenter, randomized controlled trial of HSV-2 glycoprotein D (gD2) subunit antigen vaccine administered with monophosphoryl lipid A and alum to HSV-1 and HSV-2 doubly seronegative women [6]. The primary end point was prevention of genital herpes disease caused by HSV-1 and HSV-2 between months 2 (1 month after the second of 3 doses) and 20. Disease was defined as clinically compatible signs and symptoms confirmed by viral culture, seroconversion, or both. That end point was not achieved.

Prevention of HSV-1 or HSV-2 infection, with or without disease, was a secondary end point. Infection was defined by seroconversion after the second vaccine dose (months 2–20) or after the third vaccine dose (months 7–20). Significant reduction in infection and disease was noted for HSV-1, but not HSV-2, after the second and third immunizations. The highest vaccine efficacy against HSV-1 occurred after the third immunization and was 82% among confirmed cases of genital disease. Overall, HSV-1 comprised 60% of the genital herpes infections in the placebo group, which is consistent with the changing epidemiology of infection in North America that is shifting toward HSV-1 as the most common cause of primary genital herpes [7]. It is not surprising that a gD2 vaccine protected against HSV-1 as HSV gD1 and gD2 share >80% amino acid sequence homology; however, it is surprising that the vaccine protected only against HSV-1. Ongoing studies are attempting to explain this perplexing result.

In this issue of *The Journal of Infectious Diseases*, Belshe et al evaluated the immune correlates of protection against HSV-1 genital disease in women vaccinated with the GSK gD2 vaccine. The authors present a more detailed analysis of the immune correlates of protection than reported initially in the Herpevac Trial for Women [6]. Enzyme-linked immunosorbent assay (ELISA) antibody titers, rather than CD4+ or CD8+ T-cell responses, correlated with protection against HSV-1. Among subjects who received the gD2 vaccine, HSV-1 infection rates were 2.5% in individuals with the lowest ELISA titers compared with <1% in subjects with the highest antibody responses. Higher antibody titers were associated with lower rates of both HSV-1 infection and disease. Antibody responses correlated with CD4+ T-cell responses; however, the CD4+ T-cell responses did not correlate with vaccine efficacy, suggesting that the association between antibody and CD4+ T-cell responses was relatively weak.

Many clinicians and researchers will be surprised to learn that antibodies, rather than T cells, correlated with protection against HSV-1 genital infection and disease. Severe HSV recurrent disease is common when CD4+ and CD8+ T cells decline in HIV-1–infected individuals or when subjects are treated
with immunosuppressive therapy to prevent organ transplant rejection. In contrast, severe HSV infection is seldom problematic in subjects with humoral immunity deficiencies, including immunoglobulin (Ig) and complement deficiencies. Therefore, clinical experience has taught us that T-cell responses are critical. When assessing immune correlates of protection, it is important to consider whether immunity is protecting against primary or recurrent infection. In the Herpevac Trial for Women, the gD2 vaccine was used to prevent primary genital herpes and not as therapy for recurrences. A critical role for T cells, particularly CD8+ T cells, in controlling recurrences is well supported by studies in humans and in animal models of genital herpes [8, 9]. The new information provided by Belshe et al suggests that antibodies are essential for preventing the initial infection caused by HSV-1. Additional evidence for the importance of antibody comes from studies of risks to newborns exposed to HSV during labor and delivery. Late in pregnancy, primary genital herpes poses a much greater risk to the newborn than does recurrent genital herpes, as primary infection leaves insufficient time for the newborn to benefit from passive maternal antibody [10, 11]. IgG antibodies, but not T cells, cross the placenta from mother to fetus and account for the protection of the newborn.

The gD2 antigen in the Herpevac Trial targets a glycoprotein involved in virus entry and cell-to-cell spread, activities that are blocked by antibodies and not T cells [12, 13]. ELISA titers measure antibodies that bind to gD2, but do not assess whether these antibodies neutralize virus entry, restrict plaque size as a marker of cell-to-cell spread, or lyse virus-infected cells by antibody-dependent cellular cytotoxicity or by antibody-dependent complement cytotoxicity. Therefore, it will be important to perform functional assays to assess the mechanisms of antibody action that correlate with protection. In retrospect, the fact that antibodies correlated with protection in the Herpevac Trial is not unexpected, based on the known functions of gD2 that are blocked by antibody. The gD2 immunogen did not induce CD8+ T cells; therefore, inclusion of a potent CD8+ T-cell immunogen in the vaccine formulation may improve protection. Goals for future vaccines include improving the antibody responses (neutralizing antibody titers were surprisingly low) and/or developing a vaccine with more balanced humoral and cellular immune responses.

Studies performed in murine and guinea pig models of genital herpes have assessed the importance of antibody and T cells in a prophylactic vaccine. Immunization followed by HSV-2 challenge in B-cell knockout mice with intact T-cell immunity did not protect against infection or disease, whereas immune intact mice were protected [14]. Vaginal antibodies protected at early times post-challenge; however, by 48 hours, other effector mechanisms assumed importance in controlling infection [14, 15]. In guinea pigs, passive transfer of HSV-2 antibody 24 hours after HSV-2 vaginal infection prevented severe primary disease and reduced the number of recurrent lesions and viral load in dorsal root ganglia [16]. A gD2 subunit antigen vaccine administered with HSV-2 glycoprotein C (gC2) improved antibody and complement neutralization by blocking HSV-2 immune evasion from complement and provided better protection against primary disease and recurrent infection in animal models than either antigen alone [17]. A contribution of T cells in primary disease was demonstrated in T-cell–depleted mice that had more extensive viral infection in the vaginal epithelium [18]. CD8+ T cells resident within genital tissues provided superior immunity against genital challenge than did circulating CD8+ T cells [19, 20]. Vaginal administration of chemokines to attract CD8+ T cells to the site of infection improved local vaginal T-cell immunity and provided protection against infection and disease in mice [19]. A successful HSV vaccine will likely require enhancing innate immunity and stimulating potent antibody and T-cell responses.

Seroconversion in subjects with no symptoms or signs of genital herpes is used in clinical trials to identify subjects thought to harbor latent infection [6, 21, 22]. Seroconversion is likely an accurate marker for latent infection in HSV-naive individuals based on the correlation between seroconversion and genital shedding of HSV DNA in asymptomatic subjects [23]. However, little information is available to assess the sensitivity or specificity of seroconversion as a marker for latency in immunized subjects. For example, it is possible that virus replication in genital mucosa results in seroconversion in immunized individuals without the virus ever reaching the dorsal root ganglia. In animal models, dorsal root ganglia can be excised and examined as a marker of latency in addition to measuring genital shedding of HSV DNA. Latency develops in the absence of seroconversion and seroconversion occurs in animals that do not develop recurrences, raising the possibility that seroconversion may be insensitive as a marker for latency [24]. Genital shedding of HSV DNA has not been used as an end point in HSV vaccine prevention trials; however, it may be more a more reliable end point than seroconversion, and may improve the accuracy of future studies that evaluate immune correlates of protection.

Antibodies have emerged as the key correlate of immune protection for the great majority of successful vaccines. Measles, smallpox, rubella, hepatitis A, hepatitis B, and human papillomavirus all induce protective serum or mucosal antibodies [25–27]. For pathogens such as influenza that infect at mucosal surfaces, immune correlates involve locally produced IgA or IgG that reaches the mucosa by transcytosis from serum [28]. Varicella-zoster is the only other human herpes virus in which immune correlates of protection have been evaluated. In support of antibodies, passive administration of varicella-zoster immune globulin provides protection against varicella-zoster
virus infection; however, cell-mediated responses also correlate with protection, suggesting the importance of multiple components of immunity [29–34]. Analysis of the immune correlates of protection against HIV-1 infection in the RV144 trial suggested that antibody titers to the V1/V2 loop of envelope glycoprotein gp120 correlated with protection against infection, which has led to a paradigm shift in HIV-1 vaccine research to emphasize broadly neutralizing antibodies, rather than focus on T cells [35]. The results of the study by Belshe et al in this issue perhaps will lead to a similar shift in thinking about prophylactic vaccines for genital herps.

**Note**

**Potential conflicts of interest.** Both authors: No reported conflicts.

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