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Significantly Less Anti-gC Antibody Detectable in Sera Collected After Varicella Vaccination than After the Disease Varicella

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Abstract

Varicella-zoster virus (VZV) is the first human herpesvirus to be attenuated and then approved in 1995 as a live vaccine for children. Within a few years after its administration in the United States, small outbreaks of breakthrough varicella were observed in vaccinees. Several risk factors were determined. But now a new investigation suggests another risk factor, namely, a deficiency in antibody responses to a specific individual VZV glycoprotein called gC (ORF14; gpV) in the vaccinees. Antibody concentrations to 5 VZV protein antigens were measured in children who had either wild type varicella or varicella vaccination.

These proteins included two major glycoproteins called gE (ORF68; gpI) and gC (ORF14), both constituents of the viral envelope and therefore potentially important targets of the adaptive immune response. Of particular interest, the serum antibody responses to VZV gC antigen were significantly lower in vaccinees than in children who had wild type varicella. In contrast, the serum antibody responses to VZV gE antigen were comparable in both groups. These data implied that relatively little gC antigen was produced in children who were immunized. Since abundant gC protein is produced in skin vesicles during wild type varicella, the lack of a vesicular rash after vaccination may limit the amounts of some viral antigens required for an optimal antibody response.

Physicians have measured antibody titers after varicella for the past 80 years¹⁻³. More recent studies have measured antibody responses to individual VZV protein antigens after varicella^{4,5}. Some studies have measured VZV antibody responses to VZV protein antigens after varicella vaccination⁶⁻⁹. But no study until now has measured the antibody responses to VZV glycoprotein gC antigen after both varicella and varicella vaccination. The VZV serology study included 57 vaccinees in Germany. Approximately 90% had received Varilvax (GSK) and 10% Varivax (Merck) vaccine (personal communication from Dr. Jenke). The result in the report by Jenke et al¹⁰ is both unexpected and potentially important for our understanding of the effectiveness of varicella vaccination. The investigators discovered that the VZV anti-gC titer after varicella vaccination was significantly lower than after wild type varicella (p= 0.006).

VZV is an ancient virus that was present when Lucy Australopithecus and her family lived in the Great Rift Valley of East Africa greater than 3 million years ago; today, all peoples

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around the world are infected with this virus, even the isolated tribes in Amazonia^{11,12}. The VZV genome encodes 70 open reading frames (ORFs), including 9 glycoproteins¹³. The glycoproteins are considered among the most important immunogens because they are present within the envelope of the VZ virion and therefore are prime targets for the adaptive immune system¹⁴. The predominant glycoprotein is gE (ORF68; gpI), usually present within a gE/gI complex¹⁵. Based on data from related herpesviruses, VZV gC (ORF14;gpV) is also considered a major envelope glycoprotein¹⁶. Jenke et al availed themselves of a new VZV assay prepared by Mikrogen Diagnostik (Germany) to measure individual antibody responses to 5 VZV proteins, including gE and gC.

The VZV gC product is one of the last proteins to be produced during the VZV infectious cycle¹⁷. The protein is present in abundance in the skin vesicles, the final site of virus assembly in the infected child with varicella (Fig. 1). Yet, the story during VZV infection of cultured cells is markedly different. VZV is renowned because of the difficulty to grow this virus in cultured cells. Infection spreads slowly in cell culture and viral titers are extremely low, because only 1 out of every 40,000 viral particles is an authentic virion¹⁸. Even after 48 hr, when most other major VZV proteins and glycoproteins are produced, very little gC is detectable in infected cells^{17,19}. The varicella vaccine virus (vOka) in particular expresses minimal gC in cell culture²⁰.

The low anti-gC antibody titers measured by Jenke et al in vaccinees suggest that very little gC antigen is produced after immunization of children. One explanation relates to the lack of an exanthem after vaccination, given that the vesicle is a major site of gC production after wild type varicella (Fig. 1). During an average case of chickenpox, an exanthem includes 250 or more vesicles over the entire body, each filled with gC²¹. In contrast, only a few vaccinees develop a small number of tiny vesiculopapules around the site of vaccination on the arm, a sign of limited replication of the live attenuated virus in the skin²². Up to 5% of vaccinees exhibit a viremia sufficient to cause vesicles distant from the vaccination site²³. With the assumption that gC is produced mainly within vesicles, about 5% of vaccinees would produce greater amounts in the skin while 95% would not produce much gC protein in the skin.

The investigators found no statistical differences between anti-gE titers in children following varicella or varicella vaccination. This observation fits with well documented laboratory data, which have consistently demonstrated that gE is the most abundantly expressed VZV glycoprotein in both human infection and in vitro¹⁵. In other words, even localized vaccine virus replication in the skin of one arm without a noticeable rash produces sufficient gE antigen to initiate robust anti-gE antibody production⁹.

This report will lead to further studies. Concerns to be addressed in the future include susceptibility bias, stemming from a differential baseline state between comparison groups leading to a differential (biased) outcome measure. For example, age of varicella vaccination and age of varicella infection may be quite different. Presumably many of the vaccinated subjects received their immunization between 1-2 years of life (unless they received a catch-up immunization later in childhood). For example, if children younger than 2 years mount a lesser antibody response to both varicella and varicella vaccination, a bias would ensue because few children contract wild type varicella infection at that young age while most vaccinations occur at that time. Additional steps can be taken to adjust p-values for covariate imbalance (age and duration since exposure), and to stratify antibody data by duration since wild type varicella or varicella vaccination.

In previous VZV analyses, an assumption has arisen that the failure of a single varicella vaccination to protect children from breakthrough varicella may be due to a relatively low

total immune response, as compared with immunity after natural disease^{8,9}. The new study suggests an addendum to that hypothesis, namely, an unusually low neutralizing antibody response to a single important VZV envelope glycoprotein might contribute to the failure of varicella vaccination to fully protect. Of course, at this time, we do not know the hierarchy of the most immunologically relevant B- and T-cell immune mechanisms with regard to protection. Nevertheless, the extensive varicella-zoster immune globulin (VZIG) data support the concept that antibody alone can block an initial transfer of VZV infectivity^{21,24}. (VZIG was produced from human donors with known high VZV antibody titers after recent varicella or zoster; however, the VZV protein-specific protective antibodies in VZIG have never been delineated.) In this regard, anti-gC antibody has neutralization properties²⁰. Obviously, full recovery from systemic varicella infection in healthy children also requires cell mediated immune responses²⁵. Yet, the observations by Jenke et al provide unexpected insight into the humoral immune environment of a child after varicella vaccination.

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figure 1.

Cells from a varicella vesicle immunostained for VZV glycoprotein C. Cells were collected from the vesicle of a child with wild type varicella. The cells were dried on a glass slide, fixed and stained for VZV gC with a monoclonal antibody, followed by a fluorophore, as described in ref. 19. The cells were observed by confocal microscopy. The slide includes about 7 cells, all of which contained abundant gC protein (green) in their outer membranes.