Analysis of the Persistence of Humoral and Cellular Immunity in Children and Adults Immunized with Varicella Vaccine

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The persistence of humoral and cellular immunity to varicella-zoster virus (VZV), a human herpesvirus that causes varicella (chickenpox) and reactivates as herpes zoster (shingles) [1]. The varicella vaccine was developed and evaluated in clinical trials in Japan in the 1970s [2]. In the United States, clinical trials were conducted in children with leukemia and later in healthy children [3]. The varicella vaccine elicits humoral and cellular immunity in children and adults [4, 5]. Early vaccine studies demonstrated an age-related difference in seroconversion rates, resulting in the recommendation of a two-dose regimen for adolescents and adults [6, 7]. Whether the initial administration of the varicella vaccine induces prolonged immunity or whether revaccination will be needed is an important question.

The vaccine, produced by Merck (West Point, PA), is made from the Oka isolate of VZV; it is produced in human diploid cells and given as a subcutaneous inoculation. Clinical attenuation of this vaccine has been shown in pre- and postlicensure experience [2, 8, 9]. Our analysis of the virologic basis of attenuation demonstrates that it results from impaired replication in epidermal and dermal cells [10].

The host response to primary infection with VZV involves synchronous induction of humoral and cell-mediated immune responses, which are important for the resolution of VZV infection and maintenance of latency [1]. Natural immunity is characterized by the long-term persistence of circulating T cells that recognize VZV proteins and by the persistence of IgG antibodies to VZV.

After natural infection, cellular immunity to VZV, measured as in vitro T cell proliferation, is well-preserved until the fifth decade of life or later. An age-related decline in cellular immunity to VZV then occurs, which predisposes to herpes zoster. VZV-specific T lymphocytes are found in CD4+ and CD8+ subsets. Within the CD4+ subset, T cells that recognize VZV protein are predominately of the Th1 CD4+ subset, producing interleukin (IL)-2 and interferon (IFN)-γ [11]. Cytokine profiles of elderly immune persons demonstrate that Th1 type CD4+ T cell function declines significantly with age, whereas Th2 type responses, detected by IL-4 production, do not [11]. Thus, induction and preservation of Th1 CD4+ T cell immunity appears to represent the optimal response to VZV.

The purpose of this study was to assess the long-term persistence of cellular immunity compared with that of VZV IgG antibodies in immunized children and adults and to characterize the relative preservation of Th1 and Th2 type cytokine responses.

Patients, Materials, and Methods

Study populations. Studies of the immunogenicity of the Oka-Merck varicella vaccine were done at Stanford University between 1988 and 1992 [4, 5]. The population in the current study consisted of vaccinees who seroconverted and did not develop breakthrough varicella.

Two hundred healthy children (ages 2–12 years), who were vaccinated between 1988 and 1990, received one dose of 1140–5850 pfu of infectious virus or two doses of 3315, 3625, or 9000 pfu. Sixty (30%) of these vaccinees agreed to be reevaluated [5]. Forty healthy adolescents and adults (ages 13–45 years), who were vaccinated between 1990 and 1992, were given two doses
of vaccine (905–9000 pfu/dose) [5]. Eighteen of these vaccinees (45%) agreed to be reevaluated.

Twenty-six (13%) of 200 children and 1 (2.5%) of 40 adults were excluded due to breakthrough varicella.

**Humoral immunity.** Serum was collected at the time of vaccination, 3 months after vaccination, 1 year after vaccination, and at follow-up. The presence of anti-VZV IgG antibodies was measured by ELISA to uninfected VZV- or infected cell lysates (antigen) and uninfected cell lysates (control) as previously described [5]. Initial sera were retested in parallel with follow-up sera to eliminate assay-to-assay variability.

**VZV-specific lymphocyte proliferation assay.** Peripheral blood mononuclear cells (PBMC) were isolated from whole blood and incubated with uninfected VZV- or infected cell lysates or an uninfected cell lysate using methods previously described [5]. T cell recognition of VZV antigen was determined by the stimulation index (SI), which is the ratio of mean counts per minute in antigen-stimulated wells to that in control-stimulated wells. A positive response was defined as an SI of ≥ 3.0. The method used to prepare antigen batches has been standardized to minimize variability between preparations; the antigenicity of each preparation is evaluated using naturally immune subjects.

**Assays for cytokine production.** Cytokine release by PBMC was evaluated by stimulation with VZV antigen or uninfected cell control; maximum release in supernatants harvested on days 1–8 was determined. IL-2 was detected using kits from Genzyme (Cambridge, MA); IL-10 and IFN-γ were detected using kits from Endogen (Cambridge, MA). Cytokine concentrations were determined using a series of standards and extrapolated from a standard curve. Sensitivities of detection were defined by reference standards in each assay.

**Exposure surveys.** Five (8.3%) of 60 children and 1 (6%) of 18 adults reported a household exposure to VZV since vaccination. Nine children (15%) and 3 (17%) adults reported a nonhousehold, close-contact exposure that lasted >4 h.

**Statistical analysis.** Statistical analyses were done using a computer software program (Microsoft Excel, version 5.0; Microsoft, Redmond, WA). Comparisons were made using Student’s paired and unpaired t tests.

### Results

**Study population.** 60 children and 18 adults were reevaluated in our follow-up study. Fifteen of 60 children (25%) and all adults received two doses of vaccine. For children, the mean interval after vaccination was 6.4 years (range, 2.1–8.4) and for adults was 4.7 years (range, 3.6–6.2). The mean age at vaccination was 5 years (range, 2–12) for children and 34 years (range, 23–43) for adults.

**VZV IgG antibody responses.** Three-month seropositivity rates were 100% for children and adults; 1-year seropositivity rates were 97.7% (43/44) for children and 100% (14/14) for adults. At 3 months, adults and children who received two doses of vaccine had significantly more VZV antibody than children who received one dose of vaccine (optical density ± SE: in adults, 1.06 ± 0.16; in children after two doses, 1.03 ± 0.15; in children after one dose, 0.55 ± 0.11; P < .05). Five years after vaccination, seropositivity rates were 93.3% (56/60) for children and 94.4% (17/18) for adults. An increase in antibody concentrations from 1 to 5 years was significant for both adults and children (P < .05). Antibody levels were equivalent between adults and children at 1- and 5-year time points (figure 1A).

**VZV-specific T lymphocyte proliferation.** One year after vaccination, 89.7% (26/29) of children and 94.1% (16/17) of adults had cellular immunity to VZV. At 5 years, VZV-specific T cell proliferation was maintained in 86.7% (52/60) of children and 94.1% (16/17) of adults. The mean SI ± SE at 1 year was 12.08 ± 2.03 for children and 9.89 ± 1.80 for adults, compared with a mean at 5 years of 22.08 ± 2.35 in children and 22.39 ± 4.78 in adults. Responses of children and adults were equivalent at 1 and 5 years.

**Figure 1.** A. Persistence of IgG antibodies to varicella-zoster virus (VZV). There is significant increase in 3-month antibody levels in children (black bars) and adults (white bars) who received 2 doses of vaccine, compared with levels in children who received 1 dose (P < .05). Difference between responses at 1 year and follow-up is significant (P < .05) for children and adults. B. Persistence of T cell recognition of VZV. Stimulation indices (SI) <3.0 are considered negative. Mean SI responses were increased significantly at follow-up vs. 1 year in children (black bars) and adults (white bars) (P < .05). Mos = months.
The mean SI ± SE at day 42 was significantly greater in children than in adults (15.59 ± 3.67 vs. 7.19 ± 0.95; \( P = .03 \)). The mean SI was equivalent at 3 months, by which time, all adults had received two doses of vaccine. Responses diminished in both groups between 3 months and 1 year after vaccination (\( P = .13 \) and .02 for children and adults, respectively). The SIs in individual vaccinees increased significantly between 1 year and the follow-up specimen (\( P = .04 \) for both children and adults) (figure 1B).

No statistically significant association between the infectious virus content of the vaccine and the persistence of T cell recognition of VZV antigen was demonstrated.

**Cytokine profiles.** Cytokine release profiles of proliferating T cells were evaluated when follow-up blood sampling was done. The value in picograms per milliliter was calculated as the difference between the response to the VZV antigen and control. Samples were considered positive if the level of detectable cytokine was above the sensitivity of detection defined by reference standards for each assay. Analysis of the kinetics of cytokine release from days 1 through 8 revealed no age-related differences; peak responses for IL-2 occurred at 2–4 days, and those for IL-10 and IFN-\( \gamma \) occurred at 5–7 days. The interval-to-peak response was equivalent in children and adults.

IL-2 was detected in 71% (36/51) of PBMC cultures from children and 50% (8/16) of cultures from adults. The mean IL-2 concentration in positive cultures was 496.0 ± 53.6 in children and 511.7 ± 87.7 in adults. IFN-\( \gamma \) was detected in 91% (43/47) of PBMC cultures from children and 93% (14/15) from adults. The mean value for IFN-\( \gamma \) in positive cultures was 685.4 ± 78.2 in children and 737.7 ± 163.6 in adults. IL-10 values were 214.2 ± 38.2 in children and 200.8 ± 44.0 in adults. IL-10 was at detectable levels in supernatants of PBMC cultures from 60% (30/50) of children and 38% (6/16) of adults (figure 2). Background levels (mean concentrations in control-stimulated wells) are given in figure 2. The concentrations of cytokines produced in PBMC cultures for children and adults were equivalent and did not correlate with the SI.

**Discussion**

In our study, antibodies to VZV were well-preserved at a mean of 5 years following vaccination in adults and children. Similarly high rates of antibody persistence have been observed in healthy children; surveillance studies have demonstrated that the vaccine induces antibody responses that persist for at least 20 years [2, 8]. Lower VZV IgG titers were associated with lower cellular immune responses in adult vaccinees [5]. However, with longer follow-up, VZV-specific IgG antibody concentrations increased significantly in adults and children. The increase in antibody titers with time after immunization has been observed by others [13]. Our study demonstrated that even among vaccinees with low initial responses, the antibody concentrations can be expected to increase over time and that the two-dose vaccine regimen is sufficient to achieve long-term equivalence of humoral immunity to VZV in adults and children.

The capacity to elicit cell-mediated responses is an important factor, accounting for the long-term protective efficacy of live attenuated viral vaccines. From 98% to 100% of healthy children given the varicella vaccine have T cell recognition of VZV antigens by 2–6 weeks after immunization with one dose; however, the initial T cell responses of adult vaccinees are lower [5]. In our experience with longer follow-up, cell-mediated immunity was found to increase significantly in children and adults, with the responses of adults becoming equivalent to those of children with vaccine-induced immunity.

Consistent with the significant role of the cell-mediated immune response to varicella, VZV infection elicits the proliferation of T cells that produce predominantly Th1 type cytokines [4]. A prominent IFN-\( \gamma \) response is characteristic of naturally acquired immunity to VZV [11]. In our follow-up study, the protection elicited by immunization with live attenuated varicella vaccine was characterized by antigen-specific IL-2 and IFN-\( \gamma \) release by activated T cells. No age-related differences in the cytokine secretion patterns of cultured T cells were detected with long-term follow-up in children and adults who had vaccine-induced immunity.

Surveillance studies indicate that breakthrough varicella occurs at an incidence of about 2%–3% per year in children and adults but that the incidence and severity do not increase over time [2, 14]. Our finding that immune responses to VZV are preserved well after varicella vaccination is consistent with these clinical observations.

Whether the immunologic basis for the increase of VZV immune responses in vaccinees was due to endogenous or ex-
ogenous reexposure could not be determined because the annual epidemics of varicella continued to occur during the study interval. As more of the population receives the varicella vaccine, the opportunities for exogenous exposure to wild-type VZV will decrease. If these exposures are the major mechanism for preserving high levels of immunity to VZV, it may be necessary to provide additional booster doses of varicella vaccine. Watson et al. [15] demonstrated that a booster dose of varicella vaccine given 4–6 years after the initial dose boosts both humoral and cellular immunity. Alternatively, since the immunologic basis for long-term immunity to viral pathogens in general has not been established, continued evaluation of varicella vaccinees may demonstrate that restimulation is not required to maintain memory T cell responses to VZV that sustain B cell and effector T cell populations.

References