Acyclovir Treatment for Varicella Does Not Lower gpI and IE-62 (p170) Antibody Responses to Varicella-Zoster Virus in Normal Children

JANET A. ENGLUND,^{1†*} ANN M. ARVIN,² AND HENRY H. BALFOUR, JR.¹

Departments of Laboratory Medicine and Pathology and Pediatrics, University of Minnesota Health Sciences Center, Minneapolis, Minnesota 55455,¹ and Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305²

Received 7 May 1990/Accepted 23 July 1990

The varicella-zoster virus (VZV) membrane glycoprotein gpI elicits a major immunoglobulin G antibody response after naturally acquired VZV infection; antibody to a nonglycosylated immediate-early protein, IE-62 (p170), represents a response to a nonmembrane VZV component. We evaluated antibody response to VZV gpI and IE-62 (p170) at 28 days and 1 year following infection in 34 children (ages 5 to 16 years) enrolled in a randomized placebo-controlled study of oral acyclovir for the treatment of varicella. All children were VZV antibody negative at enrollment, were previously healthy, and had laboratory-documented varicella. Compared with placebo recipients, acyclovir recipients had lower geometric mean titers by the fluorescent antibody to membrane antigen technique at 28 days (620 versus 836) but similar titers at 1 year (122 versus 122). All children had antibodies to gpI and IE-62 detectable by enzyme-linked immunosorbent assay at 28 days and 1 year. No difference in gpI at 28 days compared with 1 year was noted in acyclovir recipients. No difference in antibody to IE-62 (p170) was noted when acyclovir and placebo recipients were compared at either 28 days or 1 year. Antibody responses to gpI and IE-62 were similar when children were stratified by age (5 to 6 years, 7 to 11 years, 12 to 16 years). A short course of oral acyclovir for the treatment of varicella did not affect antibody responses to gpI or IE-62 (p170) in healthy children at 28 days and 1 year following varicella.

Varicella-zoster virus (VZV) causes varicella, the most common contagious rash disease of childhood in the United States, and is associated with substantial morbidity and mortality, especially in immunocompromised patients. The humoral and cell-mediated immunity to VZV is now being evaluated with new methods that identify viral proteins which are targets of the host responses (2, 15). Responses to specific VZV proteins are variable in healthy children and adults. The induction of antibodies to the viral envelope glycoproteins gpI, gpII, and gpIII has been described following VZV infections in the healthy host (1, 8, 9, 11, 12, 15, 17, 22). Antibodies to other VZV proteins, including a nucleocapsid-matrix p32/p36 complex and immediate-early proteins, have also been demonstrated (7, 10, 14, 16, 21, 23). The immunologic response to a single VZV protein has not been demonstrated to be either necessary or sufficient to induce lasting immunity against VZV (1). Acyclovir administered intravenously has been shown to prevent visceral dissemination of varicella in immunocompromised patients (19, 20). The immune response to VZV in such patients has not been characterized, although the humoral response to varicella in severely immunocompromised children who receive acyclovir therapy is likely to be variable because of underlying host factors. In a placebo-controlled study of otherwise healthy children with varicella (5), oral acyclovir was shown to statistically significantly accelerate time to cutaneous and clinical improvement and decrease the duration of fever. In this setting, it is important to assess VZV

immunity to determine whether the host response is diminished by antiviral therapy. Such an effect could enhance the possibility of recurrent disease later in life. The purpose of this study was to examine antibody production elicited by VZV gpI and IE-62 (p170) protein in otherwise healthy children with laboratory-documented varicella who received a short course of oral acyclovir or placebo in the study described above (5).

MATERIALS AND METHODS

Study design. Healthy 5- to 16-year-old children were recruited to participate in a randomized, placebo-controlled, double-blind investigation at the University of Minnesota Health Sciences Center (5). Children were enrolled within 24 h of onset of varicella and monitored daily for fever, rash, and complications of disease. Acyclovir was provided as a suspension containing 200 mg/5 ml, and the dose was adjusted according to age as follows: 5 to 7 years old, 20 mg/kg (body weight); 7 to 11 years old, 15 mg/kg; and 12 to 16 years old, 10 mg/kg. Drug or placebo was taken four times daily for 5 to 7 days. Venous blood samples were obtained at study entry, on day 28 following enrollment, and 1 year later in 81% of evaluable participants (83 children). Antibody titers to VZV were determined in all participants who completed the study by the fluorescent antibody to membrane antigen technique (FAMA) or enzyme-linked immunosorbent assay (ELISA). Serum samples from 34 participants, representing placebo and acyclovir recipients equally from each age group, were randomly selected for further evaluation of gpI and IE-62 (p170) antibodies.

Virologic studies. Vesicular lesions from each subject were aspirated and cultured in human foreskin fibroblast cells as

* Corresponding author.

† Present address: Influenza Research Center, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

TABLE 1. Median antibody response (GMT) to gpI and IE-62 (p170) following placebo or acyclovir (ACV) treatment of children with varicella

Age group (yr) (n)	GMT to gpI				GMT to IE-62 (p170)			
	ACV		Placebo		ACV		Placebo	
	28 days	1 yr	28 days	1 yr	28 days	1 yr	28 days	1 yr
5-6 (6)	8 ± 1.60	8 ± 5.47	8 ± 5.47	10 ± 5.47	10 ± 1.07	9 ± 1.20	8 ± 2.27	10 ± 2.27
7-11 (6)	8 ± 2.27	8 ± 2.27	8 ± 1.07	8 ± 1.60	10 ± 1.07	10 ± 1.07	10 ± 2.67	10 ± 1.07
12-16 (5)	8 ± 1.20	8 ± 2.28	8 ± 0.80	8 ± 2.80	8 ± 1.20	10 ± 1.2	10 ± 1.2	10 ± 0.80
Total	8 ± 1.74	8 ± 3.24	8 ± 2.26	8 ± 2.94	10 ± 1.03	10 ± 1.03	10 ± 1.97	10 ± 1.38

previously described (4). Serum samples were frozen at -70°C for simultaneous testing to assure a valid comparison of antibody quantitation. Serially collected serum samples were tested for varicella-zoster virus antibody titers by using the standard FAMA (24) and ELISA (Whittaker M. A. Bioproducts, Walkersville, Md.). A serologic diagnosis of varicella was defined as a fourfold or greater rise in FAMA titers or an ELISA index ratio >1.47 between paired acute- and convalescent-phase sera (4).

Specific antibody assays. Immunoglobulin G antibodies to specific VZV proteins were measured by using a solid-phase capture assay. Monoclonal antibodies to gpI (gp90/58) and IE-62 (p170) were prepared and characterized as previously reported (1). These antibodies were diluted in phosphate-buffered saline and added to flat-bottomed microtiter plates (10). After overnight incubation, the plates were washed, blocked with mouse serum, dried, coated with a detergent-solubilized extract of either VZV-infected melanoma cells or uninfected control cells, and incubated overnight. The plates were washed, and nonspecific binding was blocked with the addition of 3% bovine serum albumin in phosphate-buffered saline. After the plates were dried, serial dilutions of the test sera were added and incubated. The presence of specific antibody binding of each serum sample was determined by subtracting the mean optical density of the control wells from the mean optical density of the antigen wells. The serum dilution was considered positive if this calculated number was greater than that calculated for a standard negative serum control plus a constant derived for each assay. The constant was calculated as previously described (10).

Statistical analysis. Geometric mean antibody titers (GMT), as measured by FAMA and the gpI and IE-62 (p170) assays, were calculated for all study participants. Data were analyzed by using a nonparametric statistical test, the Wilcoxon rank sum test, to compare the median geometric mean titers in subjects receiving acyclovir therapy versus placebo recipients or at two times in the same subject group. Two-tailed P values of ≤ 0.05 were considered significant.

RESULTS

All children in this study were previously healthy and had evidence of varicella based on a diagnostic rise in VZV antibody titer. The clinical course of these children has been previously described; a statistically significant decrease in the time needed to achieve a 5% decrease in the number of lesions and fever, as reflected by the area under the time-temperature curve, was noted (5). Acyclovir recipients had FAMA titers similar to those of placebo recipients at enrollment (1.51 versus 1.22) and follow-up 1 year later (122 versus

122) but a significantly lower FAMA GMT on day 28 (836 versus 620, $P = 0.015$) (5).

Antibody to gpI and IE-62 (p170) was measured in 32 of 83 (39%) participants who completed 1-year follow-ups. Randomly selected serum samples were tested from 12 children ages 5 to 6 years, 12 children ages 7 to 11 years, and 10 children ages 12 to 16 years. Antibody responses to gpI and p170 were not measured in serum samples obtained after less than 24 h of rash, on the basis of previous studies demonstrating undetectable antibody early in acute disease (1). Equal numbers of acyclovir and placebo recipients were evaluated at 28 days and 1 year following varicella. All children had antibodies to gpI and IE-62 (p170) detectable at both 28 days and 1 year following varicella. No difference was seen in GMT antibody responses to gpI at 28 days and 1 year in either the acyclovir or the placebo recipients (Table 1); likewise, no significant difference was noted at 28 days or 1 year when acyclovir and placebo recipients were compared. No significant change in gpI antibody over time was noted in either group. The antibody response to gpI did not vary when the children were stratified by age, which was also the determining factor of the dose of acyclovir in this study. No difference in GMT to IE-62 (p170) in acyclovir versus placebo recipients was noted at either 28 days or 1 year following varicella (Table 1). Placebo recipients had a slightly but not significantly higher response to IE-62 (p170) at 1 year than at 28 days ($P = 0.096$); no change in IE-62 (p170) over time was seen in the acyclovir recipients. Again, antibody responses to IE-62 (p170) were not different when stratified by age of the subject.

DISCUSSION

In this study, we demonstrated that a short course of oral acyclovir for the treatment of varicella did not affect the antibody responses to two major proteins of VZV, gpI and IE-62 (p170), in healthy children at 28 days and 1 year following varicella. We have previously shown that although lower GMTs to VZV by FAMA and ELISA were present at 1 month after the onset of varicella, titers at 1 year were similar in the acyclovir and placebo groups (5). The finding that treated and untreated otherwise healthy children have equally high antibody levels to two specific VZV polypeptides at 1 year following varicella is reassuring but not unexpected. All children were presumably equally exposed to a high viral load prior to the development of skin rash—during the viremic phase of disease—because antiviral therapy was administered only after characteristic varicella lesions had developed. Although some evidence of antiviral response, such as defervescence, was seen as early as the first and second days of therapy, an effect on the develop-

ment of new skin lesions, which may be an indirect assessment of viral load reduction, was not seen until after the third day of therapy. On the basis of antibody levels to whole VZV and the specific peptides described here, a sufficient amount of antigen appears to be present before and during the early phase of oral antiviral therapy so that the host humoral antibody responses remain essentially intact. We postulate that this finding is desirable in the treatment of a virus known to undergo reactivation years after infection.

The effect of acyclovir antiviral therapy on the host response following primary infection with VZV has not been previously characterized. The immune response following herpes zoster has been evaluated during a placebo-controlled trial of oral acyclovir therapy in immunocompetent adults (18). No significantly different response to VZV was observed in treated versus placebo recipients as measured by FAMA and the blastogenic response of peripheral blood mononuclear cells (18).

Acyclovir is commonly used to treat infection with another member of the herpesvirus family, herpes simplex virus (HSV). The effect of acyclovir therapy on the humoral immune response to HSV proteins has been analyzed by sensitive techniques such as Western blot (immunoblot) analysis and radioimmunoprecipitation (3, 6, 13). Furthermore, functional analogies and structural homologies have been drawn between certain gene products of HSV and VZV. For example, the HSV-1 gE gene codes for a surface glycoprotein that is a major immunogen of HSV. Antibody to this glycoprotein contains the Fc receptor of HSV and can neutralize HSV only in the presence of complement. The most abundant and immunogenic of the VZV envelope glycoproteins, gpI, is a limited homolog of HSV gE: antibody to this glycoprotein can neutralize in the presence of complement, and sequence homology exists between the two (9). Short-term oral acyclovir for the treatment of primary genital herpes in the immunocompetent host has been related to a decrease in the humoral response to specific polypeptides of HSV, including HSV gE (3, 6). Acyclovir therapy also appears to delay the development of antibody to a number of different HSV-specific polypeptides, including those corresponding to glycoproteins D and E of HSV, and prolonged (6- to 12-month) daily suppression of genital herpes has been shown to decrease levels of antibodies to many major immunogens of HSV-2 (13). Although acyclovir therapy appears to influence the frequency and the magnitude of antibody response to different HSV-specific polypeptides, a similar effect was not present in the antibody response to VZV-specific polypeptides in children who received a short oral course of acyclovir. We speculate that the similar antibody responses in treated and untreated children indicate that all children were exposed to a viral load sufficient for a stimulation of the host humoral immune cascade, as reflected by the duration and magnitude of the humoral response.

ACKNOWLEDGMENTS

This study was supported by grants from the Minnesota Medical Foundation and the Burroughs Wellcome Company and Public Health Service grant AI20459 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Arvin, A. M., E. Kinney-Thomas, K. Shriver, C. Grose, C. M. Koropchak, E. Scranton, A. E. Wittel, and P. S. Diaz. 1986.

- Immunity to varicella-zoster viral glycoproteins, gpI (gp90/58) and gpIII (gp 118), and to a nonglycosylated protein, p. 170. *J. Immunol.* 137:1346-1351.
2. Arvin, A. M., C. M. Koropchak, B. R. G. Williams, F. C. Grumet, and S. K. H. Fong. 1986. Early immune response in healthy and immunocompromised subjects with primary varicella-zoster virus infection. *J. Infect. Dis.* 154:422-429.
3. Ashley, R. L., and L. Corey. 1984. Effect of acyclovir treatment of primary genital herpes on the antibody response to herpes simplex virus. *J. Clin. Invest.* 73:681-688.
4. Balfour, Jr., H. H., C. K. Edelman, C. L. Dirksen, D. R. Palermo, C. S. Suarez, J. Kelly, J. T. Kentala, and D. D. Crane. 1988. Laboratory studies of acute varicella and varicella immune status. *Diagn. Microbiol. Infect. Dis.* 10:149-158.
5. Balfour, Jr., H. H., J. M. Kelly, C. S. Suarez, R. C. Heussener, J. A. Englund, D. D. Crane, P. V. McGuirt, A. F. Clemmer, and D. M. Aeppli. 1990. Acyclovir treatment of varicella in otherwise healthy children. *J. Pediatr.* 116:633-639.
6. Bernstein, D. I., M. A. Lovett, and Y. J. Bryson. 1984. The effects of acyclovir on antibody response to herpes simplex virus in primary genital herpetic infections. *J. Infect. Dis.* 150:7-13.
7. Bogger-Goren, S., K. Bab, P. Hurley, H. Yabuuchi, M. Takahashi, and P. L. Ogra. 1982. Antibody response to varicella-zoster virus after natural or vaccine-induced infection. *J. Infect. Dis.* 146:260-265.
8. Brunell, P. W., V. M. Novelli, P. M. Keller, and R. W. Ellis. 1987. Antibodies to the three major glycoproteins of varicella-zoster virus: search for the relevant host immune response. *J. Infect. Dis.* 156:430-435.
9. Davison, A. J., C. M. Edson, R. W. Ellis, B. Forghani, D. Gilden, C. Grose, P. M. Keller, A. Vafai, Z. Wroblewska, and K. Yamanishi. 1986. New common nomenclature for glycoprotein genes of varicella-zoster virus and their glycosylated products. *J. Virol.* 57:1195-1197.
10. Diaz, P. S., S. Smith, E. Hunter, and A. M. Arvin. 1988. Immunity to whole varicella-zoster virus antigen and glycoproteins I and p170: relation to the immunizing regimen of live attenuated varicella vaccine. *J. Infect. Dis.* 158:1245-1252.
11. Dubey, L., S. P. Steinberg, P. LaRussa, P. Oh, and A. A. Gershon. 1988. Western blot analysis of antibody to varicella-zoster virus. *J. Infect. Dis.* 157:882-888.
12. Giller, R. H., S. Winistorfer, and C. Grose. 1989. Cellular and humoral immunity to varicella zoster virus glycoproteins in immune and susceptible human subjects. *J. Infect. Dis.* 160:919-928.
13. Gold, D., R. Ashley, G. Solberg, H. Abbo, and L. Corey. 1988. Chronic-dose acyclovir to suppress frequently recurring genital herpes simplex virus infection: effect on antibody response to herpes simplex virus type 2 proteins. *J. Infect. Dis.* 158:1227-1234.
14. Grose, C. 1983. Zoster in children with cancer; radioimmune precipitation profiles of sera before and after illness. *J. Infect. Dis.* 147:47-56.
15. Grose, C., and V. Litwin. 1988. Immunology of the varicella-zoster virus glycoproteins. *J. Infect. Dis.* 157:877-881.
16. Harper, D. R., and C. Grose. 1989. IgM and IgG responses to varicella-zoster p32/p36 complex after chickenpox and zoster, congenital and subclinical infections, and vaccinations. *J. Infect. Dis.* 159:444-451.
17. Keller, P. M., B. J. Neff, and R. W. Ellis. 1984. Three major glycoprotein genes of varicella-zoster virus whose products have neutralization epitopes. *J. Virol.* 52:293-297.
18. Mitchell, C. D., R. C. Gehrz, and H. H. Balfour, Jr. 1986. Varicella-zoster specific immune responses in acute herpes zoster during a placebo-controlled trial of oral acyclovir therapy. *Diagn. Microbiol. Infect. Dis.* 5:113-126.
19. Nyerges, G., Z. Meszner, E. Gyarmati, and S. Kerpel-Fronius. 1988. Acyclovir prevents dissemination of varicella in immunocompromised children. *J. Infect. Dis.* 157:309-313.
20. Prober, C. G., L. E. Kirk, and R. E. Keeney. 1982. Acyclovir therapy of chickenpox in immunosuppressed children—a collaborative study. *J. Pediatr.* 101:622-626.

21. Shiraki, K., and R. W. Hyman. 1987. The immediate early proteins of varicella-zoster virus. *Virology* **156**:423–426.
22. Weigle, K. A., and C. Grose. 1983. Common expression of varicella-zoster viral glycoprotein antigens in vitro and in chickenpox and zoster vesicles. *J. Infect. Dis.* **148**:630–638.
23. Weigle, K. A., and C. Grose. 1984. Molecular dissection of the humoral immune response to individual varicella-zoster viral proteins during chickenpox, quiescence, reinfection, and reactivation. *J. Infect. Dis.* **149**:741–748.
24. Williams, V., A. Gershon, and P. A. Brunell. 1974. Serologic response to varicella-zoster membrane antigens measured by indirect immunofluorescence. *J. Infect. Dis.* **130**:669–672.