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Immunization of Inbred Guinea Pigs with Varicella-Zoster Virus Grown in a Syngeneic Transformed Embryo Cell Line

CHARLES GROSE

Department of Pediatrics, University of Texas Health Science Center, San Antonio, Texas 78284

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Varicella-zoster virus was serially propagated in a chemically transformed and cloned line of embryo cells (designated "104 C1") derived from the inbred strain 2 guinea pig. When strain 2 guinea pigs were immunized with varicella-zoster virus subcultivated in the syngeneic cell line, they produced high-titer virus-specific antiserum which lacked antibody against cellular determinants. This immunization procedure offers both practical and theoretical advantages over prior methods which involved inoculation of outbred laboratory animals with varicella-zoster virus grown in allogeneic or xenogeneic cell cultures.

Production of high-titer antiserum to varicella-zoster virus (VZV) continues to be a difficult problem. Since VZV grown by conventional methods in cultured human cells remains cell associated, laboratory animals (guinea pigs and rabbits) immunized with infected cell extracts develop antibodies not only to the virus-specific antigens but also to various human cell determinants (7, 9). The latter antibodies often interfere with the usefulness of the antiserum and therefore must be removed by exhaustive adsorptions with uninfected cells. An alternative approach involves VZV propagation in a sensitive cell substrate derived from the same species which is to be immunized: both outbred guinea pigs (7) and outbred monkeys (8, 12) have been successfully immunized with VZV extracts obtained from homologous cells of caviid and simian origin, respectively. However, obstacles remain to those who wish to use either of the above methods; viz., only low-passage caviid cell cultures established from early gestational embryos (<2 cm in length) support the growth of VZV (B. J. Edmond, C. Grose, and P. A. Brunell, J. Gen. Virol., in press). With regard to monkeys, these species have become increasingly scarce and are often prohibitively expensive. Another unaddressed issue is that both of the above immunization techniques, which involve outbred animals, also induce the production of antibodies to major histocompatibility antigens.

This report describes a novel approach to the production of VZV immune guinea pig serum which avoids most of the difficulties encountered in the past. The cell substrate was a benzo[*a*]-pyrene-transformed and cloned line derived from strain 2 guinea pig embryos by Evans and DiPaolo (3); cells for these experiments were received from the American Type Culture Collection, Rockville, Md. (designated "104 C1", passage 23) and grown in Eagle minimal essential medium supplemented with 8% fetal bovine serum and antibiotics. The virus strain "VZV-32" was originally isolated and serially passaged in human melanoma cells (5). After 16 subcultivations, cell-free virus was obtained by sonic disruption of a VZV-infected human melanoma cells monolayer, as previously described (5). The clarified sonicate was adsorbed onto a 1-day-old culture of 104 C1 cells, and the virus was further passaged an additional eight times to remove human melanoma cell determinants. The optimal conditions for propagation of VZV in these transformed cells have been described in another publication (B. J. Edmond, C. Grose, and P. A. Brunell, J. Gen. Virol., in press). The titer of cell-free virus after the last subcultivation was 4 $\log_{10}$ plaque-forming units per ml when assayed on human melanoma cell monolayers (5).

The origin of the strain 2 line of inbred guinea pigs has been described in detail by Bauer (2). Four male strain 2 guinea pigs, 8 weeks of age, were purchased from the breeding colony at the Texas Science Park, University of Texas, Bastrop, Tex. After an initial bleeding, each animal was inoculated in each hind footpad with 0.15 ml of a 1:1 emulsion of virus sonicate and complete Freund adjuvant (Difco Laboratories, Detroit, Mich.). After 4 weeks, each animal was given a booster immunization with the same amount of virus-adjuvant emulsion in each hip muscle. Beginning 1 week after the second inoculation, the animals were periodically bled by intracardiac puncture after Innovar (Janssen Pharmaceutica, New Brunswick, N.J.) sedation.

A total of 16 serum samples were collected

from the four guinea pigs between 1 and 11 weeks after the booster immunization. Each bleeding yielded approximately 2 ml of serum from 4 to 5 ml of whole blood. The titers of antibody directed against VZV-induced membrane antigens were determined by indirect immunofluorescence on living virus-infected cells (the FAMA test) (13); the serological data are presented in Fig. 1. Of the initial four secondary sera, two each were positive at dilutions of 1:1,024 and 1:512. The mean FAMA titer fell from a peak of 1:768 to 1:192 over a 10-week period. The initial four sera also were screened for VZV-specific neutralizing activity by the semimicromethod (6) and were found to contain neutralizing antibody levels equal to or within a twofold dilution of the FAMA titer; a high concordance between results of these two serological assays has also been observed with human sera also (6).

Three assays were employed to test for antibody to nonviral cellular antigens: indirect immunofluorescence on both living and fixed uninfected cells (12, 13) and immune precipitation (4). No antibodies against human cell surface, cytoplasmic, or nuclear antigens were detected by immunofluorescence at a 1:4 serum dilution. These negative data are in contradistinction to findings in VZV immune sera obtained from guinea pigs immunized with VZV propagated in human cells (7). The results of immune precipitation are presented in Fig. 2. Antibodies to the major VZV glycoprotein antigens were present in all four of the initial sera (4, 7), whereas none of the antisera reacted with additional uninfected cell glycoproteins (4). Similar analyses of immunoprecipitates obtained with the more convalescent VZV immune caviid sera revealed the same electrophoretic profile (fluorograms not shown).

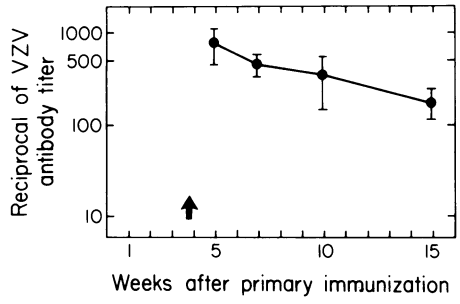

FIG. 1. *Humoral immune response in strain 2 guinea pigs inoculated with VZV emulsified in complete Freund adjuvant. Four guinea pigs were immunized and bled four times within 11 weeks after the booster (↑) inoculation. Titers of virus-specific antibody directed against VZV-induced membrane antigens were measured by indirect immunofluorescence (13) and plotted as means and standard deviations.*

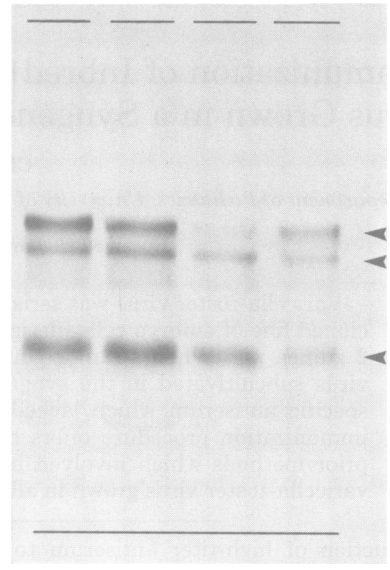

FIG. 2. *Glycoproteins precipitated by the varicella-immune guinea pig sera. Detergent-solubilized extracts of VZV-infected cultures labeled with tritiated fucose were mixed with samples of pre- and postimmune sera obtained from each of the four immunized strain 2 guinea pigs. The preimmune sera precipitated insufficient radioactivity (<0.7%) for further analysis. In contrast, 14 to 18% of the radioactivity in the antigen sample was removed by the postimmune sera; this precipitating capacity was comparable to that observed in VZV immune sera obtained from hyperimmunized outbred rabbits and guinea pigs (7). When the latter immune precipitates were analyzed by electrophoresis in a sodium dodecyl sulfate-8% polyacrylamide slab gel as previously described (4), the fluorogram demonstrated three major glycopeptides (←) with estimated molecular weights of 118,000, 98,000, and 62,000 daltons, uppermost to lowermost, respectively.*

In summary, these immunization experiments demonstrate that high-titer VZV immune serum can be raised in inbred strain 2 guinea pigs inoculated with virus propagated in a strain 2 derived and chemically transformed cell line. This antiserum, which is devoid of antibody against human cell determinants, has many uses as a viral diagnostic reagent. Since it possesses neutralizing activity, it can be employed in plaque inhibition assays to identify wild isolates. Conjugated with fluorescein isothiocyanate, it would be a sensitive and specific indicator of VZV infection in human tissue specimens (11). Other potential applications include use in radioimmunoassays or enzyme-linked immunosorbent assays for detection of VZV antigens in clinical specimens and use as an immunological

probe for precipitation of virus-specific antigens. In addition, the transformed caviid embryo cells may be an acceptable alternative substrate to primary and secondary guinea pig embryo cells for isolation and propagation of other herpesviruses and group A coxsackieviruses (cf. references 1 and 10).

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