J. gen. Virol. (1987), **68**, 2449–2454. *Printed in Great Britain*

Key words: *VZV/polypeptide antigens/immune responses*

Humoral and Cellular Immunity to Varicella-Zoster Virus Glycoprotein gpI and to a Non-glycosylated Protein, p170, in the Strain 2 Guinea-pig

By ANN M. ARVIN,[1]* SUZANNE M. SOLEM,[1] CELINE M. KOROPCHAK,[1] ELAINE KINNEY-THOMAS[2] AND SHARON G. PARYANI[3]

[1]*Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305,* [2]*Genetic Systems Inc., Seattle, Washington and* [3]*Department of Pediatrics, University Hospital, Jacksonville, Florida 32209, U.S.A.*

(*Accepted 4 June 1987*)

SUMMARY

Strain 2 guinea-pigs were inoculated with infectious varicella-zoster virus (VZV) or with immunoaffinity-purified proteins of VZV. Monoclonal antibodies to the VZV gpI (90000/58000 complex) and to a non-glycosylated protein, p170, were used to prepare the polypeptide antigens. Humoral and cell-mediated immune responses to the infectious virus were compared with those elicited by the gpI and p170 proteins. Both VZV IgG antibody production and T lymphocyte proliferation to VZV were detected after immunization with infectious VZV and with VZV proteins. The antibody and T lymphocyte responses waned after protein immunization in comparison with the responses induced by infectious VZV but were detected again immediately after reimmunization with gpI or p170.

The study of the pathogenesis of varicella-zoster virus (VZV) infection is hampered by the lack of an appropriate animal model. Simian varicella virus is antigenically related to human VZV but the disease caused by the former in primates is usually much more severe than VZV infection in the healthy human host (Arvin *et al.*, 1983). Although infection of the guinea-pig with VZV does not produce disease, Myers *et al.* (1985) observed that human VZV passaged in foetal guinea-pig tissue culture caused viraemia and nasopharyngeal infection in weanling guinea-pigs. Antibodies to viral proteins of 62000, 98000 and 118000 M_r detected by immunoprecipitation with sera from VZV-immune human subjects were also observed by immunoprecipitation with guinea-pig sera (Grose & Friedrichs, 1982). Matsunaga *et al.* (1982) showed that guinea-pigs inoculated with VZV adapted to guinea-pig cells developed delayed hypersensitivity to VZV antigen as well as VZV-specific antibodies.

In the present study, the immunogenic potentials of a major VZV glycoprotein, gpI (Davison *et al.*, 1986), and of a non-glycosylated protein, p170, were evaluated in the strain 2 guinea-pig and compared with the immune response after inoculation with infectious VZV. The strain 2 guinea-pig was used for these immunological studies because of its potential value for the study of VZV pathogenesis in relation to the immune response.

The animals were weanling strain 2 guinea-pigs obtained from the Charles River Caviary, Charles River Breeding Laboratories, Wilmington, Mass. and the National Cancer Institute, Bethesda, Md., U.S.A. and weighed approximately 250 g at the time of the initial inoculation. The animals that received infectious VZV were inoculated with guinea-pig-adapted VZV. Foetal guinea-pig cell lines were established from guinea-pig embryos of 1·0 to 1·5 cm (Edmond *et al.*, 1981). The cells were grown in MEM with 10% foetal calf serum and were used to prepare VZV-infected cells for up to six passages. A VZV isolate from a cutaneous varicella vesicle was passed ten times in guinea-pig embryo cells. Guinea-pig cell-adapted VZV was stored at −70 °C and passed once more in guinea-pig embryo cells immediately before use. Animals given

infectious VZV were inoculated with 1×10^5 to 1×10^6 VZV-infected cells subcutaneously as a single dose of 0·25 to 0·45 ml. The titration of these preparations by infectious centre assay was $1{\cdot}0 \times 10^4$ to $1{\cdot}6 \times 10^5$ p.f.u./ml. The animals were inoculated in groups of four, with three animals receiving infectious VZV and one animal given the control preparation, by subcutaneous injection. Eighteen animals received infectious VZV with six controls. These animals were bled by cardiac puncture at 3 or 10 days after the initial injection, and at 3 weeks, 6 weeks and 12 and/or 18 weeks. A final sample was taken at 18 to 24 weeks from animals inoculated with infectious VZV and their controls.

VZV protein antigens were made by immunoaffinity separation as previously described, using a monoclonal antibody (MAb) to VZV gpI (90000/58000 M_r complex) and an antibody to p170 which precipitated a protein of 170000 M_r and demonstrated a pattern of nuclear staining in VZV-infected cells (Arvin *et al.*, 1986). VZV-infected cell extracts were prepared from human melanoma cells labelled with [^{35}S]methionine and detergent-solubilized. The extract was ultracentrifuged and the supernatant was adsorbed to the MAb-coated Sepharose 4B overnight; bound proteins were eluted with 3 M-potassium thiocyanate pH 7·5. An uninfected cell control preparation was made in parallel. Each preparation was examined by SDS–PAGE and autoradiography or by immunoblot; densitometric tracing showed that the VZV-specific bands made up at least 72% of the protein preparations. Animals receiving VZV protein antigens and their controls were given an initial inoculation followed by a second injection at 1 week, with the bleeding schedule as described for animals given infectious VZV started after the second dose. A third injection was given to these animals at 6 or 18 weeks. Each injection contained 50 μg protein in phosphate-buffered saline. The animals were inoculated in groups of four, with three animals receiving the VZV protein antigen and one animal given the corresponding control preparation, by subcutaneous injection. Fifteen animals were given gpI with five controls, and 12 animals were given p170 with four controls. The morbidity associated with cardiac puncture prevented some samples from being obtained and led to the death of some animals, especially at the early time points when the animals were small.

The acquisition of humoral immunity to VZV as measured by radioimmunoassay (RIA) (Arvin & Koropchak, 1980) of sera from animals given a single inoculation of guinea-pig-adapted VZV and those given an initial injection of VZV protein antigen, gpI or p170, followed by a second dose after 1 week and a third dose at 6 or 18 weeks is shown in Fig. 1. Specific binding of VZV IgG antibodies was detected using ^{125}I-labelled monospecific goat antiserum to guinea-pig IgG (Cappel Laboratories). The antibody titre was read as the serum dilution where the ratio of mean c.p.m. of duplicate antigen wells to mean c.p.m. of duplicate control wells was above 2·5. Four of eight animals had detectable antibody to gpI by 10 days after the first injection as did four of seven animals given p170, whereas all of nine animals tested 10 days after inoculation with infectious VZV remained seronegative. However, all of the animals given infectious VZV showed seroconversion by 3 to 6 weeks and 18 of 21 samples taken from 12 to 18 weeks after inoculation had VZV antibody titres of $\geqslant 1:16$. The geometric mean titre (GMT) did not differ for animals in the groups given 1×10^5 to 6×10^5 infected cells/dose compared with those given 1×10^6 infected cells/dose. The GMT of sera from animals tested 12 to 18 weeks after inoculation with infectious VZV was 1:100 with seven of nine animals showing persistent antibody $\geqslant 1:16$, while the GMT in animals given gpI and p170 declined to $\leqslant 1:16$ during the same interval. When animals in the latter group received a third injection of the respective protein preparation, the antibody titres rose rapidly to high values, with the GMT at 3 weeks being 1:8709 for gpI and 1:7943 for p170. The titres were $<1:16$ by RIA in all of the sequential serum samples obtained from the six control animals given the uninfected cell preparation and in the four animals from each group given gpI or p170 control preparations.

Two serum samples which showed titres $\geqslant 1:1024$ by RIA following injection with gpI or p170 were tested by immunoblot to demonstrate serum antibodies reactive with specific VZV proteins. Immunoaffinity-purified VZV proteins and the control preparations made with each MAb were separated by SDS–PAGE in a 9% acrylamide gel cross-linked with methylenebis-acrylamide followed by electrophoretic transfer to nitrocellulose with a Trans-Blot chamber (Bio-Rad). The nitrocellulose strips were incubated with the test sera at a dilution of 1:20. The

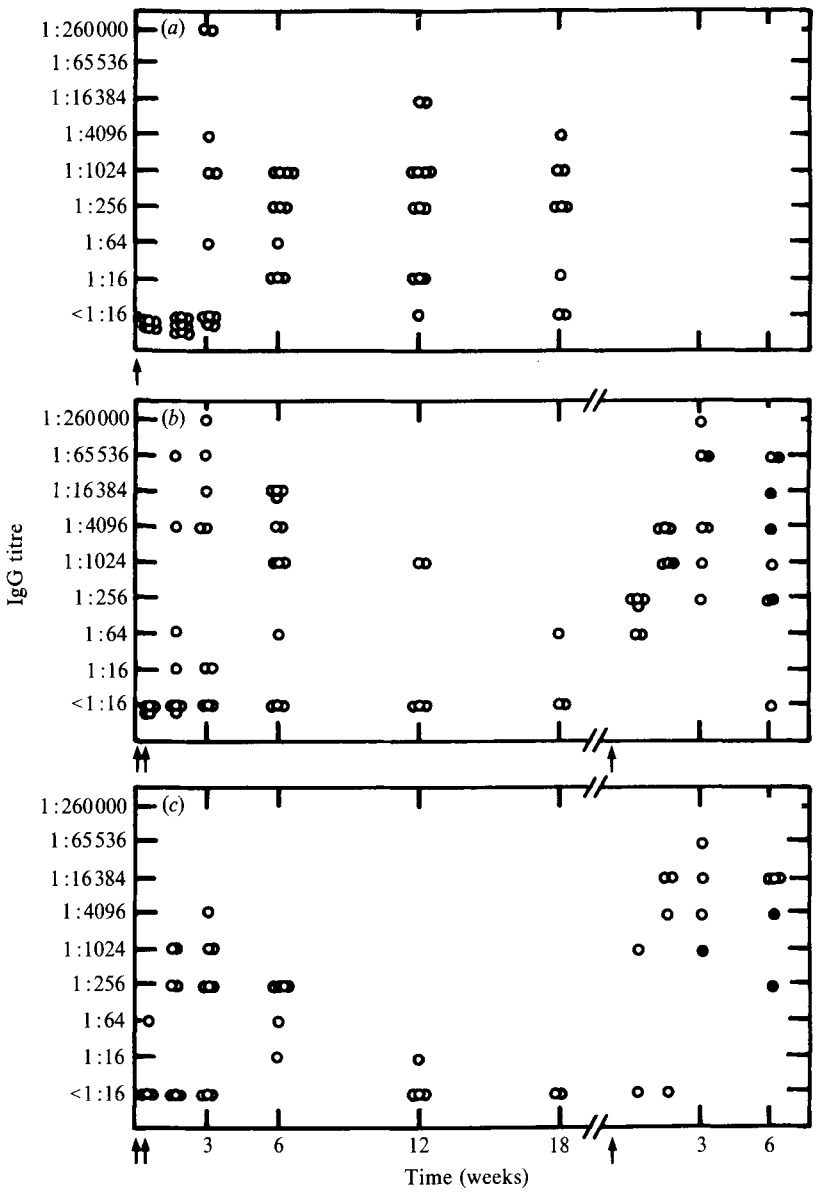

Fig. 1. VZV IgG antibody production after the inoculation of strain 2 guinea-pigs with infectious VZV, VZV gpI or VZV p170. The antibody titres were measured by RIA in serum from animals given infectious VZV adapted to guinea-pig embryo cells (*a*), immunoaffinity-purified gpI (*b*) or p170 (*c*). The titres are presented in relation to the time interval in weeks after the injections which are indicated by the arrows on the horizontal axis. The dark circles indicate the IgG titres of animals that were given the third injection of gpI or p170 at 6 weeks rather than 18 weeks after the second dose.

binding of IgG in the test serum was detected by immunoperoxidase stain using biotinylated goat antibody to guinea-pig IgG, an avidin–biotin complex solution (Vectastain ABC kit; Vector Laboratories, Burlingame, Ca., U.S.A.) and 4-chloro-1-naphthol. The M_r of visible bands was estimated using reference standards. Sera from animals that had been inoculated with uninfected guinea-pig embryo cells were included as controls. In each case, IgG binding was detected to proteins of the expected M_r based upon the protein complex which had been used for immunization, whereas no reactivity was present against the other protein preparation.

Antibody binding to specific proteins was also demonstrated by using the MAbs in a solid phase RIA to capture the VZV protein (Arvin *et al.*, 1986). The blocking of antibody binding to the specific VZV protein antigen was demonstrated in these sera when the homologous MAb was added to the plates before the test serum; the titres were reduced to $<1:16$ by capture RIA.

Sera were tested for VZV-neutralizing antibodies in a plaque reduction assay by incubating the heat-inactivated test serum with a standard inoculum of 500 p.f.u./ml of VZV (50 p.f.u./well), with and without guinea-pig complement (Hazelton Biotechnologies, Vienna, Va., U.S.A.), used at an initial dilution of 1:8. The sample was added to a freshly confluent monolayer of Vero cells in 24-well tissue culture plates, and overlaid with MEM containing 10% foetal calf serum. Plaques were counted after 10 to 14 days incubation after staining with crystal violet. The neutralization endpoint was read as the serum dilution producing 50% plaque reduction compared to control wells containing serum from an animal injected with an equivalent number of uninfected guinea-pig embryo cells. Complement-dependent neutralizing antibody against VZV, with titres of 1:16 to 1:64, was detected as late as 12 weeks in five animals tested after the initial injection of gpI. The neutralizing titres with complement rose to 1:128 in two animals tested 10 days and 3 weeks, respectively, after the third injection of gpI. Five animals given infectious VZV had low titres of complement-independent neutralizing antibodies (1:8 to 1:16) which were enhanced to 1:16 to 1:64 by complement; these antibodies persisted for 6 to 12 weeks. None of six animals given p170 had VZV-neutralizing antibodies at 3 to 6 weeks after the initial injection of p170 or after subsequent p170 injections.

VZV-specific, cell-mediated immunity was measured with a T lymphocyte proliferation assay *in vitro* by incubating peripheral blood mononuclear cells, obtained by cardiac puncture and separated with Ficoll-Hypaque, with VZV antigen or an uninfected cell control in 96-well microtitre plates. The cells were cultured at a concentration of 2×10^5 cells/0·1 ml per well in RPMI with 10% guinea-pig serum, 2-mercaptoethanol and 5% $NaHCO_3$. Duplicate wells were incubated with antigen or control at 1:4, 1:16 and 1:64. After 5 days, the wells were pulse-labelled with [^{3}H]thymidine. On day 6, the cells were harvested onto glass filter paper and radioactivity was counted in a liquid scintillation counter. The stimulation index (S.I.) was calculated as the ratio of mean c.p.m. in duplicate antigen-stimulated wells to the mean c.p.m. in duplicate uninfected cell control wells. Each assay included control wells stimulated with phytohaemagglutinin. As shown in Fig. 2, the injection of guinea-pigs with VZV gpI and VZV p170 elicited lymphocyte proliferation to VZV antigen with the peak stimulation index (S.I.) detected at 3 weeks after the initial inoculation. The mean peak S.I. was $5·2 \pm 1·03$ S.E. for gpI and $6·6 \pm 1·53$ S.E. for p170. The mean S.I. for 17 samples from gpI control animals was $1·25 \pm 0·16$ S.E. and for 11 samples from p170 controls was $0·94 \pm 0·13$ S.E. The peak S.I. did not occur until 6 weeks after inoculation of the animals with infectious VZV but it rose to $8·7 \pm 2·05$ S.E. The mean lymphocyte proliferation response decreased to less than 2·0 by 12 weeks after immunization with gpI and p170 whereas six of eight guinea-pigs tested 18 to 24 weeks after inoculation of infectious VZV maintained S.I. above 2·0. As was observed in the analysis of the humoral immune response, the third dose of gpI and p170 produced an immediate rise in cell-mediated immunity as determined by lymphocyte proliferation (Fig. 2).

Most animals that had S.I. $\geqslant 2·0$ after injection with gpI or p170 also had humoral immunity when both responses were tested on the same blood sample. Six of seven animals with S.I. $\geqslant 2·0$ after gpI also had VZV antibody titres $\geqslant 1:16$ as did five of six animals given p170.

These studies demonstrated that gpI and p170 were equally immunogenic in inducing both humoral and cellular immunity in the strain 2 guinea-pig. Herpesvirus glycoproteins have been considered likely to be important in producing virus-specific immunity because of their expression on the membranes of infected cells and because MAbs to the surface glycoproteins of human herpesviruses often have neutralizing activity (Keller *et al.*, 1984; Friedrichs & Grose, 1984; Rasmussen *et al.*, 1985). Immunization with the gpI protein prepared by immunoaffinity using a gpI reactive MAb also elicited a neutralizing antibody response *in vivo* that was complement-dependent, as was observed by Keller *et al.* (1986). Although p170 did not elicit neutralizing antibody *in vivo*, it induced both IgG antibody and T lymphocyte proliferation in the strain 2 guinea-pig which parallels our observation of persistent humoral and cellular immunity to p170 in most human subjects following VZV infection (Arvin *et al.*, 1986).

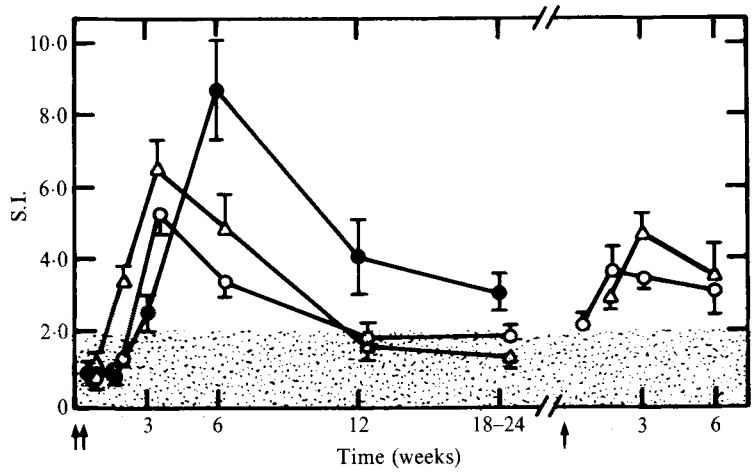

Fig. 2. VZV lymphocyte proliferation after inoculation with infectious VZV, VZV gpI or VZV p170. VZV lymphocyte proliferation is expressed as the stimulation index (S.I.) for cultures of peripheral blood mononuclear cells from animals given infectious VZV (●), VZV gpI (○) or VZV p170 (▲). The S.I. is presented in relation to the time interval in weeks after inoculation, as indicated by arrows on the horizontal axis. The stippled area represents background values.

The emphasis upon glycoproteins in viral immunology may require modification given the evidence of host responses to the non-glycosylated, internal or nucleoproteins of several other viruses (Townsend *et al.*, 1984; Yewdell *et al.*, 1986). The induction of both humoral and cellular immunity in the strain 2 guinea-pig following immunization with VZV proteins is consistent with the demonstration of both antibody and T lymphocyte proliferation to herpes simplex virus and cytomegalovirus proteins in murine and guinea-pig models (Donnenberg *et al.*, 1980; Shrier *et al.*, 1983; Bia *et al.*, 1983; Gonczol *et al.*, 1986). These observations contradict the concept that purified viral proteins are likely to induce only humoral immunity. However, the waning of VZV antibodies and T lymphocyte proliferation illustrates the general failure of immunization with herpesvirus proteins to induce long term immunity. Both peak IgG antibody production and T lymphocyte proliferation responses were delayed in animals given infectious VZV in comparison with those of animals given gpI and p170. This delay suggested that a phase of viral replication *in vivo* occurred which, by analogy with the experience with live viral vaccines, may be essential to elicit persistent immunity. One hypothesis is that local viral replication or replication in the regional lymph nodes provides a critical opportunity for antigen presentation to an essential T lymphocyte subpopulation. Nevertheless, despite the waning of VZV immunity after protein injection, our studies demonstrated a 'priming' effect upon the immune response to the third injection of gpI or p170. This phenomenon, as initially described with poliovirus peptides (Emini *et al.*, 1983), warrants further investigation in relation to the development of subunit herpesvirus vaccines.

Although a live attenuated varicella virus vaccine has been effective in selected populations, the live virus vaccine causes cutaneous lesions in some patients, suggesting the occurrence of viraemia (Gershon *et al.*, 1984). The vaccine virus can also establish latent infection, making it less desirable for use in the general population in which much of the morbidity caused by VZV is due to the reactivation of latent virus. The investigation of the immune response to VZV proteins in the guinea-pig provides background for testing methods of enhancing the immunogenicity of purified viral proteins (Ho *et al.*, 1986) which can then be compared to the immune response to infectious VZV.

The monoclonal antibodies were originally produced by Drs Elaine Kinney-Thomas and Kathy Shriver, Genetic Systems Inc., Seattle, Wash. and were supplied by Syva Inc., Palo Alto, Ca., U.S.A. This work was supported by Public Health Service Grant AI-22280; Dr Arvin is the recipient of a Research Career Development Award, AI-00624.

REFERENCES

ARVIN, A. M. & KOROPCHAK, C. M. (1980). Immunoglobulins M and G to varicella-zoster virus measured by solid phase radioimmunoassay: antibody responses to varicella and herpes zoster infections. *Journal of Clinical Microbiology* **12**, 367–374.

ARVIN, A. M., MARTIN, D. P., GARD, E. A. & MERIGAN, T. C. (1983). Interferon prophylaxis for simian varicella infection in *Erthrocebus patas* monkeys. *Journal of Infectious Diseases* **147**, 149–154.

ARVIN, A. M., KINNEY-THOMAS, E., SHRIVER, K., GROSE, C., KOROPCHAK, C. M., SCRANTON, E., WITTEK, A. E. & DIAZ, P. S. (1986). Immunity to varicella-zoster viral glycoproteins, gp I (gp 90/58) and gp III (gp 118) and to a nonglycosylated protein, p 170. *Journal of Immunology* **137**, 1346–1351.

BIA, F. J., GRIFFITH, B. P., FONG, C. K. Y. & HSIUNG, G. D. (1983). Cytomegaloviral infections in the guinea pig: experimental models for human disease. *Reviews of Infectious Diseases* **5**, 177–195.

DAVISON, A. J., EDSON, C. M., ELLIS, R. W., FORGHANI, B., GILDEN, D., GROSE, C., KELLER, P. M., VAFAI, A., WROBLEWSKA, Z. & YAMANISHI, K. (1986). A new nomenclature for the glycoprotein genes of varicella-zoster virus and their glycosylated products. *Journal of Virology* **57**, 1195–1197.

DONNENBERG, A. D., BELL, E. & AURELIAN, L. (1980). Immunity to herpes simplex virus type-2. I. Development of virus-specific lymphoproliferative and leukocyte migration inhibition factor responses in HSV-2 infected guinea-pigs. *Cellular Immunology* **56**, 526–539.

EDMOND, B. J., GROSE, C. & BRUNELL, P. A. (1981). Varicella-zoster virus infection of diploid and chemically transformed guinea-pig embryo cells: factors influencing virus replication. *Journal of General Virology* **54**, 403–407.

EMINI, E. A., KAO, S.-Y., LEWIS, A. J., CRAINIC, R. & WIMMER, E. (1983). The functional basis of poliovirus neutralization determined with monospecific neutralizing antibodies. *Journal of Virology* **46**, 466–474.

FRIEDRICHS, W. E. & GROSE, C. (1984). Glycoprotein gp 118 of varicella-zoster virus: purification by serial affinity chromatography. *Journal of Virology* **49**, 992–996.

GERSHON, A. A., STEINBERG, S. P., GELB, L., GALASSO, G., BORKOWSKY, W., LARUSSA, P. & FERARRA, A. (1984). Live attenuated varicella vaccine: efficacy for children with leukemia in remission. *Journal of the American Medical Association* **252**, 355–362.

GONCZOL, E., HUDECZ, F., IANACONE, J., DIETZSCHOLD, B., STARR, S. & PLOTKIN, S. A. (1986). Immune responses to isolated human cytomegalovirus envelope proteins. *Journal of Virology* **58**, 661–664.

GROSE, C. & FRIEDRICHS, W. E. (1982). Immunoprecipitable polypeptides specified by varicella-zoster virus. *Virology* **118**, 86–95.

HO, R. J., ROUSE, B. T. & HUANG, L. (1986). Target sensitive immunoliposomes: preparation and sensitization. *Biochemistry* **25**, 5500–5506.

KELLER, P. M., NEFF, B. J. & ELLIS, R. W. (1984). Three major glycoprotein genes of varicella-zoster virus whose products have neutralization epitopes. *Journal of Virology* **52**, 293–297.

KELLER, P. M., LONERGAN, K., NEFF, B. J., MORTON, D. A. & ELLIS, R. W. (1986). Purification of individual varicella-zoster virus (VZV) glycoproteins gpI, gpII and gpIII and their use in ELISA for detection of VZV glycoprotein specific antibodies. *Journal of Virological Methods* **14**, 177–188.

MATSUNAGA, Y., YAMANISHI, K. & TAKAHASHI, M. (1982). Experimental infection and immune response of guinea-pigs with varicella-zoster virus. *Infection and Immunity* **37**, 407–412.

MYERS, M. G., STANBERRY, L. R. & EDMOND, B. J. (1985). Varicella-zoster virus infection of strain 2 guinea-pigs. *Journal of Infectious Diseases* **151**, 106–113.

RASMUSSEN, L. R., MULLENAX, J., NELSON, M. & MERIGAN, T. C. (1985). Human cytomegalovirus polypeptides stimulate neutralizing antibody in vivo. *Virology* **145**, 186–190.

SCHRIER, R. D., PIZER, L. I. & MOORHEAD, J. W. (1983). Type-specific delayed hypersensitivity and protective immunity induced by isolated herpes simplex virus glycoprotein. *Journal of Immunology* **130**, 1413–1418.

TOWNSEND, A. R. M., MCMICHAEL, A. J., CARTER, N. P., HUDDLESTON, J. A. & BROWNLEE, G. G. (1984). Cytotoxic T-cell recognition of the influenza nucleoprotein and hemagglutinin expressed in transfected mouse L cells. *Cell* **39**, 13–25.

YEWDELL, J. W., BENNICK, J. R., MACKETT, M., LEFRANCOIS, L., LYLES, D. S. & MOSS, B. (1986). Recognition of clones of vesicular stomatitis virus internal and external gene products by cytotoxic T lymphocytes. *Journal of Experimental Medicine* **163**, 1529–1534.

(Received 13 February 1987)