IMMUNITY TO VARICELLA-ZOSTER VIRUS IN A NORMAL ADULT POPULATION

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SUMMARY. Sera from 489 trainee nurses were examined, by the ELISA technique, for the presence of varicella-zoster virus specific antibody; antibody was found in 446 (91.2%). In more detailed investigations of specific immunity in 33 healthy adults with a past history of chickenpox, 32 (97%) showed a positive lymphocyte transformation test, but only 11 out of 23 examined (48%) demonstrated mononuclear cell production of specific antibody in vitro; serum antibody was found in 30 (91%) by the ELISA and in 25 out of 26 examined (96%) by the FAMA technique. A high degree of correlation was found between both a positive lymphocyte transformation and the presence of serum antibody and a past history of chickenpox.

INTRODUCTION

Varicella-zoster virus (VZV) infection occurs predominantly in childhood, and up to 88% of children will acquire chickenpox (Ross, 1962). The resulting immunity is probably lifelong and serum antibody levels may reflect the degree of protection (Williams et al., 1974). There are, however, discrepancies in the frequency of antibody reported in adult populations in the UK, ranging from 35-5% (Faizallah et al., 1982) to 60% (Tomlinson and MacCallum, 1970); frequencies of greater than 95% have been reported from the USA (Brunell, 1977).

Chickenpox is normally a mild illness in children, but is more serious in immunocompromised patients and also in adults over 20 years old, in whom complications, such as varicella encephalitis, and death are more common (Preblud, 1981). About a third of immunocompromised patients get chickenpox (Feldman et al., 1973 and 1975), and are a source of infection for susceptible health workers. Infected health workers are then not only at risk of developing severe varicella but may themselves become a source of infection for susceptible patients. Until recently, protection was available for health workers only by passive immunisation with zoster immune globulin, and this is short-lived. A further problem is that in hospitals, such as Great Ormond Street, with large numbers of immunosuppressed patients, known susceptible nurses who have been in contact with a case of chickenpox are required to

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be off work over the incubation period. This could be avoided by availability of long-term immunisation for susceptible nurses working in high risk areas.

Two live vaccines have been developed against VZV infection, namely, the OKA strain vaccine in Japan (Takahashi et al., 1974) and the KMCC strain vaccine in the USA (Neff et al., 1981). Several trials with the OKA vaccine have demonstrated its safety and efficacy in the prevention of infection in normal and immunocompromised children (Arbeter et al., 1982 and 1984; Asano et al., 1983; Weibel et al., 1984). However, there are still insufficient data on the naturally acquired immune status of adult populations on which to base a vaccination programme.

We, therefore, examined the frequency of VZV-specific antibody in healthy entrants to a nursing school and also made a study of cellular and humoral immunity to VZV in healthy adults with a past history of chickenpox.

SUBJECTS AND METHODS

Subjects. Blood samples were collected from 489 nurses, aged 18–21 yr and a history of previous chickenpox was taken by an experienced clinician. Permission to bleed the nurses was obtained from the hospital Ethical Committee. Blood was also taken from 33 consenting healthy nurses and laboratory workers, aged 19–30 yr, with an acceptable history of chickenpox and from nine who denied having had chickenpox.

Lymphocyte transformation test. Mononuclear cells were obtained from heparinised blood by separation on ficoll-hypaque gradients, and were washed three times in Hanks's Balanced Salts Solution (HBSS) and then resuspended, to a concentration of 2 x 10^6 cells/ml, in medium RPMI-1640 (GIBCO) containing sodium bicarbonate 2g/L, HEPES buffer 25 mM, penicillin 100 IU/ml, gentamicin 50 µg/ml and autologous plasma 20% v/v. The suspension was pipetted in 100-µl amounts into the wells of a round-bottomed microtitration plate (Flow Laboratories), to which were subsequently added 100 µl of 80-fold and 160-fold dilutions of VZV CF antigen or control antigen (uninfected cells) (Flow Laboratories). Further wells containing cell suspension were inoculated with a 320-fold dilution of phytohaemagglutinin, as a non specific stimulant, and appropriate controls containing medium only were also included. All assays were performed in triplicate. The plates were incubated at 37°C in CO2 5% in air in a humidified chamber for 6 days. The cultures were then pulsed with tritiated thymidine (Radiochemical Centre, Amersham) 4 µCi/well for 6 h and harvested by an automatic harvester (Dynatech Laboratories Ltd). The incorporated radioactivity was measured in an LKB Wallac 1215 Rackbeta II liquid scintillation counter. Lymphocyte stimulation indices (SI) were calculated by dividing the tritiated thymidine uptake in stimulated lymphocytes (VZV antigen) by that in unstimulated lymphocytes (control antigen). S-values of greater than 2 were taken as positive.

In-vitro antibody production. Mononuclear cells were prepared as above and resuspended to a concentration of 2 x 10^6 cells/ml in medium RPMI-1640 (GIBCO) containing sodium bicarbonate 2g/L, HEPES buffer 25 mM, penicillin 100 IU/ml, gentamicin 50 µg/ml and horse serum 10% v/v. One-ml amounts of the cell suspension were placed in capped round-bottomed tubes containing 100 µl of a 50-fold dilution of VZV CF antigen (Flow Laboratories). All tests were done in triplicate. The tubes were incubated for 6 days at 37°C in CO2 5% in air in a humidified chamber. The cells were then washed once in medium RPMI-1640 containing HEPES buffer 25 mM and fetal calf serum 5% v/v, resuspended in 0.5 ml of the medium and incubated at 37°C without CO2 for 48 h. The supernates were harvested and assayed for VZV antibody by the ELISA technique.

ELISA technique. VZV CF and control (uninfected cells) antigens (Flow Laboratories), diluted 50-fold in carbonate-bicarbonate buffer, pH 9.6, were added in 150-µl amounts to alternate rows of wells in flat-bottomed flexible microtitration plates and allowed to adsorb at 4°C overnight. The plates were then washed three times with PBS-Tween (0.05%) and non-specific binding sites were blocked by adding bovine serum albumin (BSA) 1% in
carbonate-bicarbonate buffer and incubating for 60 min at 37°C. After further washing, three
times in PBS-Tween, a 100-fold dilution of each test serum was added, in 100-μl amounts, to
adjacent wells coated with virus antigen and control antigen and incubated for 2 h at 37°C. All
assays were performed in duplicate. The plates were again washed three times in PBS-Tween, and rabbit anti-human immunoglobulin conjugated with peroxidase (DAKO immunoglobulins
a/s, Denmark), diluted 1000-fold in PBS-Tween containing BSA 0.5%, was added, in 100-μl
amounts, to the wells. The plates were incubated for 60 min at 37°C and, after washing three
times in PBS-Tween, 100 μl of freshly prepared ortho-phenylenediamine (Sigma) 0.4 mg/ml in
citrate-phosphate buffer, pH 5, containing 30% hydrogen peroxide 0.4 μl/ml was added to each
well and the plates were incubated in the dark at room temperature. After 30 min, the reaction
was stopped by adding 50 μl of 4N sulphuric acid. The optical density (OD) (at 492 nm) was then
read on a Titertek Multiscan. Results were expressed for each serum sample as the difference
between the OD of the wells coated with VZV antigen and those coated with control antigen.
Values of greater than 0.05 were taken as positive for antibody.

In the case of the 42 adult volunteers, their serum antibody levels were expressed as arbitrary
units/ml. A standard serum, prepared by pooling the three trainee-nurses' sera with the highest
OD values, was titrated in serial dilutions at each test run to give a standard curve, from which an
arbitrary antibody titre could be calculated for each test serum. This enabled results obtained on
different days to be standardised. Each of the volunteer sera was examined for total antibody (Ig)
and for virus specific IgG, IgA and IgM, using peroxidase-labelled immunoglobulin class-speci-
fic antisera (DAKO Immunoglobulins, Denmark). The antibody titres of the standard serum, in
arbitrary units/ml, were total Ig 50 000, IgG 50 000 and IgA 10 000; values for IgM were very low
and these were expressed as either positive, if greater than 4500 arbitrary units/ml, or negative.

FAMA technique (fluorescent antibody to the membrane antigen of VZV). Human embryo
lung fibroblast (Flow Laboratories) monolayers were inoculated with VZV-infected cells, at a
ratio of one infected cell to six uninfected. When c. 90% of the monolayer showed cytopathic
effects it was trypsinised and the cells were resuspended at a concentration of 10^3 cells/ml in 0.01 M
PBS, pH 7.4, containing fetal calf serum (FCS) 2%. Cell suspension, in 250-μl amounts was
incubated with an equal volume of serial 2-fold dilutions of test sera in round-bottomed
microtitration plates (Cooke) and incubated for 30 min at 25°C in a humidified chamber. The
cells were then washed three times in PBS-FCS, mixed with 250-μl amounts of fluorescein-conju-
gated goat anti-human immunoglobulin and incubated for 30 min at 25°C in a humidified
chamber. After further washing, three times, the cells were resuspended in PBS-FCS and added,
in 10-μl amounts, to one drop of a 10-fold dilution of glycerol in PBS on a glass slide for
fluorescence microscope examination. A reading was graded as positive when more than 30% of
the cells showed membrane fluorescence. The reciprocal of the highest test dilution giving 30%
fluorescence was taken as the serum antibody titre.

RESULTS

Incidence of VZV antibody and correlation with history of past infection in nurses

Sera from 489 healthy nurses were tested for VZV-specific antibody, by ELISA, at the beginning of their training, and 43 (8.8%) were found to be without detectable antibody. Of 353 nurses who provided a reliable past history of chickenpox, only three
(0.8%) were without antibody. Of 53 nurses who denied previous chickenpox, 20
(37.7%) possessed antibody.

Humoral and cellular immunity to VZV infection in healthy adults

Humoral immunity. Among the 33 adults with a positive history of chickenpox, 30
(91%) possessed VZV-specific antibody by ELISA, and of 26 examined by FAMA, 25


### Table

Presence of VZV-specific cell-mediated immune responses and antibody in 33 adults with a past history of chickenpox

<table>
<thead>
<tr>
<th>Adults (no.)</th>
<th>Cell-mediated immunity detected by</th>
<th>Antibody detected by</th>
<th>FAMA (antibody titre)</th>
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<td>total Ig</td>
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ND = not done.

* Stimulation index values are negative if < 2.0.
† ELISA: Ig—negative if < 2000. IgG—negative if < 1000, IgA—negative if < 600.

(96%) had antibody (table). Among those examined for the immunoglobulin classes of antibody, 25 out of 26 (96%) had IgG, 17 out of 26 (65%) had IgA and 7 out of 25 (28%) had IgM (table). One adult (no. 33) had no detectable antibody, either by ELISA or FAMA, but gave a positive transformation test.

**Cellular immunity.** Of 33 adults with a positive history of chickenpox, 32 (97%) showed a positive lymphocyte transformation test with VZV antigen (table). The one adult with a negative result, however, possessed specific antibodies, IgG, IgA and IgM, detected by ELISA and FAMA.

Among 23 adults examined for mononuclear production of antibody in vitro, only 11 (48%) were positive.

None of the nine adults who denied having chickenpox in the past had detectable antibody, by any of the tests above, or gave a positive lymphocyte transformation test.
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DISCUSSION

Our finding of VZV-specific antibody in over 90% of trainee nurses and a sample population of healthy adults agrees with that of Brunell (1977) who demonstrated antibody in 95% of an urban adult population in the USA. Lower figures of 60% and 35.5%, respectively, were reported by Tomlinson and MacCallum (1970) and Faizallah et al. (1982) in studies of nurses, blood donors and other hospital workers in the UK. However, Tomlinson and MacCallum used the complement fixation test to detect antibody, which is at least eight times less sensitive than the FAMA technique (Ward et al., 1982) and the low incidence of 35.5% obtained by Faizallah et al. among 68 adult hospital workers could have been due to a sampling error in the examination of a relatively small number of individuals; in our own investigations, we detected six seronegative subjects among one batch of 22 students.

Because only 0.8% of the nurses who gave a history of chickenpox in the past did not have antibody, a positive past history would appear to be a highly reliable indicator of immunity. The finding of antibody in more than a third of those with a negative history probably reflects the incidence of asymptomatic infection in the community.

Susceptibility to VZV infection may be optimally recognised by detecting antibody by the FAMA technique (Williams et al., 1974; Ward et al., 1982). However, the requirement of tissue culture facilities and live virus makes this a tedious technique. ELISA, in our hands, gave 100% concordance with FAMA in detecting IgG antibodies, confirming the observations of Shanley et al. (1982) who obtained a similar correlation for total antibody. ELISA is a much simpler method, and our results indicate that it is highly sensitive for assessing susceptibility to VZV infection.

Cell mediated immunity is believed to be more important than humoral immunity for the control of herpesvirus infections. Patel et al. (1979) have demonstrated, for VZV infection, that the lymphocyte transformation test correlates well with active immunity, and this test provides, at present, the only practical tool for evaluating the role of cell mediated immunity in VZV infection (Arvin et al., 1978). In our investigations, 32 out of 33 adults with a past history of VZV infection had positive lymphocyte transformation results. Two adults showed somewhat discordant findings (table); subject no. 17 gave negative results in the transformation test but had low antibody levels by ELISA and FAMA, while subject no. 33 showed positive transformation, but was without detectable antibody. As the relative roles of the humoral and cellular immune mechanisms in immunity to VZV reinfection are uncertain, it is difficult to say whether these two individuals are immune or not. Certainly, overt disease after reinfection with VZV is now recognised to occur although it is rare (Weller, 1983), and the presence of maternal VZV antibody does not always prevent infection in newborn infants (Gustafson et al., 1984). Our finding of VZV-specific IgM antibody in 7 out of 25 (28%) adults with past history of chickenpox agrees with those of Gershon et al. (1982) who detected IgM in 22–40% of VZV immune persons without symptoms of zoster or known exposure to the virus. This suggests either subclinical reinfection or reactivation of latent virus.

Only 11 of the 23 adults examined demonstrated mononuclear cell production of VZV-specific antibody in vitro, and there was no correlation between in-vitro production of antibody and serum antibody titres. As the numbers of circulating T and B cells specific to VZV, even in a hyperimmune individual, would be low it is not
surprising that only a proportion of seropositive individuals responded in this assay. This, together with splenic sequestration of specific antibody-producing cells (Souhami et al., 1981), may account for the poor sensitivity of this technique for assessing susceptibility to infection. However, it may prove useful for the analysis of cellular requirements for antibody production.

Now that a live vaccine is available for clinical trial, its value for protecting susceptible adults needs to be evaluated by examining whether both cellular and humoral immunity mechanisms are adequately stimulated and whether they persist long term. We suggest that screening for susceptibility be done by the VZV-IgG ELISA assay. We have, in fact, started to immunise susceptible nurses at our hospital, with the OKA strain vaccine, and to follow their immune responses.

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REFERENCES


