Investigation of Varicella-Zoster Virus-infected Cell Proteins that Elicit Antibody Production during Primary Varicella Using the Immune Transfer Method

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(Accepted 2 August 1984)

SUMMARY

The varicella-zoster virus-infected cell proteins (VZV-ICPs) against which IgG, IgM and IgA antibodies were made in the course of primary varicella-zoster virus (VZV) infection were analysed by the immune transfer method. IgG antibodies were made against one or more of 18 VZV-ICPs by patients with varicella. IgM antibodies were produced which reacted with 21 VZV-ICPs. The spectrum of IgG antibody production during the first week after the onset of infection was limited to an average of three VZV-ICPs while IgM antibodies which reacted with an average of seven VZV-ICPs were detectable in the acute phase of varicella. Equivalent VZV IgG or IgM antibody titres by radioimmunoassay did not correlate with a similar pattern of antibody specificity for VZV-ICPs by immune transfer. A detectable immune response to all VZV-ICPs was not required for the recovery of individual patients from primary VZV infection.

INTRODUCTION

Varicella is a common infectious disease of childhood. Complications have been associated with the occurrence of primary varicella-zoster virus (VZV) infection after childhood (Preblud & D'Angelo, 1979). Varicella is also a serious illness in immunocompromised patients (Feldman et al., 1975). Despite some success in the clinical approach to VZV infections, the basic molecular virology of VZV is incompletely understood. In particular, the viral components important in eliciting an immune response in the normal host with varicella have not been well defined. In the present study, we report an investigation of the VZV-infected cell proteins (VZV-ICPs) against which IgG, IgM and IgA antibodies were made in the course of primary VZV infection as determined by the immune transfer or Western blot method (Burnette, 1981).

METHODS

Patient samples. A total of 38 serum samples were evaluated by the immune transfer method. These samples were obtained from 23 patients at periods of 2 to 49 days after the onset of acute varicella. Serum was obtained twice from 12 patients and four times from one patient. Sera obtained less than 7 days after the appearance of the varicella exanthem were considered acute, those obtained between 7 and 14 days were considered intermediate, and those obtained between 21 and 49 days were considered late samples. The subjects had no underlying diseases. Varicella was uncomplicated in all patients except for one individual who had transient pneumonia. Sera from six subjects who were seronegative to VZV by radioimmunoassay (RIA) were also tested (Arvin & Koropchak, 1980).

Immune transfer method. VZV-infected and uninfected cell extracts were prepared from human melanoma cells that were infected with VZV-32, a strain obtained from the vesicle fluid of a patient with acute varicella (kindly provided by Dr C. Grose). This strain had been passaged in vitro less than 30 times. Infected monolayers were prepared by adding infected cells which showed 90% c.p.e. to a fresh monolayer at a ratio of 1:4. When 90% c.p.e.

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was apparent, the infected cells were washed with cold phosphate-buffered saline (PBS) and harvested on ice with detergent buffer containing 10 mM-Tris-HCl pH 7.4, 150 mM-NaCl, 1% deoxycholate, and 1% Triton X-100. The extract was then clarified by centrifugation at 400 g and subjected to ultracentrifugation at 100000 g for 1 h at 4 °C. The supernatant, containing the solubilized infected cell proteins, was collected and frozen at −70 °C. Uninfected melanoma cells were processed in the same manner and used as a control uninfected cell extract.

Solubilized proteins in the infected and uninfected cell extracts were separated electrophoretically using gels that consisted of 8% acrylamide cross-linked with 2.7% N,N-diallyltartardiamide (ratio of 30:0.8) in running buffer with 25 mM-Tris-HCl, 0.2 M-glycine, 0.1% SDS. Cell extracts were diluted in sample buffer with a final concentration of 5% (v/v) 2-mercaptoethanol. The samples were boiled at 100 °C for 3 min and loaded onto a 3% acrylamide stacking gel at 40 μg protein per lane. The proteins were separated at a constant current of 25 mA for 4 to 6 h.

The separated proteins were transferred electrophoretically (100 V for 6 h) from the gel to nitrocellulose paper (0.45 μm) in a transfer chamber (Trans-Blot Cell, Bio-Rad) under transfer buffer with 20 mM-Tris–HCl, 150 mM-glycine, 20% methanol, pH 8.3. The nitrocellulose was cut into strips and incubated in Tris–NaCl buffer (10 mM-Tris-HCl pH 7.4, 0.9% NaCl) with 10% horse serum for 45 min at 37 °C. Patients' sera were diluted 1:100 in Tris–NaCl with 10% horse serum and incubated with individual nitrocellulose strips overnight at 4 °C. Following sequential washes with Tris–NaCl, and Tris–NaCl containing 0.05% NP40, the strips were incubated with 125I-labelled goat anti-human IgG, IgM or IgA (3 x 105 c.p.m./ml) (Tago, Burlingame, Ca., U.S.A.) for 75 to 90 min at room temperature. Immunoglobulins were labelled with 125I using a modified chloramine-T method (Arvin & Koropchak, 1980). The washes were repeated and the strips were autoradiographed utilizing Kodak XAR film at −70 °C for 5 to 48 h. Binding to non-VZV-specific ICPs was determined by incubating 11 sera with transferred proteins from both VZV-infected and uninfected cell extracts and 125I-labelled IgG, IgM and IgA.

The estimated molecular weights of the proteins that bound antibody were determined by extrapolating from the migration of standard proteins with known molecular weights on the same gel (Amersham).

The serum samples evaluated by immune transfer were also tested for IgG, IgM and IgA antibodies to whole VZV antigen by solid-phase radioimmunoassay as described previously (Arvin & Koropchak, 1980).

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Fig. 1. IgG and IgM antibodies to VZV-infected and uninfected cell proteins in sera from subjects with varicella. This figure illustrates the binding of IgM (a, b) and IgG (g, h) antibodies to VZV-infected (a, h) and uninfected (b, g) cell proteins as demonstrated by immune transfer using sera obtained from subjects who were convalescent from varicella. Lanes (c) and (d) and lanes (e) and (f) show the low molecular weight bands and absence of specific binding to VZV-infected cell proteins observed when non-immune sera were tested for IgM (c, infected; d, uninfected) and IgG (e, infected; f, uninfected) reactivity. The numbers to the left and right of the figure indicate the estimated mol. wt. of some of the proteins with which IgM and IgG antibodies reacted.
Fig. 2. IgG and IgM antibodies to VZV-infected cell proteins in sera from subjects with varicella. This figure provides a diagram of the bands seen with the immune transfer method when sera from (a) 37 subjects were tested for IgG reactivity and sera from (b) 31 subjects were tested for IgM reactivity. The rectangles in each vertical column represent the bands visible when serum from an individual subject was tested. The dark rectangles indicate prominent bands. The immune transfer results are diagrammed in relation to the number of days after onset of varicella when the serum was obtained (horizontal axis). Estimated mol. wt. are given on the vertical axis. The horizontal shaded bars indicate the mol. wt. of proteins in uninfected cell extracts that bound IgG and IgM antibodies.
RESULTS

Binding of IgG, IgM and IgA antibodies to VZV-infected and uninfected cell proteins

A typical pattern of IgG antibody binding to VZV-infected and uninfected cell proteins using serum obtained 22 days after the onset of varicella is shown in Fig. 1. Visible bands occurred where IgG antibody in the patient serum reacted with one of the electrophoretically separated cell proteins. IgG antibody was detected against VZV-ICPs ranging from 32K to 160K mol. wt. Apparent binding of IgG to low molecular weight proteins in the uninfected cell extract was also observed. Although these bands may have resulted from direct binding of the radiolabelled anti-IgG probe, similar bands were observed by immunoprecipitation using uninfected cell extract.

The IgG reactivity of 37 serum samples against VZV-infected and uninfected cell proteins is depicted in Fig. 2(a). This figure provides a diagrammatic representation of the bands visualized by immune transfer using the patients' sera obtained during the acute (0 to 6 days), intermediate (7 to 14 days) and late (21 to 43 days) periods after the onset of varicella. IgG antibody binding to a total of 27 proteins (32K to 188K mol. wt.) was observed. IgG antibody binding to nine proteins occurred when sera were incubated with uninfected cell extract. This reactivity which was not specific for VZV-infected cell proteins is indicated by the shaded horizontal bars on the figure. Binding to these nine proteins was also observed using sera from VZV seronegative subjects. If binding to these proteins is excluded, IgG antibodies directed against one or more of 18 VZV-infected cell proteins were detectable in sera from patients with varicella. As is evident in Fig. 1, the spectrum of IgG antibody production during the first 6 days of

![Fig. 1](image1.png)

Fig. 3. IgA antibodies to VZV-infected cell proteins in sera from subjects with varicella. This figure provides a diagram of the bands seen with the immune transfer method when sera from 11 subjects were tested for IgA reactivity. The rectangles in each vertical column represent the bands visible when serum from an individual subject was tested. The dark rectangles indicate prominent bands. The immune transfer results are diagrammed in relation to the number of days after onset of varicella when the serum was obtained (horizontal axis). Estimated mol. wt. are given on the vertical axis. The horizontal shaded bars indicate the mol. wt. of proteins in uninfected cell extracts that bound IgA antibodies.
VZV proteins that elicit antibody response

Fig. 4. IgG and IgM antibodies to VZV-infected cell proteins in sera with equivalent IgG or IgM antibody titres by radioimmunoassay. This figure provides a diagram of the bands seen with the immune transfer method (a) when sera from eight subjects which had VZV IgG antibody titres of \( 1:1 \times 10^6 \) by RIA were tested and (b) when sera from five subjects which had VZV IgM antibody titres of \( 1:2.5 \times 10^5 \) by RIA were tested. The sera were obtained at 3 to 6 days (AM, AB, GD-IgG, LB, MB), at 7 to 14 days (RV-IgG, CR, JM, DS) and > 22 days (ND, GD-IgM, RV-IgM). The dark rectangles indicate prominent bands. The horizontal shaded bars indicate the mol. wt. of proteins in uninfected cell extracts that bound IgG and IgM antibodies. Estimated mol. wt. are given on the vertical axis.

Infection was limited. IgG antibodies were detected against an average of three infected cell proteins. The initial IgG response was directed primarily against proteins of \( \leq 100 \)K mol. wt. and especially to those \( \leq 68 \)K. IgG antibodies to an average of five VZV-infected cell proteins were present in serum obtained from 7 to 14 days after the onset of varicella and to an average of seven VZV-infected cell proteins by 21 to 43 days. An increased percentage of sera showed IgG reactivity with proteins in the range of 81K to 116K in the convalescent period. All 17 of the individual patients who were tested during this period developed antibody to proteins of 107K or 116K and 75% had antibodies to both of these VZV-infected cell proteins in intermediate or late sera. IgG antibody to the proteins of lower mol. wt., especially the 35K, 42K and 68K proteins, persisted during convalescence.

An IgM profile using serum obtained from a patient 14 days after the onset of varicella is shown in the immunoblot in Fig. 1. IgM antibody reactivity with proteins of 35K to 165K was demonstrated. Prominent IgM binding with the 46K, 49K and 63K proteins occurred using both VZV-infected and uninfected cell extracts. Some direct binding of the anti-IgM probe to uninfected cell proteins may also have occurred.

A graphic representation of IgM binding to VZV-infected and uninfected cell proteins observed with 31 sera is given in Fig. 2(b). IgM antibody bound to 30 proteins with molecular weights between 32K and 215K. The binding to nine uninfected cell proteins that was observed with IgG also occurred with IgM as indicated by shaded bars on the figure. All sera showed IgM
binding to the 46K and 49K proteins from VZV-infected and uninfected cell extracts. IgM binding to these proteins also occurred with VZV seronegative sera. It has previously been established that actin co-migrates with proteins in this region (Grose et al., 1983). VZV-specific IgM antibodies were produced against several VZV-infected cell proteins during the first week after the onset of varicella infection while the IgG response was relatively restricted. IgM antibodies in the acute sera reacted with an average of seven proteins compared to IgG reactivity with an average of three VZV-ICPs. In addition to the prominent IgM binding to high mol. wt. proteins, most individuals also had IgM antibodies that reacted with VZV-ICPs of 35K, 68K and 116K.

IgA reactivity was evaluated using 11 serum samples obtained from 6 to 49 days after the onset of varicella. The binding of IgA antibody to 15 proteins with molecular weights from 35K to 188K was observed (Fig. 3). IgA binding to proteins that were present in both VZV-infected and uninfected cell extracts occurred as was observed with IgG and IgM and may be due in part to direct binding of the probe. IgA antibody was produced to an average of three VZV-infected cell proteins.

**Variable reactivity with VZV-ICPs of sera with equivalent RIA titres**

Fig. 4 displays the IgG immunoblot results from eight sera each of which had VZV IgG titres of $1.1 \times 10^6$ and the IgM immunoblot results from five sera with IgM RIA titres of $1.2.5 \times 10^5$. It is evident that sera with equivalent IgG or IgM titres by RIA contained antibodies that reacted with different patterns of VZV-infected cell proteins.

**DISCUSSION**

IgG or IgM antibodies to 21 VZV-infected cell proteins were detected by the immune transfer method in sera from otherwise healthy individuals with acute or early convalescent varicella. Sixteen infected cell proteins which ranged from 32K to 207K mol. wt. elicited an antibody response frequently. This finding was consistent with the results of other investigators who have studied the reactivity of IgG antibodies against extracts of VZV-infected cells by immunoprecipitation techniques. Grose & Friedrichs (1982) identified 16 infected cell proteins from 32K to >200K that were immunogenic in guinea-pigs. Grose (1983) also reported that sera from patients with herpes zoster had IgG antibodies which immunoprecipitated 16 VZV-infected cell proteins of 32K to 174K. Both Zweerink & Neff (1981) and Wolff (1978) demonstrated immunoprecipitation of 14 VZV proteins by human IgG in sera from individuals who were immune to VZV. However, direct comparisons of the molecular weights of the VZV-infected cell proteins which were immunogenic by immunoprecipitation and by immune transfer are difficult. Variability is observed among reports which probably reflects the use of different host cell lines, solubilization methods, and polyacrylamide gel techniques (Hyman, 1983). Our study demonstrated that the VZV-specific IgM antibody response was directed against most of the same infected cell proteins which have been found to elicit IgG antibody production except that IgM antibodies were more likely to bind the VZV-infected cell proteins of highest mol. wt.

The technique of immune transfer has only recently been used to evaluate antibody reactivity with viral proteins (Braun et al., 1983). IgG antibodies to VZV-infected cell proteins were detected by immune transfer in sera obtained immediately after the appearance of the varicella exanthem which were not demonstrated by immunoprecipitation using acute sera (unpublished observation). Zweerink & Neff (1981) also reported that no antibodies to VZV were detected in acute sera by immunoprecipitation. The immune transfer method was also useful for determining the reactivity of IgM and IgA antibodies with VZV-infected cell proteins; this is difficult to accomplish by immunoprecipitation.

At least three major glycoproteins have been identified using immunoprecipitation of VZV-infected cells labelled with radioactive glucosamine or fucose (Asano & Takahashi, 1980; Grose & Friedrichs, 1982; Shemer et al., 1980). The IgG, IgM and IgA antibodies that reacted with the 68K, 92K and 116K proteins by immune transfer were probably directed against these glycoproteins. These proteins were found by immune transfer to be quite immunogenic in most individuals with varicella. Antibodies of all three immunoglobulin subclasses were elicited against
these proteins. The 35K VZV infected cell protein has been correlated with VZV-specific thymidine kinase activity by Lopetegui et al. (1983). Kallander et al. (1982) found antibodies to this enzyme in sera from patients with herpes zoster but not from patients with varicella. Both IgG and IgM antibodies directed to the 35K protein were detected during acute varicella and early convalescence by immune transfer.

IgM antibody was produced against many VZV-infected cell proteins in the first week following the onset of varicella whereas the early IgG response was directed against only a few proteins. The restricted range of IgG reactivity could not have been judged from the VZV antibody titre by RIA since many of these sera had titres \( \geq 1:1 \times 10^6 \). It was possible to demonstrate by the immune transfer method that equivalent IgG or IgM titres measured by a standard serological assay did not imply a similar pattern of antibody specificity for individual VZV-infected cell proteins. The analysis of the humoral immune response in relation to events in the clinical course of infection with VZV and other herpesviruses is likely to require a more detailed study of antibodies generated against individual viral proteins than is possible using most serological methods. All patients in this study except one had uncomplicated varicella and all patients recovered uneventfully. A detectable immune response to all major VZV-infected cell proteins was not required to ensure the resolution of varicella in the individual subject, an observation which has significance for the possible development of a subunit vaccine against VZV.

The authors wish to thank Dr Charles Grose for kindly supplying the melanoma cells and VZV-32 strain, and Drs Michael Crowe and John Frelinger for technical advice. Dr A. M. Arvin is a recipient of a New Investigator award from the National Institute of Allergy and Infectious Diseases (AI-17421). This work received support from The Thrasher Research Fund, Salt Lake City, Utah.

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(Received 16 March 1984)