Determination of specific IgA antibodies to varicella zoster virus by immunoperoxidase assay

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SUMMARY An indirect peroxidase technique was developed for determination of IgA antibodies to varicella zoster virus (VZV). The antigen consisted of acetone-fixed trypsinised VZV-infected cells. Rabbit antihuman IgA peroxidase conjugate was used to detect human IgA antibodies bound to viral antigen. In parallel IgG antibodies to VZV were determined by an immunoperoxidase antibody to membrane antigen (IPAMA) technique. Varicella zoster virus IgA antibodies were detected in all five varicella and seven zoster patients. No VZV IgA antibodies (<2) were detected in 45 healthy control sera. Neither were they found in paired sera of five patients with herpes simplex infection, five patients with human cytomegalovirus infection and two patients with Epstein-Barr virus infection. Application of immunoperoxidase IgA technique in serodiagnosis of primary and reactivated VZV infections is discussed.

Varicella (chickenpox) is a mild, highly communicable disease caused by initial exposure to varicella zoster virus (VZV). After the patient recovers VZV persists in a latent form, and when reactivated, may cause zoster, characterised by a painful vesicular eruption limited to one or more segmented dermatomes.1 Varicella and zoster can cause severe complications in cases with malignant disease or those who are immunocompromised.2 It is, therefore, important to have a highly sensitive method for accurately detecting both primary and reactivated VZV infections.

Use of either immunofluorescent or immunoperoxidase techniques for detection of specific IgG antibodies to VZV-induced membrane antigen has been found valuable in determination of immune status, and in serodiagnosis of varicella and zoster infection when paired sera taken during acute and convalescent stages of the disease are examined.3–5 A highly sensitive radioimmunoassay (RIA) and an enzyme-linked immunosorbent assay (ELISA) for detection of specific VZV IgG antibodies have been also developed recently in a number of laboratories.6–9 As far as VZV-specific IgM is concerned, it has become apparent that it is produced regularly in varicella patients, but has been detected only in some zoster patients.7 10–12

The purpose of this study was to develop an immunoperoxidase assay for the detection of VZV-specific IgA antibodies and to evaluate the feasibility of this approach for serodiagnosis of both primary and reactivated VZV infection.

Material and methods

Cells and media
Human embryonic fibroblasts (HEF) were grown in minimum essential medium (MEM, Biolab, Jerusalem, Israel), supplemented with 10% fetal calf serum (FCS) (Gibco, Biological Co, Grand Island, NY), 1% glutamin (Biolab), 100 units of penicillin/ml and 200 µg of streptomycin/ml, pH 7.2.

VZV antigen preparation
The cells were infected with VZW (Nurit strain, isolated in 1973 from a patient with chickenpox by SLK).10–14 Infected cultures were incubated at 35°C in MEM supplemented with 2% FCS at pH 8.0 until cytopathic effects were observed in 60%-80% of the cells (24-48 h after inoculation).

Cells were removed from the glass with Versene-trypsin solution (Biolab) and washed with 0.01 M phosphate-buffered saline (PBS), pH 7.4. Drops of twice-washed cell suspension, containing 10⁸ infected cells/ml mixed with 2 x 10⁸ uninfected cells/ml, were placed on glass slides, dried at room temperature (about 24°C) and acetone-fixed. The slides were
stored at –20°C. Titres of antibody to VZV remained unchanged even when slides were examined three months after preparation.

**HUMAN SERA**

Serum samples were obtained from 12 patients with clinical diagnosis of varicella or zoster, five with cytomegalovirus (CMV) infection, five with herpes simplex virus (HSV) infection, two with Epstein-Barr virus (EBV) infection and from 45 laboratory personnel and medical students.

**IMMUNOPEROXIDASE TECHNIQUE**

Stored slides were thawed and covered with twofold dilutions of test serum or control serum. After incubation at 37°C for 30 min, followed by 15 min in PBS, slides were incubated for an additional 30 min with antihuman IgA peroxidase conjugate (specific for α chain, Dako, Copenhagen, Denmark), diluted 1/20 in PBS. After washing, enzymatic activity was detected using the method of Graham and Karnovsky, as modified by Haikin and Sarov. The freshly prepared substrate solution was composed of 4 mg benzidine (Riedel-de Haén, Seelze-Hannover) dissolved in 0.5 ml acetone, 9.5 ml PBS, and 10 μl hydrogen peroxide from a 33% stock solution. Slides were incubated with substrate at room temperature for 5 min and were washed with PBS. Each test included known positive and negative sera, and reproducibility of the titration was demonstrated by testing the same positive sera several times.

The immunoperoxidase antibody to VZV membrane antigen (IPAMA) technique for detection of virus-specific IgG has been previously described.

**Results**

**IMMUNOPEROXIDASE POSITIVE AND NEGATIVE REACTION**

The Figure demonstrates the dark blue peroxidase-catalysed positive reaction in the nuclei of infected human embryo cells after incubation with VZV-IgA-positive sera derived from varicella and zoster patients; staining was absent when VZV-IgA-negative sera were examined. Antihuman IgA peroxidase conjugate diluted 1/20 was found satisfactory for detection of specific VZV-IgA antibodies. Lower dilutions showed non-specific cytoplasmic staining while higher dilutions failed to detect low levels of VZV-IgA antibodies.

**REPRODUCIBILITY**

We examined the reproducibility of the test by testing three VZV-IgA-positive sera several times. The titre was reproducible within a twofold range.

**SPECIFICITY OF IMMUNOPEROXIDASE ASSAY**

The specificity of the method was tested with regard to cross-reactivity with other herpes group viruses. Paired sera of five patients with clinical and serological diagnosis of acute HSV infection, five patients with CMV mononucleosis and two patients with EBV infection were found to be VZV IgA negative (<2).

**VARICELLA AND ZOSTER PATIENTS**

Sera from five varicella and seven zoster patients were tested for VZV-IgG and IgA antibodies by the immunoperoxidase technique. For all the patients VZV-specific IgA was detected in one or more serum samples (Table). In sera of three patients with varicella (1, 2, 5) and two patients with zoster (9, 10) infection, peroxidase staining was observed in the nuclei of VZV-infected human embryo fibroblasts while membrane staining was observed in the other samples. In three of the varicella patients (2, 3, 5) IgA antibodies began to decline one month after the onset of the disease. Decrease of VZV IgA titre was seen 45 days after onset of rash in two of the zoster patients (10, 12). In one patient (6) a single serum sample was available after one year. This sample was still slightly positive for VZV-specific IgA.

**CONTROL-HEALTHY ADULTS**

Forty-four medical students seropositive for VZV-specific IgG (and one seronegative) were tested for VZV-specific IgA antibodies and were found negative (<2). The possibility that VZV IgA
Determination of specific IgA antibodies to varicella zoster virus by immunoperoxidase assay

Immunoperoxidase antibody titres of patients with VZV infection

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Day of illness*</th>
<th>Diagnosis</th>
<th>Antibody titre</th>
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<td>49</td>
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<td>32 8</td>
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*Days after onset of rash.
†Days after first sample.
U = unknown. ND = not done.

might be detected at titres lower than two in control sera needs to be examined.

Discussion

In the present study we have developed a simple immunoperoxidase assay for detection of specific IgA to VZV. VZV IgA antibodies were detected in all varicella and zoster patients but not in any (<2) of the control sera from 44 healthy individuals seropositive for VZV. Neither was VZV IgA detected (<2) in paired sera of patients with HSV, CMV or EBV infections. The possibility that VZV-IgA antibodies can be detected in serum of healthy persons by a highly sensitive radioimmunoassay technique recently developed in this laboratory is being investigated. Brunnel et al described VZV specific IgA antibody production in a number of varicella and zoster patients they studied by the immunofluorescent technique. We have recently shown by examination of sera from patients with CMV mononucleosis and from kidney transplant recipients that in both primary and recurrent CMV infections, IgA antibodies were detected at high titre by the ELISA technique. Thus specific IgA production seems not to be restricted to primary VZV and CMV infection, but can also be a signal of reactivation or reinfection by these viruses.

Detection of VZV-specific IgA antibodies in both varicella and zoster patients by the immunoperoxidase assay implies that this technique can be of value in the serodiagnosis of VZV infections, using a single serum sample of the "convalescent phase." In order to evaluate properly the potential application of this VZV-IgA peroxidase assay as a standard technique for serodiagnosis of VZV infection, the persistence of specific VZV-IgA antibodies needs to be examined in a large number of patients. In three varicella patients (2, 3, 5) IgA antibodies seemed to decline within a month. With regard to zoster patients VZV-IgA antibodies began to decline after about 45 days.

The persistence of IgA antibodies after other viral infections seems to depend largely on the virus involved, on individual variation among the subjects tested and probably most importantly on the sensitivity of the method used to detect specific IgA. However, with regard to herpes group
viruses which may be reactivated, when specific IgM could be detected only in some of these patients\textsuperscript{12} \textsuperscript{22} \textsuperscript{28} \textsuperscript{29} the appearance of specific IgA may be a clear indication of viral reactivation.

In summary, the present study demonstrates that specific IgA antibodies could be detected by the immunoperoxidase technique in the course of primary and reactivated VZV infections. The method is simple and rapid to perform. Moreover, it can be applied to stored target cells which can be used as and where needed. The immunoperoxidase assay is much more convenient than the immunofluorescent antibody test because there is no need for a fluorescent microscope and the results can be read with a simple light microscope. We are currently examining the interesting possibility that specific IgA may be detected in a number of neurological, autoimmune diseases and in human cancer in which an aetiological relation with herpes infections has been suggested.\textsuperscript{30} \textsuperscript{37}

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