than the fact that in two of the three who rejected (Cases 2 and 3) the influenza pursued a severe course with the development of pneumonia. The more severe course may in turn have been related to the fact that they were leucopenic at the time. Because of this they were receiving low doses of azathioprine, but in other respects the immunosuppressive regimen of the five patients was similar.

It is possible that the attacks of influenza and the onset of rejection in three of the transplanted patients were unconnected. The fact that the episodes of rejection took place within a five-day period during an outbreak of influenza, however, suggests that the rejection was precipitated by the virus infection. A similar association of rejection and virus infection has been described in three patients by David and his co-workers (1972), and infection in one was due to influenza virus A.

An explanation is required for the association between the influenza infection and rejection. Infection with the virus may have caused a general stimulation of the immune response of the patients. There is some evidence that influenza can produce stimulation of cell-mediated immunity. Thus spontaneous transformation has been observed in lymphocytes cultured from the peripheral blood of patients one to four weeks after infection with influenza virus A (Parker and Lukes, 1971; Field and Caspary, 1972). No studies of this sort were carried out on the present patients but it is of some interest that, despite immunosuppressive therapy, they all developed high levels of antibody to influenza virus. It may be that the episodes of rejection were due to a generalized stimulation of the immune mechanism, including cell-mediated immunity, after virus infection.

The apparent onset of rejection as a result of influenza raises the question of the advisability of vaccinating patients with transplants. Current influenza vaccines contain inactivated virus so that there is no risk of virus multiplication in the body. However, the postulated stimulation of the immune response by influenza virus infection might also be brought about by inocculation of influenza vaccine, and one of the patients described by David and his co-workers (1972) in fact had a rejection episode one month after being vaccinated against influenza. Present influenza vaccines contain purified virus particles but research is going on into the development of a vaccine containing purified haemagglutinin derived from the surface of the virus particles. The haemagglutinin is the main antigen involved in immunity to reinfection and it may be that it could confer protection in the absence of a general stimulatory effect on immune mechanisms. It would, however seem wise to delay a decision on vaccinating patients with transplants against influenza until more is known of effects of different types of vaccines on the immune response.

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Specific IgM Antibody in Serum of Patients with Herpes Zoster Infections

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Summary

Specific IgM varicella-zoster antibody was detected in "convalescent" sera from 20 out of 40 patients (50%) with herpes zoster infections. Since these were not primary infections with varicella-zoster virus, it seems that detection of IgM antibody specific for a particular virus may not differentiate a primary infection from secondary infections with that virus.

Introduction

Several studies have shown that after primary immunization with many protein antigens an initial transitory IgM serum antibody response is obtained, whereas after secondary immunization an IgM response is usually difficult to detect (Pike, 1967). In several human viral infections it has also been shown that specific IgM antibody may be detected during the first few weeks of a primary infection (Schluederberg, 1965; Vesikari and Vaheri, 1968; Haire and Hadden, 1972). There have, however, been few opportunities to determine if IgM specific antibody can be detected during subsequent infections with a particular virus.

Epidemiological and clinical evidence indicate that herpes zoster is a secondary infection with varicella-zoster probably due to reactivation of virus which has remained latent for many years in the sensory ganglia after the primary infecton chickenpox (Hope-Simpson, 1965). The purpose of the present study was to determine if specific IgM antibodies could be detected by indirect immunofluorescence in sera of patients with herpes zoster infections.

Materials and Methods

Paired sera for the indirect immunofluorescence test were collected by general practitioners from 40 patients aged from 33 to 80 years with clinical herpes zoster, the first specimen being taken generally during the first few days of the illness

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and the second 10-21 days later. In all 40 cases complement fixation antibody tests on paired sera had shown current infection with varicella-zoster virus; only one showed an anamnestic rising complement fixation titre to herpes simplex (Ross *et al.*, 1965). For the immunofluorescence test patients' sera were thawed from storage temperature of —10°C and diluted 1 in 5 in phosphate-buffered saline.

Antigen for the immunofluorescence test was prepared from human diploid embryonic lung fibroblast monolayer cell cultures grown in 50-oz bottles and infected with a strain of varicella-zoster initially isolated from the lesions of a child with chickenpox. Forty-eight hours after infection, when the monolayer showed widespread focal cytopathogenic effect, the culture was trypsinized (Grist *et al.*, 1966), and the cells were deposited by centrifugation at 1,000 r.p.m. for 10 minutes. After removal of the supernate the cell deposit was resuspended in 2.5 ml of phosphate-buffered saline. Control cell suspension was prepared at the same time from the same batch of uninfected cell cultures. By using a platinum loop, spots of infected and uninfected cell-suspensions were deposited on microscope slides (three of each suspension per slide). The spots were air dried, fixed in acetone for 10 minutes at 4°C, air dried for 30 minutes, and stored at —20°C until required.

The conjugate was sheep antihuman IgM (Wellcome Reagents Ltd.) conjugated with fluoroscein isothiocyanate, the specificity of which for IgM antibody had been checked (Chantler and Haire, 1972). In preliminary tests we found that the optimal dilution of this conjugate was 1 in 10. With conjugate at 1 in 10 and test sera at 1 in 5 no fluorescence or negligible fluorescence was obtained with the uninfected control cell cultures; thus no adsorption methods to remove nonspecific fluorescence were thought necessary.

The 1-in-5 dilution of each patient's serum was applied on the same microscope slide to varicella-zoster antigen smear and uninfected cell control smear for 30 minutes at 37°C in a moist chamber. A positive IgM varicella-zoster antibody control serum (from a child of 6 months with chickenpox) was included in each batch of tests. The smears were thoroughly washed with phosphate-buffered saline and the conjugate applied for 20 minutes at 37°C. Smears were again thoroughly washed in the saline, mounted in glycerol-phosphate-buffered saline, and examined for immunofluorescence with an ×40 dry lens of a Reichart zetopan microscope. In order to increase sensitivity, sera not showing IgM immunofluorescence were retested as before but by incubating the 1-in-5 serum dilution with antigen for three hours instead of 30 minutes at 37°C (Schmitz and Haas, 1972).

Results

With 30 minutes' incubation of test serum and antigen, IgM antibodies for varicella-zoster were detected by immunofluorescence in the "convalescent" sera from 16 of the 40 patients; with three hours' incubation an additional 4 sera were positive. These 20 comprised 10 males and 10 females, ages ranging from 37 to 80 years. The varicella-zoster IgM antibody titres ranged from 5 to 40 with 30 minutes' or three hours' incubation. Of two patients with the highest IgM titres one, an 80-year-old-man, was the oldest patient in the series. None of these 20 sera showed specific IgM antibodies for herpes simplex virus by immunofluorescence. "Acute" sera tested with three hours' incubation showed only nine (23%) of the 40 positive for varicella-zoster IgM; the corresponding "convalescent" sera had been positive in eight of these nine.

Sucrose density-gradient centrifugation on three of the sera with highest IgM antibody titres for varicella-zoster and collection from the bottom of the tube of six fractions from each serum (Desmyter *et al.*, 1971) showed IgM varicella-zoster antibody by immunofluorescence in the second fraction only. This was also the peak IgM fraction as assessed by single radial immunodiffusion with Hyland "immunoplates" (Mancini *et al.*, 1965).

Comment

Our finding of specific varicella-zoster IgM antibodies in a high proportion of patients with herpes zoster indicates that IgM specific immunoglobulin can often be detected during this secondary infection, even when the illness is mild or moderate as was the case in most of the patients in the present study. Thus, the finding of specific IgM antibodies would not be helpful in differentiating the secondary infection herpes zoster from the primary infection varicella. Although this differentiation is rarely necessary in varicella-zoster infections it is often important in other virus infections where clinical differentiation is more difficult—such as in cytomegalovirus infection after blood transfusion or rubella infection during pregnancy. The present study lends no support to the suggestion by other workers that the presence of IgM antibody in these infections is indicative of primary infection (Lang and Hanshaw, 1969; Boué *et al.*, 1971; Schmitz and Haas, 1972).

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