

Cellular and Humoral Immune Responses to Varicella-Zoster Virus in Immunocompromised Patients During and After Varicella-Zoster Infections

ANNE A. GERSHON* AND SHARON PROJANSKY STEINBERG

Department of Pediatrics, New York University School of Medicine, New York, New York 10016

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Humoral and cell-mediated immune responses to varicella-zoster (V-Z) virus were assessed in patients during and after V-Z infections. Ongoing V-Z infection was associated with minimal cellular immunity but not necessarily with poor humoral immunity. Recovery from V-Z infection was associated with a vigorous cellular immune response. Cell-mediated immunity to V-Z virus was demonstrable for years after varicella, but responses were lower in immunocompromised patients than in normal individuals.

It has long been thought that intact cell-mediated immunity (CMI) is crucial for recovery from varicella-zoster (V-Z) virus infections. This concept has developed largely from clinical observations on patients with agammaglobulinemia, who have been observed to recover normally from both varicella and zoster, and patients with deficiencies in CMI who are prone to develop severe V-Z infections (8). However, despite the putative importance of CMI in host defense against V-Z virus, there is relatively little published information on the subject (7, 9-11). We therefore undertook the following project, in which we measured CMI to V-Z virus in patients during episodes of varicella or zoster and after recovery from V-Z infection. In addition, we also attempted to discover whether there was any correlation between humoral and cellular immune responses to V-Z virus and the outcome of the disease. We were particularly interested in studying responses of immunocompromised persons, since they are known often to have difficulty in recovering from infections with V-Z virus. We also sought to determine whether there were any differences in CMI to V-Z virus between normal and immunocompromised persons years after an attack of varicella.

MATERIALS AND METHODS

Virus, cells, and media. V-Z virus (Ellen strain) was propagated in human embryonic lung fibroblasts using modified Eagle medium supplemented with 2% fetal calf serum (4).

Measurement of antibody to V-Z virus. The fluorescent antibody to membrane antigen procedure has been described previously (2, 12). Briefly, unfixed human embryonic lung fibroblasts infected with V-Z virus are incubated at 25°C for 30 min with the serum

being tested. The cells are then washed, incubated with anti-human globulin labeled with fluorescein, and examined by fluorescence microscopy. With sera containing antibody to V-Z virus a fluorescent halo appears around the infected cells. Titers of <1:2 are seen in persons susceptible to varicella; titers of 1:4 or greater are seen in persons immune to varicella.

Assay for CMI to V-Z virus. This assay, in which inactivation of V-Z virus *in vitro* by human leukocytes (WBC) is measured, has been previously described (4). Briefly, human WBC are isolated on a Ficoll-Hypaque gradient and washed with Hanks solution. One thousand leukocytes are then added, along with either V-Z stimulating antigen or control antigen to human embryonic lung fibroblast cultures previously inoculated with a known amount of V-Z virus. Three days later, the cultures are trypsinized and added to fresh human embryonic lung fibroblast monolayers. After another 3 days the number of V-Z plaques is counted. In cultures that contain WBC from persons immune to varicella, along with V-Z stimulating antigen, a significant reduction of V-Z plaques is observed. No decrease in V-Z plaques is seen in cultures to which control (uninfected) "stimulating antigen" is added along with WBC. When WBC from persons susceptible to varicella are used with V-Z stimulating antigen, no decrease in plaques is noted (3).

To compare tests done at different times, the following calculation was made to determine the percent V-Z plaque reduction brought about by stimulated WBC: $100 - [(\text{plaques in cultures with WBC and V-Z antigen} / \text{plaques in cultures with WBC and control antigen}) \times 100]$.

WBC were stored frozen in liquid nitrogen for several weeks without loss of their ability to inactivate V-Z virus. At times, when frozen WBC were used, the number was increased from 1,000 to 2,000 per culture, without apparent change in response.

Patients. WBC from patients with varicella (eight patients aged 1 to 32 years) and zoster (four patients aged 5 to 27 years) were studied (Table 1). All patients were examined on numerous occasions by one of the

TABLE 1. Immunocompromised patients with V-Z infections

| Infection | Therapy | No. of patients | Severity of disease | No. survived |
|---------------------|--|-----------------|---------------------|--------------|
| Primary varicella | No antiviral therapy | 2 | Mild | 2 |
| Primary varicella | Antiviral therapy ^a | 3 | Severe | 1 |
| Recurrent varicella | | 3 | Mild | 3 |
| Zoster | No antiviral therapy | 2 | Mild | 2 |
| Zoster | Antiviral therapy ^a (for dissemination) | 2 | Severe | 1 |

^a Adenine arabinoside, three patients; levamisole, one patient; transfer factor, one patient.

investigators or their assistants. Informed consent for these studies was obtained from either the patient, a parent, or both. All patients except controls were immunocompromised, usually because they were receiving chemotherapy and/or radiotherapy for malignant disease. Two patients died of varicella with pneumonia, one on day 4 and one on day 10 after the onset of the disease. Another died on day 21 after onset of zoster. She had unremitting, progressive disseminated zoster, paralytic ileus believed to be secondary to chemotherapy, and bacterial sepsis. This patient had received a 10-day course of adenine arabinoside at 10 mg/kg per day (days 5 to 15) without obvious beneficial effect on her zoster. One of the two patients who died of varicella was treated with three doses of transfer factor made from random donors; the other was given levamisole, 150 mg/day, for 3 days. Two of the patients who survived severe V-Z infection were also treated with adenine arabinoside at 10 mg/kg per day for 10 days. WBC were not tested at the time patients were receiving adenine arabinoside; however, WBC were tested when the patients were receiving levamisole or transfer factor.

One patient who survived varicella and two who died from varicella were passively immunized after exposure to the virus. The two who died are believed to have received suboptimal amounts of V-Z antibody for passive immunization.

Of the patients who died with V-Z infections, none was leukopenic at the time of admission and none had absolute lymphocyte counts of less than 1,000 per mm³ at admission.

Three of the children with varicella had what appeared to be second attacks of varicella. All three of these children had leukemia; two were on therapy, and one had been off therapy for 1 year. All these infections were mild, with few skin lesions. They were confirmed as varicella by isolation of V-Z virus from vesicular fluid.

In all these patients the diagnosis of varicella or zoster was based on the appearance and distribution of the rash, a significant rise in V-Z antibody titer, and in many instances isolation of V-Z virus from vesicular fluid. V-Z virus was also isolated from WBC of two patients with disseminated zoster.

WBC from four patients aged 2 to 27 years with lymphoid malignancy were tested from 1 to many years after varicella. WBC from one of these children had also been tested for V-Z CMI on day 6 after onset.

As controls, WBC from a group of 12 normal persons were tested to determine the percent inactivation of V-Z virus upon stimulation of the WBC with V-Z

antigen. WBC from individuals with and without a history of previous varicella were studied. Most of these persons were hospital employees, but none had frequent contact with V-Z virus. Persons considered susceptible to varicella had V-Z fluorescent antibody to membrane antigen titers of <1:2; persons considered immune had titers of ≥1:4. Immune persons had had varicella many years previously.

RESULTS

V-Z antibody titers. Sera for V-Z antibody titers were obtained from patients between day 2 and day 28 after the onset of their rash. Titers ranged between 1:2 and 1:1,024 (Fig. 1). Antibody to V-Z virus was detected in all sera within several days after onset of varicella, although in some cases this antibody was, at least in part, passively acquired. Antibody to V-Z virus was always detectable in patients with zoster. However, despite the presence of detectable specific antibody, three patients died, two with varicella and one with zoster.

CMI to V-Z virus. CMI to V-Z virus was determined during or after clinical illness and at the same time V-Z antibody titers were measured. The responses of patients with V-Z infections are shown in Fig. 2. It may be seen that during ongoing V-Z infection the CMI response was low, with a mean reduction of viral plaques

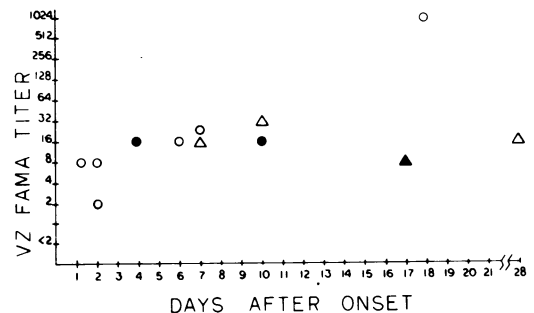


FIG. 1. V-Z fluorescent antibody to membrane antigen (FAMA) titers in 12 immunocompromised patients with varicella or zoster. (○) Varicella with recovery; (△) zoster with recovery; (●) or (▲) fatal infection.

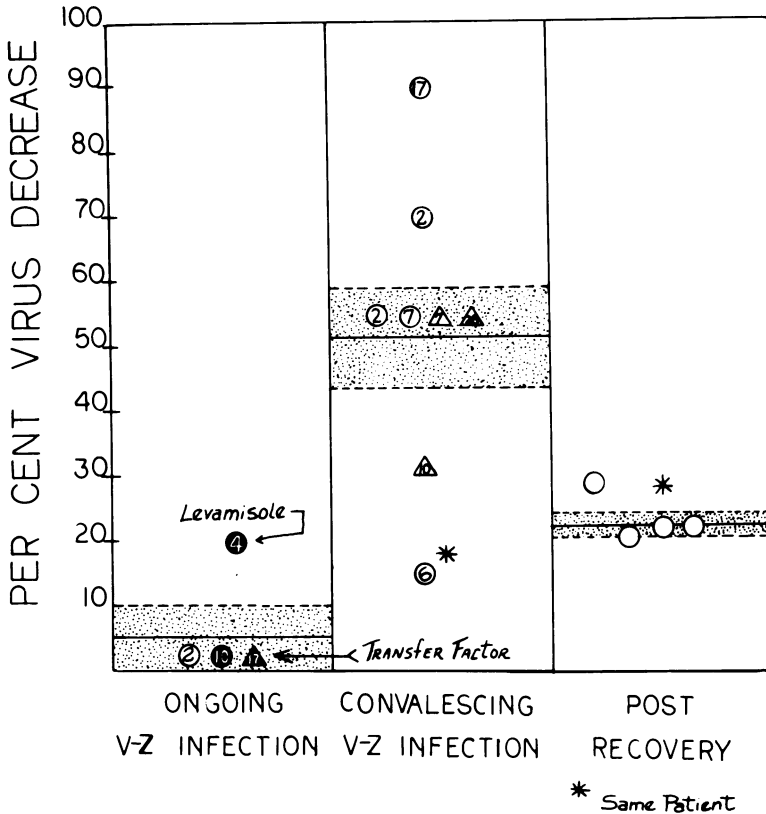


FIG. 2. CMI response of WBC from 16 immunocompromised patients to V-Z virus measured by specific inactivation of virus. Patients were tested during V-Z infection and afterwards. (○) Varicella; (△) zoster; (●) or (▲) fatal infection. The day after onset of rash is within the symbol. For ongoing infections the mean percent V-Z reduction was $5 \pm 5\%$; during convalescence it was $52 \pm 8\%$; years later it was $23 \pm 2\%$. The shaded areas represent the standard error of the mean.

of 6%. All three patients who died with V-Z infections showed low responses. Patients who were tested during resolution of their infection had a mean percent reduction of V-Z virus in vitro of 53%, which is significantly higher than that of the patients with ongoing infections.

Postrecovery responses on four immunocompromised patients showed a mean of 23% virus reduction in vitro.

WBC from six normal individuals with previous varicella and six with no history of varicella were also tested. A mean of 49% V-Z virus reduction was found for normal immunes, and 0% for susceptibles. When the CMI responses of normal and immunocompromised patients with previous varicella were compared (Table 2), the ability of WBC from these individuals to inactivate V-Z virus was found to be significantly different. WBC from both groups of patients immune to varicella were able to inactivate V-Z virus when compared with WBC from normal susceptibles. However, WBC from immunocom-

promised immune persons had a significantly less vigorous response than WBC from normal immune persons.

DISCUSSION

Clinical observations have suggested that CMI to V-Z virus plays a major role in recovery from V-Z infection (8). Our data appear to confirm this clinical impression, since patients in this study had demonstrable CMI to V-Z virus when their V-Z infection was beginning to resolve and during convalescence. In contrast, recovery did not correlate with the presence of specific antibody. Thus all three patients in this study who died had demonstrable V-Z antibody, but poor or undetectable CMI to the virus. Whether antibody plays some role in recovery from V-Z infections is not ruled out by this study, but it appears that CMI is of primary importance.

The assay for CMI to V-Z virus that we uti-

TABLE 2. *In vitro* inactivation of V-Z virus by human peripheral WBC

| Patient | Percent inactivation (mean value \pm SE ^a) |
|---|---|
| Normal: | |
| Previous varicella years ago (n = 16; six individuals) | 48 \pm 6* |
| No previous varicella (n = 6) | 0 \pm 0 |
| Immunocompromised: | |
| Ongoing V-Z infection (n = 4) | 5 \pm 5* |
| Convalescent V-Z infection (n = 8) | 52 \pm 8 |
| Years after varicella (n = 4) | 23 \pm 2* |

^a SE, Standard error of the mean. *, Significant differences between these values ($P < 0.01$).

lized detects the ability of WBC stimulated with V-Z antigen to inactivate the virus, a reaction which may theoretically be important *in vivo*. In previous studies we demonstrated the specificity of this reaction (3, 4). Only after stimulation of peripheral WBC with V-Z antigen was V-Z virus inactivated, and although WBC from persons immune to varicella were capable of this action, those from persons susceptible to varicella were not. The mechanism underlying this ability to inactivate V-Z virus requires T cells and monocytes (macrophage precursors). Secretion of immune interferon by lymphoid cells and antibody-dependent cellular cytotoxicity do not appear to play a role in this *in vitro* process (3).

Within several days after the onset of varicella or zoster, WBC from immunocompromised persons destined to recover are strongly capable of inactivating V-Z virus. This ability was found to persist after an attack of varicella, although it was less vigorous than during convalescence. However, a significantly stronger CMI response was found in normal persons immune to varicella than in immune immunocompromised persons.

Impaired CMI to V-Z virus in immunocompromised patients has been noted by others utilizing different *in vitro* techniques (9, 10), and it may in some way be related to the development of clinical zoster or recurrent varicella. One of our patients with a history of varicella several years prior to our testing of her WBC developed disseminated zoster 5 months after we determined that her WBC response to V-Z virus was low in comparison to normal immunes. The remaining three postconvalescent patients we tested have not developed clinical zoster. As in previous studies (9, 10), not all patients with

impaired CMI to V-Z virus go on to develop zoster. Thus it seems that additional, as yet unidentified factors may be important in the development of clinical recrudescence of latent V-Z infection.

The reason why certain immunocompromised patients have poor CMI responses to V-Z virus is not apparent from this study. Patients with malignant disease who are at risk to develop severe V-Z infections have been noted to be lymphopenic (1), and persons receiving therapy for malignancy may have decreased numbers of T lymphocytes as a result of chemotherapy and radiotherapy (5). Thus, a lack of sufficient immune cells that can function as killers may underlie poor CMI to V-Z virus. It is also conceivable that lymphocyte and/or macrophage killing may be impaired in these patients, possibly even due to overactivity of suppressor cells. Whereas our assay utilizes a fixed number of WBC, the quality of cells and the ratios of T, B, and null lymphocytes and monocytes may vary from patient to patient. Therefore, although our assay reflects the ability of peripheral WBC to eliminate V-Z virus, it remains unknown whether lack of this ability is related to decreased numbers or altered function of killer lymphoid cells, or both. It is also not clear from this study whether the defect is specific for V-Z virus or extended to other agents in addition. It might thus be possible that individuals incapable of mounting an effective immune response to V-Z virus would have difficulty in defense against other pathogens as well. It is also unknown whether normal individuals have active CMI against V-Z virus during active infection. Further experiments to clarify some of these points are planned.

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