Cell-Mediated and Humoral Immunity to Herpesviruses During and After Herpes Zoster Infections

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The influence of herpes zoster virus infection on cell-mediated and humoral immunity to varicella-zoster virus (VZV), cytomegalovirus, and herpes simplex virus (HSV) was followed in 17 zoster patients from the first week to 6 months after start of eruptions. The clinical responses were registered and correlated to the immune responses. A significant depression in blast transformation on stimulation of lymphocytes with all three antigens was found on days 1 to 5 compared with transformations later after zoster eruptions and compared with controls. Phytohemagglutinin exhibited the same stimulation in the different groups and controls. No significant differences in interferon production in the various groups and controls were found on stimulation with the VZV and HSV antigens. All zoster patients became seropositive by complement fixation to VZV a few days after start of the zoster eruption. Two zoster patients showed a fourfold rise in complement fixation antibodies to HSV. Three patients had changes in complement fixation titers to cytomegalovirus, which could indicate new infection or reactivation of infection with this virus. A significant lower transformation index to VZV was found during the first 9 days in zoster patients with fever compared with patients without fever. The relevance of this observation is discussed in relation to a previous similar observation from our group.

The influence of virus infections on immune responses has been dealt with in many publications (6, 8). A suppression in delayed hypersensitivity to tuberculin was first seen in children infected with measles, but later this transient depression of skin reactivity was found in infections with a variety of viruses and also in vaccine-induced infections (6, 8).

Using lymphoid cell stimulation as an in vitro correlate of cellular immunity, we observed a decreased reactivity to the different antigens and phytohemagglutinin (PHA) in patients infected with some of these viruses (6, 8).

In infections with varicella-zoster virus (VZV), a depression of skin reactivity to tuberculin has also been found (13), and a lower stimulation of lymphocytes with VZV was noticed during the first few days after the eruption (2, 12).

To further analyze the cell-mediated immunity in patients during and after herpes zoster eruptions, we studied the lymphocyte transformation reaction and the production of interferons in lymphocytes stimulated with the specific antigen, herpes simplex virus (HSV) antigen, and cytomegalovirus (CMV) antigen, together with stimulation of the lymphocytes with PHA.

The humoral immune response to the same viruses was followed by the complement fixation (CF) test. The clinical picture was followed and correlated to the immune responses.

MATERIALS AND METHODS

Persons studied. All 17 patients examined in the study were otherwise healthy persons referred to the Clinic of Infectious Diseases, Marselisborg Hospital, Aarhus, Denmark, for acute eruption of zoster. The zoster patients ranged in age from 25 to 80 years, with a mean age of 60 years. All persons with zoster were kept as inpatients the first week, and after this they were followed at the outpatient clinic.

The control group consisted of 17 persons. Eight of these were inpatients with nonimmunological, nondebilitating diseases, and the rest were laboratory technicians, physicians, and maintenance personnel. The mean age of the control group was 53 years (25 to 81 years).

None of the patients or the persons in the control group was receiving medication known to cause depression of cell-mediated or humoral immunity.

Blood samples. Fifteen milliliters of heparinized blood (20 IU of heparin per ml) was used for preparation of mononuclear cells, and 5 ml of blood without anticoagulant was used for preparation of serum samples.

Mononuclear cells. Mononuclear cells were obtained by Ficoll-Isopaque flotation and washed as previously described (7). After being washed, the cells were suspended in RPMI 1640 with buffer and antibiotics and 15% heat-inactivated human HSV- and CMV-negative sera.

Antigens for transformation reaction. (1) HSV-1. Human embryonic lung (HEL) cells grown in Eagle minimal essential medium with 2% calf serum were infected with HSV type 1 (HSV-1) strain Maclntyre
(2 to 3 plaque-forming units/cell). At the time of maximum cytopathic effect (24 to 48 h after infection), the culture was frozen and thawed twice and centrifuged at 1,000 x g for 10 min to spin down cellular debris. The supernatant fluid containing 7 x 10^6 viral plaque-forming units/ml was inactivated by ultraviolet light for 5 min at a distance of 15 cm. After inactivation no virus could be detected.

(ii) CMV. HEL cells grown in the above medium were infected with CMV strain Ad 169 (2 plaque-forming units/cell). At the time of maximum cytopathic effect (72 to 96 h after infection), the supernatant fluid was centrifuged at 1,000 x g for 10 min to spin down cellular debris. The supernatant fluid containing 10^6 to 10^7 viral plaque-forming units/ml was inactivated at 56°C for 1 h. After inactivation no virus could be detected.

(iii) VZV. HEL cells grown in the above-mentioned medium were infected with a VZV strain received from A. Godtfredsen, State Serum Institute, Copenhagen (originally isolated from a child with varicella and passed in Vero cells, and in this laboratory passed in HEL cells). Confluent HEL cells grown in Roux flasks were infected with a 1:3 split of HEL cells with 30 to 40% cytopathogenic effect. At the time of maximum cytopathic effect (72 to 96 h after infection), the supernatant was discharged, and the cells were removed from the flasks with a rubber policeman and suspended in medium without serum (10^6 cells/ml). The suspension was sonicated for 60 s, and after pelleting the cell debris by centrifugation at 3,000 x g for 15 min, the supernatant was used as antigen. The antigen prepared in this manner had CF titers of 1:16 against four units of antiserum in a two-dimensional titration. The infectivity was inactivated by ultraviolet light for 5 min at a distance of 15 cm. After inactivation no virus could be detected.

The antigens were stored in small samples at -70°C. Control antigen for all three reactions was prepared from uninfected HEL cells treated in the same way as the infected cells.

Transformation reaction. Transformation reaction was as previously described (7). Briefly, after counting, the mononuclear cell suspension was adjusted to contain 10^6 cells per ml and dispensed in 0.2 ml portions in the wells of a microplate (Linbro no. IS-MRC-96). To the cultures were added 30 or 10 µl of undiluted antigen (HSV-1, CMV, VZV, or control antigen) and 10 µl of antigen diluted 1:4, 1:16. PHA was added in 10-µl portions from solutions containing 1.0 µg/ml. All determinations were done in triplicate. The plates were closed with plastic covers, and antigen-stimulated cultures and unstimulated cultures were incubated in 5% CO₂ at 37°C for a total of 6 days. PHA-stimulated cultures were incubated for a total of 3 days.

For the final 18 h of incubation, 1 µl of a [³¹C]-thymidine solution (0.02 µCi/ml) was added to each well. The cells were harvested on glass fiber paper (Whatman CF 81) with a Skatron harvester type POB 8. The paper disks were placed in plastic vials with 2 ml of Triton-Toluol scintillation fluid and counted in a Packard Tri-Carb liquid scintillation spectrometer. Counts per minute were converted to disintegrations per minute.

Results of transformation reactions are given as the transformation index (TI), obtained by dividing the disintegrations per minute of viral antigen-stimulated cells by the disintegrations per minute of control antigen-stimulated cells.

Interferon assay. The supernatant fluid from viral antigen-stimulated lymphocytes (10 µl of antigen per 2 x 10^6 cells in 0.2 ml) was harvested after incubation for 6 days and centrifuged at 3,000 x g for 10 min. The assay was performed by a micromethod, as previously described (5). The interferon titer was the highest dilution that reduced vesicular stomatitis virus cytopathic effect by 50%.

Characterization of interferons. Interferons were characterized as type I (classical) interferon according to the description given by Valle et al. (15), i.e., partly inactivated after exposure to 56°C for 1 h, no influence by exposure to pH 2 for 24 h, and neutralization with anti-human leukocyte interferon sera.

CF antibody test. All sera were inactivated and tested for antibodies to HSV, CMV, and VZV by the CF test. CF antigen was prepared from HEL cells as described previously (1, 7).

Statistical evaluation. When groups of patients were compared, the Mann-Whitney test was used.

RESULTS

Transformation reaction in response to VZV antigen. The TI in blood samples from patients with acute and convalescent herpes zoster compared to controls is given in Fig. 1. A total of 95 blood samples from 17 patients and 17 samples from 17 controls were examined.

Of the 17 patients, fourteen were studied once during the first 5 days; two were studied twice during this period. All patients were studied once or twice during days 6 to 9, and nine patients were studied once during days 10 to 19. All 17 patients were studied during days 20 to 60, and 15 patients were studied 6 months after the episode of herpes zoster.

Results from both CF-seropositive and CF-seronegative persons are given. As will be seen from the figure, all patients, seronegative at the start of the eruption, became seropositive after a few days.

TI in blood samples from patients days 1 to 5 was found to be significantly lower than in healthy controls (p < 0.02) and in blood samples taken at days 10 to 19 (p < 0.02), at days 20 to 60 (p < 0.01), and more than 6 months after eruption (p < 0.01).

Transformation reaction in response to HSV antigen. Blood samples from the zoster patients and the controls were examined for their reactions to HSV antigen. Results from this study are given as TI in Fig. 2. Only one of the patients and five of the controls were found to be CF seronegative throughout the study. TI of the seropositive patients on days 1 to 5 was found to be significantly lower than TI in the
Fig. 1. Lymphocyte transformation of mononuclear cells from blood samples from zoster patients at various times after start of eruption, together with uninfected controls in response to VZV. Symbols: (●) blood samples from seropositive persons; (○) blood samples from seronegative persons; (□) mean ± standard error of seropositive persons; (△) mean ± standard error of seropositive and seronegative persons.

Fig. 2. Lymphocyte transformation of mononuclear cells from blood samples from zoster patients at various times after start of eruption together with uninfected controls in response to HSV. Symbols and abbreviations are as in legend to Fig. 1.
healthy seropositive controls (p < 0.02) and significantly lower than TI in the same patients on days 10 to 19 (p < 0.02), days 20 to 60 (p < 0.02), and 6 months later (p < 0.02).

Transformation reaction in response to CMV antigen. TI to CMV antigen in blood samples from patients and healthy controls is shown in Fig. 3. Five out of the 17 patients examined were CMV CF seronegative at the start of illness; one of these seroconverted 3 weeks after onset of zoster. Five out of the 17 controls were CF seronegative.

TI of the seropositive patients on days 1 to 5 was significantly lower than in the seropositive controls (p < 0.01) and significantly lower than TI in the same patients on days 6 to 9 (p < 0.05), days 10 to 19 (p < 0.01), days 20 to 60 (p < 0.05), and more than 6 months after the eruption started (p < 0.01).

Transformation reaction in response to PHA. Lymphocytes from all blood samples were stimulated with PHA. Results are given as TI in Fig. 4. No differences were found in TI at the various periods after onset of disease or between TI of patients and healthy controls.

Production of interferon in lymphocytes upon stimulation with antigens. Interferon was produced in lymphocytes upon stimulation with VZV, HSV, and CMV antigens (Table 1). Production was seen in lymphocytes from CF-seronegative, as well as from CF-seropositive patients and healthy controls. No significant differences in interferon production were found between healthy controls and patients at different times after outbreak of disease, although an increase production was found in the recently infected individuals, both with VZV and with HSV. Unfortunately, the available CMV antigens had a low interferon-producing potency; therefore, too few zoster patients and healthy controls had production of interferon to make justifiable comparisons.

Transformation reaction and fever. Eight patients had no fever at all during illness; the remaining nine patients had fever exceeding 38°C. The period of fever was from 2 to 9 days with a mean duration of 5 days. TI to VZV antigen in the group with fever tended to be lower during the first 5 days, although this difference was not significant. However, during days 6 to 9, the TI was significantly lower in the febrile group than in the nonfebrile group (p < 0.01). When examinations done on days 1 to 9 were taken together, the febrile group also had significantly lower TI to VZV than the nonfebrile group (p < 0.01). There were no differences on days 10 to 20, days 20 to 60, and more than 150 days after eruption (Fig. 5). No differences

![Graph](http://iai.asm.org/)

**Fig. 3.** Lymphocyte transformation of mononuclear cells from blood samples from zoster patients at various times after start of eruption together with uninfected controls in response to CMV. Symbols and abbreviations are as in legend to Fig. 1.
in TI of HSV- and CMV-stimulated lymphocytes were found between febrile and nonfebrile patients.

Humoral immunity to VZV, HSV, and CMV. Nearly half of the patients with eruptions of zoster were found to be CF seronegative to VZV at the start of the disease. All of these seroconverted after a few days; five were found to have an unchanged high CF antibody titer, and four had a fourfold rise in CF antibodies. Two patients had a fourfold rise in CF antibodies to HSV. One of these had unchanged high CF titer to VZV whereas the other had a fourfold rise in CF antibodies to VZV. Most of the other patients had unchanged high CF antibodies to HSV. None of the patients showed clinical signs of reactivation of HSV infections. Four patients were found to be unchanged CF seronegative to CMV. Nine patients had unchanged titer of CF antibodies to this virus, but two patients had a fourfold rise in CF titers, and one patient seroconverted. None of the patients had secondary febrile episodes or other signs of CMV infections. No attempts to isolate HSV or CMV viruses were made.

DISCUSSION
Depression of lymphocyte transformation upon stimulation with the specific antigen during the first week of a herpes zoster infection has
lymphocytes seen in more serious herpesviruses, most important phase is early mitogens other infections have been shown in vivo to accompany measles, and recent work has shown that other infections have a similar influence on this reaction (3, 4). In vitro reactivity of lymphocytes to the relevant antigens was not tested in these studies.

We found a rather unchanged response of the lymphocytes to PHA during and after the infection. This is in accordance with the findings of Russell et al. (12). Unfortunately, we did not use other mitogens or nonviral antigens to test the overall responses of the lymphocytes in the initial phase of a zoster infection. It is generally accepted that the cell-mediated immunity plays an important role in resistance to infections with most herpesviruses, and a higher frequency and more serious infections with these viruses are seen in persons with impaired cell-mediated immunity (10). Whether the low transformation reaction to VZV, HSV, and CMV is a consequence of the zoster infection or the zoster infection is a result of a low cell-mediated immunity expressed by the low lymphocyte transformation reaction cannot be deduced from the present study.

We had a positive CF reaction to VZV in half of the patients in the initial phase of their zoster, a finding which is in accordance with a previous study (14). In all cases the reaction was found positive in the later stages of the disease. A cross-reactivity in serological response is usually supposed to exist between HSV and VZV (1) and could account for the fourfold rise in CF antibodies to HSV found in two patients. It could not be excluded that the patient with a fourfold CF titer rise to HSV and no rise to VZV had a HSV infection with a zosteriform distribution. A cross-reactivity between CMV and VZV, on the other hand, has never been demonstrated and, therefore, we find it interesting that three patients had changes in CF antibodies to CMV. Two showed seroconversion, and one showed a fourfold rise. None of the patients showed clinical signs of a CMV infection, but usually this infection runs a subclinical course in patients with a normal cell-mediated immunity, and at least the lymphocyte reaction to CMV

![Graph](image_url)

**Fig. 5.** Lymphocyte transformation to VZV of mononuclear cells from blood samples from zoster patients with and without fever at various times after start of eruption. Symbols: (○) blood samples from patients with fever; (□) blood samples from patients without fever; (±) mean ± standard error.

previously been found by Russell et al. (12) and Arvin et al. (2). Arvin et al. found a normal response to HSV antigen in these patients tested two to four weeks after the onset of herpes zoster. Our findings of a depression in transformation reaction to HSV and CMV also during the first days after start of the zoster rash can be in agreement with previous in vivo observations that children during and after a varicella infection had a lower tuberculin reaction (13). Loss of cutaneous tuberculin reactivity was reported early in this century to accompany measles (16), and recent work has shown that other infections have a similar influence on this reaction (3, 4). In vitro reactivity of lymphocytes to the relevant antigens was not tested in these studies.
was found to be normal in the period when the seroconversion to this virus took place. It cannot be determined whether the possible activation of CMV was a result of initial low cell-mediated immunity to all herpesviruses likewise leading to reactivation of the zoster infection or whether it was a result of the VZV infection.

The increase in production of interferon in lymphocytes the first months after onset of herpes zoster upon stimulation with VZV is in accordance with previous studies from T. C. Merigan's group, as well as from our own group. Arvin et al. (2) found a significant increase in interferon production 1 to 2 weeks after the onset of herpes zoster. Pollard et al. (9) detected an insignificant increase in interferon production after CMV mononucleosis and Möller-Larsen et al. (7) had higher production of interferon in lymphocytes 3 to 4 weeks after HSV stomatitis than during the acute attack. We found it interesting that there was an increase in production of interferon also upon stimulation with the HSV antigen.

In zoster patients with fever during the eruption we found a significantly lower T1 for the first 9 days compared with those without fever. Previously, hyperthermic temperatures have been shown to enhance human lymphocyte transformation responses to different stimuli (11). Our findings may indicate the importance of the transformation reaction as a parameter of the ability of the organism to limit the disease. Those with a low transformation reaction might be less able to restrain virus growth which might call for a febrile response to develop. Our group has previously found an initially low incremental count on stimulation with the specific antigen in lymphocytes from children with febrile HSV stomatitis compared with those without fever (7). In the same study, we found a negative correlation between fever and antibody-dependent cell-mediated cytotoxicity cytotoxic antibodies. These antibodies were only found in children where fever had disappeared. Unfortunately, we have not so far been able to study cytotoxicity against VZV, an examination we think would be important for further understanding of the immune reactions against primary and reactivated VZV infections.

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LITERATURE CITED