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CELL-MEDIATED IMMUNITY TO VARICELLA-ZOSTER ANTIGEN IN ACUTE HERPES ZOSTER (SHINGLES)

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SUMMARY

Cell-mediated immunity to varicella-zoster antigen was assessed by lymphocyte transformation in fourteen patients with acute herpes zoster and in a group of twelve healthy controls. There was a significant dimunition in cell-mediated immunity to this antigen in the patient group when compared to the controls but no definite difference was observed in the responses to phytohaemagglutinin and PPD measured at the same time. It was suggested that this lack of cell-mediated immunity contributes to the evolution of the disease.

INTRODUCTION

Herpes zoster (shingles) is an acute infection of adults with the varicella-zoster (V-Z) virus, the same virus that in younger age groups causes chickenpox (Gold, 1965). Patients with shingles can usually be shown to have suffered from chickenpox in the past and the infection is believed to be due to activation of latent virus in a previously immunized subject (Miller & Brunell, 1970). It appears to occur in spite of normal levels of antibody and an antibody response characteristic of the secondary type is seen (Brunell *et al.*, 1969; Judelsohn, 1972). The factors, which, in an immune subject, allow persistence of live virus in the host for such prolonged periods, the reason for its emergence and renewed pathogenicity remain to be elucidated. We have investigated the possibility that a failure of cell-mediated immunity can be involved and have examined lymphocyte stimulation *in vitro* (as measured by incorporation of tritiated thymidine) to the varicella-zoster (V-Z) antigen during the acute attack. The lymphocyte reactivity *in vitro* to tuberculin and phytohaemagglutinin (PHA) was studied at the same time.

PATIENTS AND METHODS

The patients were all previously healthy and were studied within 3 days of the onset of the typical vesicular rash of shingles. As far as could be determined by clinical history,

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they had all previously had chickenpox. The control population was taken from healthy hospital personnel and while there was no age or sex matching, the ages of the two groups were similar, viz patients 23–78 yr (mean 54), controls 26–69 yr (mean 48).

Peripheral blood lymphocytes of 90–95% purity were obtained using a column of Ballotini glass beads and incubated at a concentration of 10^6/ml in 3-ml cultures of Waymouth's medium, containing 15% foetal calf serum. Triplicate cultures were set up with medium alone (control) and with varying concentrations of tuberculin-PPD (Weybridge), phytohaemagglutinin (PHA, Burroughs Wellcome), and inactivated varicella virus as well as a suitable control consisting of an extract of uninfected human embryonic lung cells in which the virus was grown. Lymphocyte stimulation was assessed at 6 days following the addition of tritiated thymidine, by liquid scintillation counting. The antigen was prepared by adding infective virus to human embryonic lung cultures maintained in Eagles' MEM containing 1% foetal calf serum. After cytopathic effects were marked, the cells were washed, sonicated and the virus inactivated by heating for 8 min at 58°C. This herpes zoster virus preparation is of proven antigenicity in complement fixation tests and was used in concentrations of 200, 20 or 2 μg of protein per ml for lymphocyte studies. In most of the control subjects and in the two convalescent patients, the optimum concentration of antigen was 20 μg/ml. Where this was not so in all cases the concentration giving the maximum response was used. A negative response was accepted only if none of the three antigen doses induced stimulation. Lymphocyte stimulation results were expressed as the ratio: cpm of the test culture (T) divided by cpm in the control culture (C). A T:C ratio of over 2 was taken to indicate significant lymphocyte stimulation. There was no significant difference between the counts of the unstimulated control cultures in the two groups.

RESULTS

Of the twelve healthy individuals studied, ten showed significant lymphocyte transformation to the V-Z antigen as judged by a T: C ratio of greater than 2, indicating a state of acquired immunity, presumably from a past attack of chickenpox. In contrast, only two of the fourteen patients with acute shingles showed lymphocyte stimulation greater than T: C of 2 (Fig. 1) and the difference was statistically significant ($P<0{\cdot}05$, Student's *t*-test). It was further noted that none of the patients with shingles had a T: C ratio greater than 5, whereas half (6/12) the healthy patients exceed this T: C ratio. An uninfected human embryonic lung cell line extract prepared to the same concentration as the virus containing extract was also tested (as a control) on lymphocytes from healthy and affected individuals and showed no significant stimulation.

The *in vitro* responses to PHA have to be interpreted with caution, as the cultures were terminated on the sixth day when sub-optimal responses are obtained, and this partly accounts for the wide spread of T: C ratios (2 to >20). There was no significant difference in the response to either PPD or PHA. However, in two patients in whom it was possible to repeat these studies 3 months after the acute infection had subsided, the PHA transformation and the response to V-Z antigen were much more marked (see Fig. 2 for the response of one of the patients).

DISCUSSION

The protective importance of cell-mediated immunity has been suggested by the enhanced

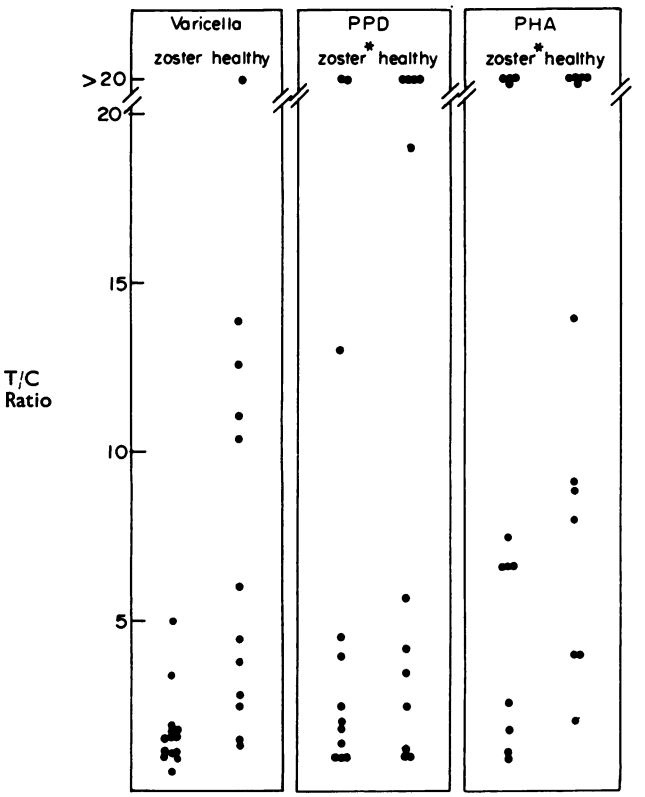

FIG. 1. Lymphocyte response in patients with acute zoster and healthy controls. * One patient not tested.

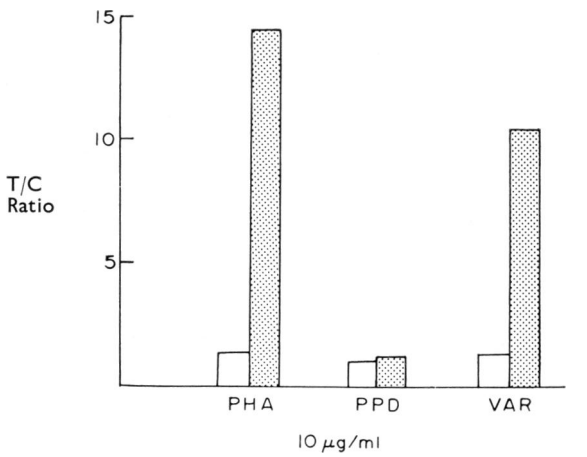

FIG. 2. Lymphocyte response in one patient with zoster during (open columns) acute and (stippled columns) convalescent stages.

susceptibility of individuals with impaired cell-mediated immunity (CMI) to both natural viral infections and to the development of vaccinia following vaccinations with smallpox (Merigan & Stevens, 1971; Good, 1968). It is generally accepted that zoster is an example of reinfection with varicella virus (Miller & Brunell, 1970; Taylor-Robinson & Slack, 1972). After the patient recovers from chickenpox—the primary infection—the virus becomes latent, perhaps residing in a doral root ganglion. Reactivation may occur after certain local exciting factors (Hope-Simpson, 1965) and is especially frequent in patients in whom there is good reason to believe that cellular immune surveillance is impaired (Chang, 1971). For example, the incidence of zoster is 18% in patients with rheumatoid arthritis treated with cyclophosphamide who were followed for 5 yr (Fosdick, Parsons & Hill, 1968); 8·4% in one series of renal transplant recipients followed up for 18 months (Rifkind, 1966); and it has been noted in 30% of patients with Hodgkin's disease of the nodular sclerotic type over a 10-yr follow-up (Chawla *et al.*, 1970). It is especially common after a splenectomy (Goffinet, Glatstein & Merigan, 1972) and is seen with an increased frequency in patients with other malignant diseases (Schimpff *et al.*, 1972).

However, shingles usually occurs in apparently normal people with an incidence of about 3 out of 10^4 persons per year (Hope-Simpson, 1965), and there is an increased frequency in older individuals (70% of patients in one study were over 50 yr (Miller & Brunell, 1970)) when immune mechanisms may be becoming defective (Burnet, 1970). Our study has demonstrated a marked diminution or lack of cellular immune reactivity *in vitro* to the V-Z antigen in patients with acute shingles compared to control normal patients. While it is possible that this is due to mopping up of antigen-sensitive cells, it is tempting to suggest that such an unresponsive state may be of importance in the evolution of the disease.

However, the relationship between virus infections and immunity is not simple, as virus infections themselves may be the cause of immune unresponsiveness in man, as observed for example in measles (Zweiman *et al.*, 1971; Dentson, 1953). In patients with shingles who are previously healthy, it is not possible to state whether the defect of cellular responses demonstrated, pre-existed or was a consequence of the virus infection. When considered with the predilection of the disease for patients in whom cell-mediated immunity is likely to be already impaired (Chang, 1971), our findings would suggest that even in the previously healthy, a depression of cell-mediated immunity to the zoster antigen might be a factor in the cause of the disease and might selectively favour the multiplication of zoster virus. There is considerable evidence that shingles is not due to reinfection from an external source, and by inference is due to activation of latent virus, however, attemps to find the virus in the skin or nerve ganglia have been unsuccessful (Meurisse, 1968).

Occasionally, the rash occurs in the peripheral nerve distribution at the level at which spinal vertebral or cord disease exist. Deep X-ray therapy or u.v. light have been similarly implicated (Hope-Simpson, 1965). Such local factors could activate the virus in some patients (Luoff, 1961). The stimulus remains obscure in a majority of otherwise healthy persons.

Interferon can readily be demonstrated in the fluid of the skin vesicles but in three patients with disseminated zoster (and neoplasia) the amount of interferon was markedly reduced (Armstrong *et al.*, 1970).

We suggest that the emergence of herpes zoster requires activation of virus with an associated state of cell-mediated immune hyporesponsiveness. In a previously healthy patient the hyporesponsive state appears to be acquired, specific and reversible, but whether special circumstances favour its development is not clear. It also seems possible that failure of

cellular mechanisms are of importance in the nature of the spread in the distribution of a sensory nerve; and that interferon and antibody may prevent widespread dissemination of the virus.

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