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Abstract

Examination was made of the in vitro response of human peripheral blood mononuclear cells (PBNMCs) to phytohemagglutinin (PHA) following treatment with varicella zoster virus (VZV) or herpes simplex virus type 1 (HSV 1). Cell proliferation was determined by colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide. The response to PHA was depressed in all cases by virus infection of PBMNCs prior to PHA treatment. When the infection with the viruses was after PHA treatment, PHA response differed. For VZV infection, the response increased in four out of six samples, but was reduced in the other two. The response to PHA was depressed in all six samples by HSV 1 infection.

KEYWORDS: virus infection, nonspecific mitogen, immune response

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Brief Note

Effects of Varicella Zoster Virus or Herpes Simplex Virus Type I Infection *In Vitro* on Response of Human Peripheral Blood Mononuclear Cells to Phytohemagglutinin

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Examination was made of the *in vitro* response of human peripheral blood mononuclear cells (PBMNCs) to phytohemagglutinin (PHA) following treatment with varicella zoster virus (VZV) or herpes simplex virus type 1 (HSV 1). Cell proliferation was determined by colorimetric assay using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide. The response to PHA was depressed in all cases by virus infection of PBMNCs prior to PHA treatment. When the infection with the viruses was after PHA treatment, PHA response differed. For VZV infection, the response increased in four out of six samples, but was reduced in the other two. The response to PHA was depressed in all six samples by HSV 1 infection.

Key words: virus infection, nonspecific mitogen, immune response

Viral infections such as the measles, which has been extensively studied, have been known to depress cell-mediated immunity in humans (1, 2). Varicella, herpes simplex (HS), rubella and mumps may also be attended by this situation (3, 4). Virus-induced immunosuppression attracts a great deal of interest because in some hyperimmune skin disorders such as atopic dermatitis immunosuppression induced by a virus infection may serve to stabilize the disorder (5). Since in measles, depression of the immune system in response to immunostimulant agents or to environmental allergic antigens such as candida comes about only when exposure to these agents/antigens precedes virus infection; otherwise, a depressed response is not observed (1). For better understanding of virus-induced immunosuppression, we evaluated the *in vitro* proliferative response of human peripheral blood mononuclear cells (PBMNCs) to a stimulating agent phytohemagglutinin (PHA) by adding VZV or HSV before and after the addition of the stimulating agent.

The wild-type VZV strain was a human isolate obtained from the Institute for Microbial Diseases and subsequetly propagated in human embryonic lung fibroblasts (HEL-FBs) (6). It was maintained in complete Eagle's minimal essential medium (MEM) supplemented with 10 % heat-inactivated fetal calf serum in a CO_2 incubator. Cell-free VZV in PSGC [5 % sucrose, 10 % fetal calf serum, 0.1 % sodium glutamate in phosphate buffered saline (PBS)] with a titer of 5.0×10^5 PFU/ml was prepared by sonicating virus-infected HEL-FBs as described previously (6). The HSV 1, strain KOS, was grown in Vero cells and maintained in complete MEM (7). A stock of cell-free HSV 1 in MEM with a titer of 4.3×10^7 PFU/ml was obtained from frozen-thawed infected cells. The cell-free viruses were kept frozen at -80°C until use.

The seven blood donors were healthy adults 26 to 45 years of age (six men and one woman), all with histories of herpes labialis and varicella, and in some cases were serologically positive for both viruses. There had been no

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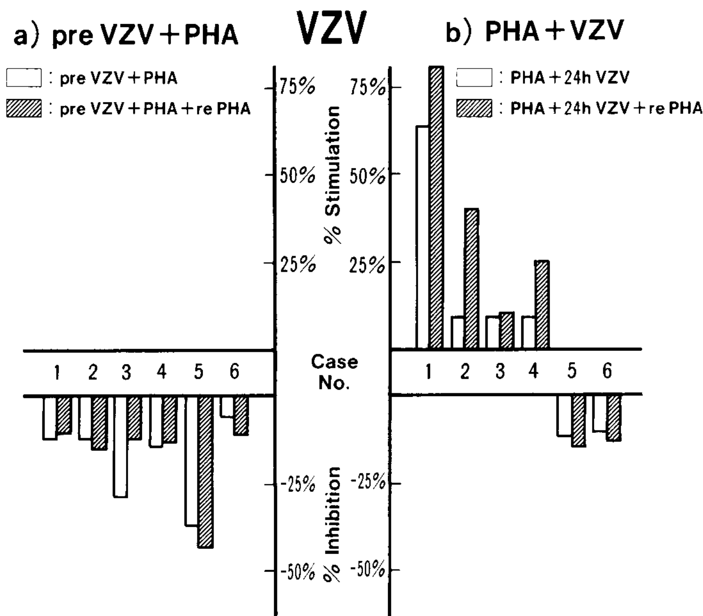

Fig. 1 Percent inhibition and stimulation of response to phytohemagglutinin (PHA) in peripheral blood mononuclear cells (PBMNCs) infected with varicella zoster virus (VZV).
a) preVZV + PHA: pretreatment of VZV and 24 h after VZV infection, PHA treatment; preVZV + PHA + rePHA: pretreatment of VZV and 24 h after VZV infection, PHA treatment and subsequent reexposure to PHA.
b) PHA + 24 hVZV: pretreatment of PHA and 24 h after PHA treatment, VZV infection; PHA + 24 hVZV + rePHA: pretreatment of PHA and 24 h after PHA treatment VZV infection and subsequent reexposure to PHA.

recent symptoms of VZV or HSV 1 infection during the 6 months leading up to the study. In the VZV and HSV experiments, only in Case 1 were PBMNC samples obtained from two different individuals. PBMNCs from each donor were obtained from heparinized venous blood by Ficoll-Hypaque gradient centrifugation. The cells were washed twice with PBS and viability was assessed by the trypan blue exclusion test. The cells were suspended in serum free medium (GIT, Nihon Pharmaceutical Co., Ltd., Tokyo, Japan) at 1.0×10^6 viable cells/ml.

An aliquot of the PBMNC suspension for each subject with the required number of cells was divided for three experimental procedures. First, some of the cells were incubated with PHA at $2.5 \mu g/ml$ dissolved in MEM and the other cells for examination of viral infection were incubated with VZV or HSV 1 at 0.05 PFU/cell or 0.1 PFU/cell, respectively, for 1 h at 37°C in a humidified atmosphere of 5% CO_2 : 95% air. After incubation, both aliquots were washed twice with PBS and resuspended in GIT medium. All cell samples were dispensed at a density of 5×10^5/ml in 96-well flat-bottom plates ($100 \mu l$/well, 5×10^4 cells/well) and incubated at 37°C for 48 h in a CO_2 incubator. Second, after virus infection, the cells washed with PBS were resuspended in GIT medium. Half of the PBMNC suspension was incubated with PHA for 24 h and the cells were washed with PBS. Subsequently, the remaining portion of the cell suspension was incubated with PHA. Third, the cell pellets initially treated with PHA for 24 h were incubated with VZV or HSV 1 at the above density for 1 h and washed twice with PBS. They were then resuspended in GIT medium and dispensed at the above cell density in plastic well plates. The remaining cell pellets were resuspended in 1.0 ml GIT medium followed by the addition of $2.5 \mu g/ml$ of PHA. They were dispensed in plastic well plates and incubated for 48 h at 37°C in the CO_2 incubator. All microtest plate cultures were prepared in triplicate.

Cell proliferation was determined by colorimetric assay (8) with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Chemicon, USA). At 72 h of culture, $10 \mu l$ of MTT, dissolved in PBS (pH 7.4) at 5 mg/ml, was added to each well and the contents were mixed by gentle tapping. The samples were incubated at 37°C for 4 h. Isopropanol/0.04 N HCl was added to each well. At 15 min, absorbance was measured at 620 nm using Dynatech MR620 reader. The mean absorbance of tripricate assays was recorded for each sample. The proliferation index was determined by the following equation:

$$\% \text{ Index} = \left(\frac{\text{experimental value}}{\text{control PHA response}} - 1 \right) \times 100\%$$

Positive index values were considered to represent stimulation (%) of the response whereas negative values were

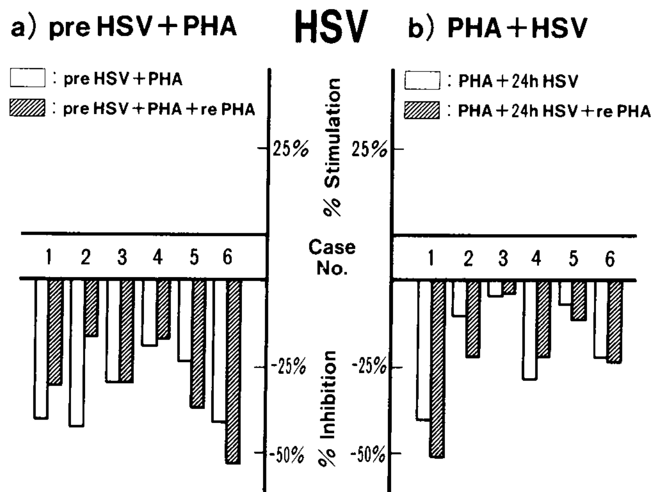

Fig. 2 Percent inhibition and stimulation of response to PHA in PBMNCs infected with herpes simplex virus type I (HSV I).
a) preHSV + PHA: pretreatment of HSV I and 24h after HSV infection, PHA treatment; preHSV + PHA + rePHA: pretreatment of HSV I and 24h after HSV infection, PHA treatment and subsequent reexposure to PHA.
b) PHA + 24hHSV: pretreatment of PHA and 24h after PHA treatment, HSV I infection; PHA + 24h HSV + rePHA: pretreatment of PHA and 24h after PHA treatment, HSV I infection and subsequent reexposure to PHA.
PHA, and PBMNCs: See Fig. 1.

taken as inhibition (%).

Infection with VZV or HSV in all PBMNC samples was confirmed to be positive in the cytoplasm and/or nucleus by immunofluorescence staining with the anti-VZV or anti-HSV 1/HSV 2 antibody. Non-treated cells and only VZV- or HSV 1-infected cells showed no proliferative response at 72h of culture according to colorimetric assay.

VZV infection. The responses to PHA in cases of virus infection prior to PHA exposure were depressed (Fig. 1). For PBMNCs infected with virus following PHA treatment, the response to PHA was moderately depressed in two cases while it was stimulated in the remaining four cases. This stimulation was especially notable in three of these four cases compared to PHA treatment alone. The samples showing stimulated response with virus infection following PHA treatment revealed marked adherent cell monocyte proliferation, suggesting that monocyte proliferation may reflect the stimulation response.

HSV 1 infection. The responses to PHA following HSV 1 infection in all subjects were depressed (Fig. 2). When virus infection was after treatment with PHA all subjects showed a depressed response to PHA. In HSV 1-infected samples, cytotoxic cell death was observed visually in some of the cells.

In the present study, PBMNC samples were reexposed to PHA following virus infection to ensure the continual presence of PHA which may have been washed out prior to the procedure for virus infection. When PHA-pretreated PBMNC cases were reexposed to PHA after infection with VZV, some individuals showed a much greater response to PHA than cases without retreatment with PHA and also showed extensive proliferation (data not shown) of adhering cells (monocytes). Monocytosis has been reported to occur in the majority of herpes zoster patients (9), though not in all. The mechanism for monocyte proliferation in VZV infection still remains unclear since it has yet to be determined whether or not VZV replication in monocytes actually promotes monocyte proliferation (10). Regardless, this would not appear to affect HSV infection. Thus, VZV may in some manner influence monocyte proliferation through mitogen stimulation and accordingly, also suppress lymphocyte response to mitogens. Monocyte proliferation, however, may serve to promote the immune response. However, it has also been proposed that monocytes promote the suppression of mitogen-induced proliferative response in measles virus infection (11).

Common viruses such as the measles virus inhibit mitogen-induced lymphocyte proliferation *in vitro* (12), and a mechanism for such inhibition has been proposed in some reports. Since viruses may hinder the adhesion of PHA to receptors on lymphocytes, this may, in turn, inhibit the responses to PHA. We used live viruses in the present study. So far, inhibition of the proliferative response in cases of HSV infection may be due to the virus-induced killing of cells and the death of infected cells became apparent during culture, even though VZV is not particularly cytotoxic. Nevertheless, inhibition of the

PHA response can not be explained by cell death alone, as pointed out by Yanagi *et al.* (12) who indicate that interactions of measles infected cells with uninfected cells may initiate intercellular signals, and hemagglutinin and fusion protein formation which affect cell proliferation (12). Whether HSV actually functions in this manner is a point that should be comfirmed. The response to even nonspecific mitogen in viral infections may thus depend on the host and its specificity, and in this study, there certainly appeared to be no influence of humoral antibodies against the viruses on PHA response since mitogen-induced proliferation was conducted *in vitro*. In VZV infection, monocytes may be more important in the stimulated response.

The defense immune response and the delayed hypersensitivity response to viruses have been extensively studied (13). However, considerable attention has not been given to the influence of virus infection on the host response to nonspecific mitogens and environmental antigens, particularly *in vitro*. No transformation in response to VZV antigens has been detected in cultures prepared from chickenpox seronegative children, but a positive response to PHA in the lymphocytes has been demonstrated (13). HSV usually exerts an immunosuppressive effect (3, 7). Data at variance with stimulation in response by HSV antigens have also been reported (14). Viruses may thus be variously involved in the response to mitogens and/or environmental antigens. The specificity of immune responses to virus and/or virus antigens, and environmental antigens may vary among individuals. Further studies with a large number of subjects, particularly those without any history of these viral infections and under conditions of changing the order of the addition of the viruses to PBMNC suspensions, are needed to confirm these preliminary findings.

Some hyperimmune skin disorders such as atopic dermatitis recover following viral infection (5) and the IgE response to Ascaris antigens is suppressed in mice infected with the HSV type 2 (15). Our previous paper (16) indicated a mild reduction of generalized erythrodermic rash induced by dinitrobenzene-sulfonic acid sodium salt in guinea pigs experimentally infected with VZV or HSV 1. Cell mediated immunity toward viral infection, by which reduction in atopic disease is induced, should be clarified in greater detail. Immune function of cytokines or monokines from monocytes activated by virus should also be further examined (17). More study is also needed of the possibility of treating atopic disorders using vaccines of these common viruses.

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