The Journal of Laryngology and Otology
October 1996, Vol. **110**, pp. 918–921

Main Articles

Impaired specific cellular immunity to the varicella-zoster virus in patients with herpes zoster oticus

MINORU IKEDA, M.D., KOUICHI HIROSHIGE, M.D., YUZURU ABIKO, M.D., KEIKO ONODA, M.D.

Abstract
The possible involvement of depression on cellular immunity in reactivation of varicella-zoster virus (VZV) in herpes zoster oticus was investigated. The subjects comprised 59 cases of herpes zoster oticus, 33 cases of herpes zoster sine herpete (ZSH) with facial paralysis, and 205 cases of Bell's palsy. The transformation rate of lymphocytes to phytohaemagglutinin in herpes zoster oticus tended to be lower than that in Bell's palsy. In skin tests with purified protein derivatives of tuberculin, the positivity rate in herpes zoster oticus was significantly lower than that in Bell's palsy ($p<0.015$). In skin tests using VZV antigen, the positivity rate in herpes zoster oticus and ZSH were significantly lower than that of Bell's palsy ($p<0.001$ and $p<0.015$, respectively). Thus, it was noted that cellular immunity, especially specific cellular immunity against VZV, was significantly depressed in herpes zoster oticus and ZSH. We consider that depression of specific cellular immunity plays an important role in triggering reactivation of VZV and onset of these diseases.

Key words: Facial paralysis; Herpes zoster oticus; Varicella-zoster virus antigen

Introduction

The mechanism of reactivation of varicella-zoster virus (VZV) which remains latently in ganglion cells after primary infection has not yet been clarified in detail. However, as one of the triggers of such reactivation, involvement of deterioration of cell-mediated immune function has been suggested (Ruckdeschel *et al.*, 1977; Miller, 1980; Burke, *et al.*, 1982). One representative example of VZV infection in the head and neck is herpes zoster oticus (Ramsay Hunt syndrome) which is known to be a causal disease of facial nerve paralyses. Cases of facial nerve paralysis without accompanying herpes in the auricle or the external ear canal, despite the fact that the symptoms are caused by VZV infection, are also well known as herpes zoster sine herpete (ZSH) (Tomita *et al.*, 1972; Djupesland *et al.*, 1976; Kukimoto *et al.*, 1988). In the present study, patients with these two types of diseases and patients with Bell's palsy (idiopathic acute peripheral facial paralysis) were compared in terms of their cell-medicated immune function, and the possible involvement of depression of cellular immunity in the reactivation of VZV in herpes zoster oticus and ZSH was investigated.

Subjects

The subjects included 297 patients with acute peripheral facial paralysis who visited our clinic (Nihon University, Itabashi Hospital, Tokyo) within two weeks after onset. The subjects comprised 59 cases of herpes zoster oticus (16–78 years of age; mean, 43 years), 33 cases of ZSH (20–74 years of age; mean 37 years), and 205 cases of Bell's palsy (16–75 years of age; mean, 43 years). No significant age difference was observed among these disease groups. Serum was collected from every subject once at the first visit and once after two or three weeks, and the titres of anti-VZV IgG and IgM, and anti-herpes simplex virus (HSV) IgG and IgM antibodies were measured by the method of enzyme-linked immunosorbent assay (ELISA). No case revealed elevation of the anti-VZV and anti-HSV IgM antibody titres in this study. Increases in the antibody titres of two-fold or more for IgG antibodies by using paired sera were judged to represent significant changes. Patients who showed no herpes zoster but significant changes in serum antibody titre to VZV were diagnosed as having ZSH, and those who showed no such changes were diagnosed as having Bell's palsy. No case that exhibited significant

From the Department of Otolaryngology, Nihon University School of Medicine, Tokyo, Japan.
Accepted for publication: 28 July 1996.

TABLE I

LYMPHOCYTE TRANSFORMATION TO PHYTOHAEMAGGLUTININ (PHA) OBSERVED IN CASES OF HERPES ZOSTER OTICUS, HERPES ZOSTER SINE HERPETE AND BELL'S PALSY

	Lymphocyte transformation
Herpes zoster oticus (n = 27)	36,447 ± 12,210 cpm
Herpes zoster sine herpete (n = 14)	43,800 ± 14,566 cpm
Bell's palsy (n = 79)	42,239 ± 13,606 cpm

elevation of the anti-HSV IgG antibody titre in the paired sera, was included in the present study.

Methods

Lymphocyte transformation to phytohaemagglutinin (PHA)

The mean transformation rates of lymphocytes to PHA were compared among the disease groups by examining a total of 120 patients (27 cases of herpes zoster oticus, 14 cases of ZSH and 79 cases of Bell's palsy). The lymphocyte transformation rate to PHA was evaluated by the ^{3}H-TdR up-take method, and the standard value in our institute was 26,000–53,000 cpm.

Delayed skin tests

For the delayed skin tests, a purified protein derivative of tuberculin (PPD) and varicella-zoster virus antigen (Kamiya *et al.*, 1977; La Russa *et al.*, 1985) were used, and positivity rates obtained with these two antigens were compared. Tests with both antigens were performed in a total of 259 patients (50 cases of herpes zoster oticus, 31 cases of ZSH and 178 cases of Bell's palsy). The skin reactions were evaluated at 48 hours after the injection. The skin reaction with PPD was judged to be positive when the major axis of erythematous change was 10 mm or longer, and that with VZV antigen was judged to be positive when the change was 5 mm or longer (Kamiya *et al.*, 1977).

Results

Lymphocyte transformation to PHA

The results are listed in Table I. All mean values of three diseases were shown within the standard value of our institute. However, the transformation rate of lymphocytes to PHA in patients with herpes zoster oticus tended to be lower than that in patients with Bell's palsy ($p<0.05$). No appreciable difference in rate was observed between the patients with ZSH and those with Bell's palsy.

Delayed skin tests

The positivity rates of the skin tests using PPD and VZV antigen are summarized in Table II. The positivity rate with PPD in patients with herpes zoster oticus was 36.0 per cent, which was significantly lower than the rate of 55.6 per cent in patients with Bell's palsy ($p<0.05$). No significant difference in rate was observed between the patients with ZSH and those with Bell's palsy.

The positivity rate with VZV antigen was 30.0 per cent in patients with herpes zoster oticus and 51.6 per cent in those with ZSH. These rates were significantly lower than the rate of 73.6 per cent in patients with Bell's palsy ($p<0.01$ and $p<0.05$, respectively).

Discussion

One characteristic of herpes viruses is their long period of latent infection after the primary infection. In the case of VZV, many of the virus particles remain latent in cerebrospinal ganglion cells after primary infection (Gilden *et al.*, 1983; Furuta *et al.*, 1992). Although neurological symptoms such as facial nerve paralysis are observable in rare cases during the course of primary infection with VZV (Watanabe *et al.*, 1994), various clinical changes comprising mostly neurological symptoms are found mainly after reactivation of the virus. Herpes zoster oticus has been considered to represent a disease in which VZV is reactivated after latently infecting the geniculate ganglion of the facial nerve (Hunt, 1907). This causes herpes zoster mainly in the external ear and the neurological symptoms seen in this disease such as facial paralysis with occasional complication by nerve disorders of the inner ear.

The mechanism of reactivation of VZV in herpes zoster oticus has not yet been clarified. Although the mechanism involved in general herpes zoster is also unclear, deterioration of cell-mediated immune function is thought to play an important role in triggering reactivation of the virus (Russel *et al.*, 1972; Miller, 1980; Burke *et al.*, 1982; Florman *et al.*, 1985). In the present study, therefore, the cellular immunity, particularly the specific cellular immune function to VZV, was examined in patients with herpes zoster oticus to clarify the possible important role of depression of specific cellular immunity against VZV in this disease.

In the present study, we examined the lymphocyte transformation to PHA as an *in vitro* test. This test

TABLE II

DETECTION RATES OF POSITIVITY FOR THE DELAYED SKIN TESTS USING PURIFIED PROTEIN DERIVATIVE OF *TUBERCULIN* (PPD) AND VARICELLA-ZOSTER VIRUS (VZV) ANTIGEN IN CASES OF HERPES ZOSTER OTICUS, HERPES ZOSTER SINE HERPETE AND BELL'S PALSY

	PPD	VZV antigen
Herpes zoster oticus (n = 50)	18 cases (36.0%)*	15 cases (30.0%)***
Herpes zoster sine herpete (n = 31)	18 cases (58.1%)	16 cases (51.6%)**
Bell's palsy (n = 178)	99 cases (55.6%)*	131 cases (73.6%)**,***

(Intergroup statistical comparisons: *,** $p<0.015$, *** $p<0.001$).

https://doi.org/10.1017/S0022215100135352 Published online by Cambridge University Press

can be regarded as a test of non-specific cellular immunity by evaluating the function of T cells. As an *in vivo* test, we undertook delayed skin tests. PPD was employed as a non-specific antigen. In order to examine the specific cellular immune function against VZV, skin tests using VZV antigen were conducted (Kamia *et al.*, 1977; Asano *et al.*, 1981; LaRussa *et al.*, 1985; Baba *et al.*, 1987). According to Kamiya *et al.* (1977), the skin test with VZV antigen was negative in 100 per cent of children who had no history of varicella infection, while it was positive in 94 per cent of subjects with a history of varicella. LaRussa *et al.* (1985) reported that the skin test with VZV antigen was positive in 96 per cent of normal subjects, and a high positivity rate of 95 per cent was also observed among relatively old subjects aged 40–69 years. Baba *et al.* (1987) demonstrated that the skin test with the virus antigen was specifically positive in subjects with VZV infection, and showed no cross reaction in subjects with HSV infection. Similarly to PPD, the skin test with this viral antigen tends to become negative when the cellular immunity is depressed (LaRussa *et al.*, 1987).

The transformation rate of lymphocytes to PHA in patients with herpes zoster oticus tended to be lower than that in patients with Bell's palsy. In the skin test, a significant decrease in the skin reaction with PPD was noted in cases of herpes zoster oticus when compared with those of Bell's palsy. When VZV antigen was employed in the skin test, the decrease in positivity rate was marked in patients with herpes zoster oticus. Such a decrease in reaction against VZV antigen was also observed in ZSH patients who were shown to be infected with VZV serologically. These findings appear to suggest the occurrence of a significant depression of specific cellular immunity against VZV in patients with facial nerve paralysis that was caused by VZV infection such as herpes zoster oticus and ZSH.

Hypofunction of cellular immunity because of aging can be one of the underlying factors causing general herpes zoster (Miller, 1980; Burke *et al.*, 1982). However, since a similarity of age structure was present among the disease groups in the present study, the incidence of herpes zoster oticus or ZSH was not necessarily elevated in elderly subjects. Thus, aging may not be an important factor for depression of cellular immune function in these diseases.

As causes of facial paralysis, the strains of work, stress, influenza or the common cold, etc. have been indicated (Koike, 1988).It is known that excessive physical fatigue and mental stress can depress cellular immune function (Bartrop *et al.*, 1977; Schleifer *et al.*, 1983). Thus is might be possible to consider that such condition could be the factors which induce reactivation of VZV in those diseases. Also, influenza viruses are known to induce depression of cellular immunity (Scheinberg *et al.*, 1976). Hence, preceding infection with influenza viruses would appear to be one of the factors for triggering reactivation of VZV. The incidence of ZSH in patients with Bell's palsy differs from report to report: a rate of 25 per cent was given by Tomita *et al.* (1972), about 15 per cent by Djupesland *et al.* (1976), and eight per cent by Kukimoto *et al.* (1988). As one of the causes of such variations in the reported incidence, we suggest that differences in the epidemicity of preceding infection with viruses that display suppressive activity on cellular immunity at the time of clinical survey can be a cause. Actually, Tomita (1977) has reported that 45 cases out of 257 Bell's palsy cases showed a rise in antibidy titres to VZV, and moreover, 20 cases out of these 45 cases exhibited a rise in the antibody titres to one or more viruses.

Conclusion

Cellular immunity in patients with herpes zoster oticus tended to be significantly depressed in comparison with that in patients with Bell's palsy. Such depression was most obvious for the cellular immunity specific against VZV. This type of depression of specific cellular immunity against VZV was also observed in ZSH, which is a very limited morbid state of herpes zoster oticus. We infer, therefore, that the present data indicate a strong association between depression of specific cellular immunity against VZV and reactivation of VZV as the cause of these diseases.

Acknowledgments

The authors thank Professor Masaaki Takahashi, the Research Institute for Microbial Diseases, Osaka University, Osaka, Japan, for allowing them to use varicella-zoster virus skin test antigen for this study. The authors also thank Dr Hideo Omori for his valuable help during the preparation of this paper.

References

Asano, Y., Shiraki, K., Takahashi, M., Nagai, H., Ozaki, T., Yazaki, T. (1981) Soluble skin test antigen of varicella-zoster virus prepared from the fluid of infected cultures. *Journal of Infectious Diseases* **143**: 684–692.

Baba, K., Shiraki, K., Kanasaki, T., Yamanishi, K., Ogura, P. L., Yabuuchi, H., Takahashi, M. (1987) Specificity of skin test with varicella-zoster virus antigen in varicella-zoster and herpes simplex virus infection. *Journal of Clinical Microbiology* **25:** 2193–2196.

Bartrop, R. W., Luckhurst, E., Lazarus, L., Kiloh, L. G., Penny, R. (1977) Depressed lymphocyte function after bereavement. *Lancet* **1:** 834–836.

Burke, B. L., Steele, R. W., Beard, O. W., Wood, J. S., Cain, T. D., Mermer, D. J. (1982) Immune responses to varicella-zoster in the aged. *Archives of Internal Medicine* **142:** 291–293.

Djupesland, G., Berdal, P., Johannessen, T. A. (1976) Viral infection as a cause of acute peripheral facial palsy. *Archives of Otolaryngology* **102:** 403–406.

Florman, A. F., Umland, E. T., Ballou, D., Cushing, A. C., McLaren, L. C., Gribble, T. J., Duncan, M. H. (1985) Evaluation of a skin test for chicken pox. *Infection Control* **6:** 314–316.

Furuta, Y., Takasu, T., Fukuda, S., Saito-Matsumura, K. C., Inuyama, Y., Hondo, R., Nagashima, K. (1992) Detection of varicella-zoster virus DNA in human geniculate ganglia by polymerase chain reaction. *Journal of Infectious Diseases* **166:** 1157–1159.

Gilden, D. H., Vafai, A., Shtram, Y., Becker, Y., Davlin, M., Wellish, M. (1983) Varicella-zoster virus DNA in human sensory ganglia. *Nature* **306:** 478–480.

Hunt, J. R. (1907) On herpetic inflammation of the geniculate ganglion: A new syndrome and its complications. *Journal of Nervous and Mental Disease* **34:** 73–96.

Kamiya, H., Ihara, T., Hattori, A., Iwasa, T., Sakurai, M., Izawa, T., Yamada, A., Takahashi, M. (1977) Diagnostic skin test reactions with varicella virus antigen and clinical application of the test. *Journal of Infectious Diseases* **136:** 784–788.

Koike, Y. (1988) An epidemiological and clinical study on idiopathic facial palsy in Japan. *Acta Otolaryngologica (Stockholm)* **(Suppl 446):** 7–9.

Kukimoto, N., Ikeda, M., Yamada, K., Tanaka, M., Tsurumachi, M., Tomita, H. (1988) Viral infections in acute peripheral facial paralysis: Nationwide analysis centering on CF. *Acta Otolaryngologica (Stockholm)* **446:** 17–22.

La Russa, P., Steinberg, S. P., Seeman, M. D., Gershon, A. A. (1985) Determination of immunity to varicella-zoster virus by means of an intradermal skin test. *Journal of Infectious Diseases* **152:** 869–875.

Miller, A. E. (1980) Selective decline in cellular immune response to varicella-zoster in the elderly. *Neurology* **30:** 582–587.

Ruckdeschel, J. C., Schimpff, S. C., Smyth, A. C., Mardiney, M. R. Jr. (1977) Herpes zoster and impaired cell-associated immunity to the varicella-zoster virus in patients with Hodgkin's disease. *American Journal of Medicine* **62:** 77–85.

Russel, A. S., Maini, R. A., Bailey, M., Dumonde, D. C. (1972) Cell-mediated immunity to varicella-zoster antigen in acute herpes zoster (shingles). *Clinical and Experimental Immunology* **14:** 181–185.

Scheinberg, M. A., Blacklow, N. R., Goldstein, A. L., Parrino, T. A., Rose, F. B., Cathcart, E. S. (1976) Influenza: response of T-cell lymphopenia to thymosin. *New England Journal of Medicine* **294:** 1208–1211.

Schleifer, S. J., Keller, S. E., Camerino, M., Thornton, J. C., Stein, M. (1983) Suppression of lymphocyte stimulation following bereavement. *Journal of the American Medical Association* **250:** 374–377.

Tomita, H., Hayakawa, W., Hondo, R. (1972) Varicella-zoster virus in idiopathic facial palsy. *Archives of Otolaryngology* **95:** 364–368.

Tomita, H. (1977) Viral etiology of Bell's palsy. In: *Facial Nerve Surgery* (Fisch, U. ed.). Kugler Medical Publications, Amstelveen, pp 356–363.

Watanabe, Y., Ikeda, M., Kukimoto, N., Kuga, M., Tomita, H. (1994) A case report of facial nerve palsy associated with chichenpox. *Journal of Larngology and Otology* **108:** 676–678.

Address for correspondence:
Minoru Ikeda, M.D.,
Department of Otolaryngology,
Nihon University School of Medicine,
30–1 Oyaguchi, Itabashi-ku, Tokyo 173,
Japan.

Fax: 03–3972–1321