Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Influence of systemic immune and cytokine responses during the acute phase of zoster on the development of postherpetic neuralgia*

Sheng-mei ZHU[1], Yong-min LIU[†‡1], Er-dan AN[2], Qing-lian CHEN[1]

([1]*Department of Anesthesiology, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China*)

([2]*Department of Anesthesiology, the Second People's Hospital, Jiaxing 314000, China*)

[†]E-mail: sx169y@163.com

Received Feb. 22, 2009; Revision accepted June 17, 2009; Crosschecked July 8, 2009

Abstract: Postherpetic neuralgia (PHN) is a severe sequela of herpes zoster (HZ). Until now, only age and pain severity were considered predisposing factors for the development of PHN. We evaluated 49 patients with acute phase HZ, 10 of whom developed PHN (Group A) and 39 of whom did not develop PHN (Group B). Twenty-five healthy volunteers similar in age and gender distribution to the study group were recruited as controls (Group C). Numbers of serum $CD3^+$ (pan-T lymphocytes), $CD4^+$ (helper/inducer), and $CD8^+$ (suppressor/cytotoxic) lymphocytes were decreased significantly in Groups A and B relative to the control group, but there were no statistical differences between Groups A and B. Interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, IL-8, and IL-10 were significantly elevated in Groups A and B relative to Group C. IL-6 was significantly higher in Group A than in Group B, and was significantly positively correlated with pain severity scored on a visual analog scale. Therefore, we suggest that the inflammatory response, especially that of IL-6, in the acute phase of HZ may be associated with hyperalgesia and the development of PHN.

Key words: T lymphocyte, Cytokines, Postherpetic neuralgia (PHN), Herpes zoster (HZ)
doi:10.1631/jzus.B0920049 **Document code:** A **CLC number:** Q813; R68

INTRODUCTION

Herpes zoster (HZ) is an often painful disease caused by varicella-zoster virus (VZV) infection. Its incidence is about 3.0 per 1000 patients who visit hospitals each year and increases with age, especially in persons over the age of 50 years (Donahue *et al*., 1995; Galil *et al*., 1997; Kost and Straus, 1996). Patients with HZ complain of painful, vesicular rashes with erythema, which usually take 3~4 weeks to heal (Opstelten *et al*., 2002).

T cells ($CD4^+$ and $CD8^+$) are induced when the patients are first infected with VZV. In the memory $CD4^+$ T cell response to VZV, T helper-1 (Th1) cells predominate, with production of pro-inflammatory cytokines including interleukin (IL)-6. It has been reported that there is reduced VZV-specific lymphocyte-mediated immunity in response to secondary challenges by the virus; however, the facts surrounding the reactivation of VZV are not well-understood (Wilson *et al*., 1992). It has also been reported that a series of Th1-like cytokines such as IL-4 and interferon (IFN)-γ are involved (Zhang *et al*., 1994). However, Webster *et al*.(1989) reported that HZ does not present in patients with a decreased T cell immunity.

Postherpetic neuralgia (PHN) is pain lasting over four weeks after the rashes of HZ have healed. Age is recognized as an important factor predicting the development of PHN (Helgason *et al*., 2000). Pain on presentation and the severity of prodromal pain are recognized as high-risk factors (Decroix *et al*., 2000). Although it has been shown that persistence of VZV in mononuclear cells following

‡ Corresponding author

* Project (No. 2008ZYC07) supported by the Zhejiang Medical Bureau of China

infection may be associated with PHN, the mechanism involved in the development of PHN remains unknown (Mahalingam *et al*., 1995). Zak-Prelich *et al*.(2003) demonstrated that although there was slight elevation of some cytokines, such as IL-6 and IL-8, in their zoster group, they found no significant differences between the group that developed PHN and the group that did not.

The aim of the present study was to investigate relationships between systemic immune and cytokine responses in the acute phase of HZ and the development of PHN by examining pain severity and serum level changes in T lymphocyte subsets and a series of cytokines in patients with acute HZ.

MATERIALS AND METHODS

Patients

The study was approved by the institutional ethics committee of Zhejiang University and informed written consent was obtained from all patients before they were enrolled in the study. Forty-nine patients (28 males, 21 females; 50~79 years of age, mean (65±6) years old) who visited the pain clinic were enrolled in the study group. All of the patients with HZ received intravenous acyclovir at 500 mg once per day for 5 d and then were switched to oral acyclovir, 800 mg once per day for 7 d. The clinical presentations of the patients were monitored for about 6 months from the acute HZ occurrence. The patients were divided into two subgroups, Group A and Group B, according to whether they developed postherpetic neuralgia (PHN) or not, respectively. Exclusion criteria were no pain during the acute phase, recent history of immunosuppressive drug or hormone therapy, other diseases of the immune system, and serious infection or complications of the central nervous system. The control group (Group C) was composed of 25 healthy volunteers (17 males, 8 females; 50~76 years of age, mean (63±7) years old) with a similar age range and gender distribution as the patient group.

Blood samples were collected via venipuncture on the patients' first visit to the hospital, within 4 d of the first presentation of zoster; blood samples were also collected from Group C via venipuncture.

Subgroups of T lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$, and the ratio of $CD4^+/CD8^+$) were determined by fluorescence-activated cell sorter analysis (Cytomics FC500, Beckman Coulter, USA) within 6 h of sampling. Monoclonal antibodies (mouse anti-human immunoglobulin G (IgG); Tritest CD4/CD8/CD3) for CD3, CD4, and CD8 determination were purchased from Becton Dickinson Ltd. (USA).

Serum for cytokine analysis was collected and stored at –20 °C immediately after sampling. Analyses of serum IL-1β, IL-6, TNF-α, IL-8, and IL-10 were done by cytometric bead array assay using kits from Bender Medsystems (FlowCytomix Human Th1/Th2 Kits, Bender Medsystems, Austria).

Statistical analysis

All data except visual analogue scale (VAS) scores are presented as mean±*SD*. VAS scores are presented as median and quartile [*M* (*Q*)]. The statistical significances of differences between groups were determined by one-way analysis of variance (ANOVA) followed by Dunnet's test for multiple comparisons. Mann-Whitney and χ^2 tests were used to evaluate differences in sex and age between the patient and control groups. The Wilcoxon test was used to evaluate differences in VAS scores between Groups A and B; Spearman rank correlation analysis was used to analyze VAS scores as a function of plasma cytokine concentrations. Pearson correlation analysis was used to evaluate correlations between cytokine levels and T lymphocyte subsets. $P<0.05$ was considered statistically significant. Statistical evaluations were performed using SPSS 13.0.

RESULTS

Clinical features

Demographic data and VAS pain scores are presented in Table 1. There were no significant differences in gender or age between the groups. The mean VAS score for Group A was significantly higher than that for Group B ($P<0.05$). Of the 49 patients with HZ, 10 developed PHN (20.4%). Three of these 10 patients had rashes on the face and 7 had rashes on the trunk. The mean age of the HZ patients with PHN was (67±5) years and that of the HZ patients without PHN was (65±6) years; this difference was not significant ($P>0.05$).

Table 1 Demographic data and visual analogue scale (VAS) scores for pain

	Age (year)	Sex, F/M	VAS
Group A	67±5	6/4	9 (8)
Group B	65±6	15/24	6 (5)*
Group C	63±7	8/17	0

Group A: patients with herpes zoster (HZ) who developed postherpetic neuralgia; Group B: HZ patients who did not develop postherpetic neuralgia; Group C: healthy controls. Age is reported and mean±*SD*; VAS scores are presented as *M* (*Q*). *$P<0.05$ as compared with Group A

T lymphocyte subgroup and cytokine analysis

The results of T lymphocyte subset ($CD3^+$, $CD4^+$, and $CD8^+$ lymphocytes) analysis and the concentrations of tumor necrosis factor (TNF)-α, IL-1β, IL-6, IL-8, and IL-10 are shown in Table 2. The percentages of $CD3^+$ (pan-T lymphocytes), $CD4^+$ (helper/inducer), and $CD8^+$ (suppressor/cytotoxic) lymphocytes were significantly lower in Groups A and B than in Group C ($P<0.05$), but there were no statistical differences between Groups A and B ($P>0.05$).

Although the levels of pro-inflammatory TNF-α, IL-1β, IL-6, and IL-8 and that of anti-inflammatory IL-10 were higher in Groups A and B than in Group C, there was only a significant difference in the concentration of IL-6 between Groups A and B ($P<0.05$).

There was a significant positive correlation between the serum levels of IL-6 and VAS scores in Group A ($r=0.8110$, $P<0.05$); there were no significant relationships between the levels of any other cytokines and VAS scores for Groups A and B ($P>0.05$; Table 3) and between cytokine levels and T cell subset populations ($P>0.05$; Table 4).

Table 2 Serum concentrations (pg/ml) of IL-6, TNF-α, IL-1β, IL-10, IL-8, and lymphocyte subset counts in patients with herpes zoster and healthy controls

	IL-6	TNF-α	IL-1β	IL-10
Group A	76.65±22.83*▽	227.72±149.15*	53.05±10.43*	109.80±40.19*
Group B	57.13±15.98*	191.89±137.85	62.04±22.06*	102.80±40.10*
Group C	45.34±10.08*	93.64±53.05*	44.69±13.33*	72.45±26.66*
	IL-8	$CD3^+$	$CD4^+$	$CD8^+$
Group A	833.75±462.23*	52.24±10.00*	29.25±5.08*	21.08±6.48*
Group B	602.16±277.99*	56.13±11.00*	33.41±8.38*	20.46±5.82*
Group C	217.76±63.33*	67.72±7.80	37.94±7.29	28.03±4.59

Data are reported as mean±*SD*. Group A: patients with herpes zoster (HZ) who developed postherpetic neuralgia; Group B: HZ patients who did not develop postherpetic neuralgia; Group C: healthy controls. *$P<0.05$ as compared with Group C; ▽$P<0.05$ as compared with Group B

Table 3 Correlation (*r*) between visual analogue scale (VAS) scores for pain and serum cytokine concentrations

Cytokine	*r*	
	Group A	Group B
TNF-α	0.2575	–0.131
IL-6	0.8110*	–0.350
IL-1β	0.5598	–0.136
IL-10	0.1730	–0.158
IL-8	0.2805	0.115

Group A: patients with herpes zoster (HZ) who developed postherpetic neuralgia; Group B: HZ patients who did not develop postherpetic neuralgia. *Significant correlation ($P<0.05$)

Table 4 Correlation (*r*) between serum cytokine concentrations and lymphocyte subset populations

Cytokine	*r*					
	Group A			Group B		
	$CD3^+$	$CD4^+$	$CD8^+$	$CD3^+$	$CD4^+$	$CD8^+$
TNF-α	–0.233	–0.187	–0.247	–0.037	–0.235	–0.077
IL-6	–0.051	0.188	–0.159	–0.063	–0.116	0.027
IL-1β	–0.148	–0.161	–0.065	–0.096	–0.145	–0.151
IL-10	–0.140	–0.410	–0.137	–0.253	0.050	–0.073
IL-8	0.250	0.164	0.391	0.031	–0.029	–0.184

Group A: patients with herpes zoster (HZ) who developed postherpetic neuralgia; Group B: HZ patients who did not develop postherpetic neuralgia

DISCUSSION

Previous reports have shown that the prevalence of PHN exceeds 50% in HZ patients older than 60 years of age, suggesting that age is a risk factor for PHN (Helgason *et al*., 2000). In the present investigation, we enrolled only patients who were older than 60 years of age.

In the present study, the populations of T lymphocyte subsets and serum levels of specific cytokines were evaluated in patients with acute phase HZ to determine whether alterations in these factors were associated with the development of PHN. We collected blood samples from patients when they visited the hospital within 4 d of HZ onset. While it might be desirable to collect the samples as soon as possible after HZ presents, this is usually impractical.

T lymphocyte subset populations and the development of PHN

Previous studies have reported different results concerning T lymphocyte subset populations in patients with HZ. Patients with deficiencies in both $CD4^+$ and $CD8^+$ lymphocytes are at high risk for VZV infection, and therefore at higher risk for PHN (Cauda *et al*., 1987; Higa *et al*., 1992; von Seidlein *et al*., 1996; Domingo *et al*., 2001). The presentation of serum VZV-specific $CD4^+$ lymphocytes reduces with age, putting aging people at elevated risk for developing PHN (Asanuma *et al*., 2000). However, a previous study detected increases in the percentages of $CD4^+$ and $CD8^+$ lymphocytes, not $CD3^+$ lymphocytes, in the acute phase of HZ, resulting in marked decreases in $CD4^+/CD8^+$ ratios (Higa *et al*., 1992). Differences in the study populations and the specific timing of the studies could have contributed to the differences in the results among the various studies. In present study, there were significant decreases in the percentages of $CD3^+$, $CD4^+$, and $CD8^+$ lymphocytes in all patients with HZ relative to patients without HZ, suggesting that depression of cell-mediated immunity in the acute HZ period was associated with development of HZ. There were no differences in T lymphocyte subset populations between Groups A and B, suggesting that changes in T lymphocyte subsets are not predictive of PHN.

Influence of cytokine production on the development of PHN

Although the populations of certain T lymphocyte subsets decreased in patients with HZ, the concentrations of five specific cytokines were elevated significantly in patients with HZ relative to those without. Furthermore, the concentrations of IL-6 in patients with PHN were higher than those in HZ patients without PHN, and there was a positive correlation between the level of IL-6 and the VAS pain score in patients with PHN, suggesting that patients with severe pain and high levels of IL-6 had a higher risk of developing PHN. Zak-Prelich *et al*.(2003) reported only slight elevations of several cytokines, such as IL-6, and no significant differences in serum cytokine concentrations between HZ patients with and without PHN. Fewer cases (only 30 patients were included) and a broader age range (14~65 years) could be the bases of the differences between Zak-Prelich's results and ours. This discrepancy should be addressed in future studies with larger sample sizes.

TNF-α is a pro-inflammatory cytokine produced by several types of cells, including inflammatory cells and some non-immune cells (Vilcek and Lee, 1991; Vassalli, 1992). TNF-α has been shown to produce hyperalgesia and to upregulate other cytokines like IL-6, IL-8, and IL-1β, but serum levels did not correlate with the severity of HZ. IL-1β is also a pro-inflammatory cytokine that can upregulate expression of substance P and nerve growth factor in the spinal cord or inhibit glutamate clearance between synapses by glia (Laughlin *et al*., 2000). TNF-α may have an important role in nocioception and induce mechanical hyperalgesia (Yabuuchi *et al*., 1996). Application of an IL-1 receptor antibody attenuated formalin-induced hyperalgesia and capsaicin-induced hyperalgesia by blocking IL-1β activation (Watkins *et al*., 1997; Davis and Perkins, 1996). It has been reported that IL-8 levels in cerebrospinal fluid, not in serum, at full crusting of herpetic rash can also predict the development of PHN (Kotani *et al*., 2004). However, in the present study, the serum concentrations of TNF-α, IL-1β, and IL-8 did not differ between the group with PHN and the group without PHN, indicating that these cytokines were involved in the development of HZ but not of PHN.

IL-6 is a pro-inflammatory cytokine and is considered a sensitive and early marker of tissue damage that is involved in series of functions, including modulation of the immune and nervous systems. This cytokine plays an important role in allodynia and hyperalgesia after peripheral nerve injury in rodents (Arruda *et al*., 2000). In the present study, only serum concentrations of IL-6 differed between the PHN group and the non-PHN group, and IL-6 levels correlated well with the VAS pain score, indicating that possible nerve injury or an excessive inflammatory

response to VZV was involved in the development of PHN. It is possible that IL-6 could predict the occurrence of PHN, but the mechanisms involved need further investigation.

IL-10 is produced by Th2 cells and is recognized as an anti-inflammatory cytokine with analgesic properties. Gene expression of IL-10 increases immediately after nerve injury and presents a second peak in 45 d. These changes in IL-10 may play an important role in nerve regeneration and the attenuation of neuralgia (Üçeyler *et al*., 2007). Similarly, the application of IL-10 reduces pain behavior in different kinds of pain models (Üçeyler *et al*., 2007). The induction of VZV-specific T lymphocytes was shown to be accompanied by transient increases in IL-10 production, suggesting that parallel increases in IL-10 may predominate in the induction of immunity to some viral pathogens (Jenkins *et al*., 1998). In the current study, serum concentrations of IL-10 in the PHN group were higher than those in controls, but did not differ from concentrations in the non-PHN group, indicating an auto-regulation of immune responses to VZV infection but also indicating that this anti-inflammatory reaction is not sufficient to prevent a patient with HZ from developing PHN.

The inflammatory response of T lymphocyte subsets and cytokines in the acute phase of HZ may result in hyperalgesia and induce limited anti-inflammatory responses, but it cannot effectively restrain the VZV and prevent damage to the skin, resulting in persistent neuralgia. It was found that VZV DNA could persist in the mononuclear cells of PHN patients, reflecting a lack of elimination of the virus in the acute phase of HZ (Mahalingam *et al*., 1995). Abendroth and Arvin (2001) reported that VZV has different types of immune evasion mechanisms allowing it to replicate locally in the skin.

In conclusion, patients with HZ who subsequently developed PHN had more severe pain and higher serum IL-6 concentrations during the acute phase of HZ than did PHN-negative patients. These two factors could predict the occurrence of PHN in patients with acute zoster.

References

Abendroth, A., Arvin, A.M., 2001. Immune evasion as a pathogenic mechanism of varicella zoster virus. *Semin. Immunol*., **13**(1):27-39. [doi:10.1006/smim.2001.0293]

Arruda, J.L., Sweitzer, S., Rutkowski, M.D., DeLeo, J.A., 2000. Intrathecal anti-IL-6 antibody and IgG attenuates peripheral nerve injury-induced mechanical allodynia in the rat: possible immune modulation in neuropathic pain. *Brain Res*., **879**(1-2):216-225. [doi:10.1016/S0006-8993(00)02807-9]

Asanuma, H., Sharp, M., Maecker, H.T., Maino, V.C., Arvin, A.M., 2000. Frequencies of memory T cells specific for varicella-zoster virus, herpes simplex virus, and cytomegalovirus by intracellular detection of cytokine expression. *J. Infect. Dis.*, **181**(3):859-866. [doi:10.1086/315347]

Cauda, R., Grossi, C.E., Whitley, R.J., Tilden, A.B., 1987. Analysis of immune function in herpes zoster patients: demonstration and characterization of suppressor cells. *J. Immunol*., **138**(4):1229-1233.

Davis, A.J., Perkins, M.N., 1996. Substance P and capsaicin-induced mechanical hyperalgesia in the rat knee joint: the involvement of bradykinin B1 and B2 receptors. *Br. J. Pharmacol.*, **118**(8):2206-2212.

Decroix, J., Partsch, H., Gonzalez, R., Mobacken, H., Goh, C.L., Walsh, L., Shukla, S., Naisbett, B., 2000. Factors influencing pain outcome in herpes zoster: an observational study with valaciclovir. *J. Eur. Acad. Dermatol. Venereol.*, **14**(1):23-33. [doi:10.1046/j.1468-3083.2000.00020.x]

Domingo, P., Torres, O.H., Ris, J., Vazquez, G., 2001. Herpes zoster as an immune reconstitution disease after initiation of combination antiretroviral therapy in patients with human immunodeficiency virus type-1 infection. *Am. J. Med.*, **110**(8):605-609. [doi:10.1016/S0002-9343(01)00703-3]

Donahue, J.G., Choo, P.W., Manson, J.E., Platt, R., 1995. The incidence of herpes zoster. *Arch. Intern. Med.*, **155**(15):1605-1609. [doi:10.1001/archinte.155.15.1605]

Galil, K., Choo, P.W., Donahue, J.G., Platt, R., 1997. The sequelae of herpes zoster. *Arch. Intern. Med*., **157**(11):1209-1213. [doi:10.1001/archinte.157.11.1209]

Helgason, S., Petursson, G., Gudmundsson, S., Sigurdsson, J.A., 2000. Prevalence of postherpetic neuralgia after a first episode of herpes zoster: prospective study with long term follow up. *BMJ*, **321**(7264):794-796. [doi:10.1136/bmj.321.7264.794]

Higa, K., Noda, B., Manabe, H., Sato, S., Dan, K., 1992. T-lymphocyte subsets in otherwise healthy patients with herpes zoster and relationships to the duration of acute herpetic pain. *Pain*, **51**(1):111-118. [doi:10.1016/0304-3959(92)90015-4]

Jenkins, D.E., Redman, R.L., Meng Lam, E., Liu, C., Lin, I., Arvin, A.M., 1998. Interleukin (IL)-10, IL-12, and interferon-g production in primary and memory immune responses to varicella-zoster virus. *J. Infect. Dis.*, **178**(4):940-948. [doi:10.1086/515702]

Kost, R.G., Straus, S.E., 1996. Postherpetic neuralgia: pathogenesis, treatment and prevention. *N. Engl. J. Med.*, **335**(1):32-42. [doi:10.1056/NEJM199607043350107]

Kotani, N., Kudo, R., Sakurai, Y., Sawamura, D., Sessler, D.I., Okada, H., Nakayama, H., Yamagata, T., Yasujima, M.,

Matsuki, A., 2004. Cerebrospinal fluid interleukin 8 concentrations and the subsequent development of postherpetic neuralgia. *Am. J. Med.*, **116**(5):318-324. [doi:10.1016/j.amjmed.2003.10.027]

Laughlin, T.M., Bethea, J.R., Yezierski, R.P., Wilcox, G.L., 2000. Cytokine involvement in dynorphin-induced allodynia. *Pain*, **84**(2-3):159-167. [doi:10.1016/S0304-3959(99)00195-5]

Mahalingam, R., Wellish, M., Brucklier, J., Gilden, D.H., 1995. Persistence of varicella-zoster virus DNA in elderly patients with postherpetic neuralgia. *J. Neurovirol.*, **1**(1):130-133. [doi:10.3109/13550289509111018]

Opstelten, W., Mauritz, J.W., de Wit, N.J., van Wijck, A.J., Stalman, W.A., van Essen, G.A., 2002. Herpes zoster and postherpetic neuralgia: incidence and risk factors using a general practice research database. *Fam. Pract.*, **19**(5):471-475. [doi:10.1093/fampra/19.5.471]

Üçeyler, N., Tscharke, A., Sommer, C., 2007. Early cytokine expression in mouse sciatic nerve after chronic constriction nerve injury depends on calpain. *Brain Behav. Immun.*, **21**(5):553-560. [doi:10.1016/j.bbi.2006.10.003]

Vassalli, P., 1992. The pathophysiology of tumor necrosis factors. *Annu. Rev. Immunol.*, **10**(1):411-452. [doi:10.1146/annurev.iy.10.040192.002211]

Vilcek, J., Lee, T.H., 1991. Tumor necrosis factor. New insights into the molecular mechanisms of its multiple actions. *J. Biol. Chem.*, **266**(12):7313-7316.

von Seidlein, L., Gillette, S.G., Bryson, Y., Frederick, T., Mascola, L., Church, J., Brunell, P., Kovacs, A., Deveikis, A., Keller, M., 1996. Frequent recurrence and persistence of varicella-zoster virus infections in children infected with human immunodeficiency virus type 1. *J. Pediatr.*, **128**(1):52-57. [doi:10.1016/S0022-3476(96)70427-4]

Watkins, L.R., Martin, D., Ulrich, P., Tracey, K.J., Maier, S.F., 1997. Evidence for the involvement of spinal cord glia in subcutaneous formalin induced hyperalgesia in the rat. *Pain*, **71**(3):225-235. [doi:10.1016/S0304-3959(97)03369-1]

Webster, A., Grint, P., Brenner, M.K., Prentice, H.G., Griffiths, P.D., 1989. Titration of IgG antibodies against varicella zoster virus before bone marrow transplantation is not predictive of future zoster. *J. Med. Virol.*, **27**(2):117-119. [doi:10.1002/jmv.1890270209]

Wilson, A., Sharp, M., Koropchak, C.M., Ting, S.F., Arvin, A.M., 1992. Subclinical varicella-zoster virus viremia, herpes zoster, and T lymphocyte immunity to varicella-zoster viral antigens after bone marrow transplantation. *J. Infect. Dis.*, **165**(1):119-126.

Yabuuchi, K., Maruta, E., Minami, M., Satoh, M., 1996. Induction of interleukin-1 beta mRNA in the hypothalamus following subcutaneous injections of formalin into the rat hind paws. *Neurosci. Lett.*, **207**(2):109-112. [doi:10.1016/0304-3940(96)12505-2]

Zak-Prelich, M., Mckenzie, R.C., Sysa-Jedrzejowska, A., Norval, M., 2003. Local immune responses and systemic cytokine responses in zoster: relationship to the development of postherpetic neuralgia. *Clin. Exp. Immunol.*, **131**(2):318-323. [doi:10.1046/j.1365-2249.2003.02061.x]

Zhang, Y., Cosyns, M., Levin, M.J., Hayward, A.R., 1994. Cytokine production in varicella zoster virus-stimulated limiting dilution lymphocyte cultures. *Clin. Exp. Immunol.*, **98**(1):128-133.