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ORIGINAL ARTICLE

Study on the T-helper cell 1/2 cytokine profile in blister fluid of patients with herpes zoster and its clinical significance

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ABSTRACT

Herpes zoster (HZ) is a Varicella zoster virus infection disease. Previous studies have presumed the connection between development of HZ and involvement of cellular immunity in peripheral blood. However, whether cellular immunity plays a role in the local skin lesion has not been addressed. To explore the levels of T-helper cell (Th)1/Th2 type cytokine profiles in the blister fluid of the skin lesions from the patients with HZ and its role in pathogenesis, we used the cytometric bead array kit to compare the levels of cytokines (interleukin [IL]-2, tumor necrosis factor [TNF]-α, IL-10 and IL-4) in blister fluid from 46 patients with those from the suction blister fluids from 20 volunteers without any infectious disease (the control group). The results indicated that the levels of Th1 cytokines, IL-2 and TNF-α in the blister fluid from the patients' skin lesions were significantly lower than those from the control group, whereas the levels of Th2 cytokines IL-10 and IL-4 were significantly higher than those in the control group. Moreover, significant variation of the levels of Th1/Th2 cytokines (IL-2, TNF-α, IL-10 and IL-4) in the blister fluid from the HZ patients' lesions was also observed among different stages of the disease. It is concluded that a cytokine imbalance was present in the local lesions of patients with HZ during disease development. Our data suggested that the Th immunity was associated with disease activity, which may play an important role in the pathogenesis of HZ.

Key words: blister fluid, cell-mediated immunity, cytokine, cytometric bead array, herpes zoster.

INTRODUCTION

Herpes zoster (HZ) is a localized disease resulting from Varicella zoster virus (VZV) infection. In general, the lesions of zoster are typically vesicular and blister,[1] affecting unilateral 1–3 dermatomes, most commonly seen at either the facial or the mid-thoracic to upper lumbar dermatomes. The lesions may last for a few days and pustulate and crust in approximately 2 weeks, but may also take several weeks to heal. The VZV-specific cellular immune is predominantly T-cell mediated,[2] with production of different cytokines. Studies have indicated that infection with VZV could induce immune cells to produce different kinds of cytokine during the whole phase of the disease.[3] Our early research reported a decline of VZV-specific T-helper cell (Th)1-mediated immunity in blood, with a decreased cytokine profile in response to the virus.[4] The events related to the cell immunity activities, however, in the skin lesion are not well understood. The aim of the current study was to analyze a variety of Th1 and Th2 cytokines in the skin lesion of patients with HZ during the whole phase of this disease. The blister fluid was collected from 46 patients with HZ

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as well as the individuals in the control group, and were evaluated for the profile of Th cytokines (interleukin [IL]-2, tumor necrosis factor [TNF]-α, IL-4 and IL-10) to comprehend a possible correlation with HZ development. These soluble cytokines were selected as a variety of T-cell immunity mediators that may contribute to cutaneous inflammation and pathogenesis of HZ.

METHODS

The study was approved by and conducted under the Shannxi Provinical People's Hospital's ethics review. All patients in the control group signed the consent form for sample collection and subsequent analysis.

Patients

The blister fluids in the lesions were taken from 46 cases of HZ patients between January 2009 and February 2010. Among them, 21 cases were male and 25 were female, with ages ranging 17–85 years (average 55.67 ± 19.78). The infected course was 2–12 days (average 6.18 ± 2.11). None of the patients received corticosteroid or immunosuppressive agents before and during the study. In the control group, the suction blister fluids were collected from uninvolved skin of 20 patients with facial vitiligo in their stable stage, of which, nine cases were male and 11 female, with ages ranging 20–77 years (average 53.52 ± 16.32). There was no statistically significant difference in the average age between the patients and the control group.

Study design and protocol

Sterilized saline was used to clean the area around the lesion and a sterilized needle was used to pierce the blister. The blister fluid was collected and kept under −80°C. The following procedure was used for sample collection of the control group. The donor skin at the abdominal area was disinfected as described by MacDonald *et al.*[5] Glass cups with appropriate orifice size were placed on the donor skin and suction was applied at a constant negative pressure of −50 to −65 kPa. Blisters were formed after continuous suction for 1 h. Blister fluids were collected, transferred into 1.5-mL Eppendorf tubes and stored under −80°C. Th cytokines were quantitatively analyzed with a cytometric bead array kit (CBA kit; Bender MedSystems, Vienna, Austria) according to the manufacturer's instructions. Briefly, beads were coated with 25 μL antibodies to human Th1/Th2 cytokine, and incubated with 25 μL blister fluid followed by incubation with 50 μL biotin-conjugate mixture for 2 h. Fifty microliters of streptavidin–phycoerythrin solution was prepared and added to all the wells in a dark room for 1 h. Sample were then analyzed using flow cytometry (FACS Calibur; BD Biosciences, Franklin Lakes, NJ, USA). The amounts (pg/mL) of respective cytokines were calculated using professional software. Standard curves were derived from the cytokine standards supplied with the kit.

Statistical analysis

The differences between patients and controls were analyzed by SPSS ver. 13.0 software. All data are presented as mean ± standard deviation and a difference in mean value was considered significant at $P < 0.05$.

RESULTS

Level of Th cytokine in skin blister fluid of patients and controls

The amount of cytokines was quantified by cytometric bead array (Fig. 1). Levels of cytokines in the blister fluids of HZ patients were compared with those in the control group. As shown in Table 1, the levels of Th1 cytokines (IL-2 and TNF-α) were markedly lower in the patients than those in the control group ($P < 0.001$ and =0.008, respectively). On the contrary, the levels of the Th2 cytokines (IL-4 and IL-10) were significantly higher in the HZ disease group than those in the control group ($P = 0.047$ and = 0.041, respectively). These results indicated that the Th2 cytokines preferentially accumulated in the skin lesion of HZ.

Level of Th cytokines in different stages of HZ

To investigate which cytokines may be involved in the improvement of disease, we evaluated the cytokine levels in HZ patients during the early and later stage. In the early stage, approximately 3–5 days, the typical rash of HZ was vesicular even bullae, and we could collect enough blister fluid to analyze Th cytokines. We examined the cytokines in the skin blister of HZ patients and compared them with those in the controls. As shown in Table 2, the concentrations of IL-2

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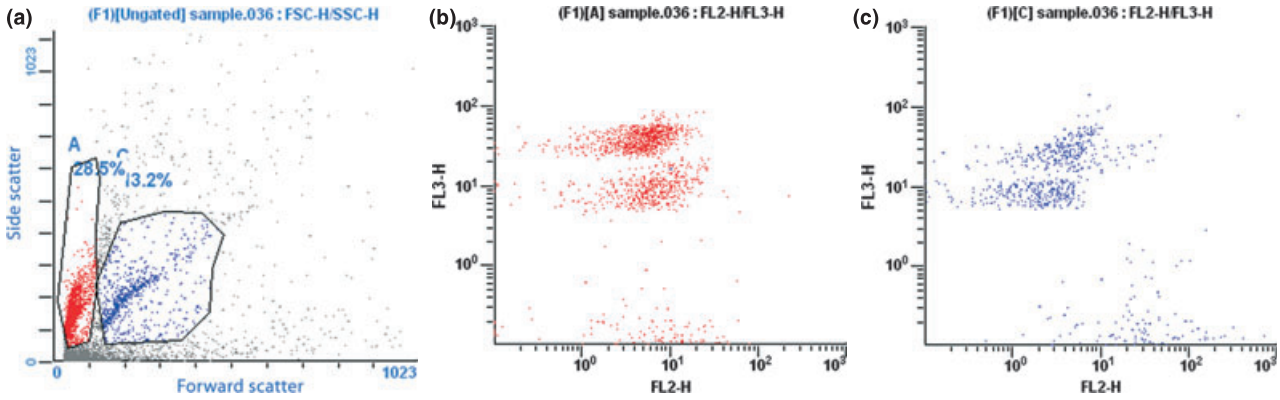

Figure 1. Select gate A in the first fluorescent dot plot. Select gate C in the second fluorescent dot plot (a). Bead populations (gate A) represent interleukin (IL)-10 and IL-2. The IL-10 bead population is in an up position and the IL-2 is in a low position (b). Bead populations (gate C) represent tumor necrosis factor (TNF)-α and IL-4. The TNF-α bead population is in an up position and the IL-4 is in a low position (c). Then, we used specific software to analyze these figures.

Table 1. Comparison of levels of IL-2, TNF-α, IL-10 and IL-4 (pg/mL) in blister fluids from the HZ patients and the control group

Group	Sample size	IL-2 (pg/mL)	TNF-α (pg/mL)	IL-10 (pg/mL)	IL-4 (pg/mL)
Patients	46	15.67 ± 4.79	35.91 ± 12.17	35.37 ± 12.42	60.29 ± 18.20
Controls	20	26.31 ± 8.32	47.63 ± 10.81	20.19 ± 7.07	19.43 ± 5.35
t		3.998	3.394	1.742	1.669
P		<0.001	0.008	0.041	0.047

HZ, herpes zoster; IL, interleukin; TNF, tumor necrosis factor.

Table 2. Comparison of the levels of IL-2, TNF-α, IL-10 and IL-4 (pg/mL) in blister fluids taken at different stages of HZ

Infected stage	Sample size	IL-2 (pg/mL)	TNF-α (pg/mL)	IL-10 (pg/mL)	IL-4 (pg/mL)
≤7 days	30	16.04 ± 2.93	34.94 ± 6.98	37.76 ± 15.2	62.64 ± 12.62
>7 days	16	17.77 ± 2.59	40.12 ± 6.14	33.67 ± 13.78	57.69 ± 11.14
Control	20	26.31 ± 8.32	47.63 ± 10.81	20.19 ± 7.07	19.43 ± 5.35
F		8.632	3.412	3.27	3.834
P		0.001	0.042	0.047	0.029

HZ, herpes zoster; IL, interleukin; TNF, tumor necrosis factor.

and TNF-α were lower in HZ patients than the controls. By contrast, the concentrations of IL-4 and IL-10 were at a higher level than controls. To determine which cytokines are involved in the outcome of the disease, we measured cytokines in the HZ patients with disease progressing; the cytokine analyses in the later stage were performed after more than 7 days. The frequency of IL-2 and TNF-α was significantly increased, and the frequency of IL-4 and IL-10 was significantly decreased. Significant difference was observed in all four cytokines between different groups. These results suggested that Th2 cytokines (IL-4 and IL-10) may play an important role in the development of HZ lesions, whereas Th1 cytokines (IL-2 and TNF-α) could be involved in the improvement of HZ.

DISCUSSION

Regarding the pathogenesis of HZ, it was shown that T cells can be stimulated *in vitro* with VZV and release different degree Th cytokines.[6] Accumulated evidence showed that exposure to VZV, peripheral blood from HZ patients, could induce expansion of

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$CD4^+$ T cells and produce Th cytokines, but VZV did not significantly induce TNF-α and interferon (IFN)-γ production, and instead induced IL-10 and IL-12 production.[7,8] Th response with cytokine production by $CD4^+$ T cells may be responsible for the host defense to VZV.

Microscopic examination of local dorsal root ganglia tissue during active HZ shows presence of lymphocytic infiltration.[9] Compromised T-cell immune function may cause reactivation of the VZV, and VZV-induced HZ may result in severe cutaneous impairment along the given dermatome by the cytopathic effect on epidermal cells and induction of a local cellular immune response.

To the best of our knowledge, the presence of Th cytokines in the blister fluids of HZ has never been reported before. In the present study, the concentrations of four cytokines from the blister fluid of the 46 patients with HZ, IL-2, TNF-α, IL-10 and IL-4, were measured and compared with those in the control group. While TNF-α and IL-2 were found at lower concentrations than those in the control group, cytokines IL-4 and IL-10 were found at higher concentrations than those in the controls. The differences were considered likely to have functional significance. Furthermore, a connection could be established between cytokine concentration and the stage of zoster or the development of blister.

Interleukin-2 is one of the cytokines responsible for immunoregulation by Th1 cells and plays an important role in promoting T-lymphocyte proliferation and differentiation, and it also stimulates T lymphocytes to express other cytokines, such as TNF-α and IFN-γ.[10] Therefore, it plays a crucial role in defense of viral infection.[11] IL-2 was released by peripheral blood leukocytes and enhanced T-cell activity against VZV-infected targets. It has been reported that IL-2 is the principal type of cytokine immune system signaling molecule in peripheral blood of HZ.[12] In this study, we were surprised to find a low concentration of IL-2 in the blisters from HZ patients. The effects of IL-2 on virus-infected epidermal cells have not been previously reported, but we found that IL-2 had an effect on VZV-infected epidermal cells. Descended IL-2 might have weakened T-lymphocyte activity against VZV-infected targets.

Recent studies have indicated that TNF-α plays an important role in several inflammatory and viral infectious diseases.[13,14] TNF-α is the macrophage-activating cytokine leading to host resistance to viruses, by inducing the secretion of IL-2 and stimulating the activation of T cells. By cytometric bead array we found that blister fluid did not express higher TNF-α than controls. This result suggested that TNF-α in the blister fluid of HZ was downregulated. The lower TNF-α is accompanied by a lower T-cell immunity action in response to VZV and a lowered production of IL-2. Zhu *et al.*[15] reported that TNF-α was higher in an early stage of HZ than controls. The age range (>60 years) and the clinical features (all the patients visited the pain clinic) could be the bases of the differences between Zhu *et al.*'s results and ours. This discrepancy should be addressed in future studies with larger sample sizes.

We compared cytokine levels in blister fluid between earlier and later stages of HZ. It was found that IL-2 and TNF-α were detectable at low levels in the earlier stage of HZ, and dropped down frequently in a majority of samples, but IL-10 and IL-4 were reduced during the later stage of diseases, which showed a trend of levels of both Th1 and Th2 cytokines in the HZ group being further away from the baseline in the control group in the earlier stage than those in the later stage. The result suggests that the concentration of these cytokines may have a correlation with the progress of HZ. These findings suggest that the weakened inflammatory response of Th1 lymphocyte may not effectively restrain the VZV and prevent damage to the skin, and measuring Th1/Th2 levels in the blister fluid of the patients may help analyzing the disease progress, which may serve as a valuable indicator for clinical treatment of HZ. The optimization time of observation, which can show maximized alternation of Th1 and Th2 cytokines in blister fluid from HZ patients, should be checked by analysis of large samples at different stages of HZ in future studies.

Previous study has reported different levels of Th1/Th2 cytokines in the peripheral blood in HZ patients.[16] Patients with deficiencies in Th1 cytokines are at a high risk for VZV infection. View from skin lesions, the production of Th cytokines in blister fluid could be interrelated with the activation of T lymphocytes in peripheral blood, inducing local immunity effects directly; furthermore, these observations suggest that cytokines secreted by Th cells in the skin

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lesions are similar to those in peripheral blood. The decline of Th1 cytokines and the increase of Th2 cytokines resulted in a Th1 to Th2 drifting at the sites of HZ skin lesions and also suppressed Th1-mediated local immunity against VZV in skin lesions. The weakened host's cellular immunity reduced virus elimination which caused the VZV in revival.

In conclusion, we have demonstrated that IL-2 and TNF-α could play a protective role in the inflammation of HZ, whereas IL-4 and IL-10 may contribute to the adverse role in local inflammation. These results proposed that a shifting of cytokine expression from Th1 to Th2 represents the key effectors of the mechanism of HZ. The decreased local secretion of IL-2 and TNF-α results in the recurrent infection of latent VZV, and release of IL-2 and TNF-α is a marker of T-cell activation and being a part of innate VZV defending mechanisms. These data may provide important information for the development of new local therapeutic strategies for HZ.

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