Analysis of Serum Cytokine Profile in Pemphigus

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Analysis of Serum Cytokine Profile in Pemphigus

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<ABSTRACT>

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Background: Pemphigus is a group of autoimmune blistering diseases affecting skin and mucous membranes. While pemphigus is an autoantibody mediated disease, the role of T cells and cytokines in the pathogenesis of pemphigus is being increasingly recognized. However, contrasting data are available regarding the serum cytokine levels, and its comprehensive role in pemphigus is not fully understood yet.

Objective: The purpose of this study was to observe alterations in the serum cytokine levels of patients with pemphigus vularis (PV), pemphigus foliaceous (PF), paraneoplastic pemphigus (PNP) and compare with bullous pemphigoid (BP) and healthy subjects.

Methods: A total of 75 subjects (28 PV, 13 PF, 7 PNP, 7 BP and 20 healthy controls) who visited the Dermatology Clinic of Gangnam Severance Hospital between 2006 and 2013 were included in this study. Serum levels of IFN-γ,

IL-4, IL-6, IL-17A, IL-10, TNF-α, and IL-8 were measured in the five groups by ELISA.

Results: The median concentration of IFN-γ was lower in PV and BP patients compared to the control group (0.765, 0.34 and 1.63 pg/ml, respectively). IL-6 and IL-10 was significantly higher in PNP patients compared to the control group (4.92 and 0.24 pg/ml for IL-6, 0.86 and <0.12 pg/ml for IL-10, respectively). IL-8 was increased significantly in PV and PNP patients compared with the control group (11.85, 31.5 and 8.31 pg/ml, respectively). For IL-4, IL-17A and TNF-α, no significant difference was observed between the five groups.

Conclusions: The decreased level of IFN-γ in PV may imply a suppressed Th1 response in the active disease stage. A Th2 predominant response is suggested in the active stage of PNP, with elevated serum level of IL-6 and IL-10. Increased level of IL-8 is observed in the sera of PV and PNP patients.

Key words : pemphigus vulgaris, pemphigus foliaceus, paraneoplastic pemphigus, autoimmunity, cytokines

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I. INTRODUCTION

Pemphigus is a group of chronic autoimmune blistering diseases of skin and mucous membranes characterized by autoantibodies against desmogleins.[1] The pathophysiology of pemphigus is that autoantibodies inhibit the adhesive function of desmoglein and lead to loss of cell-cell adhesion of keratinocytes with resultant blister formation. Pemphigus can be divided into four major subtypes; pemphigus vulgaris (PV), pemphigus foliaceus (PF), paraneoplastic pemphigus (PNP) and IgA pemphigus.[2] Each subtype can be distinguished by the target of the specific autoantibodies or by the location of blister formation. Pemphigus vulgaris is associated with autoantibodies against desmoglein (Dsg)1 and 3, while PF has autoantibodies directed to Dsg1. Paraneoplastic pemphigus, almost always associated with an underlying neoplasm, is characterized by the autoantibodies against plakin proteins such as desmoplakin, envoplakin, and periplakin, as well as Dsg1 and

Dsg3.[3]

The typical histological features of pemphigus are intra-epidermal blisters and acantholysis. Although the specific pathophysiological mechanism of acantholysis is not yet clearly understood, recent advances in basic research have provided additional insights into the process of acantholysis.[4-6] In brief, the pathogenesis of acantholysis can be explained by two different mechanisms: first, in which the pathophysiological explanation of the loss of keratinocyte intercellular adhesion is the direct inhibition of desmoglein adhesive functions by steric hindrance;[7] second, in which the disruption of cell-cell contacts is due to more complex intracellular events following from interaction between pemphigus autoantibodies and Dsg.[8-10] Such intracellular signalings include protein kinase C (PKC) activation,[11-13] heatshock protein 27 and p38 MAPK phosphorylation.[14]

While the production of pathogenic antibodies is a key to the development of pemphigus, many immunological steps are required prior to autoantibody induction. Both T and B cells with autoreactivity towards desmoglein are necessary,[15] and the role of T cell subsets and their secreted cytokines is being increasingly recognized. It is known that the T helper (Th) cells are classified into different subsets, which include Th1, Th2, Th17 and regulatory T cell (Treg), according to the cytokine production pattern. Th1 cells arise in the presence of IL-12 and IFN-γ and secrete IL-2, IL-12, IFN-γ and TNF-α. This subset plays an important role in cell-mediated immunity, cytotoxicity, and

delayed type hypersensitivity reactions. Th2 cells arise in the presence of IL-4 and are responsible for the development of B cell mediated humoral immunity, IgE production, and activation of eosinophils. Th2 cells secrete IL-4, IL-5, IL-6, IL-10 and IL-13. Another subset Th17, arises in the presence of IL-1β, IL-6 and TGF-β, and is expanded by IL-23.[16] These cells secrete several proinflammatory cytokines including IL-17A, IL-17F, TNF-α, IL-21, IL-22 and IL-26.[17] This subset is crucial in host defence against extracellular bacteria, parasites and fungi, and also drives inflammatory responses. Th17 cells have been implicated in the initiation and progression of many inflammatory and autoimmune pathologies including psoriasis, multiple sclerosis, inflammatory blowel disease and rheumatoid arthritis.[18-20] Lastly, Treg subset arises in the presence of IL-2 and TGF-β and plays a crucial role in modulating peripheral tolerance.[21] These cells secrete IL-10 and TGF-β, which help to suppress activation and cytokine secretion by the other subsets, thereby downregulating the immune response.

With the advancement of molecular techniques to identify cytokine profiles in pemphigus, several studies have been conducted trying to identify the presence of such mediators in serum, perilesional skin, and blister fluid.[22] In the majority of previous studies, upregulation in the Th2 pathway has been suggested, with increased serum level of IL-4, IL-6 and IL-10.[23] Th1 pathway on the other hand, has shown contradictory results. There are both respectable number of studies showing increased and decreased level of IFN-γ and IL-2,

as well as no significant differences in the Th1 cytokines compared to control group.[22] Only a limited number of studies are available regarding the Th17 pathway in pemphigus, and Th17 cells were increased in the skin lesions of PV in one study.[24] Studies regarding Treg cells in pemphigus are also controversial, but primarily demonstrate no significant difference between the hallmark regulatory cytokine, TGF-β, in patients versus control group.[22] Proinflammatory cytokines were also studied, which in majority of results showed elevated level of TNF-α and IL-6,[25,26] several results of elevated IL-1[27,28] and two reports of elevated IL-8 in the PV blister fluid and serum, respectively.[29,30]

In summary, results of cytokine studies in pemphigus have varied, further complicating our understanding of disease processes. Therefore, this study was conducted to assess the levels of cytokines in the serum from patients affected with PV, PF, PNP and compare with bullous pemphigoid (BP) and healthy subjects. In order to investigate the involvement of different helper T cell subsets, we evaluated IFN-γ, IL-4, IL-6, IL-17A, IL-10 as well as the proinflammatory cytokines, IL-8 and TNF-α.

II. MATERIALS AND METHODS

1. Study design

To evaluate the different T cell subsets involved in the pathogenesis of pemphigus, Th1 cytokine IFN-γ, Th2 cytokine IL-4 and IL-6, Th17 cytokine IL-17A, regulatory T cell cytokine IL-10 and proinflammatory cytokines TNF- α and IL-8 were measured. Serum levels of cytokines were measured in the individuals of five groups; PV, PF, PNP, BP and healthy individuals.

2. Patients

The sample collection for this study was conducted on patients who visited the Department of Dermatology at Gangnam Severance Hospital, Seoul, Korea between 2006 and 2013. Serum samples were obtained from 28 PV patients, 13 PF patients, 7 PNP patients, 7 BP patients, and 20 healthy subjects. All the patients enrolled in the study had clinically active stage of the disease. Active stage was defined as the de novo development of blisters/erosions on previously unaffected or healed up sites of mucocutaneous surfaces. Serum was collected on the patient's first visit or at the time of acute flare in the disease course. The clinical diagnosis of PV, PF and BP was confirmed by histopathologic findings, direct immunofluorescence (DIF) examination and/or the detection of serum autoantibodies by indirect immunofluorescence (IIF) examination.

Paraneoplastic pemphigus patients were diagnosed using the following criteria[31]: (i) clinical features, including the presence of severe mucosal involvement or polymorphous cutaneous eruption; (ii) characteristic histological features of the skin or mucosal eruption (interface dermatitis, acantholysis and apoptotic keratinocytes); (iii) the presence of autoantibodies detected in DIF or IIF studies; (iv) detection of anti-plakin or anti-Dsg autoantibodies in immunoblotting or immunoprecipitation; and (v) the presence of associated neoplasm. Diagnosis of PNP required four of five criteria, including criteria (i) and (ii).

2. ELISA

Cytokines were analyzed using a commercial assay system of immunoassay kits and panels (Millipore MILLIPLEX Human Cytokine Human Cytokine Panel I Premixed 7 Plex [HCYTOMAG60K07]) using a magnetic bead-based immunoassay kit (Luminex 200; Luminex Corp., Austin, TX, USA). Serum samples were incubated with antibody-coated capture beads overnight at 4℃. Washed beads were further incubated with biotin-labeled anti-human cytokine antibodies, followed by streptavidin–phycoerythrin incubation. Samples were read on a Luminex 200 reader with xPOTENT software. The standard curves of known concentrations of recombinant human cytokines were used to convert fluorescence units to concentrations (pg/mL). To calculate the cytokine concentrations in the serum samples, we analyzed the median

fluorescent intensity data using a 5-parameter logistic or spline curve-fitting method.

2. Statistical analyses

Owing to the limited number of subjects, the results of cytokines are expressed by employing median values and 25-75% ranges as reported by D'Auria *et al.*[32] Statistical analyses were performed using commercial software (SAS version 9.2). Levels of all parameters done were compared between groups using analysis of variance, the Kruskal–Wallis test. Multiple comparison tests were additionally performed in the parameters which showed significant difference (IFN-γ, IL-6, IL-10 and IL-8) in the Kruskal–Wallis test. Differences were defined as statistically significant at $p\text{-value} < 0.05$.

III. RESULTS

1. Baseline characteristics of study population

A total of 75 subjects (28 PV, 13 PF, 7 PNP, 7 BP and 20 healthy controls) were studied. The mean ± standard deviation of age was 55.28 ± 14.10 years (range, 24 – 83 years) in PV patients, 51.57 ± 18.97 (range, 15 – 81 years) in PF patients, 45 ± 2.19 (range, 43 – 47 years) in PNP patients, 69.71 ± 14.55 (range, 49 – 85 years) in BP patients and 24.15 ± 3.62 (range, 17 – 29 years) in the healthy controls, respectively, as shown in Table 1.

Table 1. Age and sex of study subjects

Cases	Number (Total N=75)	Male	Female	Age (Mean ± SD), Range
PV	28	13	15	55.28 ± 14.10 (24-83)
PF	13	7	6	51.57 ± 18.97 (15-81)
PNP	7	4	3	45 ± 2.19 (43-47)
BP	7	5	2	69.71 ± 14.55 (49-85)
Control	20	9	11	24.15 ± 3.62 (17-29)

2. Cytokine analysis

A. IFN-ɣ

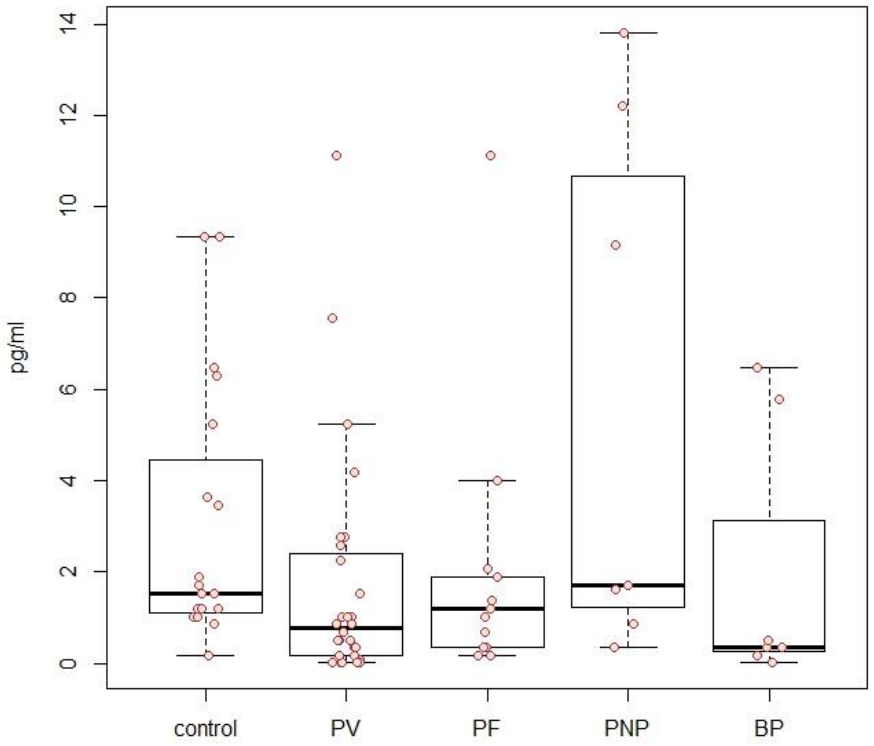
The median concentration of the Th1 cytokine, IFN-γ, was significantly decreased in PV and BP when compared to normal control (0.765 pg/ml in PV, 0.34 pg/ml in BP and 1.63 pg/ml in control; p=0.0054 and p=0.035, respectively), as shown in Table 2. Out of 28 subjects of PV, 19 samples showed concentration which were lower than 1.065, the 25% range of normal control value. Five out of seven samples showed lower value than 1.065 in the BP group. When comparing PV with BP, serum concentration of IFN-γ was not significantly different between the two groups (p=0.4302).

Table 2. IFN-γ levels detected in the serum of PV, PF, PNP, BP patients and normal controls

Patient No.	PV	PF	PNP	BP	Control
1	7.55	1.02	1.63	0.34	1.89
2	0.51	1.2	9.15	0.34	9.33
3	0.09	2.07	12.2	0.01	59.03
4	0.68	4	0.34	6.48	1.54
5	0.34	1.89	1.72	0.17	5.24
6	2.24	1.37	0.85	0.51	6.48
7	1.02	1.37	13.82	5.77	1.02
8	0.51	0.34			3.65
9	0.01	0.34			9.33
10	0.85	0.17			1.2
11	5.24	0.17			1.02
12	2.77	11.12			1.2
13	0.51	0.68			1.54
14	0.17				3.47
15	0.01				1.02
16	0.01				6.3
17	0.01				1.2
18	2.59				0.17
19	11.12				0.85
20	4.17				1.72
21	1.02				
22	2.77				
23	0.01				
24	0.85				
25	1.02				
26	1.54				
27	0.34				
28	0.17				
25-75% Range	0.170–2.502	0.34-1.98	0.85-12.2	0.17-5.77	1.065-6.035
Median	0.765	1.2	1.72	0.34	1.63
Ratio	28/28	13/13	7/7	7/7	20/20
P1	0.0054	0.1228	0.519	0.038	
P2	0.4302				

Ratio = ratio detectable/total samples; p1 = comparison between patient serum and control serum; p2 = comparison between patient serum and BP serum.
All cytokine levels are expressed as picograms per milliliter.

Figure 1. Serum IFN-γ levels shown in a dot plot



B. IL-6

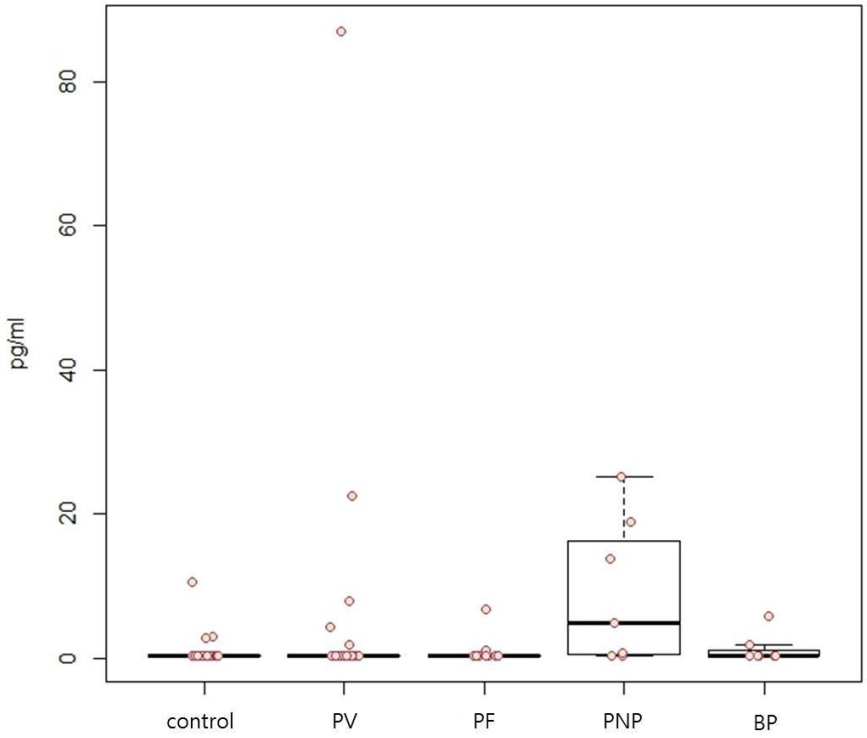
The median concentration of IL-6 was significantly higher in PNP (4.92 pg/ml in PNP, 0.24 pg/ml in control; p=0.0194), compared to healthy control (Table 3). IL-6 was detectable in 5 out of 7 PNP sera, which all showed higher concentration than the 75% value of normal control serum.

Table 3. IL-6 levels detected in the serum of PV, PF, PNP, BP patients and normal controls

Patient No.	PV	PF	PNP	BP	Control
1	UDL	UDL	18.88	UDL	UDL
2	4.28	1.14	UDL	UDL	2.99
3	1.82	UDL	4.92	UDL	UDL
4	UDL	UDL	13.68	UDL	UDL
5	UDL	UDL	UDL	5.79	10.51
6	UDL	UDL	0.61	1.82	2.82
7	UDL	6.66	25.15	UDL	UDL
8	UDL	UDL			UDL
9	UDL	UDL			UDL
10	UDL	UDL			UDL
11	22.54	UDL			UDL
12	7.9	UDL			UDL
13	UDL	UDL			UDL
14	UDL				UDL
15	UDL				UDL
16	UDL				UDL
17	UDL				UDL
18	UDL				UDL
19	UDL				UDL
20	UDL				UDL
21	UDL				
22	8.03				
23	UDL				
24	UDL				
25	UDL				
26	UDL				
27	UDL				
28	UDL				
25-75% Range	0.24-0.24	0.24-0.24	0.24-18.88	0.24-1.82	0.24-0.24
Median	0.24	0.24	4.92	0.24	0.24
Ratio	5/28	2/13	5/7	2/7	3/20
P1	0.805	0.987	0.0194	0.638	

UDL = under the detection limit (<0.24 pg/ml); Ratio = ratio detectable/total samples; p 1 = comparison between patient serum and control serum; All cytokine levels are expressed as picograms per milliliter.

Figure 2. Serum IL-6 levels shown in a dot plot



C. IL-10

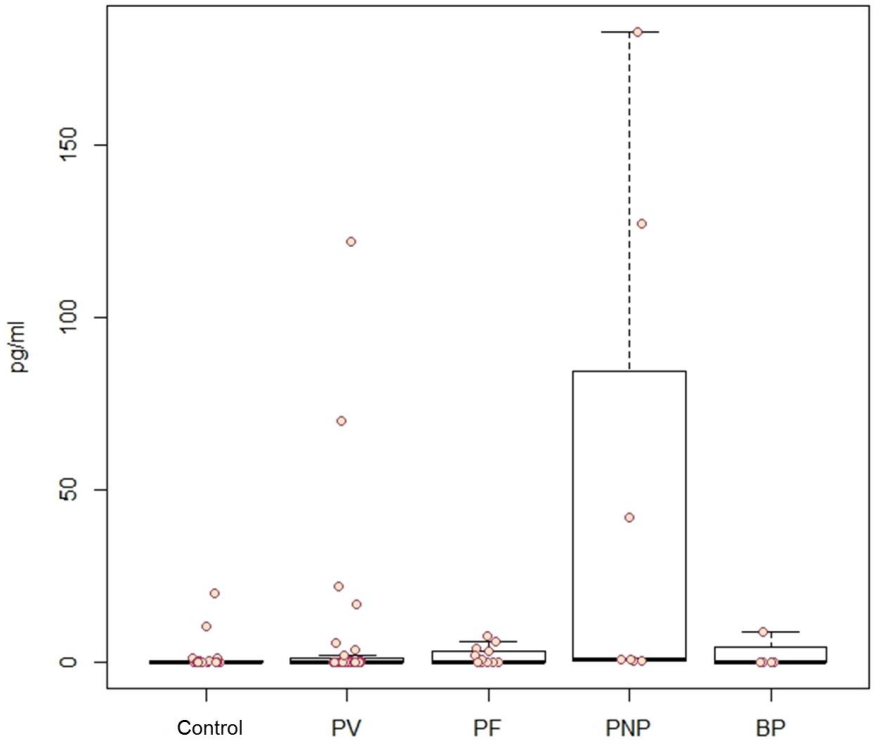
Higher concentrations of IL-10 were observed in PNP (0.86 pg/ml) when compared to normal control, which was statistically significant ($p=0.0046$). In six out of the seven PNP sera, values higher than 0.5125 pg/ml were detected, which is the 75% value of the normal control. The median concentration of IL-10 did not show statistically significant difference between normal control and PV, PF, or BP (<0.12 pg/ml in PV, PF, BP and control, Table 4).

Table 4. IL-10 levels detected in the serum of PV, PF, PNP, BP patients and normal controls

Patient No.	PV	PF	PNP	BP	Control
1	UDL	UDL	183.15	UDL	UDL
2	UDL	6.27	127.43	UDL	UDL
3	122.22	4.04	0.55	UDL	0.55
4	UDL	UDL	0.4	8.76	UDL
5	0.4	UDL	0.86	UDL	20.01
6	0.7	UDL	0.86	8.76	10.65
7	UDL	UDL	41.99	UDL	UDL
8	UDL	1.01			1.17
9	UDL	UDL			1.17
10	UDL	1.98			UDL
11	22.02	3.17			UDL
12	0.4	UDL			UDL
13	3.86	7.83			0.4
14	UDL				UDL
15	UDL				UDL
16	70.16				UDL
17	0.26				UDL
18	UDL				UDL
19	17.04				UDL
20	UDL				UDL
21	UDL				
22	5.64				
23	UDL				
24	UDL				
25	UDL				
26	1.98				
27	UDL				
28	0.12				
25-75% Range	0.12–1.66	0.12-3.605	0.55-127.43	0.12-8.76	0.12-0.5125
Median	0.12	0.12	0.86	0.12	0.12
Ratio	12/28	6/13	7/7	2/7	6/20
P1	0.535	0.499	0.0046	0.7436	

UDL = under the detection limit (<0.12 pg/ml); Ratio = ratio detectable/total samples; p1 = comparison between patient serum and control serum; All cytokine levels are expressed as picograms per milliliter.

Figure 3. Serum IL-10 levels shown in a dot plot



D. IL-8

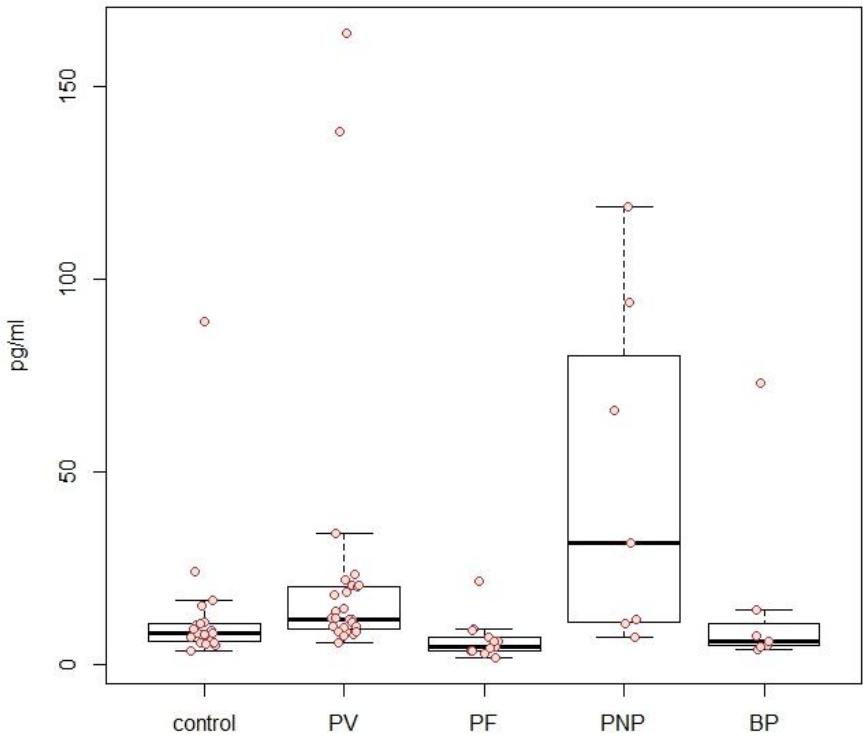
The level of IL-8 was significantly higher in the PV and PNP patient's sera than in normal control (Table 5, 11.85 pg/ml in PV, 31.5 pg/ml in PNP, 8.31 pg/ml in control; p=0.0227 and p=0.0322, respectively). In PV group, 18 out of 28 samples showed value higher than 75% range of normal control and 6 out of 28 samples showed lower value than 25% range of normal control. In PNP group, 5 out of 7 samples showed higher value of IL-8 than the 75% value of normal control.

Table 5. IL-8 levels detected in the serum of PV, PF, PNP, BP patients and normal controls

Patient No.	PV	PF	PNP	BP	Control
1	7.89	5.93	118.88	3.89	89
2	8.73	21.62	31.5	4.94	24.18
3	13.88	4.76	7.2	4.72	10.92
4	14.74	4.26	94.11	7.55	6.5
5	8.71	1.71	10.59	14.13	5.75
6	18.23	7.24	11.71	73.15	15.19
7	11.67	9.43	65.97	5.89	7.85
8	11.69	3.7			10.15
9	22.02	2.79			4.85
10	5.86	3.56			8.79
11	33.95	6.15			5.26
12	23.49	2.89			8.34
13	11.69	8.79			3.7
14	20.43				7.03
15	9.72				16.58
16	12.02				5.84
17	10.86				10.54
18	9.87				8.28
19	9.83				7.85
20	20.41				9.3
21	20.43				
22	138.43				
23	8.68				
24	8.39				
25	164.06				
26	7.63				
27	12.18				
28	18.96				
25-75% Range	8.97-20.42	3.22-8.01	10.59-94.11	4.72-14.13	5.84-10.92
Median	11.85	4.76	31.5	5.8	8.28
Ratio	28/28	13/13	7/7	7/7	20/20
P1	0.0227	0.809	0.0322	0.0859	

Ratio = ratio detectable/total samples; p1 = comparison between patient serum and control serum;
All cytokine levels are expressed as picograms per milliliter.

Figure 4. Serum IL-8 levels shown in a dot plot



E. IL-4, IL-17A and TNF-α

The level of Th2 cytokine IL-4 in the serum of patients and the control was below the detection limits (<1.86 pg/ml) except in four samples, which was in one BP patient and three healthy controls. There was no significant difference in IL-17A, the Th17 cytokine, comparing the five groups. TNF-α did not differ statistically between the five groups.

When comparing PV with PF, none of the cytokines showed significant difference (data not shown).

Figure 5. Serum IL-4 levels shown in a dot plot

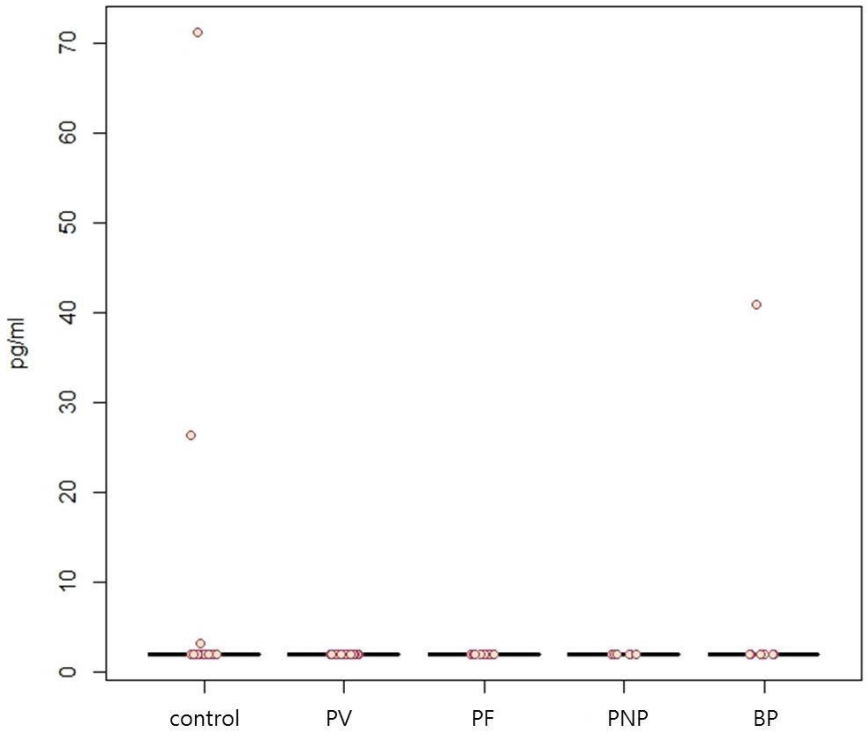


Figure 6. Serum IL-17A levels shown in a dot plot

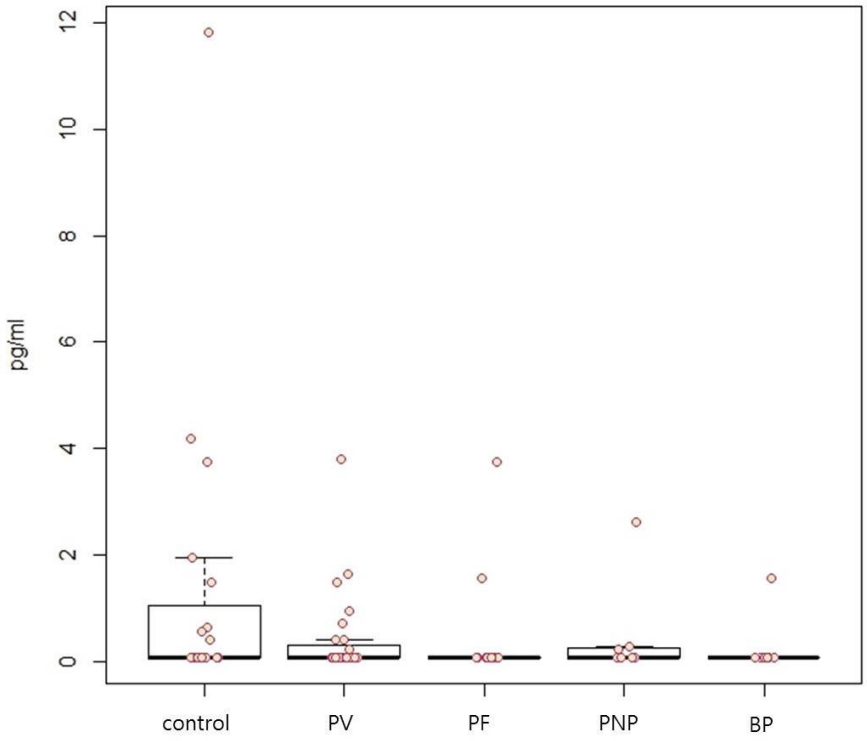
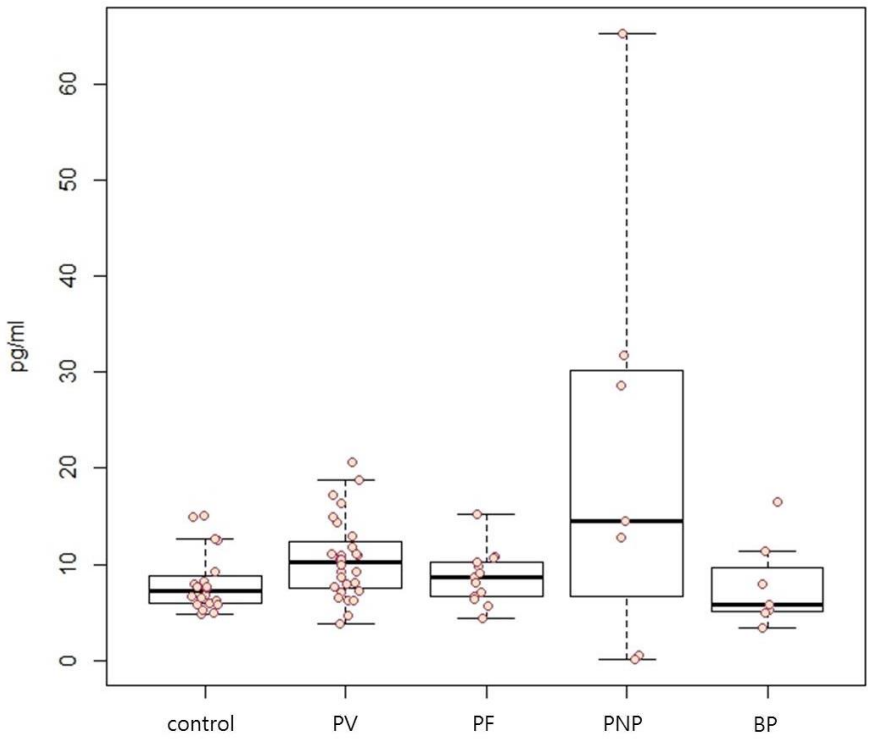


Figure 7. Serum TNF-α levels shown in a dot plot



IV. DISCUSSION

Although a number of studies have examined T cell and cytokine profiles in pemphigus, variable results have been reported so far and the role of the cytokines in the disease process remains to be clarified. The present study of serum cytokine analysis in pemphigus showed a decrease of IFN-γ in PV and BP, an increase of IL-6 and IL-10 in PNP, and an increase of IL-8 in PV and PNP compared to healthy control.

There have been studies reporting an imbalance of Th1/Th2 response in pemphigus, which ultimately can result in complex and severe impairment of immune function.[23] Once a naïve T cell differentiate to Th2 cell, specific transcription factors such as GATA-3 and c-maf are activated, which in turn activate the Th2 cytokines while down-regulating factors necessary to generate Th1 response.[33,34] In majority of previous studies, involvement of Th2 pathway has been demonstrated in the pathogenesis of pemphigus, and as a consequence, down-regulation of Th1 pathway is to be anticipated in disease.[22] However, there are both respectable number of studies showing increased and decreased level of IFN-γ, as well as studies showing no significant difference compared to the control group.[22] The reason for the variation might potentially be due to small number of samples included in some of the studies,[35] and the time of serum collection in the disease course was varied.[36] Our result of IFN-γ was significantly decreased in PV compared to control. In a previous study by Veldman *et al.*, they showed that peripheral

Dsg3-reactive Th1 cells varied according to the clinical activity of PV.[36] Among the patients with acute-onset, chronic active, and remittent PV, Dsg3-reactive Th1 cells were detected at a significantly higher frequency in chronic active PV. Sera in our study was collected in the active stage of disease, both acute and chronic, and it may be possible to assume that Th1 pathway is suppressed in the active stage of PV consistent with some of the previous findings.[23,37] In comparison of the PV sera with BP sera, which also showed decreased level of IFN-γ compared to control, there was no significant difference. In contrast to our finding, several studies reported increased level of IFN-γ in the serum and blister fluid of BP.[38-40] Giomii *et al.*[41] speculated that an early stage of BP can be characterized by an initial Th0/Th2-like response (IL-4, IL-5, low levels of IFN-γ), and a chronic Th1-skewed phase would follow. Studies in the intermediate phase would be presented by a mixed Th1/Th2 expression, this also highlighting the different cytokine profile according to the disease phases. Our result of decreased IFN-γ in BP might represent a suppressed Th1 pathway in the active disease stage. However our result of IL-4, the hallmark cytokine of Th2 pathway, did not increase in either PV or BP group, so our presumption needs careful confirmation with further studies.

Even though there are some good evidence that PV is a Th2 mediated disease and several studies have demonstrated an increase in the Th2 cytokines,[23,40,42] our result of IL-4 did not show any significant difference in

the patient groups compared to healthy control. However, limitation in the interpretation of our result exist, because most samples were under the detection limit for IL-4; 74 out of 78 sera were below the detection limit (<1.86 pg/ml), 3 samples and one sample were elevated in normal and BP group each.

Th17 is a relatively new helper T-cell subset which is generated upon IL-23 stimulation of naıve T cells, and it is characterized by the secretion of IL-17, a proinflammatory cytokine.[43] Th17 cells have been implicated in the initiation and progression of many inflammatory and autoimmune diseases such as autoimmune encephalitis, inflammatory bowel disease and psoriasis.[44] In pemphigus, on the other hand, there is only a limited number of data examining its role. Arakawa *et al.*[24] found Th17 cells in skin lesions of patients with PV, but did not find a significant correlation between the Th17 cells and disease activity or anti-Dsg3 antibody titers. Giordano *et al.*[22] mentioned their unpublished data of cytokine analysis in PV serum which showed an increase in the IL-17A level compared to controls. However, in our study, we could not find any significant difference of IL-17A level between the control group and any of the patient group. Regarding the fact that Th17 cells in lesional skin neither correlated with autoantibody level nor with the clinical severity of PV, and the fact that our study showed no significant difference in serum IL-17A level, the role of Th17 cells in pemphigus still remain questionable and needs further investigation.

Regulatory T cells play a crucial role in modulating peripheral tolerance and preventing autoimmunity. In autoimmune diseases, a relative decrease in Treg function may be involved in the pathogenesis. Data reported on Treg cells and their cytokines, TGF-β and IL-10, are quite varied in pemphigus. Cytokine studies published so far has demonstrated a lack of significant variation in TGF-β between PV and control.[32,45] As for IL-10, majority of published studies reported an increase in the serum of PV patients.[22] IL-10 does play a significant role in the Treg pathway, but it is also associated with the Th2 pathway, and some authors assumed this increase represents an activated Th2 pathway.[22] However in our study, serum IL-10 value in PV, PF, and BP did not differ from control. In PNP, on the other hand, IL-10 was elevated compared to control group. IL-10 is an anti-inflammatory cytokine with a role in preventing inflammatory and autoimmune pathologies, but it is also a potent B cell stimulator that enhances activation, proliferation, and differentiation of B cells.[46] For instance, in systemic lupus erythematosus (SLE) which is characterized by high autoantibody production and decreased cellular immune responses, levels of IL-10 in SLE patients are significantly higher and there is a correlation between IL-10 levels and clinical manifestation.[47,48] Moreover, depletion of IL-10 by anti-IL-10 antibody in vitro treatment of SLE patient–derived PBMC significantly decreased autoantibody production.[46] In PNP, no data regarding IL-10 has been published, but our result of increased IL-10 might also reflect the involvement

of humoral immunity in the disease pathogenesis.

IL-6 was also significantly increased in PNP compared to healthy control, consistent with the previous finding. There is evidence that dysregulated cytokine production by tumor cells drives the development of autoimmunity. IL-6 is known to promote differentiation and maturation of B cells, and drive immunoglobulins production. Markedly elevated serum IL-6 levels have been demonstrated in a majority of PNP patients.[49] Furthermore, in a subset of tumors associated with PNP, such as NHL, CLL and Castleman's disease, it has been observed that the tumor cells secrete large amounts of IL-6 in vitro.[50] Collectively, with our result further supporting the hypothesis, IL-6 seems to contribute to the induction of autoantibodies, taking up an important part of the autoimmune pathology in PNP.

It may be possible to speculate that Th2 pathway is upregulated in PNP, with both IL-6 and IL-10 increased in the sera of PNP patients. IL-6 and IL-10 are also the cytokines of Th2 pathway, as well as the classic Th2 cytokine IL-4, and it is known that the Th2 cells are involved in the development of humoral immunity. As extremely limited number of cytokine studies are available in PNP, a comprehensive set of cytokines including IL-5, IL-13 needs to be further examined to clarify our result.

The level of IL-8 was significantly higher in the PV and PNP serum compared to control serum. IL-8 induces chemotaxis in target cells, primarily neutrophils, and it is a product of keratinocytes and of dermal cells such as

fibroblasts, endothelial cells or macrophages. In a study by O'Tool *et al.*,[51] intensive expression of IL-8 shown by immunohistochemistry co-localized with in vivo bound IgG in the upper epidermis where the acantholysis took place. Baroni *et al.*[29] also demonstrated a high IL-8 value in the blister fluid of pemphigus patients, and Keskin *et al.*[30] showed increased serum level of IL-8 compared to control. Although controversial result does exist,[32] our result further support the possibility of IL-8 involvement in the pathogenesis in pemphigus. In PNP, IL-8 has not been studied before, but increased serum level of IL-8 might also have a role in recruitment of polymorphonuclear leukocytes to the skin lesion of PNP. However, neutrophil infiltration is not a prominent feature in PNP, and also, the small sample size of PNP sera included in our study precludes a definitive conclusion. Another possible explanation for increased IL-8 in PV and PNP is that it may not be a cause but a result of the disease, i.e., an inflammation response following the damaged epithelial barrier. It has been shown that disruptions to skin such as trauma, irritation and UVB radiation induce IL-8 in the lesional skin.[52-54] Our study was conducted with sera collected in the active stage, and multiple blisters and erosions may trigger the release of various inflammatory cytokines including IL-8. However, it should be noted that IL-8 was not increased in PF or BP, and further detailed studies will be needed to identify the precise role of IL-8 in the different pemphigus groups.

TNF-α has been widely studied in pemphigus, with majority of studies

showing an increase in the serum and in the blister fluid.[22] It has been shown in a previous study that serum levels of TNF-α correlate with disease severity in PV.[32] There were also reports of anti-TNF-α drugs with certain efficacy in PV.[55] On the contrary, a case report of spontaneous development of PV in a psoriatic patient upon infliximab was noted.[56] Also, a recently published pilot study comparing etanercept versus placebo in 8 patients with PV failed to find significant therapeutic efficacy in the etanercept group.[57] In our study, TNF-α level in the pemphigus serum failed to show any significant difference from the control serum. While a strong increase of TNF-α was observed in the most of the previous studies, heterogeneous response of anti-TNF-α drugs in pemphigus patients imply a complex mechanism of this cytokine in the disease pathogenesis.

When comparing PV with PF, none of the cytokines we evaluated were significantly different between the two groups. Although PF and PV differ in their antigens and thus represent distinct clinical entities, concerning the pathogenic role of cytokines no major differences appear to exist between the two diseases.

The reasons why cytokine studies show such variable results may be multiple. The cytokine system is extremely complex, and they can induce or suppress their release and synergize or antagonize each other. In addition, cytokines exhibit multiple effects and it is yet not possible to block only certain activities. The different expression patterns along the disease phases also

complicate the interpretation of the results. Although the direct therapeutic implications of anti-cytokine therapy may be limited, studying the role of cytokines in diseases still remains important since it will ultimately increase our understanding of the pathogenic mechanisms and thereby indirectly facilitate the development of new therapeutic approaches. The strength of our study is that we included a relatively large number of patients (n=75) from a single center, and also we analyzed the serum cytokine levels of PNP, which has not been studied much possibly due to its lower incidence compared to other pemphigus subtypes. In addition, studies regarding Th17 response in pemphigus are extremely limited, and our data provides additional information in understanding the Th17 pathway in pemphigus.

V. CONCLUSION

In conclusion, our results show decreased level of IFN-γ in PV, which may imply a suppressed Th1 response in the active disease stage. A Th2 predominant response is suggested in the active stage of PNP, with elevated serum level of IL-6 and IL-10. Increased level of IL-8 is observed in the sera of PV and PNP patients. Serum IL-17A levels were not statistically different between the five groups, and in our opinion, the pathogenic role of Th17 pathway in pemphigus seems unlikely. We think our study will be of a valuable reference data for the future studies of T cells and cytokines in pemphigus.

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<ABSTRACT(IN KOREAN)>

천포창 환자의 혈청 싸이토카인 발현 양상 분석

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이상희

천포창은 피부와 점막에 수포를 형성하는 만성 질환으로, 각질형성세포 표면에 존재하는 desmoglein에 대한 자가항체에 의해 발병하는 자가면역질환으로 알려져 있다. 조직학적으로는 각질형성세포 사이의 결합이 풀어지는 현상인 가시세포분리를 특징적으로 하며, 수포가 형성되는 위치와 임상소견, 그리고 자가항원에 따라 크게 보통천포창, 낙엽천포창, 종양수반천포창, 그리고 IgA 천포창 의 4가지 아형으로 나눌 수 있다. 천포창은 자가항체의 형성이 질환의 발생에 중요한 것으로 알려져 있으나, 자가반응 T세포가 항체의 생성과 유지에 관여하므로 이들에서 분비되는 싸이토카인의 역할이 주목되어 왔다. 그 동안의 천포창의 싸이토카인에 대한 연구들을 살펴보면, 싸이토카인의 증가 혹은 감소가 단편적으로만 보고되어 왔고 상반되는 결과들이 공존하여 전체적인 싸이토카인의 역할에 대해서 이해가 부족한 상태이다.

이에 본 연구에서는 천포창 환자의 혈청에서 일곱가지 다른 싸이토카인의 발현 양상을 분석하여, 보통천포창, 낙엽천포창, 종양수반천포창의 각 질환에서 나타나는 T세포의 종류 및 면역반응의 성격을 확인하고, 유천포창과 정상 대조군과 비교하고자 하였다.

총 75명의 혈청 (보통천포창 28개, 낙엽천포창 13개, 종양수반천포창 7개, 유천포창 7개, 정상대조군 20개)으로 분석을 시행하였다. ELISA를 통해 Th1, Th2, Th17 및 조절 T세포에서 분비되는 대표적 싸이토카인인 IFN-γ, IL-4과 IL-6, IL17A, IL-10과 염증성 싸이토카인 TNF-α, IL-8 에 대해 조사하였다.

IFN-γ의 중앙값은 보통천포창과 유천포창 환자군에서 정상대조군에 비해 낮게 나타났으며 (0.765, 0.34 vs. 1.63 pg/ml), IL-6과 IL-10은 종양수반천포창에서 정상대조군에 비해 의미있게 높은 값을 보였다 (IL-6: 4.92 vs. 0.24 pg/ml, IL-10: 0.86 vs. <0.12 pg/ml). IL-8은 보통천포창과 종양수반천포창에서 의미있게 높게 나타났다 (11.85, 31.5 vs. 8.31 pg/ml). IL-4, IL-17A, TNF-α는 통계학적으로 유의미한 차이를 보이는 군은 없었다.

보통천포창에서 보인 IFN-γ의 감소는 보통천포창의 활성기에 Th1반응이 감소된 것을 의미할 수 있을 것으로 생각된다. 종양수반천포창에서는 IL-10과 IL-6의 증가가 관찰되었으며 Th2 우세한 반응이 활성기에 나타나는 것으로 생각된다. 보통천포창과 종양수반천포창에서는 IL-8의 증가가 관찰되었다. 본 연구 결과는 천포창의 복잡한 싸이토카인 네트워크를 이해하는데 필요한 중요한 연구 자료가 될 수 있을 것이며, 더 나아가 임상적인 측면에서는 천포창 치료의 새로운 표적을 발견하는데 도움이 되는 이론적 배경을 제공할 수 있을 것으로 생각된다.

핵심되는 말 : 보통천포창, 낙엽천포창, 종양수반천포창, 자가면역, 싸이토카인