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Original Article

IMPLICATION OF RESPONSES TO
BACTERIAL HEAT SHOCK PROTEINS,
CHRONIC MICROBIAL INFECTIONS,
AND DENTAL METAL ALLERGY IN PATIENTS
WITH PUSTULOSIS PALMARIS ET PLANTARIS

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Abstract

Pustulosis palmaris et plantaris (PPP) is a chronic relapsing skin disease characterized by sterile intraepidermal pustules and, usually, by scaly erythematous skin on the palm, soles, or both. To determine the primary pathogenic factors involved in the onset of PPP, we analyzed the implications of heat shock proteins (HSPs), which are highly conserved immunogenic proteins, chronic microbial infections including marginal and periapical periodontitis, and delayed type hypersensitivity (DTH) against dental metals examined by patch tests. We found that the titers of IgG against HSPs of *Escherichia coli* GroEL and *Actinobacillus actinomycetemcomitans* DnaJ in the sera from PPP patients were higher than those in the control group (GroEL; $p < 0.05$). Two patients with PPP had more than 1,000 ng/ml serum IgE level. We found that the average serum IgG antibody level against human cytomegalovirus (HCMV) in the PPP patient group was higher than that of the healthy group ($p < 0.05$). DTH against dental metal was found in eight of 22 PPP patients. Six of eight patients reacted against more than two metals. Replacement of dental metal with resin in these patients resulted in the disappearance or remission of PPP. These findings suggest that the immune responses to HSPs produced by oral bacteria, HCMV infection, dental metal allergy, and their combinations are etiological factors for PPP. We concluded that various kinds of examinations, including oral cavity conditions and chronic infections, are essential to determine the major etiologic factors of PPP.

Key words: Pustulosis palmaris et plantaris—Heat shock proteins—
Chronic infections—Dental metal allergy

INTRODUCTION

Pustulosis palmaris et plantaris (PPP), a

chronic relapsing skin disease, is characterized by sterile intraepidermal pustules and, usually, by scaly erythematous skin on the

palms, soles or both. Its pathomechanism is not yet understood. The etiology of PPP has not yet been clarified. A close relationship between infection and PPP has been reported by many research groups; these include infections by *Streptococcus pyogenes*³⁾, *Mycobacterium*⁹⁾, human papilloma virus²²⁾, Coxsackie B virus¹³⁾, and fungi^{1,6)}.

Heat shock proteins (HSPs) are highly conserved molecules and distributed widely in nature²⁷⁾. They are also distributed in the skin; however, only limited information is available on the role of HSPs in skin diseases. Immunohistochemical studies of HSPs in the skin have demonstrated that HSPs are differently expressed in the epidermal cells of patients with systemic lupus erythematosus (SLE), atopic dermatitis, PPP, and graft versus host disease (GVHD)⁷⁾. In normal healthy skin, HSPs are constantly expressed in the epidermal cells. HSPs are also expressed in the skin in response to the influence of both the external and internal milieu of disease conditions. Cytokines released in the skin strongly affect this expression of HSPs in epidermal cells. HSPs expressed in the skin can be targets for infiltrated $\gamma\delta$ T cells and modulate the immune response to skin diseases¹²⁾.

Metal allergy is a type of allergic contact dermatitis; however, it has some other exceptional manifestations such as lichen planus in the buccal mucosa and PPP on the palm of the hand or/and the heel of the foot. Evidence for such exceptional cases include reproducible positive patch test reactions to various metals and analytical results of dental metal testing demonstrating the presence of metals to which the patients are undoubtedly hypersensitive. Dramatic curative effects of allergenic metal elimination have been reported^{19,17)}. It is valuable to examine the relationship between dental metal allergy and other humoral immune responses to various antigens in patients with PPP.

To investigate their relationships to PPP, we examined IgG responses to HSPs of *Escherichia coli* GroEL and *Actinobacillus actinomycetemcomitans* DnaJ, IgG antibody responses

against *Mycobacterium pneumoniae*, *Chlamydia pneumoniae*, herpes simplex virus (HSV), and human cytomegalovirus (HCMV), and the delayed type hypersensitivity (DTH) responses to dental metals in patients with PPP.

MATERIALS AND METHODS

1. Subjects

The study included a total of 22 patients with PPP (10 men and 12 women). All the patients were diagnosed by medical doctors who have clinics, mainly dermatology clinics, in Ichikawa and Funabashi Cities in Japan. We accessed their home pages and asked them to introduce their patients with PPP to Tokyo Dental College, Chiba Hospital, or Kosugi Dental Clinic in Funabashi City. We independently diagnosed PPP in these patients mainly according to description of previous reports^{15,16)} and based on the following criteria: pustules localized on the palms and soles, no lesions of eczema or psoriasis on any other part of the body, and negative family history of pustulosis.

2. Heat shock proteins

We used *E. coli* GroEL protein (Sigma Chemical Co., St. Louis, MO) and a recombinant DnaJ of *A. actinomycetemcomitans* HSP (generously donated by T. Koga, School of Dentistry, Kyushu University, Hakata, Japan).

3. Measurement of antibody titers

Serum IgG antibody titers against heat shock proteins were measured by enzyme-linked immunosorbent assay (ELISA) using 96-well polyethylene enzyme immunoassay plates (Coster Co., Cambridge, MA). Each HSP antigen was suspended in 0.1 M carbonate buffer (pH9.6) at 10 μ g/ml, and samples of 50 μ l of each suspension were placed in the wells of the plate. These solutions were discarded after incubation at 37°C for one hour. Then, 100 μ l of 3% bovine serum albumin in phosphate-buffered saline (PBS, pH7.2) was added, and each plate was incubated at 37°C for one hour. The wells were then washed with PBS containing 0.05% Tween-20. A 50 μ l

Table 1 Summarized data on periodontal status, serum IgE level, IgG levels against *E. coli* GroEL and *A. actinomycetemcomitans* DnaJ, serum IgG levels against human cytomegalovirus and herpes simplex virus as determined by ELISA, and serum IgG levels against *M. pneumoniae* and *C. pneumoniae*.

Patient	Gender	Number of PD>4 mm	Number of apical lesion	IgE	GroEL	DnaJ	HSV-IgG	HCMV-IgG	M.p IgG	C.p IgG
1	F	—	—	64	0.617	0.449	53.8	26.9	<40	1.28
2	F	—	—	33	0.236	0.067	73.7	33.4	<40	1.63
3	M	—	—	140	0.229	0.058	2.0	17.1	<40	0.75
4	F	—	—	11	0.292	0.193	69.9	2.0	<40	0.65
5	M	—	—	56	0.226	0.176	2.0	2.0	<40	0.58
6	M	—	—	330	0.285	0.170	11.6	35.5	<40	0.86
7	M	—	—	180	0.249	0.271	115.0	76.0	<40	1.25
8	M	—	—	350	0.222	0.200	2.0	21.5	<40	1.41
9	F	—	—	390	0.237	0.228	2.6	41.2	<40	2.21
10	F	—	—	1,100	0.350	0.182	40.3	64.7	<40	1.41
11	M	2	5	150	0.492	0.051	5.2	44.9	<40	1.41
12	F	7	0	130	—	—	77.5	25.2	<40	0.53
13	M	18	6	33	0.211	0.167	2.0	40.8	<40	0.90
14	F	0	0	89	0.100	0.137	69.1	45.2	<40	0.58
15	F	16	1	240	0.383	0.161	0.3	104.0	<40	2.40
16	F	3	2	34	—	—	46.6	53.5	80	0.31
17	F	5	3	620	0.465	0.124	41.4	8.2	<40	0.90
18	F	4	0	100	0.825	0.185	0.75	16.0	—	—
19	F	1	0	58	0.385	0.157	<2.0	<2.0	<40	—
20	M	5	0	26	0.322	0.024	32.0	16.7	<40	—
21	M	19	1	4,200	—	—	64.0	16.0	<40	—
22	M	1	2	—	—	—	<2.0	59.0	<40	—

—: Not determined, Serum IgE level: ng/ml, Serum IgG levels against *E. coli* GroEL and *A. actinomycetemcomitans* DnaJ: Absorbance at 490 nm, Serum IgG levels against HSV, HCMV and *C. pneumoniae*: ELISA titer, Serum IgG levels against *M. pneumoniae*: Passive hemagglutination titer

serum sample diluted at one to 100 in PBS containing 0.5% bovine serum albumin was added to each well. After incubation at 37°C for two hours, the plate was washed with PBS containing 0.05% Tween-20. Next, 100µl of peroxidase-conjugated goat-anti-human IgG (Fab fragment, heavy- and light-chain-specific (Cappel, INC Pharmaceuticals, Inc., Aurora, Ohio), diluted at one to 2,000 was added to each well and incubated at 37°C for one hour. After the wells had been washed three times with the buffer, 100µl of *o*-phenylenediamine-dihydrochloride dissolved in 0.1 M Na₂HPO₄, 0.05 M citric acid buffer, and H₂O₂ solution was added to each well, and incubation was allowed to proceed at room temperature for

10 min. Then, 50µl of 6N H₂SO₄ was added to stop the reaction. The absorbance at 490 nm was determined in a microplate reader. Serum samples from healthy 24 adult volunteers matched for age and gender who had no serious infectious disease were also subjected to compare their serum IgG levels against HSP antigens. After informed consent was obtained, all blood samples were collected and stocked at -80°C until use.

Serum IgE levels were determined by an enzyme immune absorbent assay (ELISA) and expressed as ng/ml. The IgG antibody levels against *C. pneumoniae*, HSV, and HCMV were determined by ELISA. The IgG levels against *M. pneumoniae* were determined by a passive



Fig. 1 A picture of typical syndrome of PPP in patient 12



Fig. 2 Before is another picture of the syndrome in patient 12 before dental treatment, and after is the same patient after dental treatments. The PPP has cleared.



Fig. 3 Before is a picture of the syndrome before treatment in patient 20, and after is the same patient after dental treatments and after he began to wear vinyl chloride gloves for collecting coins. The remission of PPP can be seen.

hemagglutination method (PA). These measurements were performed by SRL Company (Tachikawa, Tokyo). Serum samples from 10 healthy adult volunteers matched for age and gender who had no serious infectious disease were also subjected to compare the serum IgG levels.

4. Western blot analysis

To identify the antibodies to *E. coli* GroEL and *A. actinomycetemcomitans* DnaJ in the sera of PPP patients, Western blot analyses were performed. Heat shock proteins of *E. coli* GroEL and *A. actinomycetemcomitans* DnaJ were separated by SDS-PAGE and transferred onto a polyvinylidene difluoride membrane. The blotted membrane was incubated with patient sera diluted at one to 500 and washed with the buffer. It was then incubated with peroxidase-conjugated goat anti-human IgG (Cappel, Inc Pharmaceuticals, Inc.) diluted at one to 3,000, and the peroxidase reaction was initiated with Tris buffer (pH7.5) containing 4-methoxy-1-naphthol and 0.02% H₂O₂.

5. Delayed type hypersensitivity (DTH) to dental metal

To examine DTH to dental metal in patients with PPP, patch tests for 16 metals were performed in this study. One patient with PPP subjected in this study had a metal framework. We examined the oral restorative conditions in patients Number 11 to 22. All of these 12 patients had received tooth restorative treatments with metal inlays or/and crowns. The metal allergy test kit was purchased from Torii Pharmaceutical (Tokyo). The metals examined by DTH were Au, Ag, Al, Pt, Pd, Ir, Co, Cu, Zn, Sn, Mn, Ni, Fe, In, Ti, and Cr. Disks containing each metal were placed on the skin of the left arm, and the reaction was evaluated by flare on the skin, after 1, 2, 4, and 7 days.

6. Statistical analysis

The data obtained were compared statistically by the Mann-Whitney U test.

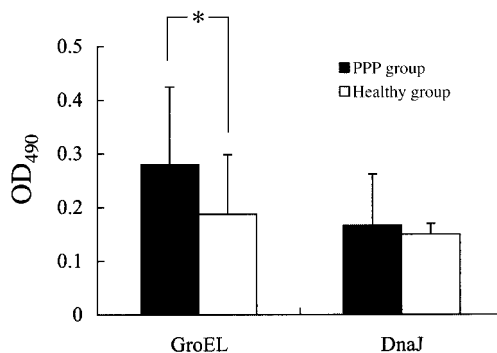


Fig. 4 Average serum IgG levels against HSPs of *E. coli* GroEL and recombinant *A. actinomycetemcomitans* DnaJ antigen in sera from 18 patients with pustulosis palmaris et plantaris and 24 healthy adults as determined by ELISA. All serum samples were diluted 100 times in phosphate buffered saline containing 0.05% Tween-20. The mean titers and standard deviations for each heat shock protein are expressed at an absorbance of 490 nm. *: Significantly different at $p < 0.05$.

RESULTS

1. Examined data from patients with PPP

Data examined included numbers of more than 4 mm periodontal pocket depth (PD), number of apical lesions, serum IgE level, IgG antibody levels to HSPs of *E. coli* GroEL and *A. actinomycetemcomitans* DnaJ, IgG antibody levels to HSV, HCMV, *M. pneumoniae* and *C. pneumoniae* in 22 patients with PPP. These values are summarized in Table 1.

A typical patient with PPP, number 12, is shown in Fig. 1. Figure 2 illustrates before and after treatment of Patient 12, respectively. Figure 3 illustrates before and after treatment of Patient 20. In Figs. 2-after and 3-after, we can see recovery or remission of the PPP syndrome.

2. Serum IgG levels against HSPs

As shown in Fig. 4, we found that the average IgG antibody titers against *E. coli* GroEL in the sera of 18 patients with PPP were significantly higher than those in 24 control subjects ($p < 0.05$). The IgG level against *A. actinomycetemcomitans* DnaJ in sera from PPP patients were higher than those in

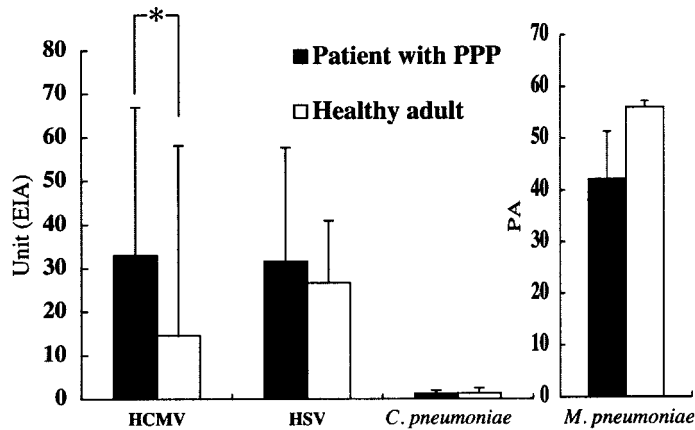


Fig. 5 Average serum IgG levels and standard deviations against HSV, HCMV, *C. pneumoniae* examined by ELISA and *M. pneumoniae* examined by passive hemagglutinin test. The mean titers and standard deviations against HSV, HCMV, *C. pneumoniae* are expressed at unit of EIA, and the titer against *M. pneumoniae* is expressed at reciprocal dilution. *: Significantly different at $p < 0.05$.

the control group, but were not statistically different.

3. Western immunoblot analysis

Western immunoblot patterns of serum samples from three PPP patients with periodontitis who had high IgG levels against *E. coli* GroEL were compared with those of three healthy subjects. We confirmed that the sera from these patients reacted against *E. coli* GroEL at 60 kDa, but sera from three control individuals did not react.

4. Serum IgE levels

As shown in Table 1, two of our 21 PPP patients, numbers 10 and 21 (9.5%), possessed Serum IgE levels of 1,100 and 4,200 ng/ml, respectively.

5. Serum IgG levels against microorganisms

Average serum IgG antibody levels against *C. pneumoniae*, HCMV, and HSV examined by ELISA and *M. pneumoniae* examined by PA in serum sample from PPP patients were compared with those from healthy control group as shown in Fig. 5. The average IgG level against HCMV in sera from the PPP patient group was significantly higher than that of the healthy control group ($p < 0.05$). However,

there was no statistical difference in the levels against *C. pneumoniae*, HSV, or *M. pneumoniae* between the two groups.

The numbers of PPP patients possessing more than the average serum IgG antibody levels against *E. coli* GroEL, *A. actinomycetem-comitans* DnaJ, *C. pneumoniae*, HCMV, or HSV in serum samples from the healthy control group are summarized in Table 2. Seven of 18 PPP patients (38.8%) possessed higher IgG antibody levels against *E. coli* GroEL, and 18 of 22 PPP patients (81.8%) had higher IgG levels against HCMV than the average titer of the healthy control group. One of 21 PPP patients examined demonstrated an IgG level against *M. pneumoniae* above 40 by the passive agglutination test.

6. Dental metal allergy

Eight patients with PPP demonstrated dental metal allergy. Patch test results and numbers of teeth treated with dental metal are summarized in Table 3. Six of these eight patients reacted against more than two metals.

Patient 11 was a 45-year-old man with chronic periodontitis who had five apical lesions and high IgG levels against *E. coli* GroEL. He underwent periodontal treatment for 24 months, and his metal fillings were

Table 2 Numbers of PPP patients demonstrating levels that were higher than the average titers in the healthy group against HSPs of *E. coli* GroEL and *A. actinomycetemcomitans* DnaJ, HCMV, HSV, *C. pneumoniae* and *M. pneumoniae*.

Antibody against	Number of higher level /Examined	Percentage
<i>E. coli</i> GroEL	7/18	38.9
<i>A. actinomycetemcomitans</i> DnaJ	9/18	50.0
HCMV	18/22	81.8
HSV	11/22	50.0
<i>C. pneumoniae</i>	2/17	11.7
<i>M. pneumoniae</i>	1/21	4.8

Table 3 Eight patients with PPP and their treatment with dental metal

Patients	Metals	Number of the teeth treated with metal materials
11	Ir	14
12	Ni	two and one framework
14	Al, Pd, Sn, Cn, Fe, Au, Cr	10
15	Zn, Ag	22
16	Pt, Au, Cr, Pd, Sn	14
17	Au, Pd, Ni	18
18	Cr, Cu, Pt, Pd, Zn, Au	11
19	Zn, Mn	5

replaced with resin materials. After this 24 months of treatment, the syndrome of PPP disappeared. Patient 12, as shown in Figs. 1 and 2, was a 45-year-old woman with a high IgG antibody against HSV and a strong reaction against Ni. Her metal denture framework was replaced by a resin plate. After three months, the PPP syndrome disappeared. Patient number 18, a 32-year-old woman, had a high IgG antibody level against *E. coli* GroEL and reacted strongly to Pd, Pt and Au and weakly to Cr, Cu and Zn. We removed the dental metals from eight teeth. Three months after the treatment, significant remission was observed. Patient 20, as shown in Fig. 3, is a 37-year-old man who had a high IgG antibody level against *E. coli* GroEL and reacted to Ni. After periodontal treatment for 12 months, the PPP syndrome went into remission, but did not disappear. During the treatment, we

found that the patient had been collecting coins for an amusement company. We advised him to wear vinyl chloride gloves when collecting coins. After he began wearing these gloves, the PPP syndrome disappeared.

DISCUSSION

HSPs are highly conserved immunogenic proteins that are often immunodominant antigens produced in bacteria and mammalian cells by a variety of stressors^{2,11,12,26}. HSPs have been implicated in the pathogenesis of several diseases^{14,18,20}. Some HSPs could be targets for T cells possessing $\gamma\delta$ -receptors, which are predominant in skin with various immune disorders. It has been demonstrated that antibodies that reacted with periodontopathic HSPs can be found in serum samples

from patients with periodontal disease^{9,14,23,24}. These data suggest that HSP-producing chronic infectious bacteria could trigger PPP. We reported that the titers of IgG against *E. coli* GroEL and *A. actinomycetemcomitans* DnaJ in the sera from PPP patients were significantly higher than those in a healthy control group¹⁰. It has been also reported that one mechanism for the onset of PPP has been considered to be focal infection¹. The present study also demonstrated that some PPP patients showed significantly higher IgG levels against *E. coli* GroEL and *A. actinomycetemcomitans* DnaJ than the average titer of a normal control group. The average IgG level against *A. actinomycetemcomitans* DnaJ was higher than that of healthy control group, but the values were not statistically different. In our previous paper, we found statistically significant higher IgG levels against not only *E. coli* GroEL but also *A. actinomycetemcomitans* DnaJ in patients with PPP than those in healthy controls¹⁰. The subjected PPP patients in that study were mainly patients with periodontitis, and we demonstrated that periodontal therapy and extraction of teeth with periapical infections resulted in remission of PPP and statistically significant reduction in the IgG antibody levels to *E. coli* GroEL in 41% of the patients examined. It has been shown that antibody against the GroEL-like HSP protein of *A. actinomycetemcomitans* shows a high level of cross-reactivity to *Porphyromonas gingivalis* and *Bacteroides forsythus*²¹. It is possible that HSPs produced by periodontal disease-associated bacteria and their cross-reactivity with HSPs in host cells are associated with the etiology of PPP. The subjected group with PPP were mainly patients who visited oral surgery or/and dental clinics. In contrast, in the present study, the titers of IgG against *A. actinomycetemcomitans* DnaJ in the sera from PPP patients were not higher than those in the control group. However, these PPP patients were introduced by medical doctors mainly working in dermatology clinics. The difference in the selection process for the PPP patients might have resulted in the difference in the results. The present and our previous studies both

showed that periodontal treatment can result in remission of PPP in some patients.

Two out of 12 patients (patients 11 and 13) with PPP examined in the present study had more than five apical lesions. Three months after extraction of the diseased teeth, the syndromes disappeared, and remission of PPP was achieved in both patients. These facts suggest that dental treatment, including periodontal treatment and diseased tooth extraction, is an essential treatment for PPP patients with chronic oral infectious diseases. However, some PPP patients did not demonstrate elevated IgG levels against various HSPs. We and other research groups have reported a relationship between skin diseases including PPP and various kinds of HSPs^{4,5,10,11}. To standardize the importance of various aspects of treatment, it will be important in the future to perform a longitudinal study using various HSPs including GroEL-like protein of *A. actinomycetemcomitans*.

In this study, we found that PPP patients possessed significantly high IgG levels against HCMV, HSV, *M. pneumoniae*, or/and *C. pneumoniae*. These data support the theory that microbial infections trigger the onset of PPP in some patients. In fact, a close relationship between microbial infections and PPP has been reported by many research groups^{1,3,6,8,19,26}. A longitudinal study of the changes in these antibody levels would clarify the linkage between these microbial infections and onset of PPP.

The etiology of PPP has been suggested to involve metal allergy as an important contributing factor²⁵. In the present study, we observed positive skin reactions against dental metals in several patients. Eight of 22 patients with PPP showed DTH positive reactions to one or more dental metals. It was found that replacement of these dental restorative metals with resin effectively induced disappearance of the syndrome in five of these eight patients. It has been reported that cobalt metal has been used for prosodontic treatment and causes metal allergy in some patients. However, we could not detect any positive skin reactions against cobalt in our PPP patients in this

study. For patch testing, the low solubility of cobalt metal in the kit used might have resulted in the negative reaction for the metal allergy. Replacement of dental metal inlay with a resin filling in some patients resulted in remission of their syndrome. These facts indicate that DTH against dental metals is the etiology in some of PPP patients.

In conclusion, we showed that there are various pathogenic factors in the oral cavity in many patients with PPP. We emphasized that various examinations such as determination of serum antibody levels against various HSPs and microorganisms as well as DTH reactivity against dental metals are very important in identifying the main etiological factors in patients with PPP.

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