

Expression of Heat Shock Protein 72 on Peripheral Blood Mononuclear Cells from Patients with Pustulosis Palmaris et Plantaris

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The expression of heat shock protein (HSP) 72 on peripheral blood mononuclear cells (PBMC) from patients with pustulosis palmaris et plantaris (PPP) was studied. PBMC isolated freshly from patients with PPP expressed HSP72, while those from psoriasis patients did not. PBMC from patients with PPP continued to express it in *in vitro* cultures at 37°C. This expression was further augmented by *in vitro* heat stimulation at 43°C. Immunofluorescence studies showed that approximately 20% of PBMC from patients with PPP were stained positively with anti-HSP72 antibody. HSP72 was expressed on both non-adherent and adherent cells of PBMC. These findings suggest that PBMC from patients with PPP may produce HSP72 spontaneously through their *in vivo* exposure to stressful agents. **Key words:** PPP; PBMC; HSP72.

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Heat shock proteins (HSP) are synthesized when cells are exposed to *in vitro* environmental stresses such as heat, ethanol, heavy metals, amino acid analogues, and certain types of metabolic poison (1, 2). The heat shock response is also induced *in vivo* by heat, ischemia, wounding, infection and inflammation (3). In humans, the 70 kD HSP family is among the most prominent classes of HSP. There are two major forms in this family: an abundant constitution member, HSP73, and a highly stress-induced member, HSP72 (4, 5). The inducible HSP72 has been reported to play an important role in intracellular protein processing and to interact with cytokines and mediators (6–8). Recent studies have shown increased amounts of HSP72 in human organs under pathologic conditions. Peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus expressed HSP72 (9). Fibroblasts from patients with systemic sclerosis and chondrocytes of patients with osteoarthritis were reported to express HSP72 in *in vitro* cultures (10, 11).

Among chronic inflammatory dermatoses, pustulosis palmaris et plantaris (PPP) is characterized by repeated attacks of sterile pustules on the palms and soles.

Some patients exhibit symptoms of osteoarthritis (12). Its etiology is suggested to be related to focal infections at the tonsils or heavy metals (13–16) known as stressful agents, which may induce an HSP response *in vitro* or *in vivo*. In view of this, the present study was designed to investigate the expression of HSP72 on PBMC from patients with PPP in order to clarify the HSP response in these patients. Here we describe the spontaneous expression of HSP72 on PBMC from patients with PPP.

MATERIALS AND METHODS

Patients

Fifteen patients with PPP (7 women and 8 men, with an age range of 36–63 years) were treated at Aichi Medical University Hospital. All had been suffering from PPP for more than 2 years. The activity of the disease had been stationary at least during the last 6 months. We also examined 10 patients with chronic plaque type psoriasis, and 9 normal healthy subjects who were matched in age and sex with the patients with PPP.

Cells and cell cultures

Heparinized peripheral blood was layered on Ficoll-Hypaque (Ficoll Trennlosung, Seromed-Biochem KG, Berlin, Germany) and centrifuged at 1,800 rpm for 20 min. PBMC were collected from the plasma-Ficoll interface and washed with RPMI 1640 medium 3 times. Freshly isolated PBMC were applied to immunoblotting and immunofluorescence for the detection of HSP. A part of PBMC was applied to *in vitro* culture systems and cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells (2 to 3×10^7 /ml) were maintained at 37°C (without heat treatment) or incubated at 43°C for 1 h and then allowed to recover at 37°C for 16 h in a 5% CO₂ incubator. PBMC from psoriasis patients and normal healthy controls were also used. Adherent and non-adherent cells were separated by incubation of PBMC (4 to 5×10^6 /ml) from PPP patients in a 35-mm plastic dish with RPMI 1640 medium, supplemented with 10% fetal calf serum at 37°C for 2 h. Non-adherent cells were removed by washing with the culture medium from the plastic dish.

Polyacrylamide gel electrophoresis and immunoblotting analysis

Freshly isolated PBMC, cultured cells and adherent or non-adherent cells were washed 3 times in PBS and suspended at a concentration of 2×10^8 /ml in a lysis buffer containing 0.5% Nonidet P-40, 0.15 M NaCl, 0.05 M Tris, and 5 mM EDTA, pH 8.0, for 30 min at 4°C. The insoluble debris was removed by microcentrifugation at $18,600 \times g$ for 10 min at 4°C. Cell lysates were diluted with an equal volume of sample buffer containing 2.5% sodium dodecyl sulfate (SDS) and 2% 2-mercaptoethanol and boiled for 2 min. Samples were separated by 4–20% gradient gels with SDS-polyacrylamide gel electrophoresis (17). Proteins in the gel were transferred to a membrane filter (Durapore, Nihon Millipore Ltd., Japan) by electroblotting (18). The membranes were blocked with 5% skim milk and then incubated with anti-HSP72 monoclonal antibody (C92F3A-5, StressGen Biotechnologies Corp., Victoria, B.C., Canada), diluted at 1:200 in PBS containing 5% calf serum and 0.05% Tween 20. They were washed and then treated with a 1:5000 dilution of goat anti-mouse IgG serum (Jackson Immuno Research Lab. Inc., Baltimore). The membranes were treated with ECL staining solution (Amersham, UK) and exposed to Fuji X-ray film, as described previously (19). A high and low MW standard kit from Nippon Bio-rad Laboratories, Tokyo, Japan, was used as reference.

Indirect immunofluorescence

Freshly isolated PBMC were fixed with acetone at 4°C for 3 min and air-dried. To block non-specific binding, samples were treated with

normal rabbit serum for 20 min at room temperature. The cells were stained with the anti-HSP72 antibody, followed by FITC-conjugated rabbit anti-mouse IgG (MBL, Inc., Nagoya, Japan) secondary antibody. An irrelevant monoclonal antibody with the same Ig class was used as negative control.

RESULTS

Detection of HSP72 on PBMC from patients with PPP by immunoblotting

The expression of HSP72 in PBMC isolated freshly from patients with PPP, patients with psoriasis, and normal healthy controls was analyzed by immunoblotting. The experimental results are summarized in Table I. Spontaneous expression of

Table I. Expression of HSP72 in PBMC from PPP patients, psoriasis patients and normal healthy controls detected by immunoblotting using anti-HSP72 monoclonal antibody

Patients (n)	Frequency of positive reaction		
	No incubation ^a	Heat treatment ^b	
		-	+
PPP (15)	14/15	14/15	15/15
Psoriasis (10)	0/10	0/10	10/10
Normal controls (9)	1/9	1/9	9/9

^a Freshly isolated PBMC were used.

^b PBMC were incubated in vitro at 37°C for 16 h with or without heat stimulation, at 43°C for 1 h before cultivation.

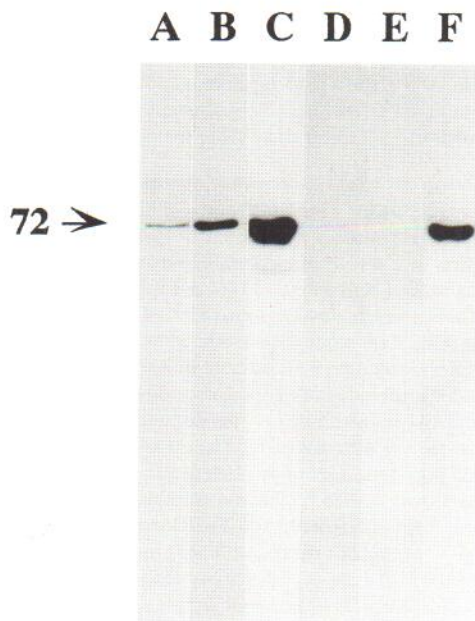


Fig. 1. The expression of HSP72 in PBMC from patients with PPP and with psoriasis was detected by immunoblotting. An extract of PBMC isolated freshly from patients with PPP (A) and with psoriasis (D) was run. PBMC from patients with PPP (B) and with psoriasis (E) were incubated at 37°C for 16 h. PBMC from patients with PPP (C) and with psoriasis (F) were exposed to 43°C for 1 h and then allowed to recover at 37°C for 16 h. Molecular weight marker (kD) is indicated to the left. A-C, PPP patients; D-F, psoriasis patients.

HSP72 in freshly isolated PBMC was detected in 14 (93%) of 15 patients with PPP, but not in any of the patients with psoriasis. However, there was spontaneous expression of HSP72 in extracts of PBMC from a normal healthy control. The typical experimental result from one patient is shown in Fig. 1. When PBMC were cultured at 37°C for 16 h, HSP72 was detected in the extract of PBMC from patients with PPP, but not from patients with psoriasis. Furthermore, exposure to heat at 43°C for 1 h clearly induced expression of HSP72 in all PBMC from patients with PPP, with psoriasis, and healthy controls.

Expression of HSP72 on non-adherent and adherent cells from patients with PPP

PBMC from patients with PPP, who spontaneously expressed HSP72, were separated into adherent and non-adherent cells, and the expression of HSP72 in each fraction was studied. HSP72 was detected on all non-adherent cell fractions tested in the case of patients with PPP on adherent cell fractions from 5 out of 6 patients (data not shown).

Immunofluorescence staining of HSP72 in PBMC from patients with PPP

The frequency of cells expressing HSP72 was examined by indirect immunofluorescence using anti-HSP72 monoclonal antibody (Fig. 2). Approximately 20% of PBMC were stained positively in most of the patients with PPP. There was one positive reaction in PBMC from 10 psoriasis patients, and one out of 9 normal healthy controls reacted positively.

DISCUSSION

In the present study we have demonstrated that the inducible HSP72, a major member of the highly conserved 70 kD HSP

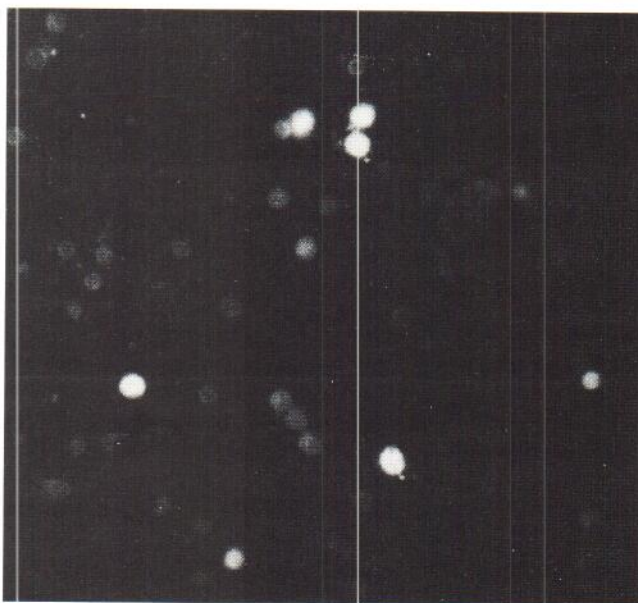


Fig. 2. Indirect immunofluorescence staining of HSP72 in PBMC from PPP patients. Freshly isolated PBMC were fixed and stained by an indirect immunofluorescence method. Note that approximately 20% of the PBMC were stained positively (original magnification $\times 200$).

family, is spontaneously expressed in PBMC isolated freshly from patients with PPP. These cells continued to express HSP72 in *in vitro* cultures without heat stimulation. This phenomenon could not be seen in the case of PBMC from psoriasis patients and normal healthy controls. This may indicate that PBMC from patients with PPP might be exposed to stressful agents *in vivo* and upregulate the HSP72 expression. There are several reports that HSP72 is induced in peripheral blood leukocytes under some pathologic conditions, such as in neutrophils from patients suffering from severe tissue injury (20). Incidentally, we found that upregulated HSP72 expression was detectable on PBMC from patients with PPP, but not on those from patients with psoriasis. This differential expression of HSP72 between PPP and psoriasis might be useful for discrimination of the two diseases. In fact, there has been a controversy as to whether PPP is a distinct disease entity from psoriasis or not (21).

It is known that heat shock response is induced *in vivo* by a variety of stresses, such as heat treatment, ischemia, wounding, infection and inflammation (3). It is also induced *in vitro* by environmental stresses such as heat, ethanol, heavy metals, amino acid analogues, and certain metabolic poisons (1, 2). The mechanism of the onset of PPP has been a subject of controversy, but the focal infection theory (14, 15) and the metal allergy theory, in which allergenic dental metals dissolve mainly by electric current in the oral cavity and provoke allergic reactions repeatedly on the oral mucosa and other various sites of the skin (16), have been suggested as possibilities. Stressful agents, such as infections and heavy metals, may induce spontaneous expression of HSP72 in PBMC from patients with PPP. Fincato *et al.* (22) reported that HSP72 mRNA was constitutively expressed in unstressed human peripheral blood monocytes and neutrophils and, at augmented levels, in bacterial lipopolysaccharide or various cytokine-treated monocytes. An amount of lipopolysaccharide was detected in the tonsillar plug of pus in patients with PPP (14). The constitutive expression of HSP in the inflammatory cells may render them more resistant to the microenvironment of inflammatory foci. This could be applied to PBMC circulating in patients with PPP. It should be clarified what kind of stressful agents induce an HSP72 response in PBMC from patients with PPP, because such stressful agents may be closely associated with the pathogenesis of PPP.

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