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Proteome analysis of proteins related to aggressive periodontitis combined with neutrophil chemotaxis dysfunction

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Abstract

Aim: Some patients suffering from aggressive periodontitis (Ag-P) also display neutrophil chemotaxis dysfunction. In this study, we attempted to identify the proteins involved in Ag-P associated with neutrophil chemotaxis dysfunction using proteome analysis.

Material and Methods: A two-dimensional fluorescence difference gel electrophoresis system was used to detect differences in protein expression between neutrophils from four patients suffering from Ag-P combined with neutrophil chemotaxis dysfunction and those from four controls. Moreover, the mRNA levels of the proteins identified by the above method were examined in neutrophils from four types of subjects using the real-time polymerase chain reaction: twenty patients suffering from Ag-P with or without the dysfunction, 15 patients with chronic periodontitis, and 15 controls.

Results: Four proteins, lactoferrin, caldesmon, heat shock protein 70, and stac, displayed a higher protein expression level in the neutrophils from the patients suffering from Ag-P combined with the neutrophil dysfunction than in those from the control group. The caldesmon mRNA levels in the neutrophils from the patients suffering from Ag-P combined with the neutrophil dysfunction were high compared with those in the neutrophils from the patients suffering from the other two types of periodontitis and those from the control group.

Conclusion: Caldesmon may be a marker of Ag-P combined with neutrophil chemotaxis dysfunction.

Key words: Aggressive periodontitis; caldesmon; neutrophil chemotaxis dysfunction; two-dimensional fluorescence difference gel electrophoresis

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Periodontitis is an inflammatory disease caused by periodontopathogenic bacteria. Periodontitis is generally classified into aggressive periodontitis (Ag-P) and chronic periodontitis (Ch-P). The age of onset of Ag-P is earlier than that of Ch-P, and Ag-P is characterized by rapid and severe periodontal tissue destruction (Manson & Lehner 1974, Hørmand & Frandsen 1979, Liljenberg & Lindhe 1980, Saxén 1980, Burmeister et al. 1984). More host factors are thought to be related to the onset and progression of Ag-P than are associated with Ch-P. Furthermore, the intrinsic factors promoting periodontal tissue destruction in Ag-P patients may differ among patients. Ag-P has been investigated in relation to neutrophil dysfunction (Van Dyke et al. 1980, 1985, Genco et al. 1986, Daniel et al. 1993, Kurihara et al. 1993, Biasi et al. 1999, Gronert et al. 2004, Yagi et al. 2009, Guentsch et al. 2009), familial background (Nishimura et al. 1990, Trevilatto et al. 2002, Llorente & Griffiths 2006), single nucleotide polymorphisms (Michel et al. 2001, Itagaki et al. 2004, Galicia et al. 2006,

Conflict of interest and source of funding statement

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Gonzales et al. 2007, Nikolopoulos et al. 2008), specific immune responses to infective agents (Shapira et al. 1991, Kuru et al. 1999, Emingil et al. 2004), and stress (Giannopoulou et al. 2003, Kamma et al. 2004).

Defective neutrophil chemotaxis is one of the dysfunctions observed in Ag-P patients. Neutrophil chemotaxis is the first step in host defense and plays a significant role in the host defense against bacterial infection. Understanding the mechanism of chemotaxis is helpful for investigating neutrophil dysfunction in Ag-P. Various mechanisms have been suggested to explain this pathology, including reduced calcium influx (Daniel et al. 1993), diacylglycerol (DAG) accumulation (Leino et al. 1994), reduced DAG kinase activity (Hurttia et al. 1997), reduced protein kinase activity (Kurihara et al. 1993), elevated nitric oxide synthase activity (Shibata et al. 2001), elevated superoxide production (Van Dyke et al. 1986), and reduced levels of calcium influx factor (Shibata et al. 2000). However, there has been no sufficiently detailed study on defective neutrophil chemotaxis in Ag-P.

We hypothesized that neutrophils from patients suffering from Ag-P combined with neutrophil chemotaxis dysfunction express characteristic proteins. To prove our hypothesis, we compared the proteins expression levels of neutrophils from patients suffering from Ag-P combined with the neutrophil chemotaxis dysfunction with those of neutrophils from a control group using a two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) system and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.

Material and Methods

Patient population

The diagnostic criteria for Ag-P and Ch-P were defined in accordance with the classification agreed at the World Workshop for Periodontics and The American Academy of Periodontology (1999). Ag-P was indicated in subjects in whom more than eight teeth demonstrated attachment loss (AL) of $\geqslant 5$ mm and a probing depth (PD) of $\geqslant 6$ mm, providing at least three affected teeth were not first molars or incisors. Ch-P was indicated in subjects with AL of $\geqslant 5$ mm in more than one tooth and more than three sites displaying a PD of $\geqslant 6$ mm including more than one tooth in each quadrant. Twenty patients with Ag-P (12 males and eight females, mean age $\pm$ SD: 32.3 ± 8.5 years) and 15 patients with chronic Ch-P (10 males and five females, mean age $\pm$ SD: 60.8 ± 7.7 years) were recruited from the patient population of Hiroshima University Hospital. The study protocol was approved by the ethical authorities at Hiroshima University. Informed consent was obtained before the collection of peripheral venous blood. Fifteen controls (11 males and four females, mean age $\pm$ SD: 30.4 ± 6.1 years) with no evidence of AL at more than one site or a PD of >3 mm were enrolled. All participants in this study were Japanese and non-smokers and had no history or current symptoms of systemic disease. The periodontal measurements of all participants in this study are shown in Table 1.

Table 1. Periodontal examination measurements

	Ag-P with neutrophil chemotaxis dysfunction	Ag-P without neutrophil chemotaxis dysfunction	Ch-P	Control group
Subject number	10	10	15	15
BOP (%)	62.4 ± 22.5	45.1 ± 30.6	29.9 ± 18.3	4.8 ± 2.7
AL (mm)	4.5 ± 1.3	4.6 ± 1.5	3.5 ± 1.2	2.2 ± 0.4
PD (mm)	3.6 ± 1.0	3.9 ± 1.1	3.0 ± 1.1	1.9 ± 0.3

Ag-P, aggressive periodontitis; Ch-P, chronic periodontitis; AL, attachment loss; PD, probing depth.

Isolation of neutrophils

Peripheral venous blood was collected into vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) containing 25 U/ml heparin. Neutrophils were separated from the peripheral blood by Histopaque (Sigma Chemical Co., St. Louis, MO, USA) gradient centrifugation, according to the manufacturer's instructions. After the neutrophil fraction had been collected and contaminating erythrocytes had been lysed, the isolated neutrophils were suspended in Hank's balanced salt solution (137 mM NaCl, 5.36 mM KCl, 1 mM KH_2PO_4, 1 mM $NaHPO_4$, 0.406 mM $MgSO_4$, and 1.26 mM $CaCl_2$). Cell viability was continuously assessed, and 99% of the cells were trypan blue negative during incubation and stimulation.

Chemotactic migration

The chemotactic migration assay was performed as described previously (Shibata et al. 2000). Neutrophils were suspended in Gey's balanced salt solution supplemented with 2% BSA at a concentration of 2.5×10^6 cells/ml. The cell suspension was placed in the upper compartment of a modified Boyden chamber separated by a 5 mm pore-size micropore filter, while the lower compartment was loaded with either the buffer solution or a chemoattractant solution of FMLP at a concentration of 2×10^{-8} M. The cell migration response was evaluated by enumeration of the cells on the distal surface of the filter after 2 h incubation in a 37°C humidified air chamber. Three representative high-power microscopic fields ($\times$ 400) were counted for each of the triplicate filters. When performed in the absence of FMLP in the lower compartment, the assay provided a measure of random neutrophil migration. The chemotactic migration assay was performed at two different times for each of the 20 Ag-P patients (Ag-P 1–20). The chemotactic migration of neutrophils from the 20 Ag-P patients was expressed as the mean percentage of neutrophils displaying chemotactic migration compared with that of the neutrophils from the matched control group. Ag-P 1–10 and Ag-P 11–20 were classified into Ag-P with neutrophil chemotaxis dysfunction and Ag-P without neutrophil chemotaxis dysfunction, respectively (Table 2). Neutrophils from Ag-P 1–4 were used as neutrophils that displayed defective chemotaxis in the 2D-DIGE.

Sample preparation for 2D-DIGE

The neutrophils were isolated from four Ag-P patients and four matched controls (their total cell numbers were as follows: Ag-P 1: 8.2×10^8, Ag-P 2: 8.5×10^8, Ag-P 3: 7.9×10^8, Ag-P 4: 6.9×10^8, control 1: 8.3×10^8, control 2: 8.1×10^8, control 3: 7.8×10^8, control 4: 8.4×10^8). The isolated neutrophils (1×10^7 cells/ml) were incubated with 4×10^{-8} M FMLP for 15 min. at

Table 2. Chemotaxis in aggressive periodontitis (Ag-P) patients

With neutrophil chemotaxis dysfunction		Without neutrophil chemotaxis dysfunction	
Patient	chemotactic migration % of control)	patient	chemotactic migration % of control)
Ag-P 1	57.1	Ag-P 11	113.3
Ag-P 2	56.5	Ag-P 12	157.1
Ag-P 3	57.2	Ag-P 13	95.5
Ag-P 4	43.3	Ag-P 14	90.9
Ag-P 5	69.2	Ag-P 15	128.3
Ag-P 6	61.1	Ag-P 16	105.2
Ag-P 7	66.7	Ag-P 17	94.6
Ag-P 8	57.1	Ag-P 18	129.4
Ag-P 9	50.0	Ag-P 19	117.8
Ag-P 10	57.9	Ag-P 20	121.4

37°C and then centrifuged at 3000 *g* for 4 min. at 4°C. The cells were then suspended in cell wash buffer (10 mM Tris-Cl and 5 mM magnesium acetate) containing complete EDTA-free protease inhibitors, kept on ice, and sonicated, before being centrifuged at 12,000 *g* for 10 min. at 4°C. The pellet fraction was then resuspended in cell lysis buffer (30 mM Tris-Cl, 2 M thiourea, 7 M urea, and 4% (w/v) CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-2-hydroxy-1-propanesulphonate) for 20 min. at 4°C. The pH of the cell lysate was checked to ensure it remained at pH 8.5, and the lysate was stored at –80°C.

CyDye labelling

Table 3 shows the experimental design for CyDye DIGE. Total proteins were measured using the 2D Quant Kit (GE Healthcare, Tokyo, Japan). Protein labelling with the three CyDye DIGE fluors, Cy2, Cy3, and Cy5 was carried out according to the manufacturer's instructions. Fifty micrograms of protein samples from the control group (control) were labelled with 400 pmol of Cy3, and 50 μg protein samples from the Ag-P patients with neutrophil chemotaxis dysfunction (treated) were labelled with 400 pmol of Cy5. An internal standard consisting of samples from the control group and Ag-P patients used in this experiment was labelled with Cy2. Labelling was performed for 30 min. at 4°C in the dark. Reactions were terminated by the addition of 10 × mM lysine. Total proteins (150 μg) were mixed and suspended in 2 × sample buffer [2 M thiourea, 7 M urea, 2% (v/v) pharmalytes 3–10, 2% (w/v) dithiothreitol (DTT), and 4% (w/v) CHAPS]. This solution was diluted in rehydration buffer [8 M urea, 1% (v/v) pharmalytes 3–10, 13 mM DTT, 4% (w/v) CHAPS, and 0.004% (w/v) bromophenol blue].

2D electrophoresis

The samples were subjected to isoelectric focusing (IEF) using an IPGphor (GE Healthcare) and Immobiline DryStrips (pH 3–10 nl, 24 cm) (GE Healthcare). The strips were then rehydrated for 12 h at 20°C, before IEF was carried out for 1 h (500 v), 1 h (1000 v), and 8.2 h (8000 v). After IEF, the Immobiline DryStrips were incubated in buffer containing 50 mM Tris-HCl (pH 8.8), 6 M urea, 30% (v/v) glycerol, 1% (w/v) sodium dodecyl sulphate (SDS), and 0.25% (w/v) DTT at room temperature for 15 min. followed by an additional 15 min. incubation in buffer containing 50 mM Tris-HCl (pH 8.8), 6 M urea, 30% (v/v) glycerol, 1% (w/v) SDS, and 4.5% (w/v) iodoacetamide.

Equilibrated strips were placed on top of a 1-mm-thick 12.5% SDS-polyacrylamide gel electrophoresis gel, and electrophoresis was carried out for 30 min. (71 V, 168 mA, 12 W) and 5 h (239 V, 399 mA, 95 V) using an Ettan DALTsix Electrophoresis System (GE Healthcare).

Image analysis and data evaluation

Gel 5 was stained using Deep Purple Total Protein Strain (GE Healthcare). All of the images were visualized using a Typhoon 9400 Variable Mode Imager (GE Healthcare). Intra-gel analysis was performed using DeCyder difference in-gel analysis, and inter-gel matching and statistical analysis were performed using DeCyder biological variance analysis (BVA) (GE Healthcare). During the data evaluation, proteins showing a mean ratio >1.5 and a p value of <0.05 on the Student t-test were considered to be significantly different and were automatically picked using an Ettan Spot Picker (GE Healthcare). The gel spots were stored at –20°C.

In-gel digestion

The collected gel spots were incubated in 25 mM ammonium bicarbonate with 50% acetonitrile for 100 min. at room temperature and dried with a vacuum desiccator. The samples were then incubated for 1 h at 56°C in 25 mM ammonium bicarbonate and 10 mM DTT, before 25 mM ammonium bicarbonate and 55 mM iodoacetamide were added. The samples were then incubated for 45 min. at room temperature, and the proteins were digested with a 10 μg/ml solution of trypsin in 50 mM acetic acid and 25 mM ammonium bicarbonate. The peptides were eluted from the gel spots with 50% acetonitrile and 5% trifluoroacetic acid.

Mass spectrometry

The samples were spotted onto an MTP 384 massive target gold-plated T (Bruker Daltonics, Billerica, MA, USA) using a saturated solution of α-cyano-4-hydroxycinnamic acid. MALDI spectra were obtained in the positive ion mode using a Bruker Daltonics Biflex IV MALDI-TOF mass spectrometer. External calibration was carried out using a peptide calibration standard (Bruker Daltonics).

Database search

The peptide mass fingerprints obtained using MALDI-TOF were analysed using Mascot Software and the Swissprot database.

Isolation of total RNA

Neutrophils from 10 Ag-P patients (Ag-P 1–10) with neutrophil chemotaxis dysfunction, 10 Ag-P patients (Ag-P 11–20) without the dysfunction, 15 Ch-P patients, and 15 controls were incubated with 4×10^{-8} M FMLP for 15 min. at 37°C. Total RNA was extracted with ISOGEN® (Wako Pure

Table 3. The experimental design for CyDye DIGE

Gel number	Cy 2	Cy 3	Cy 5	Deep purple
Gel 1	Internal standard	Control 1 (normal subject 1)	Treated 1 (Ag-P 1)	
Gel 2	Internal standard	Control 2 (normal subject 2)	Treated 2 (Ag-P 2)	
Gel 3	Internal standard	Control 3 (normal subject 3)	Treated 3 (Ag-P 3)	
Gel 4	Internal standard	Control 4 (normal subject 4)	Treated 4 (Ag-P 4)	
Gel 5				Control 1–4 Treated 1–4

Internal standard: control 1–4+treated 1–4.
Ag-P, aggressive periodontitis.

Chemical, Osaka, Japan) and quantified by spectrometry at 260 and 280 nm.

Real-time polymerase chain reaction (PCR)

Real-time PCR was performed with an ABI 7700 system (Applied Biosystems, Tokyo, Japan). The reactions were carried out using a Core Reagent Kit (Applied Biosystems), according to the manufacturer's protocol. The TaqMan probe, sense primers, and anti-sense primers used for the detection of caldesmon are listed in Table 4. Commercially available human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Applied Biosystems) was used for the real-time PCR.

Statistical analysis

The Mann–Whitney *U*-test and Student's *t*-test were used for comparative evaluations as indicated. Probability values of <0.05 were considered to be statistically significant.

Table 4. Primers and probes used in real-time PCR

Gene	Sequence
Caldesmon	F: 5′-GGTGAATGCCCAGAACAGTGT-3′ R: 5′-GCGTTTTTGGCGTCTTTCC-3′ P: 5′-AGGCCAAGACAACCACCACAAACACT-3′

F, forward; R, reverse; P, Taqman probe; PCR, polymerase chain reaction.

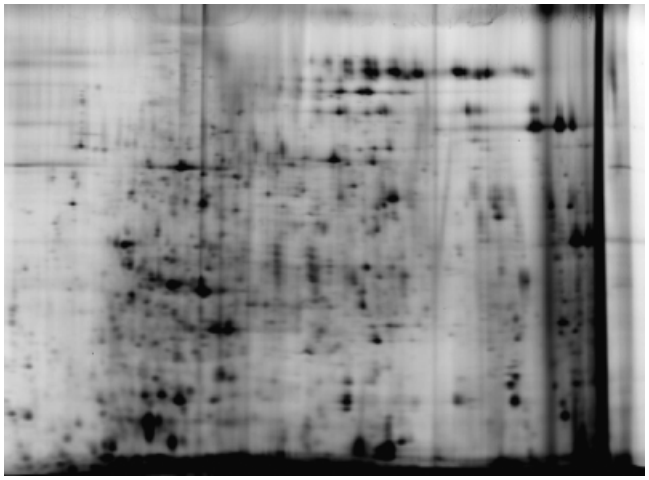

Fig. 1. Deep Purple-stained two-dimensional (2D) gel image. A 2D electrophoresis was performed using 24 cm, pH 3–10 linear IPG strips and 12.5% SDS-PAGE.

Results

Neutrophils from 4 Ag-P patients with neutrophil chemotaxis dysfunction and four controls were used for the proteome analysis. Fluorescent images were analysed using the BIA and BVA programs of the DeCyder software. Two thousand and sixty-four spots were detected using a 2D-DIGE system (Fig. 1). Seven spots were found to show statistically significant differences ($p<0.05$) in expression across all gels ($n=4$). All seven spots showed an increased expression in the neutrophils from the Ag-P patients with neutrophil chemotaxis dysfunction compared with their levels in the neutrophils from the control group. Of these seven protein spots, four spots produced a successful protein match. The four identified spots were lactoferrin, caldesmon, heat shock protein (HSP) 70, and stac, and are listed together with their mean ratio values in Table 5.

Because caldesmon was thought to be the most involved in neutrophil chemotaxis among the identified four proteins, the correlation between caldesmon mRNA levels and neutrophil chemotaxis in the Ag-P patients was analysed. The caldesmon mRNA levels in the neutrophils from the Ag-P patients were negatively correlated with chemotactic activity to FMLP ($r^2=-0.5746$) (Fig. 2).

The caldesmon mRNA expression levels were significantly higher in the neutrophils from the Ag-P patients with neutrophil chemotaxis dysfunction than in those from the Ag-P patients without the dysfunction, Ch-P patients, and control group. However, the neutrophils from some Ag-P patients with the dysfunction did not highly express caldesmon mRNA compared with those from the Ag-P patients without the dysfunction, Ch-P patients, and the control group (Fig. 3).

Discussion

We used FMLP as a neutrophil stimulant. FMLP, a synthetic molecule, can directly elicit neutrophil chemotaxis, enzyme release, and oxidant-free radical production at an inflammatory site. As the functions of neutrophils are disordered in some Ag-P patients (Van Dyke et al. 1980, 1985, Genco et al. 1986, Daniel et al. 1993, Kurihara et al. 1993, Biasi et al. 1999, Gronert et al. 2004, Yagi et al. 2009, Guentsch et al. 2009), FMLP-induced proteins may be

Table 5. Differences in protein expression and protein identification

Spot number	Mean ratio	*p*-value	Protein	No of peptides	Sequence
519	1.92	0.033	Lactoferrin	13	36
1487	1.99	0.026	Caldesmon	5	17
1967	1.50	0.04	HSP70	10	28
2045	1.50	0.017	Stac	5	16

The mean ratio value shows the degree of difference in the standardized abundance between a spot group and the control value.

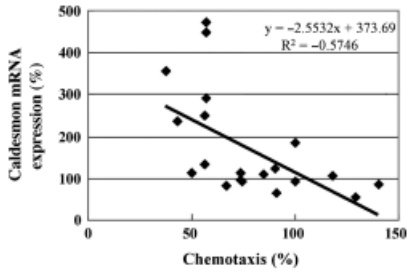

Fig. 2. Negative correlation between chemotactic activity to FMLP and caldesmon mRNA expression in neutrophils from patients with aggressive periodontitis (Ag-P). The chemotactic activity and caldesmon expression values are represented as percentages of the responses obtained from the matched controls. The correlation coefficient is shown in the lower right-hand corner.

related to the disordered functioning of neutrophils.

In this study, by including an internal standard and running neutrophil proteins from Ag-P patients with neutrophil chemotaxis dysfunction and the control group on the same gel, we were able to use the Ettan DIGE system to reduce the variability in the gel and simplify protein spot matching by amplifying the difference in the protein level (Damodaran et al. 2006, Mahnke et al. 2006, Malm et al. 2008). We identified four proteins (lactoferrin, caldesmon, HSP70, and stac) whose expression was upregulated in the neutrophils from the Ag-P patients with the dysfunction compared with those from the control group. However, in this study, we did not detect any proteins whose expression was downregulated in the neutrophils from the Ag-P patients with the dysfunction compared with those from the control group. If neutrophils from more Ag-P patients had been used, proteins whose expression was downregulated in neutrophils from Ag-P patients with neutrophil chemotaxis dysfunction might have been detected.

Lactoferrin is a member of the transferrin family of 80 kDa iron-binding proteins and is present in exocrine secretions, such as milk, tears, and saliva (Lönnerdal & Iyer. 1995, Weinberg 2001). Lactoferrin is released from neutrophils during inflammatory responses (Masson et al. 1969, Eberhard et al. 2006) and demonstrates bacteriostatic activity against a wide range of bacteria using a mechanism that is dependent on chelate iron, which is essential for bacterial growth (Arnold et al. 1977, Arnold et al. 1980). In addition, lactoferrin exhibits non-iron-dependent antibacterial, anti-inflammatory, and immunoregulatory activities (Baveye et al. 1999, Vorland et al. 1999, Conneely 2001, Elass et al. 2002). A recent study showed that lactoferrin effectively inhibits biofilm formation and reduces the size of the biofilms produced by periodontopathic bacteria at physiological concentrations (Wakabayashi et al. 2010). In the present study, the lactoferrin levels in the neutrophils from the Ag-P patients with neutrophil chemotaxis dysfunction were higher than those in the neutrophils from the control group. The present finding is not consistent with previous reports that found that lactoferrin levels were not increased in Ag-P patients (Friedman et al. 1983, Groenink et al. 1999). Further studies are necessary to elucidate a role of lactoferrin in the onset and progression of periodontal disease.

HSP are a family of highly conserved, abundant, and ubiquitous intracellular proteins. HSP are essential for the survival of cells exposed to stress (Craig et al. 1994, Jäättelä 1999). HSP70 has been shown to be an activator of the innate immune system, and the antigen-presenting function of HSP70 is important as an initiator of immune defense. In response to LPS-stimulation, the HSP70 level is increased, and thereafter, the production of pro-inflammatory cytokines is increased (Fincato et al. 1991, Nardai et al. 2006). Moreover, *Porphyromonas gingivalis* lipopolysaccharide stimulation of THP-1 human monocytic cells upregulated the expression of HSP70 (Saba et al. 2007). This phenomenon may explain our finding that the expression of HSP70 was upregulated in the neutrophils from the Ag-P patients with the dysfunction compared with those from the control group. HSP has been shown to be present in periodontal tissues from patients with periodontitis. Some studies have reported that these bacterial HSP are recognized by the immune system (Choi et al. 2004). The extensive homology between human and periodontal pathogen HSP may play a role in autoimmune reactions during periodontitis.

Stac was cloned as a neuron-specific gene encoding a 47 kDa cytosolic protein with a cysteine-rich domain and an src homology three domain, suggesting that it functions as an adapter in a signal transduction cascade (Suzuki et al. 1996, Hardy et al. 2005). However, its exact function remains unclear.

Caldesmon is an actin- and myosin-binding protein that participates in the regulation of actomyosin ATPase (Hayashi et al. 1991, Huber et al. 1998). Caldesmon is an essential component of the cytoskeleton in smooth muscle and non-muscle cells (Sobue & Sellers 1991). It has been reported that caldesmon is related to cell migratory responses, contraction, and division (Yamboliev & Gerthoffer 2001). Intriguingly, caldesmon overexpression inhibits VEGF-stimulated endothelial cell migration into the wound breach and delays wound closure (Mirzapoiazova et al. 2005). A recent study showed that caldesmon plays a pivotal role in cell migration via reorganization of the actin cytoskeleton in response to glucocorticoids (Mayanagi et al. 2008, Sobue & Fukumoto 2010). The binding of caldesmon to actin-inhibited actomyosin interactions and resulted in the inhibition of many cellular processes (migration, adhesion, and proliferation) (Lin et al. 2009). In neutrophils from Ag-P patients with neutrophil chemotaxis dysfunction, caldesmon may suppress their motility by stabilizing structures composed of filamentous actin.

From the reports mentioned above, among the four identified proteins, caldesmon is most likely to be involved in neutrophil chemotaxis. In this study, the mRNA levels of caldesmon in neutrophils from Ag-P patients were negatively correlated with their chemotactic activity to FMLP. Furthermore, the caldesmon mRNA expression levels in neutrophils from Ag-P patients with

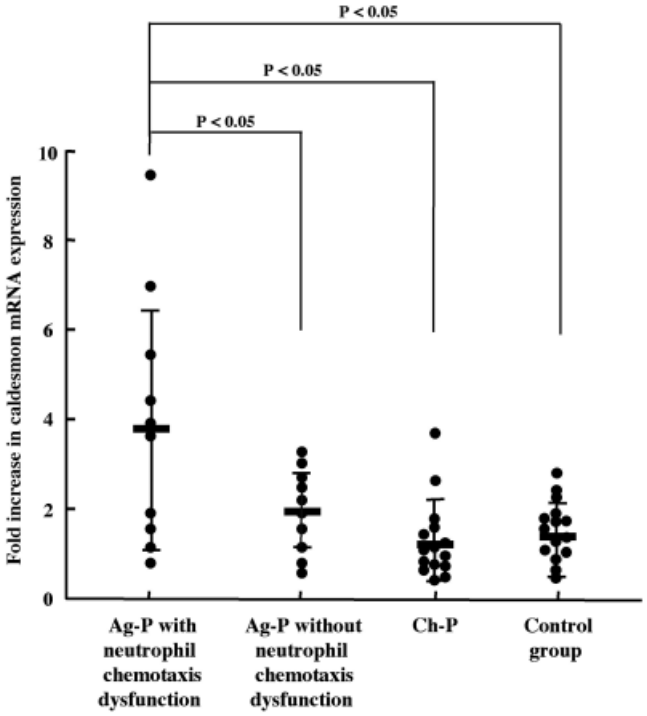

Fig. 3. Caldesmon expression in neutrophils from aggressive periodontitis (Ag-P) patients with neutrophil chemotaxis dysfunction, Ag-P patients without the dysfunction, chronic periodontitis (Ch-P) patients, and the control group. Total RNA was extracted from neutrophils that had been stimulated with 10^{-7} M FMLP for 15 min. The results are shown as the fold increase in the expression of the individual mRNA normalized to the GAPDH expression level and were compared with the target-internal control using the cycle threshold method. Values are means ± SD (bars).

neutrophil chemotaxis dysfunction were higher than those in neutrophils from Ag-P patients without the dysfunction, Ch-P patients, and the control group. A high expression level of caldesmon may be an important determinant of neutrophil dysfunction in Ag-P patients. However, some Ag-P patients with the neutrophil chemotaxis dysfunction did not show a high level of caldesmon mRNA expression. It is assumed that the levels of other proteins are also related to neutrophil chemotaxis dysfunction in Ag-P patients. Moreover, it remains unclear whether abnormal caldesmon production is the primary pathogenic mechanism in Ag-P patients and how increased levels of caldesmon are involved in other signal transduction abnormalities in neutrophils.

In summary, we have examined protein production in neutrophils from Ag-P patients with neutrophil chemotaxis dysfunction and a control group using proteome analysis. The levels of caldesmon, lactoferrin, HSP70, and stac in neutrophils from Ag-P patients with the dysfunction were higher than those in neutrophils from the control group. In particular, caldesmon is a candidate marker of Ag-P combined with neutrophil chemotaxis dysfunction.

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Clinical Relevance

Scientific rationale for the study: Patients with Ag-P show rapid and severe periodontal tissue destruction. Some patients suffering from Ag-P display neutrophil chemotaxis dysfunction. However, the pathogenic mechanism of this Ag-P has not been fully elucidated. Therefore, the establishment of a diagnostic marker for Ag-P is desired.

Principal findings: Using a 2D-DIGE system and MALDI-TOF mass spectrometry, four proteins (lactoferrin, caldesmon, HSP70, and stac) related to Ag-P combined with neutrophil chemotaxis dysfunction were identified.

Practical implications: The four identified proteins may be useful markers of Ag-P.