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Original Article

Identification of 62-kDa Protein as an Immunogenic Antigen of *Vibrio vulnificus* for Humans

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Abstract *Vibrio vulnificus* infection can cause necrotizing fasciitis and sepsis and can develop within a few days despite intensive care. The mortality rate is up to 60% in vulnerable people. Most patients infected with this microbe have chronic liver disease, especially liver cirrhosis or cancer, as an underlying disease. *V. vulnificus* infection is opportunistic, and there is an urgent need to develop an anti-*V. vulnificus* vaccine. Thus, it is important to identify immunogenic antigens. We collected human sera from three subject groups : patients with *V. vulnificus* infection, patients with chronic liver disease but without *V. vulnificus* infection, and healthy volunteers with normal liver function. Immunoblots of cytosolic and membrane proteins of seven strains of *V. vulnificus* and one of *V. parahaemolyticus* were performed with sera from these groups. Although we could not demonstrate differences in antibody response between the groups, all sera showed a strong antibody response to a 62-kDa protein that was common to all strains examined. Immunoblots of *Escherichia coli* and *Klebsiella pneumoniae* also showed strong antibody response to this 62-kDa protein, and the possibility of cross-reaction cannot be denied. We identified this 62-kDa protein as an immunogenic antigen of *V. vulnificus* for humans.

Key words : *Vibrio vulnificus* · Immunogenic antigen · Immunoblotting

Introduction

Vibrio vulnificus is found globally in marine coastal waters. It prefers low salinity and warm water temperatures for optimum growth. Infection with this organism, which can occur through ingestion of raw shellfish or exposure to marine water, can cause necrotizing fasciitis and sepsis and can develop within a few days despite intensive care. The mortality rate is up to 60% in vulnerable people[1)]. Most patients infected with this microbe have chronic liver disease, especially liver cirrhosis, as an underlying disease[2)]. *V. vulnificus* infection is opportunistic, and there is an urgent need to develop an anti-*V. vulnificus* vaccine.

The outer membrane proteins (OMPs) are highly immunogenic components because of their exposed epitopes on the cell surface. Some OMPs of *Vibrio* species such as OmpW, OmpV, OmpU, and OmpK can induce protective immunity[3)4)]. However, it is important to identify immunogenic antigens for the development of a reliable vaccine against *V. vulnificus*. We performed immunoblotting to identify immunogenic antigens and analyzed IgG antibody production with immunoblots of

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V. vulnificus proteins from the sera of 10 *V. vulnificus*-infected patients (VUL). VUL might be expected to have developed immunologic responses to *V. vulnificus* proteins as a result of subclinical infection. Therefore, we compared two groups without *V. vulnificus* infection, chronic liver disease patients (CLD) as the high-risk control and normal liver function subjects (NLF) as the low-risk control, against VUL. Immunoblotting of cytosolic and membrane proteins of seven strains of *V. vulnificus* and one of *V. parahaemolyticus* was performed with sera from these three groups. We analyzed differences in antigen-antibody reaction according to origins of the *V. vulnificus* strain or host liver function, respectively.

Materials and Methods

Subjects and sera

Sera were collected from the VUL (n = 10), CLD (n = 26), and NLF (n = 10), all of whom were patients of our hospital or our associated hospitals. We collected patient information, including sex, age, and underlying disease, from patient medical records. Information regarding critical infections and outcomes was also obtained for the VUL group. Between the VUL, 90% were men, and the mean age (± standard deviation) of the group was 65.1 (± 17.7) years. Eight of the 10 VUL had underlying liver disease ; particularly, 7 of these 8 patients had liver cirrhosis. The remaining 2 patients had malabsorption syndrome or diabetes mellitus as their underlying disease, respectively. The critical infection was primary septicemia in 7 patients, wound infection in 1, and unknown in 2. Four patients died. For the VUL, 42 sera collected on different days were available from the 10 patients (Table 1). The day of onset of *V. vulnificus* infection was termed day 1. Both the sera from the onset phase (day 1 or day 2) and immune phase (2 weeks after onset) were available from 4 VUL. Sera from the onset phase only were available from 4 other patients, and sera from the immune phase only were available from the remaining 2 patients. Sera from VUL-1 and VUL-10 were especially valuable because a number of serial sera were available from these 2 patients. Sera were also collected from each of the CLD and NLF. Men comprised 76.9% of CLD and 60% of NLF. The mean ages (± standard deviation) of the CLD and NLF groups were 64.7 (± 9.2) and 62.7 (± 9.4) years, respectively, and no significant difference was observed between the three groups ($p = 0.876$). Between the CLD, 88.5% had liver cirrhosis. All samples were stored at-80℃ until use. This clinical study was approved by the Institutional Ethics Board of our university (Unique registration number : 17-14, 20-32), and informed consent was obtained from all subjects prior to their inclusion in the study.

Table 1 Outcomes and serum collection days of *V. vulnificus*-infected patients

Patient no.	Outcome	Collection day
1	Dead	1, 2, 5, 7, 8, 12, 15, 18, 25, 31, 33
2	Dead	1, 4
3	Dead	2
4	Dead	1, 2, 3
5	Cured	1, 2, 3
6	Cured	2
		1 and 2 months after onset
7	Cured	2
		1 month after onset
8	Cured	1 month after onset
9	Cured	2 and 3 months after onset
10	Cured	1, 2, 3, 4, 5, 6, 7, 9, 11, 12, 14, 16, 17, 19

Bacterial strains

Seven strains of *V. vulnificus* and one strain of *V. parahaemolyticus* were used in this study (Table 2-1). The *V. vulnificus* strains comprised five clinical strains, one environmental strain isolated from the Ariake Sea, Japan, and the type strain (T) of the species (ATCC27562$_T$). The five clinical strains were isolated from patients in our hospital : two strains were isolated from patients who died (vul1d, vul2d), and three were isolated from cured patients (vul5c, vul6c, vul7c). The one strain of *V. parahaemolyticus* was ATCC17802$_T$. Genomic DNA sequence analysis of the 16S rRNA genotype of each strain except those in the ATCC$_T$ was determined as described by Nilsson et al. with minor modifications[5]. Briefly, chromosomal DNA for each strain was prepared with the QIAmp DNA Mini Kit (QIAGEN, Germany). A 492-bp segment of 16S rDNA of *V. vulnificus* was targeted for amplification by PCR using primers 8-519-S : 5'-AGA GTT TGA TCC TGG CTC AG-3' and 8-519-A : 5'-ATT ACC GCS GCT GCT G-3', and Taq DNA Polymerase (Promega Corporation, WI, USA). The amplified PCR products were separated in gel and purified using the QIAquick PCR Purification Kit (QIAGEN). Nucleotide sequences of the products were BLASTed against the database in GenBank. Besides *Vibrio, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia,* and *Pseudomonas aeruginosa* were used as controls (Table 2-2).

Sample preparation

All strains were grown on sheep blood agar plates at 37℃ overnight. To recognize the location of antigens with *Vibrio*, the strains were separated into cytosolic and membrane proteins. Briefly, bacterial cultures were washed three times with 20 mM Tris-HCl (pH 7.5) and disrupted by sonication. After centrifugation at 5000 × g for 10 min, the supernatants were collected as cytosolic protein by centrifugation at 230000 × g at 4℃ for 1 h. The pellets were resuspended in 20 mM Tris-HCl (pH 7.5) and recentrifuged as above. After resuspension in 20 mM Tris-HCl (pH 7.5) and 1% TritonX-100 (Nacalai Tesque, Kyoto, Japan), bacterial cultures were shaken gently at 4℃ for 12 h. The supernatants were collected as membrane protein by centrifugation at 330000 × g at 4℃ for 1 h. To recognize the differences in antigen-antibody reaction with microbes besides *Vibrio*, whole proteins of *E. coli, S. aureus, K. pneumonia, P. aeruginosa,* and ATCC27562$_T$ were prepared. The protein concentrations were measured using a bicinchoninic acid (BCA) protein assay reagent

Table 2-1 Bacterial strains of *Vibrio* used for immunoblotting

Strain name	Source	16S rRNA
vul1d	Clinical (from VUL-1, dead patient)	*V. vulnificus*
vul2d	Clinical (from VUL-2, dead patient)	*V. vulnificus*
vul5c	Clinical (from VUL-5, cured patient)	*V. vulnificus*
vul6c	Clinical (from VUL-6, cured patient)	*V. vulnificus*
vul7c	Clinical (from VUL-7, cured patient)	*V. vulnificus*
Marine	Environment (from Ariake Sea, Japan)	*V. vulnificus*
ATCC27562$_T$	Clinical (from ATCC, *V. vulnificus*)	
ATCC17802$_T$	Clinical (from ATCC, *V. parahaemolyticus*)	

VUL/vul = *V. vulnificus*-infected patient, d = dead, c = cured, T = type strain

Table 2-2 Bacterial strains besides *Vibrio* used for immunoblotting

Strain name	Source
ATCC25922$_T$	Clinical (from ATCC, *Escherichia coli*)
ATCC29213$_T$	Clinical (from ATCC, *Staphylococcus aureus*)
ATCC13883$_T$	Clinical (from ATCC, *Klebsiella pneumoniae*)
ATCC27853$_T$	Clinical (from ATCC, *Pseudomonas aeruginosa*)

T = type strain

kit (Pierce Biotechnology, IL, USA).

To analyze differences in antigen-antibody reaction according to origins of the *V. vulnificus* strain or to host liver function, we performed immunoblots of cytosolic and membrane proteins of all eight *Vibrio* strains with all sera with the E-PAGE™ 48 protein electrophoresis system (Invitrogen Corporation, CA, USA). To analyze detailed molecular weight (MW) and differences in the appearance of IgG antibody production in VUL, we also performed immunoblots of cytosolic and membrane proteins of ATCC27562$_T$ with VUL sera with the NuPAGE® Bis-Tris gel system (Invitrogen). To analyze differences in antigen-antibody reaction to origins of the microbe, we also performed immunoblots of whole proteins of *E. coli, S. aureus, K. pneumonia, P. aeruginosa,* and ATCC27562$_T$ with sera with the NuPAGE® Bis-Tris gel system.

Immunoblotting with the E-PAGE™ 48 protein electrophoresis system and iBlot™ dry blotting system

Samples were mixed with E-PAGE™ loading buffer 4 × (Invitrogen) and NuPAGE® sample reducing agent 10 × (Invitrogen) and heated for 5 min. Ten micrograms of protein was applied per well and subjected to electrophoresis according to the manufacturer's directions[6]. SeeBlue® Plus2 Pre-Stained Standard (Invitrogen) and MagicMark™ XP Western Protein Standard (Invitrogen) were used as size markers for Western blotting. Standard proteins in MagicMark™ XP Western Protein Standard contain repetitive units of a fusion protein forming the size variation and have an IgG binding site that allows direct visualization of protein standard bands on a blot without the need for protein modification or special detection reagents. For immunoblotting, protein was transferred onto nitrocellulose membranes with the iBlot™ dry blotting system (Invitrogen). The membranes were then blocked with 0.05% Tween-20 in Tris buffered saline (TBS-T) containing 5% skimmed milk at room temperature for 2 h and washed three times with TBS-T. Sera diluted 1 : 1000 were added as primary antibodies and incubated at 4℃ overnight. The membranes were washed as above and rabbit-anti-human IgG HRP conjugate (Chemicon International, CA, USA) diluted 1 : 10000 was added as secondary antibody and incubated for 2 h. The membranes were washed and then developed for 5 min at room temperature with ECL Plus Western Blotting Detection Reagents (GE Healthcare Bio-Sciences Corp., NJ, USA). The chemiluminescence signals were detected with the VersaDoc™ imaging system model 5000 (Bio-Rad, CA, USA). The relative densities of the bands were densitometrically quantified with Quantity One software (version 4.4 ; Bio-Rad) and normalized to the density of the 60-kDa band in MagicMark™ XP Western Protein Standard.

Immunoblotting with the NuPAGE® Bis-Tris gel system with *Vibrio*

Samples were mixed with NuPAGE® LDS sample buffer 4 × (Invitrogen) and NuPAGE® sample reducing agent 10 × and heated for 5 min. Electrophoresis was performed using the NuPAGE® Bis-Tris gel 1.0 mm × 10 well with the strain of ATCC27562$_T$. Ten micrograms of protein was applied per well, and MagicMark™ XP Western Standard was added. Electrophoresis was performed with the XCell *SureLock*™ mini-cell system (Invitrogen) in NuPAGE® MOPS SDS running buffer (Invitrogen) and NuPAGE® antioxidant (Invitrogen) at 200 V for 50 min. For immunoblotting, protein was transferred at 20 V for 60 min onto PVDF membranes with Trans-blot SD semi-dry transfer cell (Bio-Rad). VUL sera diluted 1 : 1000 were added as primary antibodies, and the membranes were scanned using an LAS-3000 image reader (FUJIFILM, Tokyo, Japan). The relative densities of the bands were densitometrically quantified with Multi Gauge (version 3.1 ; FUJIFILM) and normalized to the density of the 60-kDa band in MagicMark™

XP Western Protein Standard.

Immunoblotting with the NuPAGE® Bis-Tris gel system with microbes besides *Vibrio*

Samples were mixed with NuPAGE® LDS sample buffer 4 × and NuPAGE® sample reducing agent 10 × and heated for 5 min. Mark12™ Unstained standard (Invitrogen) and MagicMark™ XP Western Protein Standard were used as size markers for Western blotting. Some sera diluted 1 : 1000 were added as primary antibodies, and the membranes were scanned using an LAS-3000 image reader.

Statistical analysis

Statistical differences between the three groups were analyzed by analysis of variance following one-way ANOVA, and p values of < 0.05 were taken to indicate statistical significance. These analyses were conducted with StatView version 5.0J (SAS Institute Japan, Tokyo, Japan).

Results

Differences in antigen-antibody reaction according to origins of the *V. vulnificus* strain or to host liver function with the E-PAGE™ 48 protein electrophoresis system and iBlot™ dry blotting system

The raw data of immunoblots with sera prepared from VULs in whom sera from both the onset and immune phases were available are shown in Fig. 1-1. The patterns observed by immunoblotting with sera from the onset phase were almost identical in all samples. All four onset-phase sera reacted with an approximately 60-kDa band (major band) produced by all eight strains with cytosolic and membrane proteins, irrespective of the origin of the strain. Onset-phase sera also reacted with an approximately 80-kDa band produced by several strains with cytosolic and membrane proteins. Immune sera from VUL-1 and VUL-7 reacted with an approximately 30-kDa or just below a 20-kDa band present in membrane protein preparations (arrows in Fig. 1-1).

The raw data of immunoblots with sera of VULs in whom sera were available from either the onset or the immune phase are shown in Fig. 1-2. All sera commonly reacted with an approximately 60-kDa band (major band) produced by all strains with cytosolic and membrane proteins. Onset-phase sera also reacted with an approximately 80-kDa band produced by several strains with cytosolic and membrane proteins. Immune sera from VUL-8 reacted with an approximately 30-kDa and just below a 20-kDa band present in membrane protein preparations (arrows in Fig. 1-2).

Typical immunoblots with sera from CLD and NLF are shown in Fig. 1-3. All sera commonly reacted with an approximately 60-kDa band (major band) produced by all strains with cytosolic and membrane proteins. Sera also reacted with an approximately 80-kDa band produced by several strains with cytosolic and membrane proteins.

We compared the normalized density of the 60-kDa major band in immunoblots between VUL, CLD, and NLF (Table 3). For analysis of VUL, we selected eight samples from the initial onset phase. No significant differences in the density of the 60-kDa major band were seen between the three groups.

Detailed MW and the appearance of IgG antibody production to ATCC27562$_T$ in VUL with the NuPAGE® Bis-Tris gel system

We analyzed the kinetics of IgG antibody production to ATCC27562$_T$ in immunoblots with sera from VUL. The raw data of immunoblots of cytosolic and membrane proteins from ATCC27562$_T$ with VUL-1 sera are shown in Fig. 2A. Detailed MWs of the major bands of approximately 80, 60, and below 20 kDa were 82, 62, and 16 kDa, respectively. VUL-1 sera reacted with several minor bands including those of 125, 94, 72, 52, 48/46, 40, 38, 36, 32, 31, 29, 28, and 20 kDa. Kinetic analysis of normalized band densities revealed that VUL-1 had increased levels of antibodies to the 62-kDa protein that is present

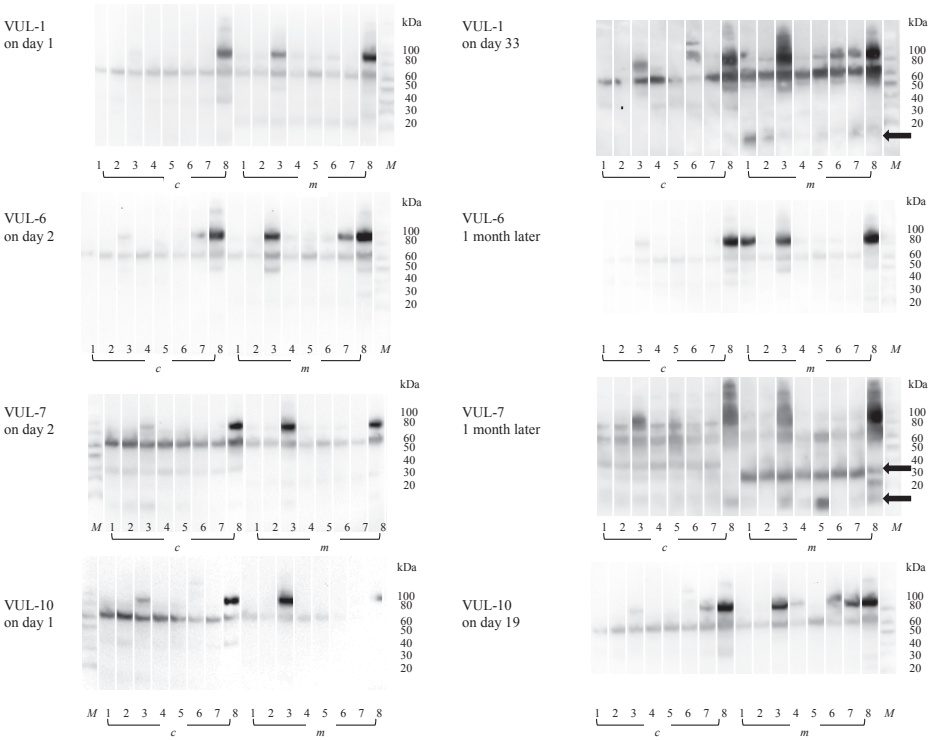

Fig. 1-1 Immunoblots of proteins with sera from *V. vulnificus*-infected patients (VUL) in whom sera from both the onset and immune phases were available. Ten micrograms of cytosolic and membrane proteins from *V. vulnificus* and *V. parahaemolyticus* were applied per well and subjected to immunoblotting with the E-PAGE^{TM} 48 protein electrophoresis system and with the iBlot^{TM} dry blotting system. Arrows indicate that IgG antibody production appeared. The bands were at approximately 30 or below 20 kDa. Numbers at the bottom of lanes indicate strains : 1, vul1d ; 2, vul2d ; 3, vul5c ; 4, vul6c ; 5, vul7c ; 6, Marine ; 7, ATCC27562$_T$; 8, ATCC17802$_T$. *c* = cytosolic proteins, *m* =membrane proteins, *M* = molecular marker.

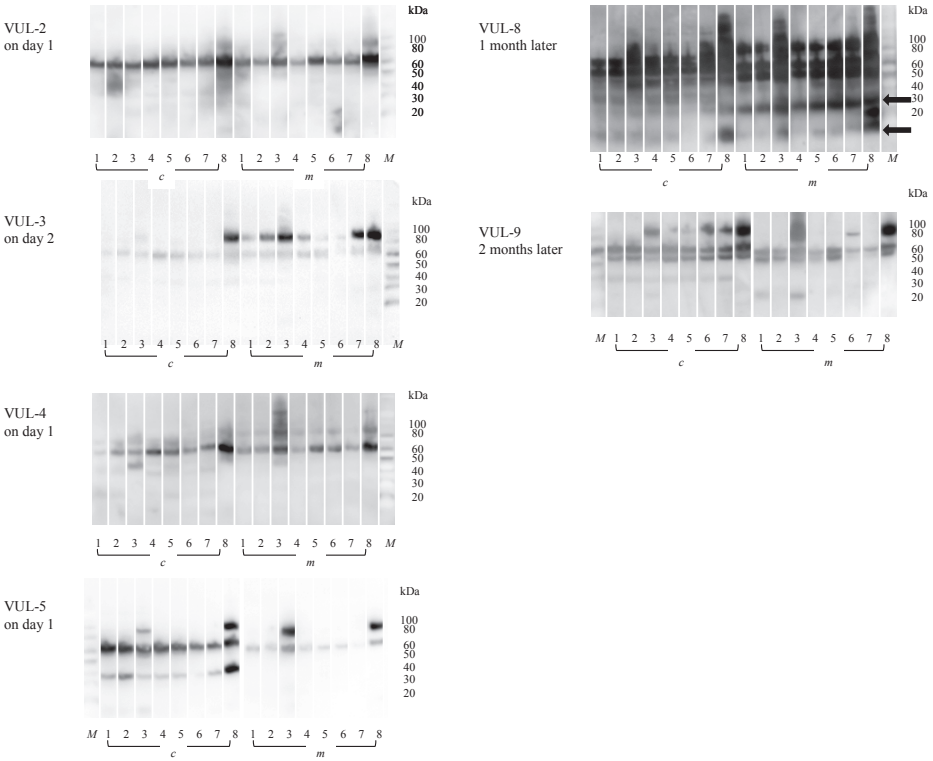

Fig. 1-2 Immunoblots of proteins with sera from *V. vulnificus*-infected patients (VUL) in whom sera from either the onset or the immune phase were available. Description and abbreviations as in Fig. 1-1.

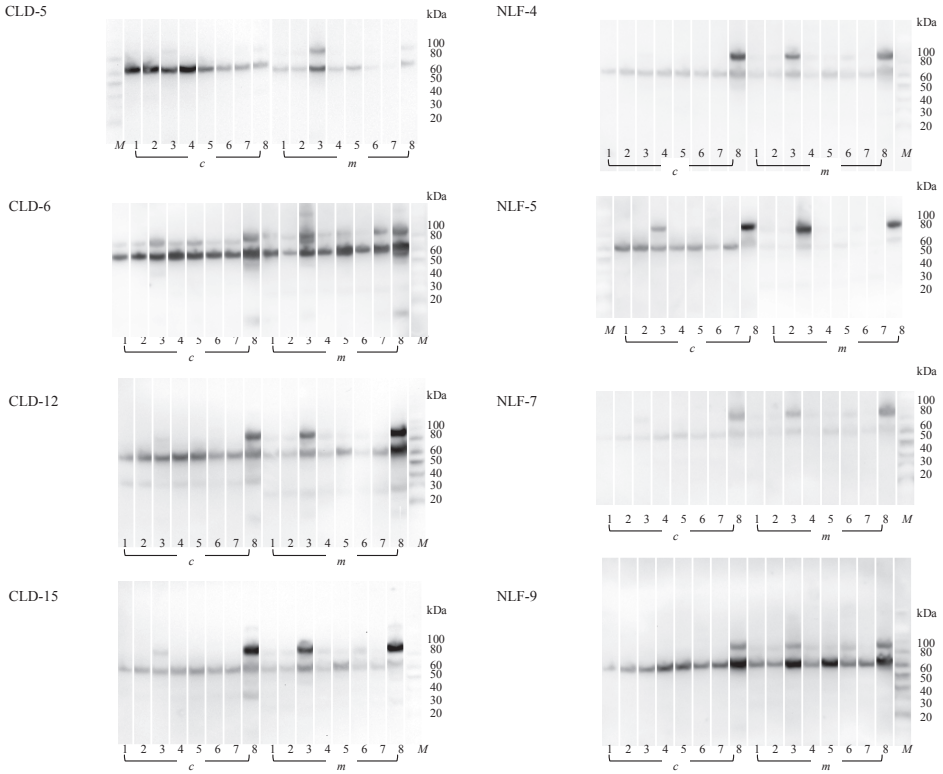

Fig. 1-3 Immunoblots of proteins with sera from chronic liver disease patients (CLD) and normal liver function (NLF) subjects. Abbreviations as in Fig. 1-1. There are no bands at approximately 30 or below 20 kDa with any CLD or NLF sera.

Table 3 Densities of the approximately 60-kDa major band on immunoblots of VUL, CLD, and NLF

	Strain name	VUL (unit)	CLD (unit)	NLF (unit)	*p* value
Cytosolic proteins	vul1d	12437	12702	8801	0.471
	vul2d	16480	14710	10205	0.346
	vul5c	15634	15237	10101	0.303
	vul6c	18274	17869	10298	0.166
	vul7c	15792	17963	10739	0.22
	Marine	12235	15743	8988	0.232
	ATCC27562$_T$	11487	14547	7888	0.157
	ATCC17802$_T$	15830	20196	11772	0.259
Membrane proteins	vul1d	9676	15107	7400	0.062
	vul2d	8840	13985	6972	0.058
	vul5c	15491	24642	11459	0.157
	vul6c	8805	13547	6835	0.093
	vul7c	11588	17192	8656	0.225
	Marine	9960	12354	7695	0.441
	ATCC27562$_T$	8650	11925	7120	0.324
	ATCC17802$_T$	14968	19508	9553	0.304

Immunoblotting with sera from VUL, CLD, and NL F was performed with the E-PAGE^{TM} 48 protein electrophoresis system and iBlot^{TM} dry blotting system. Relative densities of the approximately 60-kDa major bands were quantified after being normalized to the density of 60-kDa band in MagicMark^{TM} XP Western Protein Standard. Values of VUL, CLD, and NLF are expressed as means. VUL include the sera of VUL-1 on day 1, VUL-2 on day 1, VUL-3 on day 2, VUL-4 on day 1, VUL-5 on day 1, VUL-6 on day 2, VUL-7 on day 2, and VUL-10 on day 1.
VUL/vul = *V. vulnificus*-infected patients, CLD = chronic liver disease patients, NLF = normal liver function subjects, d = dead, c = cured, T = type strain

both in the cytosol and the membrane and to the 16-kDa protein present in the membrane, especially from day 12 after onset (Fig. 2A, B). Increases in the levels of antibodies to other bands were unclear. In VUL-10, increases in the levels of antibodies to any bands were unclear (data not shown). Detailed MWs from immune sera of VUL-7 and VUL-8, which reacted with the approximately 30-and below 20-kDa bands, were 29 and 16 kDa, respectively (data not shown).

Differences in antigen-antibody reaction according to origins of microbes

We performed immunoblots of whole proteins of *E. coli, S. aureus, K. pneumonia, P. aeruginosa,* and ATCC27562$_T$ with some sera. Typical immunoblots with sera CLD-25, NLF-4, and NLF-9 are shown in Fig. 3. The 62-kDa major bands (black circles) indicated for *E. coli* and *K. pneumonia* are similar to those for ATCC27562$_T$.

Discussion

We performed immunoblotting of *V. vulnificus* proteins with human sera including that from 10 patients with *V. vulnificus* infection to identify immunogenic antigens. We considered that origins of *V. vulnificus* strains or host liver function might be expected to affect differences in antigen-antibody reaction.

Because clinical strains might be expected to have specific antigens, we selected clinical strains that included the type strain and environmental strains. Clinical strains were isolated from patients who both died from and who were cured of *V. vulnificus* infection. To detect the location of antigenic proteins, cytosolic and membrane proteins were separated. Biosca et al. reported antigenic response to OMPs of 66, 60, 48, 46, and 44 kDa in immunoblots using antibodies raised in rabbits injected intravenously with formalin-killed whole cells of several *V. vulnificus* strains[7]. The bands were common to all *V. vulnificus* strains examined, independent of origin. Wright et al. found that immune sera from a patient with septicemia produced by *V. vulnificus* biotype 1 showed a strong antigenic response to 66-kDa outer membrane proteins, which were common to all biotype 1 strains examined[8]. Although we could not detect apparently different proteins between the immunoblots in any of the *V. vulnificus* strains, we found the 62-kDa protein commonly detected by all groups of human sera independent of strain origin.

Most patients with *V. vulnificus* infection have chronic liver disease, especially liver cirrhosis, as an underlying disease. In patients with liver cirrhosis, it was reported that reticuloendothelial system functions and sera opsonic activities are decreased[9][10] and that bacterial translocation occurs according to portal hypertension, and endotoxin outflow to the systemic circulation is dependent on portal vein-hepatic vein shunt. In addition, systemic iron overload leads to conditions susceptible to infection[11]. VUL might be expected to have developed immunologic responses to *V. vulnificus* proteins as a result of subclinical infection, and as control groups with no history of clinical *V. vulnificus* infection, CLD might also be expected to have developed an immunologic response, whereas NLF would not. Although we could not demonstrate differences in antibody response between the three groups, all sera showed a strong antigenic response to the 62-kDa protein that was common to all strains examined. Indeed, VUL-1 had increased levels of antibodies to the 62-kDa band. Immunoblots of *E. coli* and *K. pneumonia* also showed strong antibody response to the 62-kDa protein, and the possibility of cross-reaction cannot be denied. We identified the 62-kDa protein to likely be an immunogenic antigen in *V. vulnificus* for humans. Except in VUL-1, increases in the levels of antibodies to the 62-kDa major bands with VUL sera were unclear. We speculate that IgG antibody production was suppressed by antibiotics that were administered to all VUL.

Some of the immune-phase sera in the VUL reacted with the 29-and 16-kDa membrane

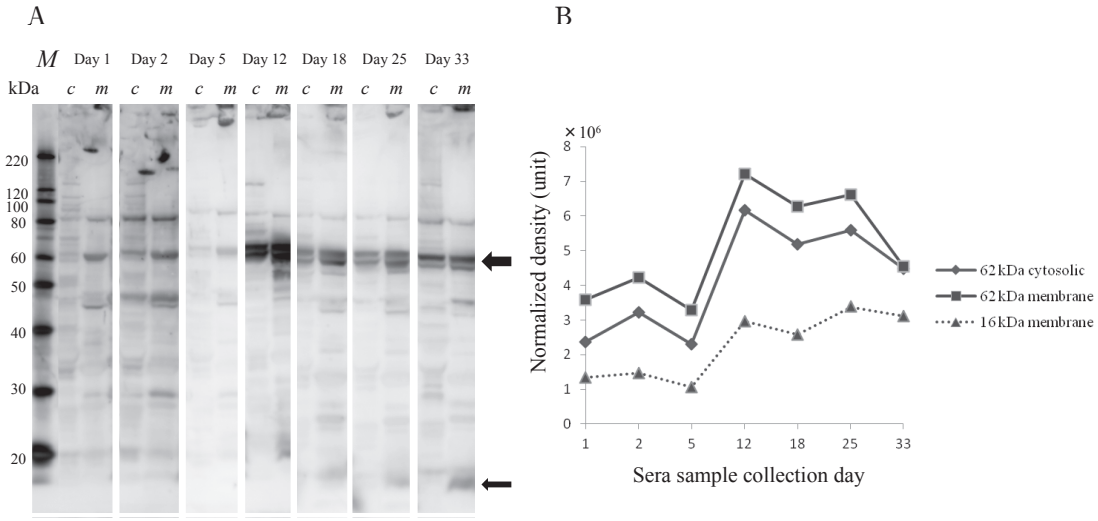

Fig. 2 (A) Immunoblots of proteins with VUL-1 sera. Ten micrograms of cytosolic and membrane proteins from ATCC27562$_T$ were applied per well and subjected to immunoblotting with the NuPAGE® Bis-Tris gel system. Arrows indicate that IgG antibody production appeared. The 62-kDa major band (thick arrow) and the 16-kDa band (thin arrow) are indicated. (B) Kinetic analysis of IgG antibody production in VUL-1 sera against the 62-and 16-kDa proteins of ATCC27562$_T$. Density of the bands in immunoblots was normalized to the density of the 60-kDa band in MagicMark™ XP Western Protein Standard in each of the blots. *c* = cytosolic proteins, *m* = membrane proteins, *M* = molecular marker.

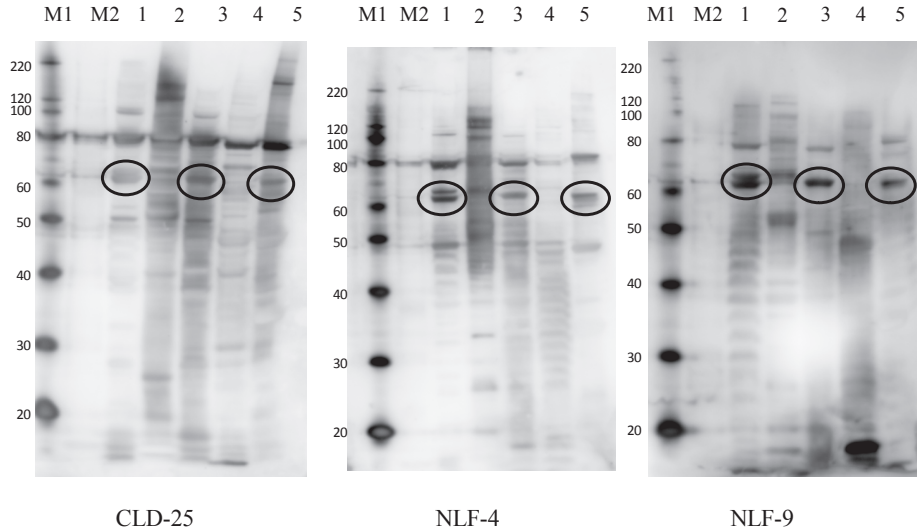

Fig. 3 Immunoblots of proteins with sera CLD-25, NLF-4, and NLF-9. Ten micrograms of whole proteins from ATCC27562$_T$ and other microbes besides *Vibrio* were applied per well and subjected to immunoblotting with the NuPAGE® Bis-Tris gel system. 1, *Escherichia coli* ; 2, *Staphylococcus aureus* ; 3, *Klebsiella pneumonia* ; 4, *Pseudomonas aeruginosa* ; and 5, ATCC27562$_T$. M1 = molecular marker, MagicMark™ XP Western Protein Standard ; M2 = molecular marker, Mark12™ Unstained standard. The 62-kDa major bands (black circles) are indicated.

proteins, which could be promising as immunogenic antigens. It is known that some OMPs of *Vibrio* species such as OmpW, OmpV, OmpU, and OmpK can induce protective immunity. OMPs change immediately and dramatically to adjust to external conditions. OmpW and OmpV are related to osmoregulation in *V. parahaemolyticus* and *V. alginolyticus* and appear immunogenic in *V. cholerae*[12)~14)]. OmpU is a porin that plays a critical role for survival and colonization in *Vibrio* species in relation to resistance to bile salts in the intestine and to adherence to host cells[15)16)] and has been considered one of the important protective antigens in *V. cholerae*. OmpK is likely to be a genus-specific antigen of *Vibrio* species and is recognized as the receptor of broad-host-range vibriophage KVP40[17)~19)]. Amino acid sequence alignments showed that OmpW, OmpV, and OmpU of *V. parahaemolyticus* shared high identity of greater than 60% with those of *V. alginolyticus, V. cholerae,* and *V. vulnificus,* suggesting that such proteins may play similar roles during infection and have the same opportunities to be exposed to the host immune system[20)]. The patterns of the immunoblots of *V. vulnificus* and *V. parahaemolyticus,* including the bands of the 62-, 29-, and 16-kDa proteins, were almost identical in the present study. Estimated relative mobilities of OmpW, OmpV, OmpU, and OmpK varied between 23 to 37 kDa. Therefore, the 29-kDa protein that we found could be one of the Omps.

To our knowledge, immunoblots of proteins from *V. vulnificus* from many human sera samples including those from VUL have not been reported previously. The present results suggest that the 62-kDa protein is likely to be an immunogenic antigen of *V. vulnificus* for humans.

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Conflict of interest : None.

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（和文抄録）

Vibrio vulnificus の 62-kDa の蛋白がヒトにおける免疫原性の高い抗原の一つと考えられた

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Vibrio vulnificus（*V. vulnificus*）は，世界中の沿岸海水中に広く生息し，本菌に汚染された魚介類の生食や創傷からの菌の侵入により感染する．発熱，四肢の壊死性筋膜炎等の症状を呈し，短期間で死に至る極めて予後不良の疾患である．*V. vulnificus* 感染症患者の多くは基礎疾患に肝硬変や肝癌等の肝機能障害を有する．日和見感染症である本症において有効なワクチンの開発が重要であり，免疫原性の高い抗原の同定が必要である．今回我々は，*V. vulnificus* のヒト血清における免疫原性の高い抗原を確認することを目的とし，*V. vulnificus* 感染症患者 10 名の血清を用いた菌溶解液（*V. vulnificus* 7 株，*V. parahaemolyticus* 1 株）の immunoblotting を施行し，感染による抗体産生を分析した．発症時からすでに見られる 62-kDa の band は，患者のみならず *V. vulnificus* 感染症未発症の慢性肝機能障害者及び肝機能正常者のいずれの血清を用いた immunoblotting においても共通して認められた．また，*Escherichia.coli* や *Klebsiella pneumoniae* を用いた immunoblotting においても同様の反応を認め，交差反応を起こしている可能性も考えられた．今回の実験から，*V. vulnificus* の 62-kDa の蛋白がヒトにおける免疫原性の高い抗原の一つであることが予想された．