J. Microbiol. Biotechnol. (2000), **10**(2). 137–142

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Expression of Heat Shock Protein 70 in Umbilical Vein Endothelial Cells Infected by *Staphylococcus aureus*

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Received: April 13, 1999

Abstract Environmental stress is known to induce heat shock proteins (HSPs) in eukaryotic cells. However, the induction of HSPs in host cells by microbial infection has not yet been well explained. *Staphylococcus aureus* (*S. aureus*) is one of the major pathogens in the pathogenesis of endovascular diseases such as infective endocarditis. In this study, the synthesis of stress-inducible 70 kDa HSP was investigated in the endothelial cells (ECs) after 3 h to 20 h of incubation with *S. aureus*. The effect of *S. aureus* infection on the expression of HSP70 in cultured ECs was analyzed using laser scanning confocal microscopy (LSCM). The increase of HSP70 expression was found to be dependent upon the incubation time and inoculum size of the *S. aureus* infection. The HSP70 expression in ECs infected by *S. aureus* (10^4 cfu/ml) for 20 h was 1.1-fold higher than that in heat shock treated ECs and 2.2-fold higher than that in untreated cells. Heat shock is known to induce intranucleus HSP70 expression in mammalian cells, whereas the *S. aureus* infection induced perinuclear expression in ECs as observed by LSCM. Consequently, the expression of HSP70 in ECs plays an important role in the pathogenesis of endovascular infection.

Key words: *Staphylococcus aureus*, endothelial cells, heat shock protein70, endovascular infection

S. aureus is gram positive and facultatively anaerobic. This bacterium, which is a natural inhabitant of mammalian skin and mucous epithelia, is one of the pathogens causing various diseases in humans and animals. In humans, *S. aureus* causes two main types of infection: mucosal and septicaemic (abscesses, endocarditis, lung infections, osteomyelitis) [20, 27]. In particular, the adherence of *S. aureus* to cardiac ECs may initiate infective endocarditis [1].

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The endothelium occupies a unique location at the interface between blood vessels and flowing blood, and accordingly, it is often the major target of cardiovascular diseases such as infective endocarditis and atherosclerosis. Vascular endothelium plays a critical role during the initiation of hematogenous infections, since microorganisms likely adhere to and penetrate through the EC lining of the blood vessels to reach to the tissue parenchyma. Thus, blocking the ability of microorganisms to escape from the intravascular compartment is a potential method to enhance the host defense against these organisms and prevent the development of infectious diseases [2].

The structural or surface characteristics of the vascular endothelium are altered by external conditions: infection, fluid shear stress, and thrombus. These structural alterations are induced by a change of microfilament. The polymerization of the microfilament may prevent hydrodynamic damage to the endothelium and thus protect the vascular wall from certain pathological stimuli [3].

During infection, the vascular EC undergoes important immunologic alterations leading to increased leukocyte-EC adherence and the initiation of a host inflammatory response [4, 6]. HSP is the stress protein which is the major antigen leading to leukocyte activation. Heat shock treatment or other physiological stress conditions lead to the expression of HSP in many organisms [15, 19]. Microbial invasion also represents a form of stress to the host, and infections represent a particularly interesting situation, since invading pathogens have the potential of both inducing HSP expression by the host cells and eliciting immune responses to their own HSPs [12]. It has been reported that HSP70 protein levels are elevated by bacterial infections with Mycobacterium or phagocytosis of heat-killed *Staphylococcus aureus*, but the kinetics of the induction were not determined yet [14, 16]. This study examines the bacterial adherence, cell viability, and polymerization of the actin microfilament in *S. aureus* infected ECs. Furthermore, the heat shock responses of ECs in an *in vitro* culture system

have been characterized and the effect of infection with *S. aureus* on the expression of ECs HSP70 was analyzed.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Staphylococcus aureus (ATCC 27735) was used in this experiment. The organism was grown in a nutrient broth (Difco Lab., U.S.A.) in a 37°C shaking incubator for 18 h, and harvested in a log phase.

Isolation and Culture of Human Umbilical Vein Endothelial Cells (HUVECs)

Human umbilical vein endothelial cells were obtained by a modification of the method of Jaffe *et al.* [11]. The cells were harvested using collagenase and were grown in Medium 199 (Gibco BRL, U.S.A.) containing 20% fetal bovine serum (Gibco BRL.), 100 IU/ml penicillin/streptomycin (Gibco BRL.), 2 mM L-glutamine (Gibco BRL.), and a 25 mM HEPES buffer (Sigma Chemical Co., U.S.A.). The HUVECs were seeded on fibronectin (Boehringer Mannheim, Germany)-coated glass at a concentration of 5×10^4 cells per cm^2 and grown for 4 days before the experiment.

Bacterial Adherence Assay for ECs

The adherence assay was a modification of a previously described technique [23]. Cultured ECs were seeded onto human fibronectin-coated glass. The bacteria were grown in a nutrient broth for 18 h and were then resuspended in a phosphate buffered saline (PBS). *S. aureus* (initial inoculum level: 5×10^8 cfu/ml) was inoculated into the cell wells, and incubated for 3, 7, and 20 h at 37°C. After incubation, the unbound bacteria were removed with PBS. The remaining adherent bacteria were removed with the ECs using 0.25% trypsin-0.02% ethylenediamine tetra-acetic acid (EDTA, Sigma Chemical Co.). The released bacteria and ECs were harvested and treated with distilled water to disrupt the ECs. The bacteria and cell extractions were centrifuged at 12,000 ×g for 5 min. The pellet was added to PBS and the bacteria grown on a nutrient agar (Difco Lab.). After 24 h of incubation at 37°C, the bacterial colonies were counted. The results were expressed as the number of adherent bacteria/endothelial cells.

Viability of ECs Infected with *S. aureus*

The viability of ECs infected with *S. aureus* was determined by a trypan blue assay [13]. Confluent ECs grown on a 96-well micro-tissue culture plate were infected with 10^3, 10^6, and 10^8 cfu/ml *S. aureus*. After 3 h and 7 h of incubation at 37°C, the non-cell-associated *S. aureus* and dead cells were washed and the monolayers were rinsed with warm PBS. The resulting ECs suspension was stained with a 0.4% trypan blue solution (Sigma Chemical Co.) and the number of viable ECs was counted using a hemacytometer. Nonviable cells were stained with trypan blue.

Indirect Immunofluorescent Staining Studies

The ECs with the added bacteria were incubated at 37°C for various lengths of time and then heat shocked at 43°C for 1 h. They were fixed with 3.7% formaldehyde (Merck, Germany) at room temperature (RT) for 10 min, washed with PBS, and permeabilized with 0.5% Triton X-100 (Boehringer Mannheim) containing 20 mM Hepes (Sigma Chemical Co.), 300 mM sucrose (Sigma Chemical Co.), 50 mM NaCl (Kanto Chemical Co. Japan), and 3 mM $MgCl_2$ (Sigma Chemical Co.) at pH 7.4, on ice for 10 min. After blocking the nonspecific staining by incubation in PBS with 1% bovine serum albumin (BSA, Sigma Chemical Co.) and 5% normal serum (Gibco BRL.) for 30 min, the cells were incubated in a 1:200 dilution of a mouse monoclonal antibody (Santa Cruze Inc., Germany) for the inducible form of HSP70 at room temperature for 30 min, washed with PBST (0.02% Tween20 in PBS) and labeled with a 1:1000 dilution of fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Sigma Chemical Co.) as a secondary antibody of the HSP70 antibody at 37°C for 30 min, and finally stained with rhodamine-phalloidin (Sigma Chemical Co.) to produce an actin microfilament for mounting on microscopy slides.

Laser Confocal Microscopy Study for Distribution of HSP70

S. aureus-infected ECs were examined using a LSCM (Bio-Rad. Co., U.S.A., MRC 600) with an argon laser for illumination. The wavelength of the excitation was 488 nm.

Image Processing of Immunofluorescence Light for Distribution of HSP70

The relative distribution of HSP70 was determined by LSCM. The relative intensity was obtained by using the histogram function of a PV-WAVE (Visual Numerics Inc., Boulder, Co., U.S.A.) running on a Sun workstation. This software displays the intensity of the fluorescence of a selected area in terms of pixel values per unit area.

RESULTS

Binding of *S. aureus* to ECs at Various Lengths of Incubation Time

The ECs were infected through incubation with 5×10^8 cfu/ml of the growth medium for 3, 7, and 20 h. Bacterial adherence to the EC monolayers was assayed at various times (Table 1). After 3 h of incubation, the concentration of adherent bacteria to ECs was approximately 2.5×10^7 cfu/ml (5.0×10^2 cfu/ml per EC), and 22.0×10^7 cfu/ml (4.4×

Table 1. Binding of *S. aureus* to ECs at various incubation times.

Incubation time (h)	Initial bacterial inoculum (cfu/ml)	The number of bacteria bound per EC (cfu/ml)
3	5×10^8	500
7	5×10^8	800
20	5×10^8	4,400

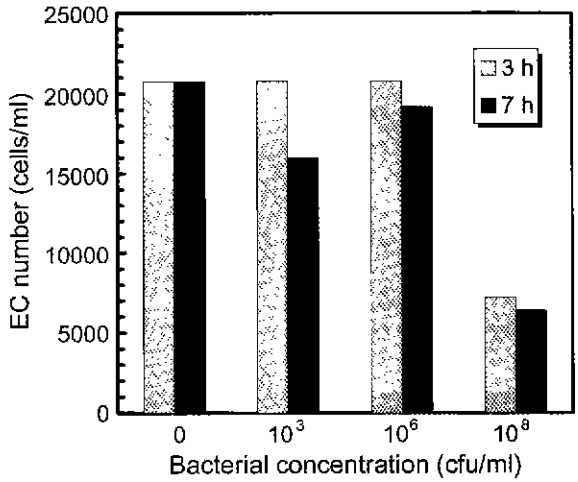

Fig. 1. Viability of ECs infected with various *S. aureus* concentrations.

10^3 cfu/ml per EC) after 20 h. The bacterial adherence increased as the incubation time increased.

Viability of ECs Infected with Various Concentrations of *S. aureus*

A trypan blue assay was used to determine the viability of ECs infected with *S. aureus* (Fig. 1). After infection with 10^3 or 10^6 cfu/ml *S. aureus* for 3 h or 7 h at 37°C, the degrees of viability of the ECs were not different to uninfected ECs. However, the viability of the ECs infected with 10^8 cfu/ml *S. aureus* for 3 h or 7 h decreased by 25% compared to that of uninfected ECs.

Actin Polymerization in ECs Infected by *S. aureus*

To investigate the relationship between cell damage and the cytoskeletal changes of ECs infected with *S. aureus*, the actin microfilamental change was investigated using LSCM (Fig. 2). When the ECs were incubated with *S. aureus* (10^4 cfu/ml) for 3 h at 37°C, an increased actin polymerization of ECs in peripheral regions was detected (Fig. 2B), however, the actin polymerization with 10^6 and 10^8 cfu/ml *S. aureus* for 3 h decreased compared with that of cells infected with 10^4 cfu/ml (Figs. 2C and 2D).

Expression of HSP70 in ECs Infected by *S. aureus*

To examine whether HSPs were expressed during infection, infected ECs were analyzed using indirect immunofluorescent staining methods. As shown in Fig. 2, the expression of

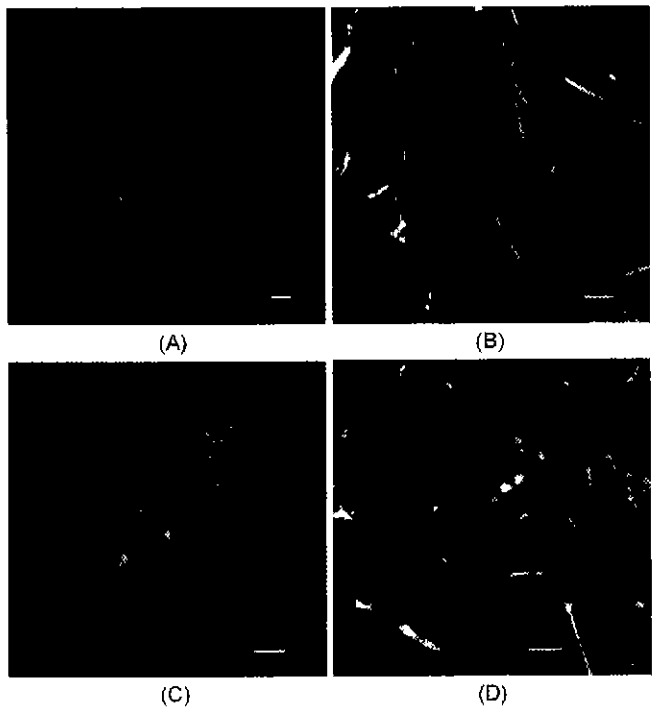

Fig. 2. Actin cytoskeletal changes on ECs infected by *S. aureus* with different inoculum sizes.
Bar represents 10 μm. Incubation time: 3 h. (A) untreated cell: incubated at 37°C for 1 h; (B) inoculum size: 10^3 cfu/ml; (C) inoculum size: 10^6 cfu/ml; (D) inoculum size: 10^8 cfu/ml

HSP70 in *S. aureus* (10^4 cfu/ml)-infected ECs for various lengths of time was detected using LSCM. The ECs displayed a weak fluorescence level (Fig. 3A) when probed with anti-HSP70 antibody in an uninfected condition. This may be due to the low expression level of HSP70 that has been reported for other cell types in the absence of stress. However, after 3 h of incubation with *S. aureus* (Fig. 3B), an increased level of HSP70 was observed in the infected ECs. Furthermore, after 7 h or 20 h of infection, the fluorescence level of HSP70 increased compared with those of the uninfected or 3 h-infected cells (Figs. 3C and 3D). Therefore, HSP70 induction by *S. aureus* infection appears to be dependent on infection period. The HSP70 expression of HUVECs incubated with 10^4, 10^6, and 10^8 cfu/ml *S. aureus* for 3 h was observed using LSCM (Fig. 4). In the infected cells, an increased cytoplasmic fluorescence was observed compared to the untreated 37°C cells. Localized nuclear fluorescence was not observed in the ECs after infection for either 3 h or 7 h (Fig. 3). This indicates that the expression of HSP70 in ECs depends on the size of the *S. aureus* in the inoculum and the length of time after exposure to *S. aureus*. The amount of expressed HSP70 in the infected ECs was calculated by measuring the image intensities from the laser confocal micrographs using a PV-WAVE software (Table 2). Therefore, the fluorescent light in the ECs was proportional to the amount

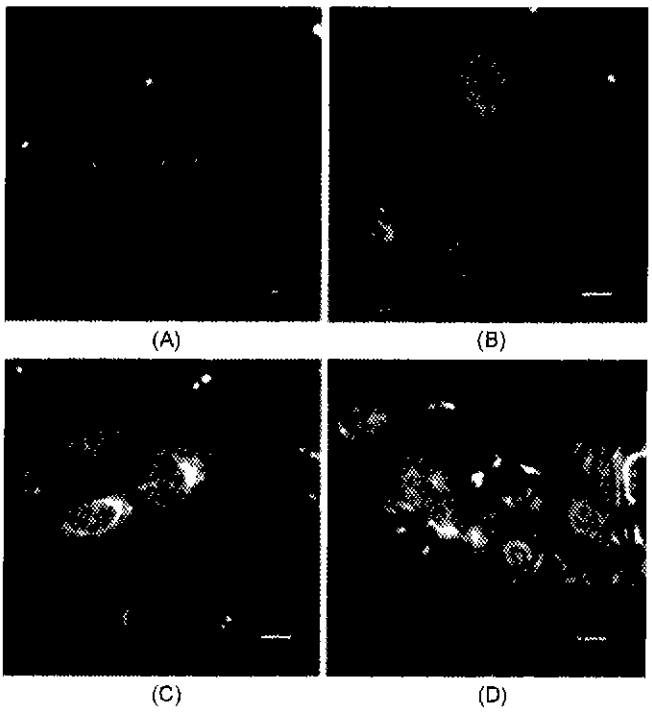

Fig. 3. Laser scanning confocal microscopic localization of HSP70 in HUVECs infected by *S. aureus* (10^4 cfu/ml) after different incubation times.
Bar represents 10 μm. (A) untreated cells; (B) incubated for 3 h, (C) incubated for 7 h, (D) incubated for 20 h.

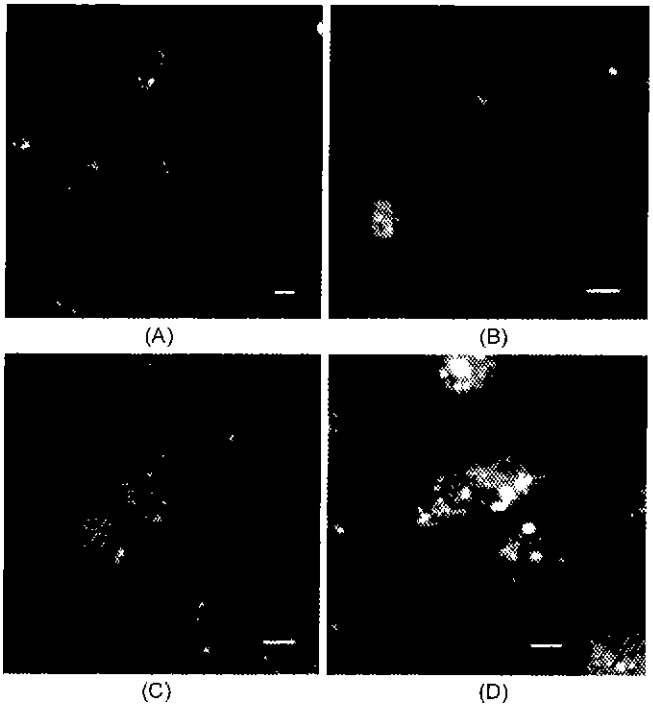

Fig. 4. Laser scanning confocal microscopic localization of HSP70 in HUVECs infected with different inoculum sizes of *S. aureus* for 3 h.
Bar represents 10 μm (A) untreated cells; (B) inoculum size: 10^4 cfu/ml; (C) inoculum size: 10^6 cfu/ml; (D) inoculum size. 10^8 cfu/ml.

of HSP70 expression. In the untreated cells, HSP70 was expressed at a very low basal level. The HSP70 expression in ECs infected by *S. aureus* (10^4 cfu/ml) for 20 h was 1.1-fold higher than that in heat shock treated ECs and 2.2-fold higher than that in the untreated cells. White spot in

Table 2. The amount of HSP70 in ECs treated by heat and infected by *S. aureus* measured using the PV-WAVE software.

			Cell infected by *S. aureus*				
	*UC	*HC	Incubation time (h) at constant inoculum sizes (10^4 cfu/ml)			Inoculum size (cfu/ml) at constant incubation times (3 h)	
			3	7	20	10^6	10^8
Flurorescene intensity	40.9	77.6	58.3	79	88.3	67.7	78.1

*UC (Untreated cells) was incubated at 37°C for 1 h; *HC (Heat shocked cells) was incubated at 43°C for 1 h.

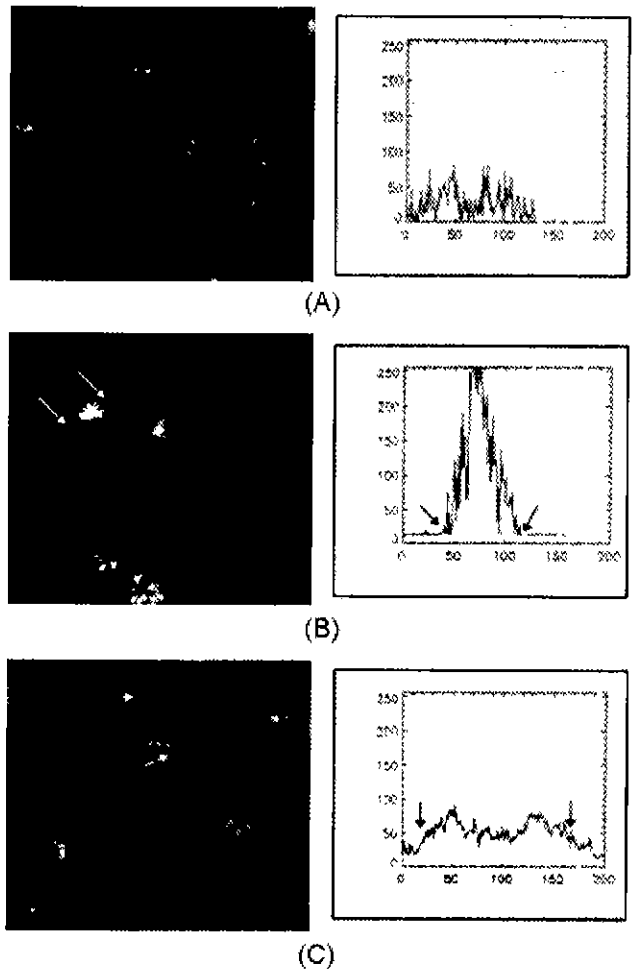

Fig. 5. Distribution of HSP70 in ECs.
(A) untreated cells incubated at 37°C for 1 h; (B) heat shocked cells at 43°C incubated for 1 h; (C) cells infected by *S. aureus* (10^4 cfu/ml) and incubated at 37°C for 3 h.

Fig. 4D was shown as a result of nonspecific binding of antibody.

Distribution of HSP70

The subcellular location of the 70 kDa stress protein was observed using LSCM and the data was analyzed using the PV-WAVE software (Fig. 5). The fluorescent intensity of a target cell was measured along a calculation line (white line). Accordingly, increased HSP70 within ECs could be detected as peaks (right panel). The staining of heat shocked cells, shown as a positive control, indicated that HSP70 increased after heat shock and was mainly localized in the nucleus (Fig. 5B), as reported for other cells. No accumulation of HSP70 in the nucleus was observed in the infected cells, and the accumulation pattern of HSP70 in the nucleus was distinct from that of HSP70 after heat shock treatment (Fig. 5B). However, in the infected ECs. HSP70 was mostly located in the cytoplasm (Fig. 5C). In the control conditions, the expression of HSP70 was even lower than that after exposure to infection and heat shock (Fig. 5A).

DISCUSSION

Many environmental stresses, such as heat shock, mechanical stress, and heavy metals cause morphological changes in eukaryotic and prokaryotic cells [3, 8]. Huh and Choi [8] reported morphological transition of *Hansenula anomala* B-7 from a unicellular yeast to a pseudohyphae-like coagulation when subjected to prolonged heat shock treatment. The pseudohyphae-like cells overexpressed several proteins. Synthesis of such stress-responsible proteins are induced very rapidly to protect the cells against toxicity caused by the stresses, thereby enabling the organisms to survive in harmful environmental conditions. Many heat shock and heavy metal resistance in bacteria are known to be conferred by plasmids [7, 17].

In infected ECs, microorganisms may induce cytoskeletal changes and endothelial cell injury. Filler *et al.* [2] reported that endothelial cell actin microfilaments polymerized around *Candida albicans* when the organisms were phagocytosed [20]. Also, one of the physiological changes in host cells with infection may be the induction of HSP70 [16]. Recently, it has been reported that the expression of HSP70 in a host cell during infection may be relevant to understanding the responses in the various stages of the bacterial invasion process. In particular, the infection-related overexpression of HSP in the host cell is reported in many conditions: *Mycobacterium leprae* [15], *Listeria monocytogenes* [19], *Leishmania donovani* [18], and *S. aureus* [12, 14]. It is known that upregulation of HSP70 by infection is part of the cellular protective mechanism against phagocytosis-related oxidative injury to a host cell, and yet the detailed mechanism is still not well understood [5. 9. 10]. Members of the HSP65 and HSP70 families have been found to be predominantly immunogens in intracellular infections, including leprosy, tuberculosis. and atherosclerosis. These findings provide substantial evidence to implicate the HSP60 and HSP70 families as important elements in the pathogenesis of intracellular infection [18].

To investigate the role of HSP70 in the pathogenesis of infections with *S. aureus*, an important pathogen of infective endocarditis, the expression of HSP70 during the infection of ECs with *S. aureus* was examined. The synthesis of HSP70 in infected ECs was monitored using indirect immunofluorescent staining and detected using LSCM. It was shown that *S. aureus* infection was capable of inducing HSP70 expression in ECs. and that this induction was dependent on the incubation time and also on the inoculum size of *S. aureus*. Accordingly, when the number of organisms adhered to the EC increased, the HSP70 expression in the EC increased. The initiating event in the establishment of many types of infection by bacteria is adherence of bacteria to cell surfaces [12, 15]. The binding of *S. aureus* to ECs appeared to be dependent on incubation time (Table 1), and the damage to the ECs [24]. Bacterial binding to a host cell is likely to be an important early event in the pathogenesis of several infections and a cause of host cell injury [2, 21].

The "*Ex novo*" synthesis of HSP70 is a defense response triggered in cells by a variety of stimuli [28], and HSP initially localizes in sites of major injury [22, 25]. Welch *et al.* [26] reported that 72 kDa HSP localizes within the nucleus and accumulate in the nucleoli of a cell after heat shock. Interestingly, the effect of infection was different from that of heat shock. Instead, the cytoplasmic location of HSP70 was observed during the infective injury by *S. aureus* (Fig. 5). Therefore, it is likely that the induction of HSP70 in ECs during *S. aureus* infection is a response to cellular damage by a toxic component and phagocytosis.

REFERENCES

1. Campell, K. M. and C. M. Johnson. 1990. Identification of *Staphylococcus aureus* binding proteins on isolated porcine cardiac valve cells. *J. Lab. Clin. Med.* **115:** 217–223.
2. Filler S. G., N. Jonathan, C. H. Swerdloff, and M. L. Peter. 1995. Penetration and damage of endothelial cells by *Candida albicans. Infection and Immunity* **63:** 976–983.
3. Franke. R. P., M. Gräfe, H. Schnittler, D. Seiffge, and C. Mittermayer. 1984. Induction of human vascular endothelial stress fibers by fluid shear stress. *Nature* **307:** 648–649.
4. Gimbrone, M. A. and M. R. Buchanan. 1982. Interaction of platelets and leukocytes with vascular endothelium: *In vitro* studies. *Ann. N. Y. Acad. Sci.* **401:** 171–183.
5. Hamill. R. J., J. M. Vann, and R. A. Proctor. 1986. Phagocytosis of *Staphylococcus aureus* by cultured bovine

aortic endothelial cells: Model for postadherence events in endovascular infections. *Infection and Immunity* **54:** 833–836.
6. Hickey, M. J., K. A. Sharkey, E. G. Sihota, P. H. Reinhardt, J. D. Macmicking, C. Nathan, and P. Kubes. 1997. Inducible nitric oxide synthase-deficient mice have enhanced leukocyte-endothelium interactions in endotoxemia. *FASEB. J.* **11:** 955–964.
7. Hinteregger, C., R. Leiter, M. Loi, A. Ferschl, and F. Streichsbier. 1992. Degradation of phenol and phenolic compounds by *Pseudomonas putida* EKII. *Appl. Microbiol. Biotechnol.* **37:** 252–259.
8. Huh, N. E. and N. S. Choi 1999. Effect of environmental stress on morphological change of an extremely cadmium-tolerant yeast, *Hansenula anomala* B-7. *J. Microbiol. Biotechnol.* **9:** 70–77.
9. Jä, M. and W. Dorte. 1993. Heat shock proteins protect cells from monocyte cytotoxicity: Possible mechanism of self-protection. *J. Exp. Med.* **177:** 231–236.
10. Jacquier-Sarlin, M. R., L. Jornot, and B. S. Polla. 1995. Differential expression and regulation of hsp70 and hsp90 by phobol esters and heat shock. *J. Biol. Chem.* **23:** 14094–14099.
11. Jaffe, E. A., R. L. Nachman, C. G. Becker, and R. Minick. 1973. Culture of human endothelial cells derived from umbilical veins. *J. Clin. Invest.* **52:** 2745–2756.
12. Johnson, C. M. 1993. *Staphylococcus aureus* binding to cardiac endothelial cell is partly mediated by a 130 kilodalton glycoprotein. *J. Lab. Clin. Med.* **121:** 675–683.
13. Kaltenbach, J. P., M. H. Kaltenbach, and W. B. Lyons. 1958. Nigrosin as a dye for differentiating live and dead ascites cell. *Exp. Cell. Res.* **15:** 112–117.
14. Kantengwa, S. and B. S. Polla. 1993. Phagocytosis of induced a selective stress response in human monocyte-macrophage: Modulation by macrophge differentiation and by iron. *Infection and Immunity* **61:** 1281–1287.
15. Kauffman, S. H. E. 1990. Heat shock proteins and the immune response. *Immunol. Today* **11:** 129–136.
16. Mistry, Y., D. B. Young, and R. Mukherjee. 1990. Hsp70 synthesis in Schwann cells in responses to heat shock and infection with *Mycobacterium leprae*. *Infection and Immunity* **60:** 3105–3110.
17. Nies, D. H. and S. Silver. 1989. Metal ion uptake by plasmid-free metal sensitive *Alcaligenes eutrophus*. *J. Bacteriol.* **171:** 896–900.
18. Rey-Ladino, J. A. and N. E. Reiner. 1993. Expression of 65 and 67 kilodalton heat regulated proteins and 70-kilodalton heat shock cognate protein of *Leishmania donovani* in macrophages. *Infection and Immunity* **61:** 3265–3272.
19. Schlesinger, M. 1990. Heat shock proteins. *J. Biol. Chem.* **265:** 12111–12114.
20. Schwan, W. R. and W. Goebel. 1994. Host cell responses to *Listeria monocytogenes* infected include differential transcription of host genes involved in signal transduction. *Proc. Natl. Acad. Sci. USA* **91:** 6428–6432.
21. Stra, L. and B. Poutrel. 1994. Virulence factors involved in the pathogenesis of bovine intramammary infections due to *Staphylococcus aureus*. *J. Med. Microbiol.* **40:** 79–89.
22. Tetsuya, A. E., T. Konishi, and K. Higashi. 1995. Possible correlation between DNA damage induced by hydrogen peroxide and translocation of heat shock 70 protein into the nucleus. *Biochem. Biophys. Res. Commun.* **206:** 548–555.
23. Tompkins, D. C., V. B. Hatcher, D. Patel, G. A. Orr, L. L. Higgins, and F. D. Lowy. 1990. A human endothelial cell membrane protein that binds *Staphylococcus aureus in vitro*. *J. Clin. Invest.* **85:** 1248–1254.
24. Vann. J. M. and R. A. Procter. 1987. Ingestion of *Staphylococcus aureus* by bovine endothelial cells results in time- and inoculum-dependent damage to endothelial cell monolayers. *Infection and Immunity* **55:** 2155–2163.
25. Velazquez, J. and S. Lindquist. 1984. HSP70; nuclear concentration during environmental stress and cytoplasmic storage during recovery. *Cell* **36:** 655–662.
26. Welch. W. J. and J. R. Feramisco. 1984. Nuclear and nucleolar localization of the 72,000-dalton heat shock protein in heat-shocked mammalian cells. *J. Biol. Chem.* **259:** 4501–4513.
27. Wijngaerden, E. V., W. E. Peetermans, S. V. Lierrde, and J. V. Eldere. 1997. Polyclonal *Staphylococcus endocarditis*. *Clinical Infectious Disease* **25:** 69–71.
28. Zhu. W., P. Roma, and A. L. Catapano. 1994. Oxidized-LDL induce the expression of hsp70 in human endothelial cells. *Biochem. Biophys. Res. Commun.* **200:** 389–394.