INFECTION AND IMMUNITY, Oct. 1994, p. 4140–4144
0019-9567/94/$04.00+0

Vol. 62, No. 10

In Vivo Production of Heat Shock Protein in Mouse Peritoneal Macrophages by Administration of Lipopolysaccharide

YUE-HUA ZHANG, KAZUKO TAKAHASHI, GUO-ZHI JIANG, XIAN-MIN ZHANG, MAKOTO KAWAI, MASAKO FUKADA, AND TAKASHI YOKOCHI*

Department of Microbiology, Aichi Medical University, Nagakute, Aichi 480-11, Japan

Received 8 April 1994/Returned for modification 10 May 1994/Accepted 24 June 1994

The in vivo production of heat shock protein was studied by administration of bacterial lipopolysaccharide (LPS) into mice. Heat shock protein 70 was detected in the extract of adherent peritoneal cells from mice injected intraperitoneally with LPS by using the immunoblotting method. The expression of heat shock protein 70 was found 2 days after injection of LPS and reached its peak 4 days after injection. The intraperitoneal injection of LPS induced the expression of heat shock protein 70, whereas its subcutaneous injection did not. The in vivo production of heat shock protein 70 was inhibited by administration of LPS together with quercetin, an inhibitor of accumulation of heat shock protein 70 mRNA. Tumor necrosis factor alpha enhanced LPS-induced heat shock protein production in vivo. There was a decrease of γδ T cells in the peritoneal cavity of mice injected intraperitoneally with LPS. It was suggested that bacterial LPS is a stressful agent which induces the in vivo heat shock protein response, and its administration leads to the production of heat shock protein 70 in peritoneal macrophages.

Heat shock proteins (HSP) or stress proteins are produced by a broad spectrum of pathogens and have been shown to be among the dominant target antigens recognized in immune responses to pathogens (6, 16, 24, 25). The stress exerted by phagocytes may induce increased HSP synthesis in microbial pathogens in an attempt to protect themselves from the host effector mechanism (1, 6, 11). On the other hand, host cells also need to protect themselves from the noxious molecules which pathogens produce (6). This may be achieved in part by HSP synthesis in host cells. In fact, it has been reported that host cells express increased HSP levels after in vitro infection with a variety of viral pathogens (3, 9, 13). However, less is known about the in vivo HSP response in the host infected with microbial pathogens, although HSP are almost certainly involved in protecting cells from their deleterious effects (24). Lipopolysaccharide (LPS), one of the noxious products of gram-negative organisms, may certainly alter the metabolism of various cells in the host, and host cells may exhibit the HSP response to LPS with similar behavior. However, there are no reports of LPS stimulation of in vivo HSP production by the host.

The HSP70 family is probably one of the best-studied stress proteins found in all prokaryotes and eukaryotes, and it is, in particular, the most highly conserved of HSP families. The highly inducible mammalian HSP70 is hardly detectable under normal conditions but becomes one of the most actively synthesized proteins in cells in response to stress (19, 24). In the present study, we investigated whether and how the administration of LPS to mice caused in vivo induction of the HSP70 family in the host. Here we describe the in vivo production of HSP70 in peritoneal macrophages from mice injected intraperitoneally (i.p.) with LPS.

* Corresponding author. Mailing address: Department of Microbiology, Aichi Medical University, Nagakute, Aichi 480-11, Japan. Phone: 0561 62 3311, ext. 2269. Fax: 0561 63 9187.

MATERIALS AND METHODS

Animals. Inbred SMA mice, approximately 6 to 8 weeks of age, were supplied by the Institute for Laboratory Animal Research at Aichi Medical University.

Reagents. Anti-HSP70 monoclonal antibodies (SPA-810 and SPA-820) were purchased from StressGen Biotechnologies Corp., Victoria, British Columbia, Canada. Hamster anti-mouse γδ T-cell receptor monoclonal antibody (GL3) conjugated with fluorescein isothiocyanate was purchased from Pharmingen Corp., San Diego, Calif. Recombinant mouse gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin 1α (IL-1α) were purchased from Genzyme Corp., Cambridge, Mass. Quercetin was obtained from Wako Pure Chemical, Osaka, Japan.

LPS. The LPS preparation extracted by the phenol-water method from *Escherichia coli* O111:B4 or *Salmonella enteritidis* was obtained from Difco Laboratories, Detroit, Mich. LPS from *Klebsiella pneumoniae* O3 was also prepared by the phenol-water method (20, 22). All experiments were carried out by i.p. administration of LPS from *E. coli* O111 (100 μg) unless stated otherwise. Separation of the free lipid A and polysaccharide fraction from *K. pneumoniae* O3 LPS was performed by heating at 100°C for 1 h in 1% acetic acid as described previously (5). Rough-type LPS was prepared from *K. pneumoniae* LEN-113 ($O3^-$:$K1^-$) (14).

Preparation of peritoneal cells and adherent cells. Peritoneal cells were obtained by washing out the peritoneal cavity with RPMI 1640 medium. Two mice per experimental group were used. Separation of nonadherent cells and adherent cells was performed by incubating peritoneal cells in plastic petri dishes for 1 h at 37°C with RPMI 1640 medium containing 5% fetal calf serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml.

Immunoblot analysis. Cells were obtained 4 days after injection of LPS unless stated otherwise and washed three times in 0.01 M phosphate-buffered saline at pH 7.2 (PBS). Cell pellets were suspended at a concentration of 2×10^7/ml in a lysis buffer containing 0.5% Nonidet P-40, 0.15 M NaCl, 0.05 M Tris, and 5 mM EDTA at pH 8.0 for 30 min at 4°C. The insoluble debris was removed by microcentrifugation at 18,600

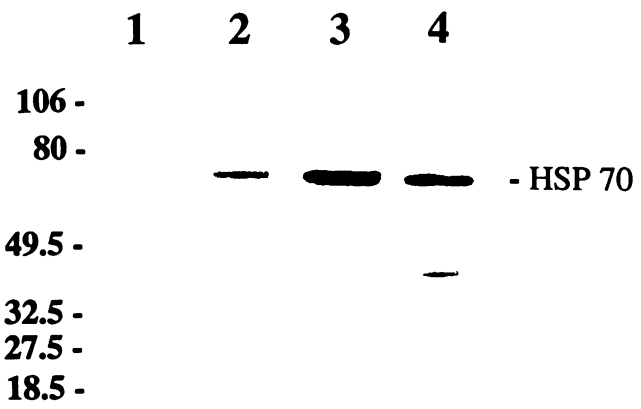

FIG. 1. Time course of the expression of HSP70 in mice injected i.p. with LPS. Peritoneal cells were collected 2, 4, or 9 days after injection of LPS (100 μg). The expression of HSP70 was estimated by the immunoblotting method. Lanes: 1, untreated control; 2, 2 days; 3, 4 days; 4, 9 days. Molecular size markers in kilodaltons are on the left.

× *g* for 10 min at 4°C. Cell lysates were diluted with an equal volume of sample buffer containing 0.5 M Tris, 10% glycerol, 2% sodium dodecyl sulfate (SDS), 2% 2-mercaptoethanol, and 0.05% bromophenol blue and boiled for 2 min. Samples were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with a 5 to 20% gradient gel. Proteins separated by SDS-PAGE were transferred to a membrane filter (Durapore; Nihon Millipore Ltd., Tokyo, Japan) by electroblotting. The filters were blocked with 5% skim milk in PBS. After the blots were washed in PBS containing 0.05% Tween 20 (PBS-T), they were treated with a 1:1,000 dilution of anti-HSP70 monoclonal antibody and then washed with PBS-T three times. Resulting immune complexes were reacted with a 1:2,000 dilution of horseradish peroxidase-conjugated protein A (Nippon Bio-Rad Laboratories, Tokyo, Japan) in PBS-T. Finally, labeled antigen bands were detected by an ECL Western blotting (immunoblotting) detection reagent (Amersham, London, United Kingdom). A prestained low-molecular-weight standard kit from Nippon Bio-Rad Laboratories was used as a reference.

Flow cytometric analysis. Mice were injected i.p. with LPS (100 μg), and peritoneal cells were collected 3 or 5 days after injection. Peritoneal cells were stained with fluorescein isothiocyanate-conjugated anti-mouse γδ T-cell receptor antibody (GL3) by a direct immunofluorescence staining method. The stained cells were fixed with 1% paraformaldehyde and analyzed by a FACS-400 flow cytometer (Becton Dickinson, Mountain View, Calif.). The intensity of fluorescence was expressed in a log scale from 1 to 10^4.

RESULTS

Detection of HSP70 on peritoneal cells from mice injected i.p. with LPS. The peritoneal cells, thymocytes, and spleen cells were collected from mice various days after i.p. injection of LPS from *E. coli* O111 (100 μg), and the induction of HSP70 was studied by the immunoblotting method. As shown in Fig. 1, HSP70 was found in the extract of peritoneal cells 2 days after injection of LPS. The expression of HSP70 increased and reached its peak 4 days after injection. The high expression of HSP70 lasted longer than 9 days. The qualitative and quantitative time course in the induction of HSP70 was essentially reproducible in every experiment. On the other hand, there was no significant induction of HSP70 in either thymocytes or spleen cells from mice injected with LPS (data not shown).

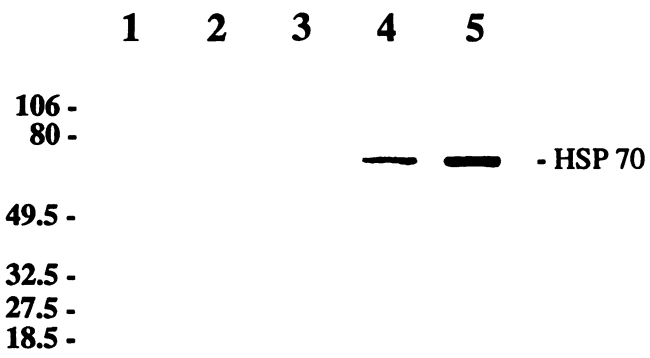

FIG. 2. Dose dependency of the expression of HSP70 on injected LPS. Peritoneal cells were collected 4 days after i.p. injection of various doses of LPS. The expression of HSP70 was estimated by the immunoblotting method. Lanes: 1, untreated control; 2, 1 μg; 3, 10 μg; 4, 50 μg; 5, 100 μg. Molecular size markers in kilodaltons are on the left.

Induction of HSP70 on peritoneal cells from mice injected i.p. with various doses of LPS. The dose dependency of injected LPS on the expression of HSP70 in peritoneal cells was studied. The experimental results are shown in Fig. 2. The level of HSP70 expression was roughly dependent on the dose of LPS injected. The highest expression of HSP70 was found with injection of 100 μg of LPS, whereas the injection of 1 or 10 μg of LPS did not cause in vivo production of HSP70.

Comparison of HSP70 induction in peritoneal cells by i.p. and subcutaneous routes of LPS injection. The inductions of HSP70 in peritoneal cells by i.p. and subcutaneous routes of LPS injection were compared. The experimental results are shown in Fig. 3. The i.p. injection of LPS definitely induced the expression of HSP70, while subcutaneous LPS injection did not induce it in peritoneal cells.

Expression of HSP70 on adherent peritoneal cells from mice injected i.p. with LPS. Which cell type in peritoneal cells produced HSP70 by i.p. administration of LPS was studied. Adherent and nonadherent cell fractions were separated by incubating peritoneal cells in plastic dishes. The experimental results are shown in Fig. 4. Injection of LPS induced produc-

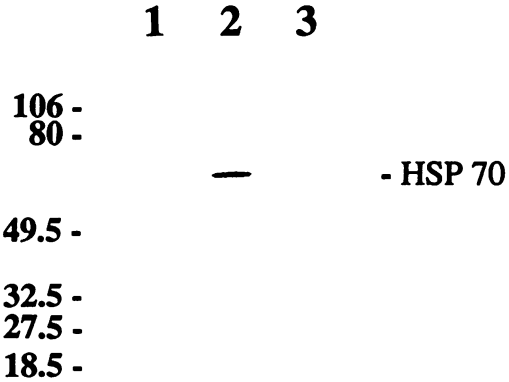

FIG. 3. Comparison of HSP70 production in peritoneal cells induced by i.p. or subcutaneous injection of LPS. Peritoneal cells were collected 4 days after injection of LPS (100 μg). The expression of HSP70 was estimated by the immunoblotting method. Lanes: 1, subcutaneous route; 2, i.p. route; 3, untreated control. Molecular size markers in kilodaltons are on the left.

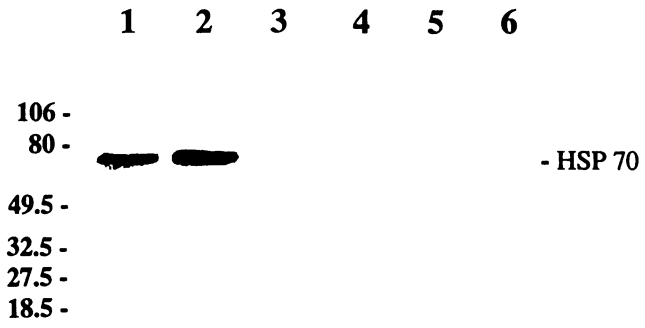

FIG. 4. Expression of HSP70 on adherent peritoneal cells from mice injected i.p. with LPS. Peritoneal cells were collected 4 or 8 days after injection of LPS (100 μg). Adherent and nonadherent cells were separated by incubating peritoneal cells in plastic dishes. The expression of HSP70 was estimated by the immunoblotting method. Lanes: 1 to 3, adherent peritoneal cells; 4 to 6, nonadherent peritoneal cells; 1 and 4, 8 days; 2 and 5, 4 days; 3 and 6, untreated control. Molecular size markers in kilodaltons are on the left.

tion of HSP70 only in adherent peritoneal cells, and the expression of HSP70 continued at high levels from 4 to 8 days after injection. On the other hand, there was no significant expression of HSP70 in nonadherent peritoneal cells 4 to 8 days after injection of LPS.

Inhibitory effect of quercetin on induction of HSP70 in peritoneal cells by administration of LPS. Quercetin is known to inhibit the induction of HSP, especially the accumulation of HSP70 mRNA (4). To ascertain the HSP70 expression, whether quercetin inhibited the production of HSP70 on peritoneal cells by LPS was tested. LPS with or without quercetin was injected i.p. into mice (50 μg). As shown in Fig. 5, the induction of HSP70 by LPS in peritoneal cells was significantly blocked 4 days after injection with quercetin.

Effects of various cytokines on induction of HSP70 in peritoneal cells by administration of LPS. Whether a variety of cytokines affected the induction of HSP70 in peritoneal cells by in vivo administration of LPS was determined. IFN-γ, TNF-α, or IL-1α (100 U each) was injected i.p. into mice together with LPS. A typical experimental result is shown in Fig. 6. There

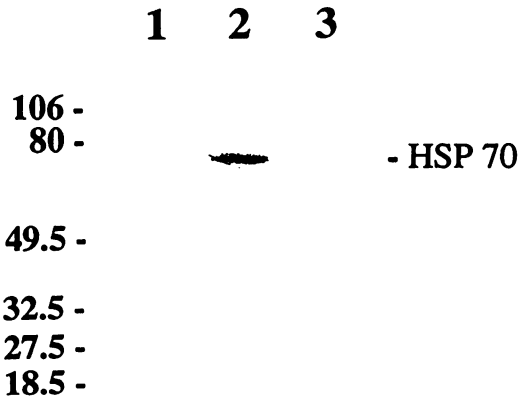

FIG. 5. Inhibitory effect of quercetin on induction of HSP70 in peritoneal cells by administration of LPS. Mice were injected i.p. with LPS (100 μg) alone or together with quercetin. Peritoneal cells were collected 4 days after i.p. injection of LPS (100 μg). Lanes: 1, untreated control; 2, LPS alone; 3, LPS and quercetin. Molecular size markers in kilodaltons are on the left.

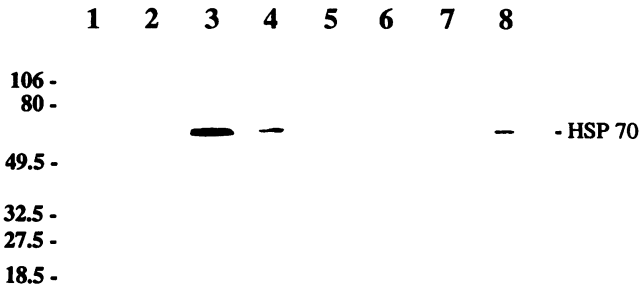

FIG. 6. Effect of various cytokines on induction of HSP70 in peritoneal cells by administration of LPS. Mice were injected i.p. with IFN-γ, TNF-α, or IL-1α (100 U each) in the presence or absence of LPS (100 μg). Peritoneal cells were collected 4 days after injection. Lanes: 1, untreated control; 2, LPS and IL-1α; 3, LPS and TNF-α; 4, LPS and IFN-γ; 5, IL-1α; 6, TNF-α; 7, IFN-γ; 8, LPS alone. Molecular size markers in kilodaltons are on the left.

was a marked increase in induction of HSP70 by administration of TNF-α together with LPS. The administration of IFN-γ together with LPS slightly enhanced the expression of HSP70, compared with that of LPS alone. The injection of IL-1α plus LPS inhibited the induction of HSP70 (Fig. 1). In some cases, however, IL-1α did not show such inhibition. In addition, HSP70 was not induced by in vivo administration of IFN-γ, TNF-α, or IL-1α (100 U each) alone.

Induction of HSP70 in peritoneal cells by administration of various preparations of LPS. A comparison of the abilities of various preparations of LPS (100 μg) to induce HSP70 in peritoneal cells was done. Administration of LPS from *K. pneumoniae* O3 or *S. enteritidis* could induce expression of HSP70 as well as LPS from *E. coli* could (Fig. 7). Strong expression of HSP70 was found in the case of i.p. injection of rough-type LPS from *K. pneumoniae* O3. The injection of free lipid A from *K. pneumoniae* O3 did not cause the production of HSP70.

Disappearance of γδ T cells from the peritoneal cavity of mice injected i.p. with LPS. It has been reported that γδ T cells respond to HSP and have a particular preference for it (6–8). We investigated the appearance of γδ T cells in the peritoneal cavity after injection of LPS. Peritoneal cells were collected 3 or 5 days after i.p. injection of LPS. Peritoneal cells were stained directly with fluorescein isothiocyanate-conjugated an-

FIG. 7. Induction of HSP70 in peritoneal cells by administration of various preparations of LPS. Mice were injected i.p. with various preparations of LPS (100 μg). Peritoneal cells were collected 4 days after injection. Lanes: 1, *S. enteritidis* LPS; 2, *K. pneumoniae* O3 LPS; 3, rough type LPS from *K. pneumoniae* O3; 4, free lipid A from *K. pneumoniae* O3. Molecular size markers in kilodaltons are on the left.

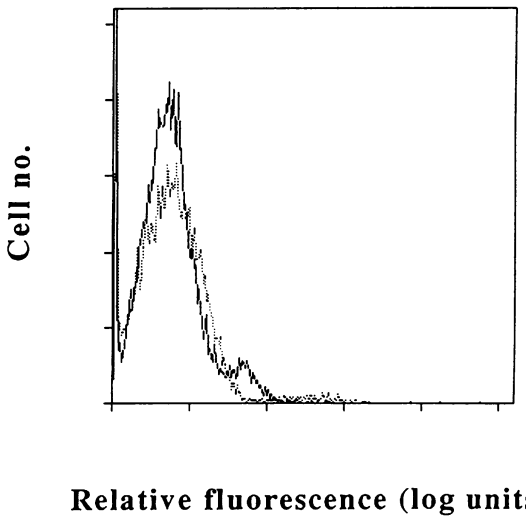

FIG. 8. Flow cytometric analysis of γδ T cells in the peritoneal cavities of mice injected i.p. with LPS. Mice were injected with LPS (100 μg), and peritoneal cells were collected 5 days after injection. The histograms of untreated control mice (——) and LPS-treated mice (– – –) are shown. The fluorescence intensity is expressed on a log scale.

ti-γδ T-cell receptor antibody (GL3), and the frequency of positively stained cells was analyzed by laser flow cytometry. The experimental results are shown in Fig. 8. No positive cells were detected in peritoneal cells from mice injected i.p. with LPS, although a small percentage of γδ T cells (approximately 10%) was found in peritoneal cells of untreated control mice.

DISCUSSION

In the present study, we have demonstrated that HSP70 was produced in vivo by peritoneal cells in mice injected i.p. with LPS. The evidence for this conclusion is based primarily on the induction of proteins electrophoretically and immunologically identical to HSP70. HSP70 might be produced by peritoneal macrophages in particular because it was detected only in the extract of adherent peritoneal cells from mice injected i.p. with LPS. No production of HSP70 in nonadherent peritoneal cells might be consistent with the failure of its production in thymocytes and spleen cells. Because purified LPS was injected, the HSP70 detected was definitely derived from the host cells and not from infectious agents. Therefore, it was concluded that LPS was a stressful agent which induces in vivo HSP responses in mice. HSP is induced in in vivo response to a range of different stresses, including heat, ischemia, reperfusion injury, wounding, and inflammation (15). To our knowledge, this is the first report that the i.p. administration of LPS can lead to the in vivo HSP response and the production of HSP70 in peritoneal macrophages of the host.

There are several reports that LPS causes an HSP response of cells in an in vitro culture system. Previously, it was reported that human monocytes cultured in vitro with LPS showed an increase of in vitro HSP70 transcripts and expression of HSP70 (2). On the other hand, it was found that the synthesis of the HSP70 family was reduced if the cells were cultured in vitro with LPS with heating at 43°C (18). It is still unclear how LPS affects the production of HSP even in an in vitro culture system of cells.

HSP70 is probably one of the best-studied stress proteins found in all prokaryotes and eukaryotes, and it is, in particular, the most highly conserved of the HSP family. The highly inducible mammalian HSP70 is hardly detectable at normal temperatures but becomes one of the most actively synthesized proteins in the cell upon stress response (19, 24). Actually, HSP70 was undetectable at normal conditions in the present study but was highly inducible by injection of LPS. It has been proposed that HSP70 serves a role in the assembly and disassembly of protein complexes and translocation of certain proteins (6, 10, 24). It is possible that peritoneal macrophages produce HSP70 to preserve their cellular functions under the stressful condition of LPS administration.

On the basis of the observation that i.p. injection of LPS resulted in the in vivo production of HSP70, it is likely that production may require direct contact between the peritoneal macrophages and LPS at a relatively high concentration. It is also possible that the induction is a localized phenomenon at the site of injection. Furthermore, rough-type LPS induced more HSP70 than smooth-type LPS. At a given weight, rough-type LPS may contain more LPS molecules than smooth-type LPS. Physicochemical characteristics, such as solubility, of LPS preparations may also affect the induction of HSP70 through the interaction with macrophages.

The administration of TNF together with LPS markedly enhanced the expression of HSP70. This suggested that TNF cooperates synergistically with LPS because the in vivo administration of TNF alone did not cause the production of HSP. Since many effects of TNF are mediated by oxygen free radicals, oxygen free radicals might play some role in the enhancement of LPS-induced HSP induction by TNF (12, 15). From the present study, it is very likely that cytokines such as TNF may modulate the production of HSP70 in peritoneal macrophages by administration of LPS. The study of the action of cytokines on the in vivo production of HSP70 by LPS is now in progress.

T lymphocytes frequently recognize microbial HSP and could provide an early defense mechanism against pathogenic microbes. It was reported that certain γδ T cells seem to have a particular preference for this molecule (6–8). Skeen and Ziegler have reported that i.p. administration of LPS induced the increase in γδ T cells in the peritoneal cavity at day 3 after injection (17). In the present study, however, there was a decrease of γδ T cells in the peritoneal cavities of mice injected i.p. with LPS when HSP70 was expressed on adherent peritoneal cells. The frequency of γδ T cells might decrease somewhat because the injection of LPS results in the accumulation of a large number of polymorphonuclear leukocytes in the peritoneal cavity (23).

Recently, evidence that HSP are major antigens of many pathogens has been accumulating. The stress imposed by the host may lead to increased HSP synthesis by microorganisms. HSP becomes a prominent antigen that triggers a major portion of the immune repertoire. In particular, members of the HSP70 and HSP60 families are major targets for antibodies and T cells in many bacterial infections. On the other hand, the present study demonstrates that the stress imposed by LPS, a major component of the outer membrane of gram-negative bacilli, leads to the production of HSP by the host. Such host-derived HSP might also become the target of antibody or T cells directed toward bacterial HSP and could trigger an autoimmune reaction (21, 24, 25).

ACKNOWLEDGMENTS

This work was supported by a grant from the Ministry of Education, Science and Culture of Japan.

We are grateful to Makoto Naruse for technical assistance in the laser flow cytometry.

REFERENCES

1. **Christman, M. F., R. W. Morgan, F. S. Jacobson, and B. N. Ames.** 1985. Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. Cell **41**:753–762.
2. **Fincato, G., N. Polentarutti, A. Sicca, A. Mantovani, and F. Colotta.** 1991. Expression of a heat-inducible gene of the HSP70 family in human myelomonocytic cells: regulation by bacterial products and cytokines. Blood **77**:579–586.
3. **Garry, R. F., E. T. Ulug, and H. R. Bose.** 1983. Induction of stress proteins in Sindbis virus- and vesicular stomatitis virus-infected cells. Virology **139**:319–332.
4. **Hosokawa, N., K. Hirayoshi, H. Kudo, H. Takechi, A. Aoike, K. Kawai, and K. Nagata.** 1992. Inhibition of the activation of heat shock factor in vivo and in vitro by flavonoid. Mol. Cell. Biol. **13**:3490–3498.
5. **Kato, N., N. Kido, M. Ohta, and S. Naito.** 1985. Comparative studies on adjuvanticity of *Klebsiella* O3 lipopolysaccharide and its lipid A and polysaccharide fractions. Immunology **54**:317–324.
6. **Kaufmann, S. H. E.** 1990. Heat shock proteins and the immune response. Immunol. Today **11**:129–137.
7. **Kaufmann, S. H. E.** 1991. Gamma/delta T lymphocytes and heat shock proteins. Curr. Top. Microbiol. Immunol. **167**:191–207.
8. **Kaufmann, S. H. E.** 1992. The cellular immune response to heat shock proteins. Experientia **48**:640–643.
9. **Khandjian, E. W., and H. Turler.** 1983. Simian virus 40 and polyoma virus induce synthesis of heat shock proteins. Mol. Cell. Biol. **3**:1–8.
10. **Langer, T. W., and E. Neupert.** 1991. Heat shock proteins hsp60 and hsp70: their roles in folding, assembly and membrane translocation of proteins. Curr. Top. Microbiol. Immunol. **167**:3–30.
11. **Morgan, R. W., M. F. Christmas, F. S. Jacobson, G. Storz, and B. N. Ames.** 1986. Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc. Natl. Acad. Sci. USA **83**:8059–8063.
12. **Muller, J. M., H. W. Ziegler-Heitbrock, and P. A. Baeuerle.** 1993. Nuclear factor kappa B, a mediator of lipopolysaccharide effects. Immunobiology **187**:233–256.
13. **Nevins, J. R.** 1982. Induction of the synthesis of a 70,000 dalton mammalian heat shock protein by the adenovirus E1A gene product. Cell **29**:913–919.
14. **Ohta, M., M. Mori, T. Hasegawa, F. Nagase, I. Nakashima, and N. Kato.** 1981. Further studies of the polysaccharide of *Klebsiella pneumoniae* possessing strong adjuvanticity. I. Production of the adjuvant polysaccharide by noncapsulated mutant. Microbiol. Immunol. **25**:939–948.
15. **Pola, B. S.** 1988. A role for heat shock proteins in inflammation? Immunol. Today **9**:134–137.
16. **Shinnick, T. M.** 1991. Heat shock proteins as antigens of bacterial and parasitic pathogens. Curr. Top. Microbiol. Immunol. **167**:145–165.
17. **Skeen, M. J., and H. K. Ziegler.** 1993. Induction of murine peritoneal γ/δ T cells and their role in resistance to bacterial infection. J. Exp. Med. **178**:971–984.
18. **Tomasovic, S. P., and J. Klostergaard.** 1991. Bacterial endotoxin lipopolysaccharide modulates synthesis of the 70 kDa heat stress protein family. Int. J. Hyperthermia **7**:643–651.
19. **Welch, W. J., and J. P. Suhan.** 1985. Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. J. Cell Biol. **101**:1198–1131.
20. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides—extraction with phenol water and further application of the procedure, p. 83–91. *In* R. L. Wistler (ed.), Methods in carbohydrate chemistry, vol. 5. Academic Press, Inc., New York.
21. **Winfield, J. B., and W. N. Jarjour.** 1991. Stress proteins, autoimmunity, and autoimmune disease. Curr. Top. Microbiol. Immunol. **167**:161–189.
22. **Yokochi, T., Y. Inoue, J. Yokoo, Y. Kimura, and N. Kato.** 1989. Retention of bacterial lipopolysaccharide at the site of subcutaneous injection. Infect. Immun. **57**:1786–1791.
23. **Yokochi, T., I. Nakashima, N. Kato, J. Asai, and S. Iijima.** 1980. Adjuvant action of capsular polysaccharide of Klebsiella pneumoniae on antibody response. IX. Its effect on the histology of the regional lymph node and other lymphoid organs. Microbiol. Immunol. **24**:933–944.
24. **Young, R. A.** 1990. Stress proteins and immunology. Annu. Rev. Immunol. **8**:401–420.
25. **Young, R. A., and T. J. Elliott.** 1989. Stress proteins, infection, and immunosurveillance. Cell **59**:5–8.