Cell Stress & Chaperones (2000) **5** (5), 471–480

Article no. csac. 2000.262

Fever and the heat shock response: distinct, partially overlapping processes

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Abstract The heat shock response is an ancient and highly conserved process that is essential for surviving environmental stresses, including extremes of temperature. Fever is a more recently evolved response, during which organisms temporarily subject themselves to thermal stress in the face of infections. We review studies showing that fever is beneficial in the infected host. We show that core temperatures achieved during fever can activate the heat shock response and discuss some of the biochemical consequences of such an effect. We present data suggesting 4 possible mechanisms by which fever might confer protection: (1) directly killing or inhibiting growth of pathogens; (2) inducing cytoprotective heat shock proteins (Hsps) in host cells; (3) inducing expression of pathogen Hsps, an activator of host defenses; and (4) modifying and orchestrating host defenses. Two of these mechanisms directly involve the heat shock response. We describe how heat shock factor-1, the predominant heat-induced transcriptional enhancer not only activates transcription of Hsps but also regulates expression of pivotal cytokines and early response genes. The relationship between fever and the heat shock response is an illuminating example of how a more recently evolved response might exploit preexisting biochemical pathways for a new function.

INTRODUCTION

The heat shock response is an ancient and highly conserved biological process that is essential for surviving environmental stresses, including extremes of temperature, toxic chemicals, and high levels of radiation, conditions that cause denaturation of essential cellular proteins. During heat shock, the transcriptional and translational machinery of the cell is reprogrammed to express preferentially a set of stress-inducible heat shock proteins (Hsp). The Hsps interact with denatured proteins, either preserving them until the stress has ended or targeting the denatured protein for degradation and removal from the cell. While the heat shock response is a fail-safe mechanism for coping with unavoidable environmental stresses, fever is a complex physiologic response to infection or injury, during which organisms temporarily subject themselves to thermal stress. This paper will discuss whether fever is beneficial, if so, whether its benefit is achieved in part by inducing a heat shock response, and identify the physiological and biochemical consequences of increasing core temperature in the face of infection.

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Received 7 August 2000; Revised 29 August 2000; Accepted 31 August 2000.

HEAT SHOCK RESPONSE AND FEVER, AN EVOLUTIONARY PERSPECTIVE

Genes encoding Hsps, the principal mediators of the heat shock response, are highly conserved and found in every species studied (Feder and Hofmann 1999). The presence of homologous Hsp genes in archebacteria, eubacteria, and eukaryotes suggests that they first arose at least 2.5 billion years ago (Feder and Hofmann 1999). The Hsp genes have persisted during evolution despite the appearance of alternative protective mechanisms against stress, including the ability of higher animals to withdraw from stressful environments. The persistence of Hsps argues for their fundamental importance in all organisms. A review of the molecular mechanisms through which Hsps exert protection is beyond the scope of this paper, but Hsp biochemistry has been comprehensively reviewed (Craig et al 1994; Morimoto et al 1997).

In the setting of a conserved heat shock response, fever arose as an additional response to infection in higher animals. Classically, fever is defined as an elevation in core temperature brought about by alterations in firing rate of thermoregulatory neurons by endogenous pyrogenic mediators. Such a change in neural output regulates the integrated behavioral, physiological, and biochemical processes that determine the balance between heat generation and elimination. This response is generally considered to be limited to endothermic animals. However, if one broadens the definition of fever to an increase in core temperature stimulated by infection or injury and achieved solely by seeking external sources of heat, the prevalence of fever expands to include many ectothermic vertebrates, arthropods, and annelids (Klatersky 1971). The ability of the same antipyretic drugs that are effective in mammals to block the heat-seeking behavior in infected fish (Reynolds 1977) and reptiles (Bernheim and Kluger 1976) suggests that the mechanisms of fever in ectothermic and endothermic animals might be related. Starks (Starks et al 2000) recently reported that honeybees increase hive-wide temperature following infection of the hive with the heat-sensitive pathogenic fungus *Ascosphaera apis.* This temperature increase, achieved through a communal increase in wing muscle activity by the adult bees, kills the fungal pathogen and preserves viability of the bee larvae. In this case, the febrile response has been adapted to a hive animal in which survival of the species depends on survival of the hive rather than viability of individuals. For the remainder of this paper, we will use the broader definition of fever.

The prevalence of fever in modern day members of the 2 major animal divisions, Deuterostomia (vertebrates) and Protostomia (arthropods and annelids), suggests that it must have first appeared approximately 600 millions years ago. The persistence of fever for over 600 million years despite its considerable metabolic cost offers persuasive evidence that it is protective in the infected host.

EXPERIMENTAL EVIDENCE THAT FEVER IS PROTECTIVE IN THE INFECTED HOST

The assumption that fever is protective during infections is well supported by studies in diverse animal species. In 2 studies of ectothermic vertebrates, increases in core temperature from 38 to 40°C in the lizard *Diposaurus dorsalis* (Kluger et al 1975) and from 28 to 32.7°C in goldfish (Covert and Reynolds 1977) improves survival from 25% to 67% and from 64% to 100%, respectively, during infection with the same gram-negative pathogen, *Aeromonas hydrophila.* Similar results were found in infected sockeye salmon, rainbow trout, crickets, and grasshoppers (reviewed) (Kluger 1991). In a lethal Herpes simplex-infected mouse model, housing infected mice at 38°C for 6 days increased both their core temperature (by approximately 2°C) and their survival rate (from 0% to 85%) compared with infected mice housed at 23 to 26°C (Armstrong 1942). Schmidt et al confirmed these results in a similar model of Herpes simplex-infected mice (Schmidt and Rasmussen 1960). Bell and Moore (1974) reported a survival benefit of warming mice infected with rabies virus. In our own study of mice with experimental *Klebsiella pneumoniae* peritonitis, the survival rate improved from 0% to 50%, and the intraperitoneal bacterial load decreased 100 000-fold when core temperature (measured with a colonic thermistor probe) was raised from basal levels (36.5–37.5°C) to 39–39.5°C by housing mice at 35.5°C rather than 24°C (Fig 1) (Jiang et al 2000).

In several other animal models, administration of antipyretic agents blocked fever and decreased survival (Bernheim and Kluger 1976; Esposito 1984; Kurosawa et al 1987; Van Miert et al 1978; Vaughn et al 1980) during bacterial infections. In *A hydrophila*-infected lizards, treatment with sodium salicylate blocked fever in 7 of 12 animals, all of which died, while all febrile animals survived (Bernheim and Kluger 1976). In experimental murine *Streptococcus pneumoniae* pneumonia, treatment with aspirin impairs bacterial clearance and reduces the LD_{50} from 6.3×10^6 colony-forming units (CFU) to 3.3×10^5 CFU (Esposito 1984). While these studies demonstrate that an elevation in core temperature improves survival in several experimental models of infection, the mechanisms through which this occurs are incompletely understood. Four possible mechanisms are discussed below.

DIRECT EFFECTS ON PATHOGEN VIABILITY

Exposure to febrile temperatures might be directly cytotoxic or cytostatic for invading microbial pathogens, thereby accelerating pathogen clearance and shortening disease duration. This is the case for some human pathogens, including *Cryptococcus hominis* (Kuhn 1949), *S pneumoniae* (Rich and McKee 1936), *Neisseria gonorrhoeae* (Carpenter et al 1933), and *Mycobacterium leprae* (Rodbard et al 1980). However, the growth rate of most pathogenic bacteria, including *Staphylococcus aureus, Escherichia coli* (Mackowiak 1991), *K pneumoniae* (Jiang et al 2000), *Pasteurella multocida* (Kluger and Vaughn 1978), and *A hydrophila* (Covert and Reynolds 1977) varies little with temperature changes within the normal febrile range.

INDUCTION OF HEAT SHOCK IN HOST CELLS

The second way in which fever might confer protection during infection is by inducing Hsp expression in host cells, thereby increasing their resistance to the chemical stresses generated in the infected microenvironment (Perdrizet 1995). Cytoprotection conferred by exposure to su-

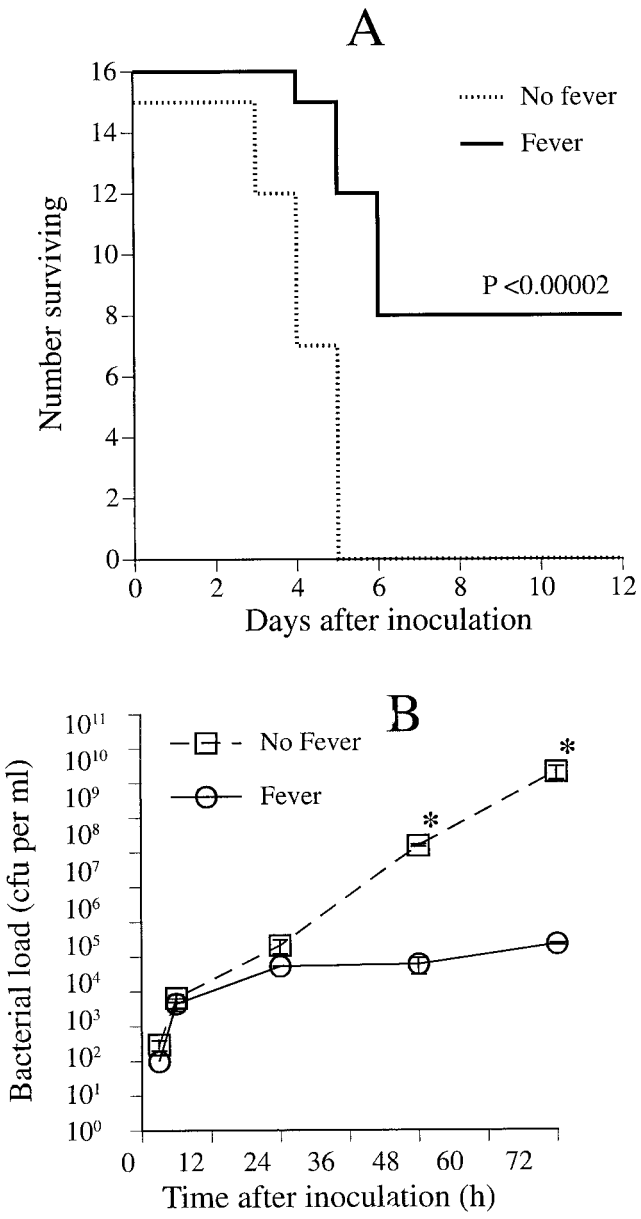

Fig 1. Influence of core temperature on experimental *K pneumoniae* peritonitis. (A) Survival after inoculation with *K pneumoniae.* Mice were inoculated intraperitoneally with 100 CFU Caroli strain of *K pneumoniae*, then placed in 23°C (no fever) or 35.5°C (fever) ambient temperatures and survival was followed over 12 days. Core temperatures were maintained at 36.5–37°C and 39–39.5°C in the 2 groups. (B) Influence of core temperature on bacterial clearance after inoculation with *K pneumoniae.* Mice were inoculated intraperitoneally with 100 CFU *K pneumoniae* strain 5055, then placed in 23° (no fever) or 35.5°C (fever) ambient temperatures. Six mice in each group were sacrificed at the indicated times and the bacterial CFUs in peritoneal lavage fluid were determined by plating on MacConkey agar. Mean ± SE; n = 6. **P* < 0.05 compared with the controls at 23°C ambient temperature.

praphysiological temperatures (42–45°C) is mediated in part by 4 families of Hsps. These proteins, induced by thermal and biochemical stresses, preserve essential cellular components. The reader is referred to 2 recent reviews for a description of the biological activities of these proteins (Craig et al 1994; Moseley 1998). In addition to Hsps, heat shock also activates at least 2 other cytoprotective genes, Cu/Zn superoxide dismutase (SOD) (Yoo et al 1999) and hemoxygenase-1 (Ewing and Maines 1991). Many studies have clearly demonstrated associations between the magnitude of Hsp expression following heat shock (41–42°C) and resistance to a subsequent injury, including 2 models of sepsis, the lipopolysaccharide (LPS)-challenged mouse (Hotchkiss et al 1993), and the rat cecal ligation and puncture model (Villar et al 1994). Directly increasing intracellular concentrations of Hsp using genetic techniques have consistently conferred protection against cellular injury. For example, overexpressing Hsp70 protected rat coronary endothelial cells from hypoxia/reoxygenation injury (Suzuki et al 1998) and the H9C2 rat cardiomyocyte cell line from oxidative injury (Chong et al 1998). Transgenic mice that overexpress Hsp70 suffer smaller cerebral infarcts following experimental cerebral ischemia (Rajdev et al 2000). While these data clearly show that Hsps are cytoprotective in the face of injuries, including those encountered during infections, the relevance of these effects to fever depends on whether Hsp generation is activated by temperatures within the febrile range.

Most studies show that induction of heat shock in mammals requires >4°C increase in temperature above basal levels (reviewed) (Feder and Hofmann 1999; Ray 1999). However, smaller increases in temperature can activate the stress response in some in vivo models. For example, lysergic acid diethylamide-induced hyperthermia activated the heat-inducible transcription factor, heat shock factor, HSF-1, in brain and kidney while increasing core temperature by only 2.5°C (Brown and Rush 1996); however, Hsp expression was not measured in this study. Work in our own laboratory using an externally warmed mouse model showed that maintaining a 3°C increase in core temperature for 3 hours stimulated the appearance of Hsp70 protein in kidney and liver (Jiang et al 1999a). Su (Su et al 1999) showed that exposing isolated rat myoblast cultures to hyperthermia in vitro (39°C for 24–48 h) also confers protection against subsequent oxidative injury, but induces expression of the constitutive Hsp, HSC73, rather than the inducible Hsps. The lower thermal threshold for heat shock in some in vivo models may reflect the capacity of some inflammatory mediators, including interferon (IFN)α/β (Morange et al 1986) and arachidonic acid (Jurivich et al 1994), to reduce the threshold for inducing the stress response. This interaction between inflammatory mediators and body temperature in

Table 1 Basal and febrile temperature ranges in modern animals

Animal	References	Basal temperature (°C)	Febrile temperature (°C)
Endotherms			
Human	Mackowiak and Boulant 1996	36.0–37.8	37.9–41
Horse	Longworth et al 1996	38.0–38.4	38.3–39.3
Dog	LeMay et al 1990	38.1–39.2	39.3–42.2
Pig	Parrott et al 1997	39.3–39.9	40.5–41.1
Rat	Romanovsky et al 1998	37.9–38.2	38.6–39.4
Mouse	Habicht 1981	36.5–37.2	37.8–39.3
Pigeon	Strouse 1909	39.7–40.7	41.0–41.5
Ectotherms			
Lizard	D'Alecy and Kluger 1975 Kluger 1978	34.0–37.0	39–42
Edible frog	Myhre et al 1977	25–28	39–35
Goldfish	Covert and Reynolds 1977	27.9	32.7 (mean)

activating the stress response might allow animals to activate selectively the response during fever rather than during episodes of hyperthermia caused by extreme exertion or exposure to elevated environmental temperatures.

The core temperature increase generated during febrile responses to infection is remarkably consistent in higher animals. Despite having distinct basal temperatures, most mammals and birds, as well as many ectothermic vertebrates generate similar 1.5 to 5°C elevations in core temperature during infections (Table 1), a range that overlaps with the threshold for the heat shock response. These data suggest that fever is capable of activating at least some components of the heat shock response.

INDUCTION OF HSP GENERATION IN THE PATHOGENS

Another way in which fever might protect the infected host is by stimulating the pathogens to generate Hsps, many of which are potent activators of host innate immune defenses. For example, *Legionella pneumophilia* Hsp60, *E coli* GroEL, *Mycobacterium tuberculosis* Hsp70, *M leprae* HSP65, and *Mycobacterium bovis* Hsp65 each induced mouse peritoneal macrophages to express interleukin (IL)-1α, IL-1β, IL-6, tumor necrosis factor (TNF)α, and granulocyte–macrophage colony-stimulating factor mRNA (Retzlaff et al 1994). Recently, Kol et al (2000) reported that chlamydial Hsp65 and human Hsp60 apparently activate mononuclear phagocytes through the same pattern recognition receptor, CD14, that mediates macrophage activation by bacterial endotoxin. However, because CD14 is a phosphatidylinositol-anchored surface protein, Hsp must be either cell free or expressed on the bacterial surface to be accessible to this macrophage protein. This has been shown to occur during in vitro culture of 2 bacterial pathogens, *Helicobacter pylori* (Phadnis et al 1996) and *Haemophylus ducreyi* (Frisk et al 1998). Asea (Asea et al 2000) reported that CD14 is also essential for macrophage activation by human Hsp70. Hsp65 from *M bovis* also directly activates human endothelial cells to increase adhesiveness for monocytes and granulocytes (Verdegaal et al 1996), an early step in the recruitment of these inflammatory cells to sites of infection. By increasing synthesis and release of Hsps from bacterial pathogens, fever might provide an early signal for activation of innate defenses. Furthermore, by stimulating Hsp generation in pathogens, fever might help target-activated host defenses to sites of infection and thus limit a potentially counterproductive systemic inflammatory response.

THE IMMUNOREGULATORY EFFECTS OF FEVER

A fourth way in which fever might improve survival during infections is by helping to orchestrate and optimize the host immune response through mechanisms that may utilize components of the heat shock response. Exposure to elevated temperatures has been reported to exert both stimulatory and inhibitory effects on components of the immune response.

Human polymorphonuclear cell (PMN) motility (Bryant et al 1971; Nahas et al 1971) and phagocytosis (Ellingson and Clark 1942; van Oss et al 1980) are potentiated at temperatures within the human febrile range, but PMN chemotaxis is not enhanced, and bactericidal capacity is only weakly and inconsistently augmented by exposure to these temperatures (Roberts and Steigbigel 1977; Sebag et al 1977). At temperatures above the human febrile range (>41°C) bacterial phagocytosis and killing by PMNs are reduced (Leijh et al 1979; Peterson et al 1976, 1977; Sebag et al 1977; van Oss et al 1980). Thus, enhancement of human PMN functions in vitro and optimal survival in clinical infections occur at similar temperatures (Hodgin and Sanford 1965; Bryant et al 1971; Mackowiak et al 1980).

In murine macrophages, several functions that are required for microbicidal activity are enhanced at febrile range temperatures, including expression of Fc receptors, phagocytosis, pinocytosis, and reduction of nitroblue tetrazolium (Yoshioka et al 1990; Bruggen et al 1991), a measure of oxygen radical generation and killing of intracellular bacteria (Berman and Neva 1981). However, like PMNs, murine and human macrophages have markedly reduced function at temperatures >41°C (Leijh et al 1981; Yoshioka et al 1990; Bruggen et al 1991).

Exposing human lymphocytes to febrile-range temperatures (38–41°C) in vitro enhances their L-selectin-mediated binding to lymphatic endothelium (Wang et al 1998),

an important early step in lymphocyte recruitment. Several groups have shown that exposing human T lymphocytes to febrile temperatures also enhances their proliferative response to nonspecific mitogens (Ashman and Nahmias 1977; Roberts and Steigbigel 1977; Manzella and Roberts 1979; Narvanen et al 1986), and allogeneic lymphocytes (Smith et al 1978), IL-1, and IL-2 (Duff and Durum 1982; Lederman et al 1987). However, like PMNs and macrophages, human T lymphocytes exhibit a reduced proliferative response when exposed to temperature ≥41°C (Roberts et al 1985; Lederman et al 1987). In mice, T helper cell potentiation of the B cell antibody response (Jampel et al 1983a, 1983b; Saririan and Nickerson 1982) and generation of cytotoxic T lymphocytes against allogeneic cells (Smith et al 1978) and virus-infected cells (Mullbacher 1984; Owen et al 1988) are also enhanced by exposure to febrile temperatures. Taken together, the studies of neutrophils, macrophages, and lymphocytes in mice and humans demonstrate a similar pattern of temperature-responsiveness in which many of the functions that are essential for microbicidal activities are enhanced at febrile temperature (38–41°C) but are attenuated at temperatures >41°C.

Antimicrobial defenses are orchestrated, at least in part, by a structurally and functionally diverse group of proteins called cytokines. Such cytokines have complex biological activities, sometimes overlapping and sometimes antagonistic, that influence immune cell functions. Some cytokines, notably IL-1, TNFα, and the IFNs, are required for optimal host defense (Cross et al 1989, 1995; Marino et al 1997), and yet, when dysregulated, appear to participate in the pathogenesis of sepsis (Ward and Lentsch 1999). Thus, the net effect of these cytokines on survival during sepsis is determined by the magnitude, timing, and pattern of their collective expression.

A growing body of literature has shown that expression of these cytokines is modified by exposure to elevated temperatures, but these effects are complex and are influenced by the magnitude and timing of the temperature change and the cellular source of the cytokines. For example, exposing murine peritoneal macrophages to 42–43°C for 1 hour beginning immediately after LPS stimulation caused profound inhibition of TNFα secretion, while a similar exposure to 40.5°C had no effect on TNFα secretion (Tomasovic et al 1989). In contrast, if the 1-hour warming to 42°C was delayed until 2 to 4 hours after LPS stimulation, TNFα secretion was increased 3- to 6-fold. If the 1-hour exposure to 42°C was followed by a 4-hour recovery at 37°C before adding LPS, the inhibition of TNFα secereton was reversed, but the same 37°C recovery failed to restore TNFα expression after exposure to 43°C. In the U-373 astrocytoma cell line, continuous exposure to 40°C coincident with LPS stimulation reduced the accumulation of TNFα and IL-1β mRNA and attenuated cytokine secretion (Velasco et al 1991). In human monocyte-derived macrophages (Ensor et al 1994), the Raw 264.7 murine macrophage cell line (Ensor et al 1995; Jiang et al 2000; Singh et al 2000), murine Kupffer cells, liver slices, and peritoneal macrophages (Jiang et al 1999b), continuous exposure to 39.5–40°C beginning 30 minutes prior to stimulation with LPS reduced TNFα secretion. We recently reported that the temperature-dependent inhibition of TNFα expression in Raw 264.7 cells and murine peritoneal macrophages was caused by an early and abrupt termination of TNFα transcriptional activation in the warmer (39.5°C) cells (Fig 2A) (Singh et al 2000).

The direct effects of elevated temperature on IFN generation are variable and appear to depend on the type of IFN studied, the magnitude of the temperature increase, and the stimulus used to induce IFN production. Incubating human peripheral blood mononuclear cells (PBMC) at 39°C for 24 hours reduces IFNγ in LPS-stimulated cells (Kappel et al 1991), but warming the cells to 40.7°C for 24 hours failed to inhibit IFNγ secretion (Roberts 1986). Exposing human PBMC to 42.7°C for 24 hours reduces generation of IFNα in mitogen-stimulated, but not in influenza virus-infected cells (Roberts 1986). By contrast, increasing in vivo core temperature of humans and monkeys to febrile levels by external warming prior to collecting PBMC increases their capacity for generating IFNγ after stimulation with phytohemagglutinin in vitro (Downing et al 1987, 1988).

A CENTRAL ROLE FOR HSF-1 IN MEDIATING THE EFFECTS OF FEVER

Hsp genes are regulated by a family of transcription factors called HSFs. A family of 4 HSFs has been described in vertebrates, of which HSF-1 is the predominant stress-activated member (Tanabe et al 1997). The biology of HSF-1 relevant to its function as a transcriptional activator has been recently reviewed (Morimoto 1998). HSF-1 is a complex molecule comprising several domains, including an amino-terminal transcription activation domain, a carboxy-terminal DNA binding domain, and a trimerization domain mediating its homotypic polymerization. Under basal conditions, HSF-1 exists as a monomer bound to Hsp90 in a multichaperone complex (Zou et al 1998) and is incapable of stable DNA binding. Following stress, HSF-1 trimerizes, translocates to the nucleus, and is phosphorylated. Once activated, HSF-1 attains the capacity to bind to its cognate heat shock response element (HRE) binding site comprised of inverted dyad repeats of the pentanucleotide nGAAn (Perisic et al 1989) and to activate transcription (Sarge et al 1993). Phosphorylation of HSF-1 can either activate or inactivate its DNA binding and transactivating activities depending on which serine

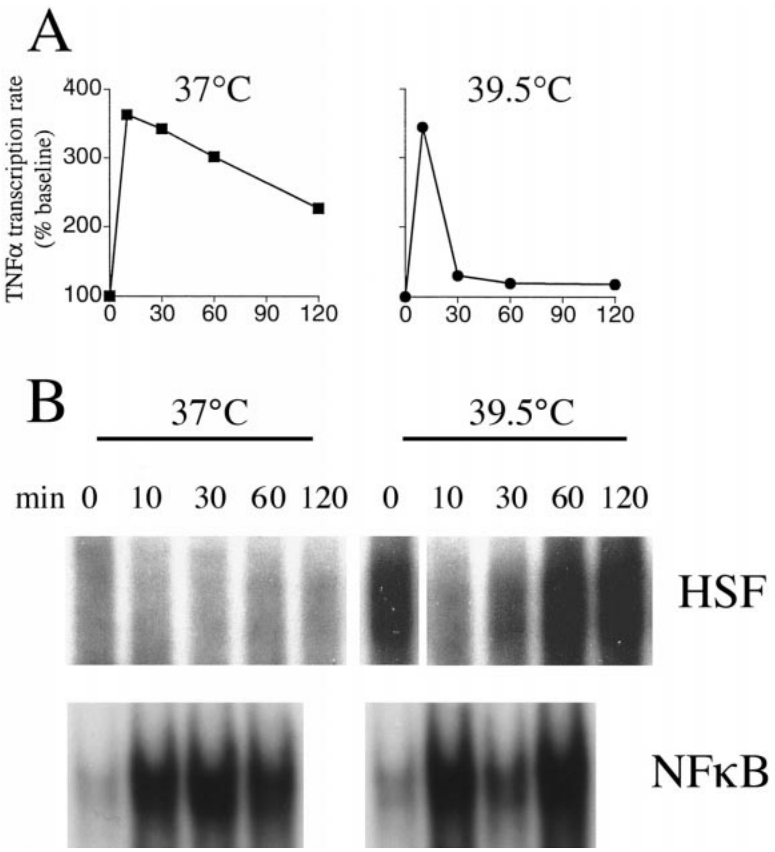

Fig 2. Effect of incubation temperature on TNFα transcription and HSF-1 and NFκB activation. (A) TNFα transcription in Raw 264.7 cells. Cells were preincubated for 30 minutes and then stimulated with 100 ng/ml LPS at the indicated temperature and the TNFα and glyceraldehyde phosphate dehydrogenase (GAPDH) transcription rates were measured by nuclear run-on assay. Background signal (insertless plasmid) was subtracted, and the TNFα/GAPDH ratio was calculated for each time point and normalized to pre-LPS levels at each temperature. Mean ± SE; $*P < 0.05$ compared with 37°C. (B) An electrophoretic mobility shift assay (EMSA) analysis of HSF and NFκB. EMSA was performed using an HRE sequence probe from the human Hsp70 promoter (5′-GATCTCGGCTGGAATATTCCCGACCTGGCAGCCGA-3′) (upper) or an NFκB response element probe from the murine TNFα promoter (5′-ACAGGGGGCTTTCCCTCCT-3′). Raw 264.7 nuclear extracts were obtained after incubation with 100 ng/ml LPS at the indicated temperature and for the indicated time. For the 39.5°C extracts, cells were preincubated at 39.5°C for 30 minutes prior to adding LPS.

and threonine residues are phosphorylated. While the protein kinases mediating stress-induced HSF-1 activation have not been clearly identified, several members of the mitogen-activated protein (MAP) kinase family have been shown to inactivate HSF-1, including extracellular signal-regulated kinase (ERK)-1 (Mivechi and Giaccia 1995; Knauf et al 1996). HSF-1 can also be inactivated by binding to Hsp90 and p23 (Zou et al 1998), Hsp70 (Kim et al 1995), and HSFBP (Satyal et al 1998).

Recent studies of HSF-1 indicate that besides activating transcription of Hsp genes, it also inhibits transcription of certain cytokine and early response genes, including IL-1β (Cahill et al 1996), *c-fos* (Chen et al 1997), and TNFα (Singh et al 2000). In the case of IL-1β, the access of the C/EBP-β/NF-IL-6 transcriptional enhancer to its cognate binding site in the IL-1β promoter is blocked by HSF-1 binding to a contiguous HRE in the heat-shocked THP-1 human promonocyte cell line (Cahill et al 1996). In the case of *c-fos*, the ability of HSF-1 mutants that are deficient in DNA binding and transactivation activities to inhibit its transcription and the absence of an HRE in the c-fos promoter indicates that HSF-1 represses *c-fos* and IL-1β through different molecular mechanisms (Chen et al 1997). In studying how febrile temperatures inhibited TNFα expression in the murine Raw 264.7 macrophage cell line, we found (1) that HSF-1 is partially activated at febrile temperatures (39–39.5°C) to a DNA binding form that lacks transactivating activity, (2) that this heat-activated form of HSF-1 can bind to the TNFα promoter and/or 5′ untranslated region sequence (−85 to +138 bp of the murine TNFα gene), and (3) that overexpression of HSF-1 represses TNFα transcription (Singh et al 2000). Although this region of the TNFα gene does not contain HREs, it does contain multiple HRE half-sites (nGAAn) in critical locations, including sites (1) adjacent to an essential Sp1 binding site, (2) at the transcription start site, and (3) a 5 half-site array 35–75 nucleotides downstream of the transcription start site. These observations suggest

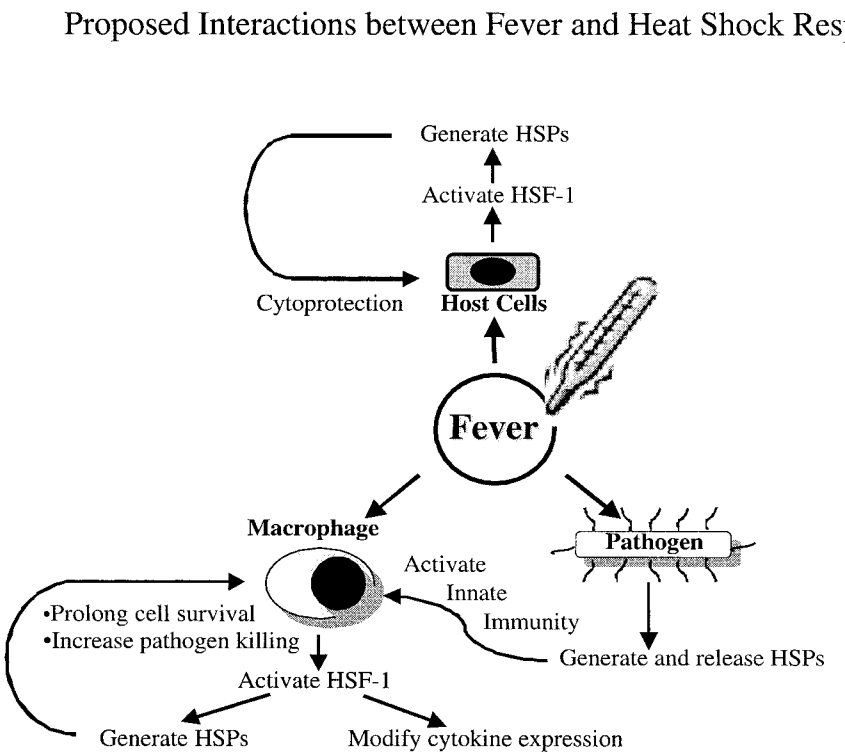

Fig 3. Proposed interactions between fever and heat shock response through which protection is conferred to the infected host.

that HSF-1 activated during febrile states might repress TNFα transcription by blocking the binding of Sp1, by interfering with assembly of the general transcription complex, and by preventing transcript elongation. This proposition was further supported by Xiao (Xiao et al 1999), who showed that mice deficient in HSF-1 have exaggerated TNFα production and increased mortality following endotoxin challenge.

In Raw 264.7 macrophages, LPS treatment in the presence of activated HSF-1 (achieved by preincubating the cells at 39.5°C for 30 min) stimulates TNFα transcriptional activation of comparable magnitude but of much shorter duration than in 37°C Raw 264.7 cell cultures (Fig 2A) (Singh et al 2000). This raises the question of how transcriptional activation could occur at all in the presence of this putative repressor. A parallel analysis of HSF-1 by electrophoretic mobility shift assay shows that activated HSF-1 is lost within 10 minutes of adding LPS (Fig 2B). This loss is coincident with activation of the transcriptional enhancer NFκB and with transcriptional activation of TNFα. Activated HSF-1 subsequently reappeared coincident with a rapid inactivation of TNFα transcription and a reduction in levels of NFκB. The mechanisms through which LPS causes the loss of activated HSF-1 are unkown, but immunoblot analysis suggests that it is caused by inactivation of HSF-1 rather than reduction in the level of HSF-1 protein (Singh et al 2000). LPS is a potent inducer of MAP kinases, including ERK-1, which in turn can inactivate HSF-1 (Mivechi and Giaccia 1995), suggesting at least one way in which LPS may transiently derepress TNFα transcription in the presence of HSF-1.

Based on these data, we propose the hypothesis that once the febrile state is established the presence of activated HSF-1 in macrophages represses TNFα transcription. The interaction between LPS-induced signaling pathways and HSF-1 changes the kinetics of subsequent TNFα expression to one characterized by shorter bursts rather than sustained TNFα synthesis. Such a change in cytokine expression pattern might retain the immunostimulatory activity of TNFα while reducing the risk of host tissue injury that can be caused by sustained exposure to high levels of TNFα (Tracey et al 1986).

In summary, we have reviewed studies showing that fever is beneficial in the infected host. We showed that fever can activate the heat shock response and discussed some of the biochemical consequences of such an effect. We presented data suggesting 4 possible mechanisms through which fever might confer protection. Two of these mechanisms directly involve the heat shock response in host cells or by the pathogen, and a third mechanism, the regulation of immune defenses, that might share some components of the heat shock response (Fig 3). We described how HSF-1, the predominant heat-induced transcriptional enhancer not only activates tran-

scription of HSPs but also regulates expression of pivotal cytokines and early response genes. The relationship between fever and the heat shock response is an intriguing example of how a more recently evolved biological response might exploit preexisting biochemical pathways for a new purpose.

ACKNOWLEDGMENTS

We thank Drs Rose Viscardi, Phil Mackowiak, and Sheldon E. Greisman for their intellectual input and for reviewing this manuscript. This work was supported by PHS grant RO1 AI42117 (J.D.H.) and a VA Merit Review grant (J.D.H.).

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Erratum

The first author of the article ''Immobilization stress induces c-fos accumulation in liver,'' Vol 5(4), is Guillermo Fernández-Varo, not Guillermo Fernández.