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Physiological Fever Temperature Induces a Protective Stress Response in T Lymphocytes Mediated by Heat Shock Factor-1 (HSF1)

Patience Murapa,* Siva Gandhapudi,* Hollie S. Skaggs,† Kevin D. Sarge,† and Jerold G. Woodward2,*

Heat shock factor-1 (HSF1) is a transcription factor that serves as the major temperature-inducible sensor for eukaryotic cells. In most cell types, HSF1 becomes activated to the DNA binding form at 42°C and mediates the classical heat shock response, protecting the cells from subsequent lethal temperatures. We have recently demonstrated that HSF1 is activated at a lower temperature in T lymphocytes than in most other cell types (39°C vs 42°C), within the physiological range of fever. In this study, we show that T cell activation at fever temperatures not only activates HSF1 but induces the up-regulation of the HSF1 protein and the HSF1-regulated protein, HSP70i. T cells from HSF1 knockout mice proliferate normally under optimal conditions but are impaired in proliferation at physiological fever temperatures and low CO2 concentrations, conditions that do not impair wild-type T cells. This defect in proliferation appears to be mediated by a block in the G1/S transition of the cell cycle and is independent of HSP70. Elevated temperature and low CO2 concentrations resulted in a dramatic reduction of the intracellular reactive oxygen species (ROS) levels in both normal and knockout T cells. Wild-type T cells were able to restore ROS levels to normal within 5 h, whereas HSF1−/− T cells were not. These results suggest that the proliferation defect seen in T cells from HSF1−/− mice at fever temperatures was because of dysregulated ROS levels and that HSF1 is important in maintaining ROS homeostasis and cell cycle progression under the stressful conditions encountered during fever. The Journal of Immunology, 2007, 179: 8305–8312.

The febrile response is an adaptive physiological response in animals that is well conserved throughout evolution. In mammals, the febrile response involves an increase in cytokines and acute phase proteins as well as activation of the central nervous and endocrine systems, all of which participate in the elevation of core body temperature. In ectotherms, elevation of core body temperature is achieved by moving to warmer environments (1). In both endotherms and ectotherms, survival after experimental infection is reduced drastically if temperature elevation is inhibited, indicating that temperature elevation is critical for survival from pathogens (2–4). How temperature elevation translates into increased resistance to infection is poorly understood. It is generally accepted that temperature elevation acts to enhance the host immune response rather than pathogen growth or survival, and a large number of studies generally support this (reviewed in Refs. 2 and 4). The best examples of fever-enhancing effects have been in the innate immune system (5–10). The effects of fever temperature on the adaptive immune system are less clear. Some studies have shown increases in T or B cell responses whereas other studies have shown no change or reduced responses (2, 11–16). Because of the variations in experimental systems and the complex cellular interactions involved, it has been difficult to study temperature effects at the mechanistic level. Thus, our current knowledge of how fever temperature affects immune cell function is still very limited.

The cellular response to increased temperature has been studied extensively. Exposure of cells to elevated temperatures, typically 42°C, causes rapid induction of heat shock proteins that serve a variety of important protective roles for the cell (17–19). This is typified by the classical heat shock response in which cells exposed to 42°C are found to be protected from the lethal effects of a subsequent 45°C exposure. This effect is primarily mediated by the transcription factor known as heat shock factor-1 (HSF1)3 (20, 21). In response to elevated temperature or other stressors, cytoplasmic monomeric HSF1 trimerizes and translocates to the nucleus where it binds to heat shock-responsive elements found in numerous heat-inducible genes, in particular heat shock protein (HSP) genes (reviewed in Ref. 22). The temperature at which HSF1 becomes activated in different cell types has been studied extensively using EMSAs to measure activated HSF1 binding to heat shock-responsive elements. These cell types include cell lines such as HeLa and K562 (23–25) and tissues such as liver (26), lung, kidney, and heart (27). In all of these cases, activation of HSF1 is not apparent until the cells or tissue are warmed to 42°C, a temperature at the very high end of the physiological fever range. When physiological fever temperatures are used, little or no HSF1 activation in these tissues is apparent. One notable exception to

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Abbreviations used in this paper: HSF1, heat shock factor-1; DCFH-DA, 2,7’-dichlorodihydrofluorescein diacetate; HSP, heat shock protein; HSP70i, inducible HSP70; ROS, reactive oxygen species.
this is the male germ cell. The temperature of the testes is tightly regulated at 30°C, and HSF1 activation in the male germ cell occurs at a temperature of 35°C (26, 28). Nonetheless, even in the testes neighboring interstitial somatic cells show a 42°C activation temperature for HSF1 (20). Because of this, HSF1 activation has largely been viewed as a protective mechanism in response to severe thermal stress, but not fever (29–31). However, we recently demonstrated that in T lymphocytes HSF1 becomes activated at 39°C, well within the physiological range of fever (27). This lower threshold for HSF1 activation does not occur in B lymphocytes, which show the more typical 42°C activation threshold. Thus, the only somatic cell type to date showing HSF1 activation at fever temperature is the T lymphocyte. This led us to hypothesize that HSF1 serves a protective and/or function-enhancing role in T cells at fever temperatures (27).

One of the most widely studied genes controlled by HSF1 is the inducible form of HSP70 (HSP70i). HSP70i has been shown to be regulated by HSF1 whereas a related constitutive form of HSP70 (HSC70) is not (20). HSP70 has numerous effects on the immune system. As a chaperone, HSP70 binds a wide variety of self and foreign peptides, resulting in greatly enhanced uptake of Ags by APC and enhanced cross-presentation (32–34). HSP70 also directly activates APC, possibly by direct interaction with TLRs (32, 35–37). Fever temperatures cause the up-regulation of HSP70i in a variety of leukocytes including T cells, leading several groups to propose that fever temperatures provide an innate “danger” signal to the immune system (8, 9, 27, 38). These inducible effects can largely be attributed to HSP70i, because HSC70 is constitutively expressed in all cell types including T cells (38). Because HSP70i appears to be strictly regulated by HSF1, these latter studies support the idea that HSF1 plays an important role in the enhancement of immune responses at fever temperatures. However, the role of HSF1 in T cell function at fever temperatures has not been examined.

Recently, HSF1−/− mice have provided additional insight into the biological role of HSF1. HSF1−/− mice generally survive to adulthood, but there is some embryonic lethality mediated by defects in the choroiallantoic placenta that is dependent on the background genotype (39). HSF1−/− females are also sterile because of a requirement for maternal HSF1 in early postfertilization development of the zygote (40). Cells from HSF1−/− mice lack the classical heat shock response, underscoring the key role of HSF1 as the major temperature-inducible transcription factor in cells (20). Adult HSF1−/− mice have normal frequencies of T and B cells and normal T cell proliferative responses following immunization with nominal Ag (34). However, they show an impairment in IgG1 and IgG2a Ab in response to immunization (41). HSF1−/− mice are also deficient in a variety of HSP70-mediated immunologic effects, primarily at the level of the APC (9, 34). However, there have been no reports demonstrating a role for HSF1 in T cells.

In this study we show that T cells from HSF1−/− mice were profoundly deficient in proliferation and cell cycle progression at fever temperatures. The inhibitory effect of elevated temperature on proliferation was more pronounced at low CO2 concentrations and appeared to be due to a block between the G1 and S phase of the cell cycle. The modulating effect of CO2 led us to investigate intracellular reactive oxygen species (ROS) levels in T cells at fever temperatures. Surprisingly, we found that fever temperature significantly lowered ROS levels in T cells, and the levels were further reduced in low CO2 environments. T cells normally increase their ROS levels upon activation, and wild-type T cells could overcome this temperature-induced depression in ROS. In contrast, HSF1−/− T cells were unable to recover from this effect, suggesting that the inability of HSF1−/− T cells to proliferate at fever temperatures is because of depressed ROS levels. Finally, this proliferation defect in HSF1−/− T cells was not because of a lack of HSP70i expression. These results suggest that HSF1 plays a key role in facilitating the proliferation of T cells and maintaining ROS homeostasis at fever temperatures.

Materials and Methods

Mice

HSF1−/− mice on the 129 × BALB/c mixed background were colonized from breeding pairs provided by I. Benjamin (University of Utah, Salt Lake City, UT). Mice were maintained by breeding heterozygotes. Homozygous HSF1−/− mice were obtained by intercrossing heterozygous males and females or homozygous males and heterozygous females and selecting HSF1−/− mice by PCR. Heterozygous or wild-type littermates were used as controls. HSP70−/− mice on a B6 × 129 mixed background (B6;129S5-Hspalatm1Dix Hspltb1m1Dix/UCD) were obtained from the Mutant Mouse Regional Resource Center (University of California, Davis, CA). These mice are deleted for both hspa1a and hspb1 genes encoding the inducible HSP70i proteins (42). Homozygous knockout mice were obtained by intercrossing heterozygotes or homozygotes and selecting by PCR. Wild-type littermates were used as controls. Mice were maintained under specific-pathogen-free conditions at the Division of Laboratory Animal Resources, University of Kentucky Medical Center (Lexington, KY). All animal experiments have been reviewed and approved by the University of Kentucky Institutional Animal Care and Use Committee.

Lymphocyte cultures

T cells were purified from spleen and lymph nodes using anti-CD3 magnetic beads and the autoMACS (Miltenyi) Biotech. For proliferation assays, 2 × 105 T cells were cultured in RPMI 1640 medium supplemented with HEPES (1 mM), 1 mM glutamine, 1 mM sodium pyruvate, 1× MEM nonessential amino acids, 50 μM 2-ME (except where indicated), 100 U/ml penicillin, and 100 U/ml streptomycin (complete medium), alone or with plate bound anti-CD3, Con A (2 μg/ml), or PMA/ionomycin for 3–4 days at the indicated temperature. [3H]Thymidine was added during the last 18 h and proliferation was determined by scintillation counting. For CFSE experiments, purified T cells were first labeled with CFSE as previously described (43) and cultured at 1 × 106 cells per well in 24-well plates, coated or not coated with anti-CD3 at the indicated temperature and CO2 concentration. Cells were harvested, stained with anti-CD4 or anti-CD8, and analyzed on a FACScan flow cytometer. For cell cycle analysis, cells were harvested from 24-well plates, fixed, permeabilized, and stained with propidium iodide to determine DNA content. DNA synthesis was determined by the addition of [3H]thymidine for 18 h. Cell viability was determined by trypan blue exclusion.

Western blot analysis

CD4+ T cells were purified by using anti-CD4 magnetic beads and autoMACS (Miltenyi) according the manufacturer’s directions. Purified CD4+ T cells were incubated in complete medium in the presence or absence of anti-CD3 for 6 h at either 37°C or 39.5°C.

Cells were harvested for 10 min at 4°C in 5 volumes of buffer C (25% (v/v) glycerol, 0.42 M NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 20 mM HEPES (pH 7.9), 1 mM PMSF, and 1 mM DTT) and centrifuged at 13,000 rpm at 4°C. Five microliters of the supernatant was boiled for 5 min at 4°C. The supernatant was loaded into the cells in PBS and incubated for 30 min at 37°C before being boiled for 5 min before being boiled for 5 min. The supernatant was loaded into the cells in PBS and incubated for 30 min at 37°C before being boiled for 5 min. The supernatant was loaded into the cells in PBS and incubated for 30 min at 37°C before being boiled for 5 min. The supernatant was loaded into the cells in PBS and incubated for 30 min at 37°C before being boiled for 5 min. The supernatant was loaded into the cells in PBS and incubated for 30 min at 37°C before being boiled for 5 min.
HSF1 protein expression. Purified CD4+ T cells from either HSF1+/+ or HSF1−/− mice were cultured for 6 h in medium alone or with plate-bound anti-CD3 (α-CD3) at either 37°C or 39.5°C. Shown are Western blots of cell extracts probed with anti-HSP70i, anti-HSF1, or anti-actin Ab as a loading control.

10 mM HEPES, and 10 mM PIPES). After staining, cells were washed once and resuspended in PBS. Cells for the standard curve were resuspended in the pH standards containing 20 μM of nigericin (Catalog no. N1495; Molecular Probes) to equilibrate intracellular pH with the extracellular medium. The cells were analyzed at 37°C using a MoFlo flow cytometer and the fluorescence intensity ratios of two wavelengths, λex/λem (FL3/FL2), were calculated. A value of p for the samples was determined using a linear regression equation derived from the standard curve.

**Statistical analysis**

Results are expressed as mean values ± SD. Comparisons were made using a paired t test. Statistical indications in the figures are defined as follows: *p ≤ 0.01; **p ≤ 0.005; and ***p ≤ 0.001. All experiments were repeated at least three times.

**Results**

**Physiological fever temperatures and T cell activation up-regulate and activate HSF1 in T lymphocytes**

Our previous studies demonstrated that HSF1 is activated to the DNA binding form in T cells at physiological fever temperatures (39°C) in contrast to 42°C for B cells and other nonlymphoid cells (27). To further understand the role of HSF1 in T cells, we examined the expression of HSF1 protein and the product of an HSF1-responsive gene, HSP70i, in T cells by Western blotting (Fig. 1). This method detects total HSF1 protein in cells regardless of whether it is active or not, whereas the presence of HSP70i is an indication of transcriptionally active HSF1. HSF1 protein was constitutively expressed but inactive in resting T cells, as revealed by the lack of HSP70i. After incubation of resting T cells at 39.5°C, HSF1 protein levels were slightly reduced but HSF1 activation was apparent through the induction of HSP70i. The reduction in HSF1 protein levels was most likely because of its activation, localization in the nucleus, and binding to nuclear material. In contrast, anti-CD3 activation of T cells resulted in up-regulation of HSF1 protein and HSF1 transcriptional activity. This effect was observed at 37°C but was much more pronounced at 39°C. No HSF1 protein was detectable in T cells from HSF1−/− mice, indicating that temperature- and activation-induced HSP70i expression is totally dependent on HSF1. Thus, T cell activation, even at 37°C, results in the up-regulation of HSF1 protein and conversion to an active transcription factor, and this is further enhanced at fever temperatures. Previous studies have documented the induction of HSP70i in lymphocytes at febrile temperatures (8, 38). Our results further demonstrate that this induction is entirely dependent on HSF1 and that HSF1 activation occurs in stimulated T cells even at 37°C.

**HSF1 is required for optimal T cell proliferation under conditions of elevated temperature in the fever range and low CO2 concentrations**

Because HSF1 is strongly activated in T cells at physiological fever temperatures, and given the critical role of T cell function during fever, we hypothesized that HSF1 plays an important role in either the protection of T cells and/or the enhancement of T cell function at fever temperatures. To test this, T cells from HSF1+/+ or HSF1−/− mice were stimulated in vitro with anti-CD3, anti-CD3 plus anti-CD28, Con-A, or PMA/ionomycin at either 37°C or 39.5°C (Fig. 2, A–F). For all four stimuli, HSF1+/+ T cells proliferated extensively at both 37°C and 39.5°C. Occasionally we noted some reduction in proliferation of HSF1+/+ T cells at 39°C
and 3% CO2, this effect was selective for the sor. Given that wild-type cells still proliferated normally at 39.5°C. In many cases proliferation was almost completely shut down, whereas in others the reduction was highly significant. This effect was observed over a wide range of anti-CD3 and anti-CD28 concentrations and under optimal T cell stimulation conditions. Superoptimal anti-CD28 stimulation may partially reverse this effect, but this was not consistently observed. The block in proliferation of HSF1−/− T cells at 39.5°C was also observed after stimulation with Con A or PMA/ionomycin (Fig. 2, E and F). Because PMA/ionomycin stimulation bypasses the proximal TCR signal transduction machinery, the block in proliferation must occur downstream from these events. Furthermore, because these experiments were performed with purified T cells, this effect is T cell intrinsic.

During the course of our experiments we noticed that the 39.5°C incubator had been running at a lower CO2 percentage than the 37°C incubator (3 vs 5%, respectively) due to the fact that the higher temperature setting altered the calibration of the CO2 sensor. Given that wild-type cells still proliferated normally at 39.5°C and 3% CO2, this effect was selective for the HSF1−/− T cells. We therefore performed a series of experiments to determine the effect of CO2 concentration on cell proliferation of HSF1−/− T cells. A series of incubators were set up at either 37°C or 39.5°C and CO2 concentrations of 8, 5, or 3%, carefully calibrated using the Fyrite CO2 sensor. Purified T cells from either wild-type or HSF1−/− mice were then stimulated with plate-bound anti-CD3 under different temperatures and CO2 conditions. Wild-type T cells proliferated well at 39.5°C and all CO2 concentrations. In contrast, T cells from HSF1−/− mice proliferated well at 39.5°C and 8 or 5% CO2, but failed to proliferate at 39.5°C and 3% CO2 (Fig. 2E). In a separate experiment, purified T cells from HSF1−/− mice proliferated well at 37°C and 7% CO2 or 37°C and 3% CO2, but not at 39.5°C and 3% CO2. T cells from heterozygous HSF1+/− mice proliferated normally at all temperatures and CO2 concentrations, indicating that this phenotype requires a homozygous HSF1 mutation (Fig. 2F). Thus, the proliferation defect observed in HSF1−/− T cells was only observed under conditions of elevated temperature in the fever range and low CO2 concentrations, conditions that do not impair T cells from wild-type or HSF1 heterozygous mice. These data demonstrate that HSF1 and/or downstream target genes play a protective role in facilitating T cell proliferation at fever temperatures.

The proliferation defect in HSF1−/− T cells is due to a block between the G1 and S phase of the cell cycle

The previous CFSE experiments suggest that the majority of T cells from HSF1−/− mice fail to enter into cell division when cultured under nonpermissive conditions. To further determine the phase of the cell cycle at which these cells were blocked, purified T cells were activated with plate-bound anti-CD3 under permissive or nonpermissive conditions for 48 h, stained for DNA content with propidium iodide, and analyzed for the percentage of cells in either G0/G1 or S/G2/M phases of the cell cycle by flow cytometry. Roughly 50% of the T cells from HSF1−/− mice had entered into the S/G2/M phase of the cell cycle after 48 h of activation at either 37°C and 7% CO2 or 39.5°C and 3% CO2. In contrast, T cells from HSF1−/− mice showed normal entry into the S phase of the cell cycle at 37°C and 7% CO2 but minimal entry at 39.5°C and 3% CO2 (Fig. 4A). To measure the transition from the G0 to the G1 phase of the cell cycle, incorporation of [3H]uridine was measured as an indication of RNA synthesis in the first 24 h of activation (Fig. 4B). Both HSF1+/+ and HSF1−/− T cells showed high and comparable levels of uridine incorporation at 37°C and 7% CO2. Although uridine incorporation in T cells from both HSF1+/+ and HSF1−/− mice was somewhat diminished at 39.5°C/3% CO2, these differences were not statistically significant. Furthermore, 24 h activated T cells from HSF1−/− showed an increase in cell size.
39.5°C and 7% CO₂ for the first 24 h and then switched to 39.5°C and 3% CO₂, proliferating these cells after 48 h allowed completely normal proliferation. HSF1 is not impaired in their early activation from the G₀ to the G₁ phase of the cell cycle. Cell viability measurements during the first 24 h of culture showed no significant reduction in viability in the HSF1⁻/⁻ T cells cultured at 39.5°C and 3% CO₂ (Fig. 4). Individual cultures of purified CD3⁺ T cells were stimulated with anti-CD3 for 24 h and the percentage of viable cells was determined by trypan blue dye exclusion. D, Individual cultures of purified CD3⁺ T cells were stimulated with anti-CD3 for 4 days at either 37°C and 5% CO₂, 39.5°C and 3% CO₂, or combinations thereof on different days as indicated. [3H]thymidine ([H Tdr]) was added for the final 18 h of culture. Data represent the mean cpm ± SD of triplicate wells.

comparable to that of their wild-type counterparts (data not shown). These data indicate that T cells from HSF1⁻/⁻ mice were not impaired in their early activation from the G₀ to the G₁ phase of the cell cycle. Cell viability measurements during the first 24 h of culture showed no significant reduction in viability in the HSF1⁻/⁻ T cells cultured at 39.5°C and 3% CO₂ (Fig. 4), indicating that these cells were not undergoing apoptosis. To determine the reversibility of the HSF1⁻/⁻ T cell proliferation defect at different time points, T cells from HSF1⁺/+ or HSF1⁻/⁻ mice were cultured at either 37°C and 7% CO₂ or 39.5°C and 3% CO₂ for the entire 4 days or with switching between temperatures at various time points. As previously shown, T cells from HSF1⁻/⁻ mice proliferated well at either temperature or combination thereof. Also as expected, T cells from HSF1⁻/⁻ mice proliferated well when cultured for all 4 days at 37°C and 7% CO₂ but poorly when cultured for all 4 days at 39.5°C and 3% CO₂. When T cells from HSF1⁻/⁻ mice were cultured at 37°C and 7% CO₂ for the first 24 h and then switched to 39.5°C and 3% CO₂, proliferation was still almost completely inhibited, whereas switching these cells after 48 h allowed completely normal proliferation. In contrast, when T cells from HSF1⁺/+ mice were cultured at 39.5°C and 3% CO₂ for the first 24 h and then switched to 37°C and 7% CO₂, substantial proliferation occurred at ~50% of the level seen when these cells were cultured for all 4 days at 37°C. These results point to a critical period between 24 and 48 h when the block in proliferation occurs in HSF1⁻/⁻ T cells. Collectively, these results point to a block in the G₁/S phase transition in the cell cycle in HSF1⁻/⁻ T cells when activated at 39.5°C and 3% CO₂.

FIGURE 4. The block in proliferation of T cells from HSF1⁻/⁻ mice at fever temperatures occurs at the G₁/S phase transition of the cell cycle. A, Purified T cells from HSF1⁻/⁻ or HSF1⁺/+ mice were cultured at either 37°C/5% CO₂ or 39.5°C/3% CO₂ with plate-bound anti-CD3. The cells were harvested after 48 h and intracellular DNA was stained with propidium iodide. The percentage of cells in each phase of the cell cycle was quantitated by flow cytometry and analysis using ModFit. The bar graphs show the average percentages of three mice ± SD. This experiment was repeated at least three times with the same result. B, To measure RNA synthesis, [3H]uridine deoxyribose ([H UdR]) was added during the last 12 h of a 24-h culture of purified T cells from HSF1⁺/+ or HSF1⁻/⁻ mice at either 37°C and 5% CO₂ or 39.5°C and 3% CO₂. Data are expressed as cpm ± SD of triplicate cultures. C, Purified CD3⁺ T cells from HSF1⁺/+ or HSF1⁻/⁻ mice were stimulated with anti-CD3 for 24 h and the percentage of viable cells was determined by trypan blue dye exclusion. D, Individual cultures of purified CD3⁺ T cells were stimulated with anti-CD3 for 4 days at either 37°C and 5% CO₂, 39.5°C and 3% CO₂, or combinations thereof on different days as indicated. [3H]thymidine ([H Tdr]) was added for the final 18 h of culture. Data represent the mean cpm ± SD of triplicate wells.

FIGURE 5. The intracellular pH of HSF1⁺/+ or HSF1⁻/⁻ T cells remains within normal levels at 3% CO₂. A, Intracellular pH (pHi) standards were generated by incubating T cells in buffers of known pH and equilibrating extracellular and intracellular pH with nigericin. Cells were then harvested and stained with the intracellular pH dye SNARF-1. A pH standard curve was generated by plotting pH vs FL3/FL2 ratios. B, Purified T cells from HSF1⁺/+ or HSF1⁻/⁻ mice were stimulated with anti-CD3 and cultured at 37°C with 5% CO₂ or 39.5°C with 3% CO₂ for 3 days. The FL3/FL2 ratios were plotted over time to insure stability of the measurement. The numbers above the graph represent pH values calculated using the linear equation generated in the standard curve.

T cells from HSF1⁻/⁻ mice show a dysregulation in levels of ROS
To account for the effect of low CO₂ concentrations on the proliferation defect of HSF1⁻/⁻ T cells, we considered two possible explanations. First, because the CO₂ percentage regulates the bicarbonate buffer system in the medium, the lower CO₂ could raise the pH of the medium and possibly the intracellular pH, thus exacerbating the effect of temperature on proliferation of the HSF1⁻/⁻ T cells. Second, dissolved CO₂ levels can affect ROS levels in cells, potentially altering cell signaling pathways (44, 45). Several experiments were performed to evaluate the effect of 3% CO₂ on the pH of the anti-CD3-stimulated cultures. As expected, medium alone had a higher pH at 3% CO₂ than at 5% (pH 7.6 vs 7.3). However, when the pH of the cultures containing metabolizing cells was measured, the pH at both CO₂ concentrations was similar at ~7.2 (data not shown). To determine whether the intracellular pH was altered at the lower CO₂ concentration, we used a flow cytometric method to measure the intracellular pH of the activated T cells (46). Purified CD3⁺ T cells were activated for 48 or 72 h at either 5 or 3% CO₂ and then stained with SNARF-1 to measure intracellular pH. Contrary to expectations, T cells from both wild-type and HSF1⁻/⁻ mice had a slightly lower intracellular pH at 39.5°C and 3% CO₂ compared with cells cultured at
37°C and 5% CO₂, but still at a level well within physiological intracellular pH levels (Fig. 5). Finally, using a bicarbonate-free medium in which pH is independent of the CO₂ concentration, the low CO₂ effect was still observed in the HSF1+/− T cells (data not shown). Thus, it is unlikely that a change in intracellular pH was the cause of the proliferation defect of HSF1−/− cells at 39.5°C and 3% CO₂. Because CO₂ concentrations also have the potential to alter the redox status of cells (44), we evaluated the effect of temperature and CO₂ concentrations on the intracellular ROS levels in T cells using the ROS-sensitive fluorochrome DCFH-DA and flow cytometry. DCFH-DA is relatively nonfluorescent unless oxidized by ROS and thus the level of fluorescence is proportional to the total level of intracellular ROS. Studies using DCFH-DA and other redox assays have established that T cells undergo a delay in the G1/S phase transition of the cell cycle. These conditions and CO₂ concentrations (Fig. 7). These results indicate that an absence of HSP70i is not responsible for the HSF1−/− T cell proliferation defect observed at fever temperatures.

Discussion

This study reveals an important new function for HSF1 in T lymphocytes. T cells from HSF1+/− mice show a profound defect in proliferation at fever range temperatures that is exacerbated by low CO₂ concentrations. This defect is T cell intrinsic and does not affect early T cell activation events but rather manifests as a block in cell cycle progression at fever temperatures. This effect of HSF1 in T cells is in agreement with a variety of studies showing an important role for HSF1 in cell cycle progression in other cell types. In yeast, HSF plays a critical role in cell cycle progression, partly through the induction of HSP90 (48). Similar to our results in T cells, in the absence of functional HSF, yeast cell cycle progression is temperature sensitive and blocked at the G₂/M phase (48). Likewise, deletion of HSF1 and HSF3 in a chicken B cell line resulted in a temperature-sensitive block in G₂/S and G₂ phases of the cell cycle. As in yeast, the block was attributed to a lack of HSP90 in the mutant cells with Cdc2 as one of the molecular targets (49). Thus, there appears to be an evolutionarily conserved fundamental role for HSF1 in the stabilization of cell cycle progression at elevated temperatures. We have not yet examined the role of HSP90 in HSF1−/− T cells but have confirmed that the block in cell cycle progression is not because of a lack of HSP70i expression. Thus, it is possible that HSP90 is a critical HSF1 target gene controlling on HSF1. Furthermore, HSP70 has been shown to have a wide range of protective effects on cellular function, as well as immunostimulatory effects (32). To determine whether a lack of HSP70i was responsible for the proliferation defect observed in HSF1−/− mice, we tested the proliferation of T cells from HSF1−/− mice at permissive and nonpermissive conditions (Fig. 7). As previously shown, proliferation of T cells from two different HSF1−/− mice was significantly impaired at 39.5°C and 3% CO₂ compared with 37°C and 5% CO₂. In this experiment, proliferation of HSF1−/− cells was also impaired at 39.5°C and 5% CO₂ (Fig. 7), indicating that the requirement for low CO₂ is not absolute and that elevated temperature is perhaps the dominant effect. In contrast, T cells from HSF70−/− mice proliferated equally at both temperatures and CO₂ concentrations (Fig. 7). These results indicate that an absence of HSP70i is not responsible for the HSF1−/− T cell proliferation defect observed at fever temperatures.

The proliferation defect of HSF1−/− cells is independent of HSP70

Our own data in T cells (Fig. 1) and other studies in non-T cells (20) clearly show that HSP70i is largely, if not entirely, dependent
cell cycle progression in T cells at fever temperatures, and we are currently examining this possibility.

Our original studies showed that HSF1 was activated to the transcriptionally active form in T cells at fever temperatures. However, those studies were done using resting T cells (27). In the present study, we showed that HSF1 became transcriptionally active (as assessed by the expression of HSP70i) even at 37°C upon T cell activation. Activation of T cells at fever range temperatures resulted in enhanced expression of HSF1 and enhanced transcriptional activation of target genes. Our studies of T cell activation in vitro show “normal” proliferation of HSF1/+/− T cells at 37°C, indicating that HSF1 is dispensable for T cell proliferation in vitro at normal temperatures. However, we have recently found that in vivo T cell expansion in response to staphylococcal enterotoxin B administration is blunted in HSF1−/− mice in the absence of fever (S. Gandhapudi and J. Woodward, manuscript in preparation). Thus, HSF1 has demonstrable effects in T cells even at normal temperatures and may play a role in T cell clonal expansion in vivo, even in the absence of obvious “stress.”

Indeed, recent data challenge a conventional view of HSF1 as a strictly stress-inducible transcription factor. It is becoming apparent that HSF1 plays important roles in a number of cellular functions not normally associated with stress. Although HSF1+/− mice are viable, they exhibit a number of phenotypic effects. The mice are often smaller than their wild-type littermates and embryonic lethality of HSF1−/− embryos is common, particularly as the mice are backcrossed onto a pure inbred strain, indicating a role for HSF1 in normal development (39) (J. G. Woodward, unpublished observations). Also, HSF1−/− females are sterile because of a requirement for maternal HSF1 in the postfertilization embryo, presumably to facilitate optimal early cell division of the embryo (40). HSF1 also appears to be important in regulating redox homeostasis in mouse cardiac muscle under normal physiological conditions in vivo (50). Thus, there are likely a host of more subtle stressors encountered in the in vivo environment for which HSF1 plays an important role. During a typical immune response, the T cell is likely to encounter many different potential stressors, including fever range temperatures, changes in ROS levels, changes in O2 and CO2 levels, changes in osmolarity, and exposure to cytokines. Therefore, it is likely that HSF1 plays an important role in optimizing T cell cycle progression during immune responses in vivo.

In vitro, increasing the temperature to the fever range and lowering the CO2 concentration created a set of conditions that resulted in our ability to visualize a proliferation defect in HSF1−/− T cells. These conditions did not affect HSF1+/+ or HSF1+/− mice. The critical question is, how do these conditions compare with those encountered by T cells in vivo? Certainly, fever range temperature is encountered by T cells in vivo during the proliferation phase in response to many viruses and bacteria. The local concentration of CO2 in blood and tissues can also vary, although there are limited data available allowing direct comparison of incubator CO2 levels with the partial pressures of CO2 encountered in blood, lymph, and tissues. Our data ruled out pH as an explanation for the low CO2 levels that were encountered in HSF1−/− mice. Thus, we conclude that HSF1 is important for both the protection of T cells or enhances the T cell response during fever. Our current results suggest that HSF1 is critical for optimum T cell clonal expansion during fever. The implication of this finding is that fever range temperature is inherently detrimental to cell division and that HSF1, through the induction of HSP and other genes, acts to facilitate cell cycle progression. Our data suggest that ROS dysregulation may be one factor affecting optimum cell cycle progression during fever, but many more studies are needed to understand exactly how fever range temperature affects cell division. Likewise, it will be important to identify the target genes induced by HSF1 that confer this protection. Our data rule out HSP70i as playing a role in cell cycle protection, but HSP90 and HSP25 remain strong candidates. In contrast, HSP70i is strongly induced in activated T cells in an HSF1 dependent manner. Given that HSP70i augments both Ag presentation and costimulatory functions (8, 9, 32–37), HSF1 is therefore involved in more than cell cycle protection. Thus, we conclude that HSF1 is important for both the protection of T cells as well as the enhancement of immune responses at fever temperatures.

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Disclosures

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8312 HSF1, ROS, AND T CELL PROLIFERATION

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