The induction of heat shock protein gene expression in response to stress is critical for the ability of organisms to cope with and survive exposure to these stresses. However, most studies on HSF1-mediated induction of hsp70 gene expression have utilized immortalized cell lines and temperatures above the physiologically relevant range. For these reasons much less is known about the heat shock response as it occurs in mammalian cells within tissues in the intact organism. To gain insight into this area we determined the temperature thresholds for activation of HSF1 DNA binding in different mouse tissues. We have found that HSF1 DNA binding activity and hsp70 synthesis are induced in spleen cells at significantly lower temperatures relative to cells of other tissues, with a temperature threshold for activation (30 °C) that is within the physiological range for fever. Furthermore, we found that the lowered temperature set point for induction of the stress response in spleen is specific to T-lymphocytes residing within this tissue and is not exhibited by B-lymphocytes. This lowered threshold is also observed in T-lymphocytes isolated from lymph nodes, suggesting that it is a general property of T-lymphocytes, and is seen in different mouse strains. Fever is an early event in the immune response to infection, and thus activation of the cellular stress response in T-lymphocytes by fever temperatures could serve as a way to give these cells enough time to express hsps in anticipation of their function in the coming immune response. The induced hsps likely protect these cells from the stressful conditions that can exist during the immune response, for example increasing their protection against stress-induced apoptosis.

When cells are exposed to elevated temperature, they respond by rapidly increasing the expression of heat shock proteins (hsps), which act to protect essential cellular functions from the adverse effects of increased temperature (1–5). This phenomenon, known as the cellular stress response, is mediated by a transcriptional regulatory protein called heat shock factor 1 (HSF1), which exhibits heat-inducible DNA binding activity. Upon exposure of cells to elevated temperature, HSF1 is converted from an inactive monomeric form to a trimeric DNA-binding form, which then interacts with specific sequences in the promoters of hsp genes and induces their transcription (5–11).

Fever represents a physiological example of elevated temperature in an organism. Our previous studies analyzing HSF1 activation in a limited set of mouse tissues in response to whole body hyperthermia at different temperatures revealed that the threshold temperature for HSF1 activation can vary between tissues. The temperature threshold for activation of HSF1 DNA binding in male germ cells is 35 °C, consistent with the known temperature sensitivity of this cell type (12). However, in the somatic testis cell types and liver cells of these heat-treated mice, HSF1 DNA binding was not activated until a temperature of ~42 °C was reached (12, 13). This is well above the temperature of most fevers, leading us to question whether any cell types in mammalian species exhibit HSF1 activation at lower temperatures (e.g. 39–40 °C) more consistent with the typical fever.

To test this we repeated our experiment and analyzed HSF1 activation temperature thresholds in a larger group of mouse tissues, including lung, spleen, kidney, and heart. Similar to previous results on liver and somatic testis cells, lung, pancreas, and heart exhibited an HSF1 activation threshold of ~42 °C. However, HSF1 DNA binding was activated in spleen beginning at a temperature of 39 °C, well within the range of normal febrile temperatures. This was associated with induction of hsp70 expression in this same temperature range. Two major cell types present in spleen are B-cells and T-cells. Interestingly, the lowered temperature threshold for HSF1 activation is exhibited by T-cells but not B-cells, the latter exhibiting activation at ~42 °C. T-lymphocytes isolated from lymph nodes also show this lowered threshold. Since appearance of fever is one of the first events associated with infection, activation of the cellular stress response in T-cells at febrile temperatures could serve to ratchet up the level of stress protection in these cells in anticipation of the coming immune response, ensuring their ability to function under the stressful conditions that can exist during this response.

**EXPERIMENTAL PROCEDURES**

**Experimental Animals and Whole-body Hyperthermia—C3H/HeNcr male mice were obtained from NCI (Charles River) and maintained under a controlled light cycle (14 h light:10 h dark). These studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and University of Kentucky Guidelines. For whole-body hyperthermia, 2-month-old mice were placed in modified Falcon tubes in water baths of various temperatures for 60 min. Core body temperatures taken using a Digisense thermometer (Cole-Parmer 8528–20) showed that core body temperatures reached the temperature of the water bath within 15 min and then remained at that temperature for the rest of the treatment period. Following incubation, mice were sacrificed by cervical dislocation, and...
Altered Stress Response of T- lymphocytes

A 104-bp fragment of mouse ribosomal protein S16 cDNA was amplified and then protein extracts prepared from lung (A) and spleen (B) tissues of these mice were analyzed by gel shift assay using a specific 32P-labeled HSF-binding oligonucleotide. Temperatures at which mice were incubated are indicated at the top of the panels. Results for control animals, which were treated exactly as the experimental animals except that they were not subjected to heat treatment, are shown in the lane marked C.

Isolation of T-lymphocytes and B-lymphocytes—For isolation of T-lymphocytes, mice were sacrificed by cervical dislocation and the spleen removed. A spleen cell suspension was made in RPMI medium + 5% fetal calf serum (FCS) using a Stomacher 80 for 60 s on high speed, and the cells were collected by centrifugation at 3,000 × g for 10 min. Red cells were lysed by incubation in Tris-NH4Cl for 4 min on ice, after which 9 × volume of fresh medium was added. Following centrifugation, the cell pellet was washed with medium and resuspended in 4 ml of medium (1 ml/spleen). The spleen cell suspension was passed over a nylon wool column. Non-adherent cells were collected and incubated with α-antibody for 30 min on ice, then complement was added and the cells incubated at 37 °C for 45 min. T-lymphocytes were pelleted and washed three times with fresh medium. For isolation of B-lymphocytes (14), a single cell suspension was prepared from spleens in 5% FCS/Hanks’ balanced salt solution (FCS/BSS) using a Stomacher 80. After centrifugation, cells were washed with FCS/BSS and pelleted, then resuspended in 10 ml of FCS/BSS. T-cells were removed by treating with monoclonal antibody α-Thy-1.2, α-CD4, and complement. After washing twice with FCS/BSS, cells were centrifuged on Percoll gradients. The resting B-cell layer was placed in RPMI medium containing 5% FCS. Isolated T-cells and B-cells were aliquotted and incubated at various temperatures for 60 min. Cells were pelleted, washed with phosphate-buffered saline, and frozen. CD4+ T-cells from spleen and lymph node of BALB/c mice were purified using Dynabeads (Dynal Inc., Lake Success, NY) according to the manufacturer’s protocol. Briefly, spleen and LN cells were isolated separately from BALB/c mice, and spleen cells were depleted of red blood cells by ammonium chloride lysis. Cells were incubated on ice in biotinylated anti-CD4 monoclonal antibody (L3T4, Pharmingen) followed by the addition of streptavidin-Dynabeads (M-280). CD4+ T-cells were isolated, washed by magnetic separation, and lysed in lysis buffer. Flow cytometry revealed that the cells were greater than 95% pure CD4+ T-cells (data not shown).

Native Gel Mobility Shift Analysis—Protein extracts were prepared and gel mobility shift analysis performed as described previously (15). For identification of DNA binding activity, extract from purified T-lymphocytes incubated at 39 °C (10 μg) was mixed with 1 μl of a 1:50 dilution of polyclonal antibodies specific to HSF1 or HSF2 polypeptides (15) and incubated at 25 °C for 10 min before subjecting to gel shift analysis.

RT-PCR Analysis—Total RNA was prepared from mouse tissues by homogenization in guanidinium isothiocyanate and centrifugation through 5.7 M CsCl. Reverse transcription and PCR were performed as described previously (16). Two oligonucleotide primers (5'-ATCACCAT-CACCCAAGCAAG-3' and 5'-TGCCCAAGAGCTATCAAGTGC-3') were used to amplify a 497-bp product from mouse HSP72 cDNA (17). A 104-bp fragment of mouse ribosomal protein S16 cDNA was amplified as an internal control (5'-TCCAGGTCGCCGCTGACGTC-3' and 5'-ATCACCAT-

RESULTS

To determine whether there was any variation in the temperature set point for induction of the cellular stress response between different somatic cell types, we first compared the temperature profiles for activation of HSF DNA binding in cells of various mouse tissues. Mice were subjected to whole-body hyperthermia at various temperatures from 38 to 42 °C for 60 min. Measurements indicated that core body temperatures of...
the animals reached the temperature of the water bath within 15 min and thereafter stabilized at that temperature for the duration of the treatment. Following the treatments, tissues were collected and analyzed by gel shift assay employing a specific HSF-binding oligonucleotide probe. Most of the tissues tested, including heart, liver, and kidney, displayed an HSF activation profile very similar to that displayed by lung (Fig. 1A). In these tissues, significant HSF activation is not observed until temperatures of 41 °C are reached, with higher levels observed in animals treated at 42 °C. In contrast, spleen displayed an HSF activation profile that is shifted to significantly lower temperatures relative to the other tissues (Fig. 1B). High levels of HSF DNA binding activity are observed in spleens of animals treated at a temperature of 39 °C, with similar levels present at 40 and 41 °C and diminishing levels at 42 °C.

To verify that the HSF DNA binding activity induced in spleen at the lowered temperature threshold of 39 °C does in fact mediate a productive cellular stress response, we performed RT-PCR analysis to measure levels of hsp70 mRNA in total RNA preparations of lung and spleen of animals treated at the various temperatures. To allow a good comparison to the HSF DNA-binding profiles in each tissue, the samples of tissue used for the RT-PCR analysis were from the same animals used for the gel shift analysis whose results are shown in Fig. 1. hsp70 amplification products were quantitated and normalized to S16 ribosomal protein mRNA internal controls to facilitate accurate comparison of hsp70 mRNA levels between tissue samples. Very little induction of hsp70 mRNA is observed in lung at temperatures below 41 °C, with maximal induction at 42 °C (Fig. 2, A and C). However, significant induction of hsp70 mRNA is first observed in spleen at the temperature of 39 °C (8-fold), with similar levels at 40 °C (8.6-fold) and slightly higher levels at 41 °C (14.6-fold) and 42 °C (23-fold) (Fig. 2, B and C).

We next determined whether the lowered temperature set point we observed for HSF activation in spleen was a general property of spleen cell types or whether it was specific to one or more of the cell types contained within this tissue. T-lymphocytes and B-lymphocytes were isolated from spleens of C3H/HeNCr mice, heated in vitro at various temperatures, and then extracts made from these cells were subjected to gel shift analysis. Significant HSF DNA binding is observed in B-lymphocytes only at temperatures of 41 °C or higher (Fig. 3A). This temperature profile for activation of HSF DNA binding is very similar to that observed for cells of most somatic tissues (e.g., Fig. 1A). In contrast, purified T-lymphocytes exhibit a profile of HSF activation that is shifted to significantly lower temperatures relative to B-lymphocytes (Fig. 3B). High levels of HSF DNA binding activity are induced in these cells by treatment at 39 °C, with slightly higher levels induced at 40 °C and decreasing levels at 41 °C.

To determine whether this lowered temperature threshold of
HSF activation in T-lymphocytes could be specific to this strain of mice, we repeated this experiment using CD4⁺ T-lymphocytes isolated from spleens of BALB/c mice. The results (Fig. 4A) show a similar profile of HSF activation at temperatures in the range of 39–41 °C, indicating that reduced HSF activation temperature in splenic T-lymphocytes is likely a general property and not mouse strain-dependent. Another possibility is that this temperature profile may be a property unique to T-lymphocytes found in the spleen, not shared by T-lymphocytes found outside this tissue. This possibility was tested by examining HSF activation temperature of CD4⁺ T-lymphocytes isolated from lymph nodes. As shown in Fig. 4B, the HSF activation temperature profile of these cells is very similar to that observed for cells isolated from spleen (Figs. 3D and 4A), suggesting that the phenomenon of lowered HSF activation temperature is a general property of T-lymphocytes.

Our previous studies showed that mouse cells express two different members of an HSF protein family, HSF1 and HSF2, and demonstrated that the DNA binding activity of HSF1 is heat-inducible while that of HSF2 is not (37). In most cell types studied to date, HSF1 DNA binding is activated only at temperatures of 41 °C and higher. Fig. 5 shows that the DNA binding activity induced in purified T-lymphocytes by heat treatment at 39 °C is composed of HSF1 and not HSF2, as antibodies specific to HSF1 perturb this activity while antibodies to HSF2 do not. These results indicate that some mechanism specific to T-lymphocytes functions to lower the temperature set point for activation of HSF1 DNA binding in this cell type relative to other cell types.

**DISCUSSION**

Our results show that when an organism experiences fever-like temperatures, T-lymphocytes exhibit HSF1 activation while other cell types, including B-lymphocytes, do not. We do not know the specific function of activation of the cellular stress response in T-cells at fever temperatures. However, in light of the known cytoprotective functions of hsps, one possibility is that hsp expression at fever temperatures provides T-lymphocytes with an enhanced ability to withstand the adverse cellular environments that can exist during an immune response. In support of this possibility, previous studies showed that elevation of hsp70 expression in T-cells by transfection decreases stress-induced apoptosis, and thus one possibility is that the mechanism we have uncovered could serve to protect T-cells against apoptosis during an immune response when they are often exposed to stressful environments (19, 20). Hsp also play essential roles as molecular chaperones in the folding, assembly, and transport of newly translated proteins (1–5). Therefore, another possibility is that T-lymphocytes require higher levels of hsps to handle the large increase in protein synthesis that occurs in those T-cells that become activated and undergo rapid proliferation. Up-regulation of hsps could also be necessary to chaperone the receptors that function in signaling events between T-lymphocytes and other cells of the immune system (21, 22).

One or more of the mechanisms above could explain previous observations that fever temperatures enhance a number of important functional properties of T-lymphocytes, including mitogen- and cytokine-induced proliferation and cell-mediated responses (23–30). On a higher level, these mechanisms could contribute at least partially to the long known observation that the presence of fever increases the ability of organisms to mount an immune response (Refs. 31–38 and reviewed in Refs. 39–42). Therefore, fever-induced hsp expression in T-lymphocytes may represent an important molecular mechanism by which fever acts to boost the immune response of an organism.

These results also indicate that cell type-dependent regulation of the temperature set point for activation of the cellular response may be a more widespread phenomenon than was previously thought. Our previous studies showed that male germ cell types of mouse testis activate HSF1 DNA binding at a threshold temperature of 35 °C, a temperature 7 °C lower than the temperature at which HSF1 is activated in cell types such as liver cells (42 °C) (12, 13). These studies also revealed that somatic cell types present in the testis do not share the lowered HSF activation temperature exhibited by the male germ cell types of this tissue, and instead display an HSF temperature set point (42 °C) that is identical to that of other somatic cell types such as liver. On the basis of these results, we had hypothesized that alteration in the temperature set point for the cellular stress response is a property unique to male germ cell types and that all somatic cell types would exhibit the “normal” set point of 42 °C. However, the results presented in this paper show unequivocally that this is not the case and that the temperature set point for inducible hsp expression can also be differentially regulated in somatic cell types. Indeed, another study found that HSF1 DNA binding activity is induced in cells of the nervous system of rabbit, particularly the cerebellum, at temperatures in the fever range (43). The goal of future studies is to elucidate the mechanism(s) that regulate cell type-dependent differences in HSF activation temperature, which will also increase our understanding of the fundamental mechanism of heat-induced HSF activation itself.

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Lowered Temperature Set Point for Activation of the Cellular Stress Response in T-lymphocytes
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