Dysregulation of Temperature and Liver Cytokine Gene Expression in Immunodeficient Wasted Mice

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Wasted mice bear the spontaneous autosomal recessive mutation wst/wst; this genotype is associated with weight loss beginning at 21 days of age, neurologic dysfunction, immunodeficiency at mucosal sites, and increased sensitivity to the killing effects of ionizing radiation. The pathology underlying the disease symptoms is unknown. Experiments reported here were designed to examine thermoregulation and liver expression of specific cytokines in wasted mice and in littermate and parental controls. Our experiments found that wasted mice begin to show a drop in body temperature at 21–23 days following birth, continuing until death at the age of 28 days. Concomitant with that, livers from wasted mice expressed increased amounts of mRNAs specific for cytokines IL-6 and IL-1, the acute phase reactant C-reactive protein, c-jun, and apoptosis-associated Rp-8 when compared to littermate and parental control animals. Levels of β-transforming growth factor, c-fos, proliferating cell nuclear antigen, and ornithine amino transferase transcripts were the same in livers from wasted mice and controls. These results suggest a relationship between an acute phase reactant response in wasted mice and temperature dysregulation. © 1996 Academic Press, Inc.

INTRODUCTION

Mice bearing the autosomal recessive gene wst/wst develop a series of abnormalities that include weight loss, neurologic dysfunction, and immunodeficiency, all of which are evident in the mice as early as 21 days of age (1–4). Lymphocytes from these mice also manifest an increased sensitivity to the killing effects of ionizing radiation (5) and enhanced radiation-induced apoptosis

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MATERIALS AND METHODS

Mice. The wst/wst mice were bred in the sterile, hooded animal facility in the Center for Mechanistic Biology and Biotechnology at Argonne National Laboratory from wst/+ breeding pairs obtained from the Jackson Laboratory (Bar Harbor, ME). Because the wasted trait is inherited as an autosomal recessive disorder, approximately 25% of the animals in each litter can be diagnosed (by neurologic examination) as having the syndrome. The remainder of the litter appears normal. Normal littermates of wst/wst mice are labeled wst/•. These wst/• mice should be a mixture of 67% wst/
and 33% +/+; they serve as age-matched controls. All results reported here are for animals 22–28 days of age. For each experiment, livers from 3–5 animals (mixed male and female) were pooled. Preliminary experiments have not shown differences between male and female mice or between individual animals.

Previous work from our laboratory has suggested that wst/- mice may not be totally normal (2, 3, 5–7; G. E. Woloschak et al., unpublished information). Therefore, as an additional control, we included BCF1 mice (C57BL6 × C3H/HeN F1) bred in the same animal facility at Argonne. All BCF1 mice were age-matched (within 2 days) with wst/wst mice within a single experiment. This mouse strain was chosen as a control because it is the parental strain from which wst mice were derived (1) and is designated in this work as the parental control. Mice were given a nonpurified diet (Purina Laboratory Chow, Cincinnati, OH) ad libitum. All mice were killed by cervical dislocation; tissues were harvested and stored at –70°C before use.

All animal treatments were approved by Argonne National Laboratory Animal Care and Use Committee prior to implementation.

Temperature determinations. Daily rectal temperatures were taken between 10 A.M. and noon; wst/wst mice and littermates from a single litter were individually housed beginning at Day 18 of age. During this time, the body temperatures of wst/wst mice dropped from 36.5 to 34.5°C. It is likely that huddling in a litter is required for maintenance of body temperature during this time interval; animals that failed to do so died.

mRNA preparation. Frozen tissues were thawed in homogenization buffer (0.075 mol/liter sodium chloride, 0.025 mol/liter disodium EDTA, 0.02 mol/liter Tris, pH 8.0, and 0.5g/liter sodium dodecyl sulfate) and homogenized in a Waring blender with an equal volume of fresh phenol. Following phenol extraction, samples were precipitated from ethanol overnight at −20°C. The pellet was dissolved in water and RNA was precipitated from ethanol overnight at 0°C. Following phenol extraction, samples were derived (1) and is designated in this work as the parental control. Mice were given a nonpurified diet (Purina Laboratory Chow, Cincinnati, OH) ad libitum. All results reported here are for animals 22–28 days of age. During this time interval; animals that failed to do so died.

mRNA Northern blots and hybridizations. Northern blots were performed as described previously (12). Prior to hybridization, filters were soaked for 15 min in 450 mmol/liter NaCl, 45 mmol/liter sodium citrate, and for 2–4 hr with shaking at 43°C in hybridization mix as described previously (12). 32P-labeled probes were denatured at 90°C for 5 min and cooled on ice before use. Hybridizations were carried out at 43°C in the hybridization buffer. Hybridized blots were exposed to X-ray film at −70°C. Relative quantitation was determined using a Hirshman microdensitometer (Frankfort, Germany).

The probes were removed by incubation overnight in 43°C water. They were checked for total removal of the probe by overnight exposure to X-ray film. Those blots showing total removal of the initial probe were then rehybridized to a different labeled cDNA clone.

All results reported here were derived from three independent sets of experiments; each experiment used pooled tissues derived from three to five mice. All experiments provided similar results. Student's t test was used for statistical analysis. The microdensitometric results presented in Table 1 were derived from those exposures of blots that were within the linear range of the X-ray film. Only blots showing equal amounts of RNA capable of hybridizing to the rRNA probe were used in these experiments. It should be noted that while RNA in these experiments is poly(A+), there was sufficient contaminating rRNA to detect hybridization following short exposures to labeled pHRR rRNA probe. This control permits checks on both RNA loading onto nitrocellulose filters and also on purity of poly(A+) RNA preparations used in all experiments.

### RESULTS

Body temperature studies. Litters from wst/+ parents were monitored for rectal temperature beginning at Day 18 of age. During this time interval; animals that failed to do so died.

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>OAT</th>
<th>PCNA</th>
<th>RP-8</th>
<th>IL6</th>
<th>c-fos</th>
<th>IL1</th>
<th>C-reactive protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCF1</td>
<td>1.0 (0.05)†</td>
<td>1.0 (0.02)</td>
<td>1.0 (0.10)</td>
<td>1.0 (0.09)</td>
<td>1.0 (0.02)</td>
<td>1.0 (0.05)</td>
<td>1.0 (0.20)</td>
</tr>
<tr>
<td>wst/-</td>
<td>0.7 (0.05)</td>
<td>1.3 (0.07)</td>
<td>1.1 (0.15)</td>
<td>1.1 (0.04)</td>
<td>1.4 (0.20)</td>
<td>1.1 (0.08)</td>
<td>1.1 (0.09)</td>
</tr>
<tr>
<td>wst/wst</td>
<td>1.1 (0.10)</td>
<td>1.3 (0.10)</td>
<td>1.8 (0.20)</td>
<td>1.8 (0.01)</td>
<td>1.1 (0.07)</td>
<td>2.2 (0.20)</td>
<td>9.7 (0.31)</td>
</tr>
</tbody>
</table>

Note. Microdensitometric results for c-jun experiments are not presented (Fig. 2E) since a hybridization signal was not detected in livers from control mice.

† As measured by Northern blot hybridization.

‡ Expression of all transcripts in BCF1 liver was set at 1.0. All other results are expressed relative to that. Values in parentheses are standard error of the mean (S.E.M.).

§ Statistically different from controls of P < 0.05.
at Day 18 and ending at the death of the wst/wst littermates (Day 28). Results of the temperature patterns of all mice from two different litters are presented in Figs. 1A and 1B. These results show that near Day 22 of age, the body temperatures of the wst/wst mice begin to drop while those of the normal littermates (a mixture of wst/+ and +/+ offspring) display only small variations in body temperature during this same timeframe. A difference in body temperature was observed as early as 4–5 days prior to death.

Note that all animals were separated into individual cages at Day 18 and therefore were not able to help increase body temperature through “huddling” in the cage.

Gene expression studies. A number of genes have been implicated in the regulation of body temperature (9, 13, 14). Studies of the expression of some of these transcripts in liver tissues from wst/wst and age-matched control mice were carried out by Northern blot hybridization. Results of blots are evident in Fig. 2 and microdensitometric quantitation of some of these blots is presented in Table 1. These results show that expression of ornithine amino transferase (OAT; a liver-specific marker), proliferating cell nuclear antigen (PCNA), c-fos, and transforming growth factor-beta (TGF-β) transcripts was no different in livers from control and wasted mice. The expression of cytokines IL6 and IL1 and of the apoptosis-associated transcript Rp-8 was approximately twofold higher in wst/wst mice relative to controls, while expression of the acute-phase reactant C-reactive protein-specific transcripts was over 10-fold higher in wasted mice than in controls. c-jun mRNA was detected in livers from wst/wst mice but not in livers from control mice, demonstrating increased expression of c-jun in wasted mice relative to controls. The relative increases of expression of c-jun could not be determined because the transcript was undetected in control tissues. The expression of any of these genes in wst/− littermates was not significantly different than was detected in age-matched BCF1 strain-specific controls.

It should be noted that the c-jun, IL6, and C-reactive protein-specific transcripts appear to be somewhat degraded in Fig. 2. This degradation was observed on the same membranes that revealed intact transcripts for OAT, β-TGF, and other transcripts not showing degradation. This has lead to the conclusion that these transcripts (which all have short half-lives; 10, 14) exist in a partially degraded state in the cell.

**DISCUSSION**

Inappropriate regulation/expression of various cytokines (or extracellular mediators) has been associated with disease and even death in mammals. Abnormal levels of IL6 have been found to be associated with recovery from surgery, rheumatoid arthritis, and AIDS (13–16), while IL1 and TNF have been shown to play a role in the progressive wasting, weakness, and anorexia associated with cancer cachexia (17–19). The results reported here demonstrate an increased expression of hepatic mRNA specific for IL1 and IL6 in the wasted mouse relative to controls. The enhanced expression of these two cytokines may be responsible for some of the pathology and symp-
TEMPERATURE/CYTOKINE DYSREGULATION IN WASTED MICE

FIG. 2. Northern blots of liver Poly(A+) RNA from BCF1, wst/+, and wst/wst mice hybridized to the following labeled probes: (A) PCNA, (B) TGF-β, (C) IL6, (D) C-reactive protein, and (E) c-jun.

Our results demonstrate that the liver expression of C-reactive protein, one of the numerous acute phase reaction proteins, was increased in wasted mice relative to littermate and parental controls. This, as well as the previously reported low albumin expression in the strain (6, 7), could be attributed to an IL6 increase. Experiments are underway to determine whether wasted mice in fact undergo a reaction similar to the acute phase reaction in response to physiological stimuli. It remains unclear whether the cascade of events is in response to direct stimuli or is a consequence of some secondary signal(s). The acute phase reaction may be involved in returning the body to homeostasis after responding to infection, since the wasted mice are immunocompromised. The pleiotropic nature of IL6 (14–16) also suggests that other cells or tissues in the wasted mouse may be undergoing pathogenesis due to the increased levels of the two cytokines.

RP-8, IL6, and c-jun transcript induction is associated with the onset of an apoptotic response in a variety of different cell types (10, 20, 21). Previous work from our group has suggested that wst/wst mice express in-
increased spontaneous apoptosis in lymphocytes relative to controls (G. E. Woloschak et al., unpublished information). Experiments are aimed at determining whether hepatocytes from wasted mice are more susceptible to spontaneous or radiation-induced apoptosis than controls. It is interesting that Staphylococcos enterotoxin B, which induces acute phase reactants when administered in vivo, has also been reported to induce apoptosis (22). In addition, a recent report by Soloff et al. (23) has demonstrated induction of the apoptotic response in cultured cells exposed to low temperature conditions. We have not yet established a clear relationship between specific gene induction, thermal dysregulation, and apoptosis, but the wasted mouse model may provide a unique opportunity for such studies.

The c-fos and c-jun gene products make up the AP-1 transcription factor, which regulates cell growth and differentiation. It is interesting that c-jun is expressed at high levels in livers from wasted mice, while c-fos is expressed at control levels. Regulation of these genes is similar, as is regulation of IL6 expression; all three genes share nucleotide similarities in the enhancer region, suggesting similar regulatory patterns (24, 25). The differences, then, for our observation that c-fos mRNA expression is no different, may be due to a more complex regulatory pattern for c-fos. Several reports have documented that c-fos uses both transcriptional and posttranscriptional regulation for gene expression (25, 26).

Recent work from our laboratory has also documented lymphocyte-specific cytokine dysregulation in wasted mice relative to controls (6, 7). The work reported here combined with these previous reports suggests the presence of overall cytokine dysregulation in wasted mice.

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