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**Subnormal Albumin Gene Expression
is Associated with Weight Loss in
Immunodeficient/DNA-repair-deficient Wasted Mice¹**

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1 ABSTRACT Mice bearing the autosomal recessive mutation wst
2 express a disease syndrome of immunodeficiency, neurologic
3 dysfunction, and increased sensitivity to the killing effects
4 of ionizing radiation. The mice were originally
5 characterized as "wasted" because of their dramatic weight
6 loss that begins at 21 days of age and progresses until death
7 at 28-32 days of age. Because of the reported association
8 between abnormal liver status and weight loss, we examined
9 expression of a variety of liver-specific genes in wst/wst
10 mice relative to littermate (wst/•) and parental strain
11 (BCF₁) controls. Interestingly, the results revealed a
12 greater than 67% reduction in albumin mRNA expression in
13 livers derived from wst/wst mice relative to both controls.
14 Expression of alpha-fetoprotein as well as a variety of other
15 liver-specific genes (secretory component, metallothionein,
16 cytochrome P₁450, transferrin receptor, tumor necrosis
17 factor, and Ia antigen) was unaffected. These results
18 suggest a relationship between low albumin expression and
19 wasting syndromes in mice. In addition, we believe that our
20 data suggest the wasted mouse as a unique model for subnormal
21 albumin expression in humans.

22 INDEXING KEY WORDS:

- 23 • *albumin* • *ataxia telangiectasia*
24 • *wasting syndromes* • *wasted mice* • *gene expression*

1 Wasted mice were first identified by the expression of a
2 disease syndrome characterized by neurologic abnormalities,
3 immunodeficiency at mucosal sites, and increased sensitivity
4 to the killing effects of ionizing radiations (Inoue et al.
5 1986b; Shultz et al. 1982). The wasted (wst) trait is
6 inherited in an autosomal recessive manner and is identified
7 by whole-body tremors and subsequent rapid wasting evident as
8 early as 21 days of age (Goldowitz et al. 1985; Shultz et al.
9 1982). This mutant was originally considered to be a model
10 for the human disease ataxia telangiectasia (AT) (Inoue
11 et al. 1986a; Shultz et al. 1982; Tezuke et al. 1986), though
12 several reports have documented differences between the
13 animal and the human disease syndromes (Inoue et al. 1986a;
14 Woloschak et al. 1987). Genetic studies have identified a
15 single gene or several tightly linked genes on chromosome 2
16 which seem to be responsible for all of the manifestations of
17 the wst abnormality (Beechey and Searle 1982).

18 The most notable feature of the wasted mouse is the
19 failure to gain weight by 28-32 days (Shultz et al. 1982;
20 Kaiserlian et al. 1986; Woloschak et al. 1987). On the day
21 before death, wasted mice usually weigh one-third of the
22 weight of unaffected littermates. Because of the association
23 between abnormal liver status and weight loss (Oratz et
24 al. 1967; Rothschild et al. 1969, 1972a) we examined
25 expression of genes encoding liver carrier proteins in wasted
26 mice to determine (Shultz et al. 1982) a possible role for

1 carrier protein deficiency in the wasting syndrome of wst/wst
2 mice. Experiments were also designed to examine possible
3 dysregulation of alpha fetoprotein(α FP)/albumin expression
4 associated with the wst mutation (such dysregulation is also
5 associated with the human counterpart, ataxia telangiectasia)
6 (Ishiguro et al. 1986; Waldman 1982).

MATERIALS AND METHODS

Mice. The wst/wst mice were bred in the sterile, hooded animal facility in the Biological and Medical Research Division at Argonne National Laboratory from wst/+ breeding pairs obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were fed *ad libidum* a nonpurified diet (Purina Laboratory Chow, Cincinnati, OH.). Because the wasted trait is inherited as an autosomal recessive disorder, approximately 25% of each litter can be diagnosed (by neurologic examination) as having the syndrome. The remainder of the litter appear 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. Unless otherwise noted, all results reported here are for animals 25-28 days of age. For each experiment, livers from 3-5 animals (mixed male and female) were pooled. Preliminary experiments had not shown differences between male and female mice.

Previous work from our laboratory has suggested that wst/+ mice may not be totally normal (Inoue et al. 1986b; Woloschak et al. 1987). Therefore, as an additional control, we included BCF₁ mice (C57BL6 x 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

1 parental strain from which wst mice were derived (Shultz et
2 al. 1982) and designated as the parental control. All mice
3 were sacrificed by cervical dislocation; tissues were
4 harvested and stored at -70 °C before use. Mice were weighed
5 and checked for food/fluid intake daily; differences between
6 wst/wst mice and their littermates were not observed. All
7 animal treatments were approved by Argonne National
8 Laboratory Animal Care and Use Committee prior to
9 implementation.

10 mRNA preparation. Frozen tissues were thawed in
11 homogenization buffer (0.075 mol/L sodium chloride, 0.025
12 mol/L disodium EDTA, 0.02 mol/L Tris, pH 8.0, and 0.5g/L
13 sodium dodecyl sulfate) and homogenized in a Waring blender
14 with an equal volume of fresh phenol. Following phenol
15 extraction, samples were precipitated from ethanol overnight
16 at -20 °C. The pellet was dissolved in water and RNA was
17 precipitated from 3 mol/L sodium acetate (pH 6.0) for 2 h at
18 4 °C. Poly A+ RNA was separated from total RNA using
19 oligodeoxythymidylate affinity column chromatography. mRNA
20 was quantitated by monitoring absorbance at 260 nm (Inoue
21 1986b; Woloschak et al. 1990).

22 mRNA dot blots and hybridizations. Nitrocellulose
23 filters were wetted in water and soaked in 3M NaCl, 0.3M
24 sodium citrate for 15 min prior to dotting. Each RNA sample

(0.5 μ g to 2 μ g) was dissolved in water. All blots were baked under vacuum at 80 °C for 1 h (Woloschak et al. 1990).

Prior to hybridization, filters were soaked for 15 min in 450 mM NaCl, 45 mM sodium citrate and for 2-4 h with shaking at 43 °C in hybridization mix as described previously (Woloschak et al. 1990). 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 3-5 mice. Student's t-test was used for statistical analysis. The microdensitometric results presented in Tables 1 and 2 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.

1 It should be noted that while RNA in these experiments is
2 poly A+, there was sufficient contaminating rRNA to detect
3 hybridization following short exposures to labeled pHRR rRNA
4 probe. This control permits checks on both RNA loading onto
5 nitrocellulose filters and also on purity of poly A+ RNA
6 preparations used in all experiments.

7 cdna clones. We gratefully acknowledge the following
8 individuals who provided us with cDNA clones: α -fetoprotein
9 from Dr. S. Tilghman (Princeton Univ., Princeton, NJ);
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11 pHRR rRNA from Dr. E. Wieben (Mayo Clinic, Rochester, MN);
12 secretory component (SC/p21,53) from Dr. K. Mostov (Whitehead
13 Institute, Boston, MA); Ia (pCA12) from Dr. C. David (Mayo
14 Clinic, Rochester, MN); transferrin receptor (pCTR-1) from
15 Dr. F. Ruddle (Yale, New Haven, CT); cytochrome P₄₅₀ from
16 Dr. D. Nebert (NIH, Bethesda, MD); and human metallothionein-
17 2 (hMTII) and albumin probes from American Type Culture
18 Collection (Rockville, MD).

RESULTS

Sequential weights were recorded on individual mice from a single litter before and after establishing the expression of the wasted phenotype (increased tremulousness). Figure 1 presents results from one litter (confirmed independently in four separate litters) demonstrating that wst/wst mice lose weight while unaffected littermates are gaining weight daily. This weight loss is associated with the wasting of the wst/wst mice. The dot blots in Figure 2A demonstrate less albumin mRNA in the pooled livers from 3-5 wst/wst mice than normal littermates and parental controls. α FP expression in these mice was at control levels (Figure 2B). Hybridization of each blot to an rRNA probe (Figure 2C) similarly revealed that equal amounts of RNA had been loaded onto the filter. Analyses (by microdensitometry) of the levels of expression are presented in Table 1. The wst/wst mice expressed only one-third the level of albumin mRNA as that found in BCF₁ control mice. Northern blot analyses demonstrated that the albumin mRNA expressed in wst/wst mice was similar to that in controls (not shown).

We also examined a series of other genes known to be expressed preferentially in liver. Table 2 presents results of micro-densitometric analyses of dilution dot blot hybridization for RNA quantitation (similar to those presented in Figure 2). No significant differences in

- 1 expression of any of these genes were seen in wasted mice
- 2 relative to both parental and littermate controls.

DISCUSSION

The results of this study demonstrated that liver-specific albumin gene expression is reduced by 67% in mice demonstrating the wasted phenotype as compared to parental controls. This suggests an association between physical wasting (as demonstrated by weight loss) and albumin expression. Albumin is a major protein produced by the liver that maintains osmotic pressure and functions as a carrier of metals, ions, drugs, and hormones in the plasma. Because a delicate balance must exist between albumin synthesis, distribution, and degradation, serum concentrations of the protein are frequently used as a marker of disease. Many human syndromes that are associated with wasting, such as malnutrition, cirrhosis of the liver, and liver carcinomas, are associated with decreased serum albumin levels (Oratz et al. 1967; Rothschild et al. 1969, 1972a). Nutrition, temperature changes, and levels of cortisone and thyroid hormones have also been shown to affect albumin protein synthesis (Munro et al. 1965; Rothschild et al. 1957, 1958; 1972b). Several reports have shown no correlation between albumin levels and neurologic disease alone (Krzalic, 1982; Shoji et al., 1983), suggesting that the neurologic abnormality as a direct cause of low albumin gene expression in wasted mice is not likely. While the mechanism underlying the low albumin expression in wasted mice needs to be determined, this mouse may prove to be an interesting model

1 for studying nutritional syndromes as well as associated
2 interventive therapies that may influence the course of the
3 wasting itself.

4 Past work by several groups has documented elevated
5 levels of α FP in sera from patients with AT (Ishiguro et al.
6 1986; Kaiserlian et al. 1986). The wasted mouse, which has
7 been suggested as an animal model for the human disease, has
8 been shown by Kaiserlian et al. (1986) to express normal
9 levels of serum α FP. Therefore, it was not surprising that
10 our results demonstrated equal amounts of α FP mRNA in liver
11 tissue from wasted mice and controls. It should be noted
12 that wst/wst mice express low albumin mRNA levels with no
13 apparent compensatory increase in α FP mRNA expression.

14 These findings suggest a molecular model for independent
15 regulation of the albumin and α FP genes in the mouse (Belayew
16 and Tilghman 1982; Tilghman and Belayew 1982). Repression of
17 the α FP gene is initiated at birth while the albumin gene
18 remains transcriptionally active throughout life (Tilghman
19 and Belayew 1982). Only during regeneration (Belayew and
20 Tilghman 1982; Jalanko 1989) or hepatocarcinogenesis can α FP
21 be reactivated in the hepatocyte. The differential
22 regulation of these liver-specific genes occurs by binding
23 liver-specific transcription factors to promoter sequences
24 conserved in both α FP and albumin genes (Papaconstantinou
25 et al. 1990). The dominant liver-specific factor responsible

1 in directing liver-specific albumin transcription in the
2 adult liver is hepatic nuclear factor-1 (Maire et al. 1989).
3 CCAAT/enhancer core binding protein h20 as also been
4 identified as a positive transcription factor for the albumin
5 gene, but not as the predominant one (Papaconstantinou et al.
6 1990).

7 Our results also suggest that at least some liver
8 functions in wasted mice are normal, as evidenced by
9 expression of a variety of liver-specific transcripts (Table
10 2) at the same level in wasted mice as in controls. Since
11 albumin expression is specifically altered in the wasted
12 mouse and many regulatory steps are involved in the rate of
13 synthesis of albumin, experiments are underway to examine
14 transcriptional control of the albumin gene in isolated liver
15 cells from these mice. The mechanisms responsible for low
16 albumin synthesis in mice are not yet fully characterized;
17 further study of this model would increase this
18 understanding.

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Figure Legends

Figure 1. Weight of mice in a single litter composed of both wst/wst and control wst/• mice. Similar results have been documented independently for four such litters. Each line represents a different mouse. Measurements were made every two days.

Figure 2. RNA dilution dot blot hybridization examining expression of (a) albumin, (b) α -fetoprotein, and (c) rRNA in liver tissues derived from BCF₁, wst/• (w/•), and wst/wst (w/w) mice. The blot shown in Figure 2C is a rehybridization of the blot shown in Fig. 2A.

Table 1. Liver-Specific Albumin/ α -Fetoprotein
mRNA Accumulation¹

Strain	Alpha fetoprotein	Albumin
BCF ₁	1.0 [±] (0.05)	1.0 [±] (0.05)
<u>wst</u> / [•]	0.9 [±] (0.10)	0.8 [±] (0.10)
<u>wst</u> / <u>wst</u>	0.9 [±] (0.05)	0.3 [±] (0.10)

¹Amounts of mRNA in parental control BCF₁ tissues were set at 1.0, and all other mRNA were expressed relative to that. Values are means $\pm$ SD, n = 3 independent observations, and are based on three independent experiments; each experiment includes pooled tissues from 3-5 mice.

[±]Significantly different from either control at p<.005.

Table 2. Liver-Specific Gene Expression*

Mouse Strain	SC	MT-2	Cyt P ₁ -450	TfRec	TNF	Ia
BCF ₁	1.0(.20) [‡]	1.0(.07) [‡]	1.0(.10) [‡]	1.0(.10) [‡]	1.0(.10) [‡]	1.0(.05) [‡]
<u>wst</u> / [•]	0.8(.20) [‡]	0.9(.10) [‡]	1.0(.20) [‡]	0.8(.10) [‡]	0.8(.10) [‡]	0.8(.20) [‡]
<u>wst/wst</u>	1.1(.10) [‡]	1.0(.20) [‡]	1.0(.60) [‡]	1.3(.20) [‡]	1.0(.05) [‡]	1.0(.10) [‡]

*Determined by dilution dot blot hybridization of immobilized RNA to labeled gene-specific cDNA probes. Amounts of mRNA in parental control BCF₁ tissues were set at 1.0, and all other mRNAs were expressed relative to that. Standard deviations are indicated following [‡] and are based on three independent experiments; each experiment examined pooled tissues derived from 3-5 mice.

WEIGHT VARIATION IN WASTED MICE

Figure 1

Liver Tissue Sample

albumin
Liver

Fig. 2A

Fig. 2B

Fig. 2C

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