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ADENOSINE DEAMINASE ACTIVITY IN RECIPIENTS OF BONE MARROW FROM IMMUNODEFICIENT MICE HOMOZYGOUS FOR THE WASTED MUTATION

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Mice homozygous for the mutation *wasted (wst/wst)* have been postulated to be a model for the form of human severe combined immunodeficiency disease (SCID) that is secondary to a genetic deficiency of adenosine deaminase (ADA). To test this hypothesis more critically, we transplanted marrow from *wst/wst* and littermate control mice into lethally irradiated normal recipients. The Vmax and Km values for ADA in recipient's hematologic and non-hematologic tissues did not differ significantly. These results indicate that the *wasted* mouse is not a model for ADA deficiency and SCID.

Homozygotes for the autosomal recessive mutation *wasted* (*wst*) are distinguished by a number of neurological and immunological abnormalities that in some respects resemble the multifaceted, genetically determined human syndrome ataxia-telangiectasia (1,2). The pathological changes found in the

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lymphoid systems of *wst/wst* mice include significantly decreased spleen, thymus, and lymph node to body weight ratios, marked lymphocytopenia affecting both T- and B-cell populations, and increased levels of spontaneous and gamma-ray induced chromosomal damage. Affected mice develop progressive paralysis and die by 30 days of age with degeneration of neurons in the brain and spinal cord.

Immunologic abnormalities found in *wst/wst* mice are also similar to those of the genetically determined human disorder, severe combined immunodeficiency disease (SCID) (3). This syndrome can be inherited in an X-linked recessive or autosomal recessive fashion (3). Approximately one-half of the autosomal recessive cases are due to a deficiency in the purine metabolic enzyme adenosine deaminase (ADA) (4). The murine gene coding for ADA has been mapped to chromosome 2, and likewise, the gene for *wst* has been shown to reside in the distal region of the same chromosome (5).

Recently, Abbott *et al.* have reported low levels and altered kinetic parameters of erythrocyte ADA in *wst/wst* mice, and postulated that *wst* is a mutation within the ADA structural gene locus (6). In the present work we transplanted marrow from *wst/wst* and littermate control mice into normal, gamma-irradiated recipients in order to investigate the possible association between the *wst* mutation and ADA. The results indicate that the *wst* gene is not a defective gene coding for ADA.

MATERIALS AND METHODS

Mice: The original autosomal recessive mutation to *wst* occured on the inbred HRS/J strain at the Jackson Laboratory. To increase viability the autosomal recessive mutation was crossed onto a segregating background (C57BL/6JxC3HeB/FeJ). Subsequently, *wst* was backcrossed 6 generations onto the C57BL/6J strain background by ovary transplantation from *wst/wst* females to histocompatible recipients and mating to C57BL/6J males.

Cell Suspensions: Bone marrow cells from 26 day-old C57BL/6J *wst/wst* and +/- sex-matched littermate control mice were obtained by flushing the medullary cavities of femurs and tibias with RPMI 1640 medium (M.A. Bioproducts, Walkersville, MD). Marrow clumps were passed gently through a 25 gauge needle to prepare single- cell suspensions. The marrow cell suspensions were then strained through 100-mesh nylon cloth, washed, and resuspended in RPMI. Nucleated cells were counted in a Coulter ZB1 electronic cell counter, and percent viability determined by trypan blue exclusion.

Irradiation and Bone Marrow Injection: C57BL/6J mice at 16 weeks of age were exposed to 1000R whole body gamma irradiation at a rate of 230R per minute

from a Shepard Mark 1 irradiator loaded with 10,000 Ci of cesium-137. Groups of 4-5 irradiated mice were injected in the lateral tail veins with either $5x10^6$ *wst/wst* or +/- littermate control cells. At 89 days after injection, the recipients were sacrificed. Thymuses, spleens, livers, brains, and packed erythrocytes were collected and frozen immediately at -20°C.

Adenosine Deaminase Assay: The enzyme was assayed spectrophotometrically as described previously (7), with modifications. Briefly, tissue extracts were prepared by homogenizing individual organs using a Serval Omni-Mixer with a Mini-Max attachment. Homogenization buffer consisted of 50 mM Tris/HCl, pH 7.4 with 0.5 mM dithiothreitol (DTT) at 4°C. Homogenates were centrifuged at 25,000 x g for 60 min, and supernatants dialyzed overnight against 25 mM Tris/HCl with 1 mM DTT. Ten microliters of tissue extract was added to a 1 ml cuvette containing 0.99 ml adenosine solution buffered with 50 mM potassium phosphate, pH 7.4, and the change in absorbance at 265 nm, reflecting the conversion of adenosine to inosine, was recorded. Enzyme activity was expressed per milligram protein, as determined by the method of Bradford (8), using bovine serum albumin as standard.

For the determination of kinetic constants, assays were conducted at five adenosine concentrations between 0.003 mM and 0.243 mM. Kinetic constants were determined graphically from Eadie-Hofstee plots. Differences in mean values were tested for significance with a two-tailed t-test.

RESULTS

In order to examine the possible relationship between the mutation *wst* and alterations in the ADA gene, bone marrow transplants were performed after lethal irradiation of normal (+/+) recipients. The normal irradiated mice were transplanted either with marrow from two *wst/wst* or from two littermate control mice. If there was an inherent ADA deficiency in the hematopoietic stem cells of the *wst/wst* mutant, one would expect this defect to be passed on to the irradiated marrow chimeras, resulting in decreased Vmax values for ADA and/or a change in the Km for adenosine. The results in Table 1 show no significant change in the Vmax, nor a change in the apparent Km for adenosine, between recipients of littermate +/- marrow and recipients of *wst/wst* marrow.

Confirming these results, we also found no significant differences in ADA activities between lymphoid and liver tissue from untreated 24-28 day old *wst/wst* and normal littermate control mice. Tissue ADA activities of both *wst/wst* and control mice were inhibited to non-detectable levels with the addition of 5uM deoxycoformycin, a potent inhibitor of ADA (9).

DISCUSSION

The murine *wasted* mutant recently described by Shultz *et al* (1) has immune abnormalities which are similar to the genetically determined human

TABLE 1

KINETIC PARAMETERS OF ADA IN TISSUE HOMOGENATES FROM IRRADIATED RECIPIENTS OF *WST/WST* OR CONTROL BONE MARROW

Donor Marrow	+/-		*wst/wst*	
	Km (mM)	Vmax (nmol/min·mg)	Km (mM)	Vmax (nmol/min·mg)
Thymus	27.7± 2.6	362± 112	29.2±6.9	387± 83
Liver	27.7± 5.1	12.9± 5.7	32.0±3.9	5.8± 2.6
Spleen	31.9± 3.9	97± 18.0	27.8±6.6	112± 17
Brain	51.3±14.7	9.8± 6.1	32.1±7.9	5.8± 1.6
Erythrocytes	26.6± 8.5	1.9± 0.6	16.1±6.3	1.3± 0.2

All values are expressed as mean ± S.E.M.

disease SCID (6). The present study was conducted to re-examine the reported deficiency of ADA in tissues of *wst/wst* mice, and to investigate more carefully any association between the *wst* mutation and ADA.

We have shown on the basis of specific activity measurements of tissue homogenates, no significant changes in ADA activity in the thymus, spleen, liver, brain, and red blood cells of *wst/wst* mice. This is in agreement with findings of Geiger *et al.* (10) except that they observed slightly elevated levels of ADA, expressed per mg protein, in *wst/wst* spleens. Abbott *et al.* (6) found significantly decreased levels of erythrocyte ADA in *wst/wst* homozygotes. However, the latter authors used six week old mice as controls, and adult levels of ADA in erythrocytes may not be reached in mice prior to the age of weaning. In addition, quantitation and characterization of ADA activity in red cells is imprecise due to the normally low enzyme activity.

To assess more conclusively the association between the *wst* mutation and alterations in ADA, bone marrow was removed from *wst/wst* and littermate control mice and transplanted into lethally irradiated recipients. If hematopoietic stem cells from the mutant have reduced ADA activities and altered kinetic parameters, one would expect these defects to be passed on to the irradiated marrow chimeras. Our results show no significant difference in

the Vmax nor Km between recipients of *wst/wst* and normal marrow in spleen, thymus, and red blood cells. An analysis of the donor mice similarly showed no significant differences in the Vmax or Km values for ADA, using adenosine as a substrate.

Our findings of normal ADA activity and unaltered kinetic parameters in *wst/wst* mice suggest that the animal is not a model for ADA deficiency and SCID. Previous observations of cerebral nerve fiber degeneration in *wst/wst* mice (1), prior to progressive hypoplasia of lymphoid organs, also imply that the immune deficit is not the sole or even primary abnormality. It is possible that the immune dysfunction may occur as a consequence of the neural degeneration, with accompanying stress, and corticosteroid release (11).

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