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ABSTRACT

Mice recessive for the autosomal gene "wasted" (wst) display a disease pattern which includes increased sensitivity to the killing effects of ionizing radiation, immunodeficiency, and neurologic dysfunction. The recent cloning and characterization of recombinase genes (*Rag-1*/*Rag-2*) expressed in lymphoid and possibly central nervous system tissues prompted us to examine expression of these genes in DNA repair-deficient/immunodeficient wasted mice. Our results revealed that in thymus tissue, a small *Rag-1* transcript (1.0 kb) was detected in wst/wst mice that was not evident in thymus from control mice. In wst/• mice, a two-fold increase in *Rag-1* mRNA was evident in thymus tissue. *Rag-2* mRNA could only be detected in thymus tissue from wst/• and not from wst/wst or parental control BCF_1 mice. Southern blots revealed a rearrangement or deletion within the *Rag-1* gene of affected wasted mice that was not evident in known strain-specific parental or littermate controls. These results support the idea that the *Rag-1* gene may map at or near the locus for the wasted mutation. In addition, they suggest the importance of recombinase function in normal immune and central nervous system development as well as the potential contribution of this gene family to the normal repair of radiation-induced DNA damage.

INTRODUCTION

Initial reports describing the wasted mouse mutation defined an autosomal recessive defect that resulted in premature death (by 28 days of age), neurologic dysfunction, and

increased sensitivity of lymphocytes to damage by ionizing radiation as measured by chromosome breaks.[1] This strain was considered a model for the human disease ataxia telangiectasia, although later reports documented that not all features of the mouse disorder were similar to those found in the human disease.[2,3,4] In recent work, Oettinger *et al.*[5] identified *Rag-1* and *Rag-2* recombinases that synergistically activate V(D)J recombination in both B- and T-lymphocytes. Chun *et al.*[6] determined that the *Rag-1* gene is expressed in both lymphoid and central nervous system (CNS) tissue. The precise functions of these genes are still undetermined, although their roles both in gene recombination and in gene conversion have been suggested.[5,6,7]

MATERIALS AND METHODS

Mice.

All experiments reported here used wst/wst mice bred from wst/+ breeders obtained from the Jackson Laboratory (Bar Harbor, ME). All wst/wst mice expressed neurologic symptoms (whole body tremors, failure to gain weight, inability to navigate an inclined plane) at 21–22 days of age and were sacrificed at days 25–28 of age.

Hybridizations.

Northern blots and Southern blots were done as previously described.[8,9]

cDNA clones.

We gratefully acknowledge Dr. D. Baltimore's (The Rockefeller University) generous gift of *Rag-1* (M6) and *Rag-2* (MR2-1) DNA, Dr. D. McKean (Mayo Clinic, Rochester, MN) who provided murine IL2 cDNA, and Dr. C. Veneziale (Mayo Clinic, Rochester, MN) who provided α-tubulin cDNA.

RESULTS

Experiments were designed to examine *Rag-1* and *Rag-2* expression in lymphoid and CNS tissues of wasted mice as well as in tissues from littermate and parental controls. Northern blots for *Rag-1* and *Rag-2* mRNA expression demonstrated that thymus tissue of wst/wst mice expressed a small *Rag-1* transcript (1 kb) while age-matched parental and littermate controls expressed significant amounts of the normal sized mRNA in thymus. In addition, wst/• mice expressed at least twofold more *Rag-1* mRNA than parental controls (BCF_1 mice) or affected wst/wst littermates. Low levels of *Rag-1* mRNA were found in spleen of BCF_1 and wst/• mice, but *Rag-1* transcripts were undetected in spleen from wst/wst mice. In addition, *Rag-1* mRNA was not detected in brain of any mouse strains examined (BCF_1, wst/• or wst/wst); it was evident in high abundance in spinal cord of BCF_1 mice, but not in spinal cord from wst/• or wst/wst mice.

Southern blots were performed to determine the status of the *Rag-1* gene in wst/wst mice relative to controls. Figure 1 presents the results of such a Southern blot done with the *Rag-1* clone using liver DNA derived from wst/wst mice, wst/• littermates, and BCF_1 controls as well as BALB/c and C57BL/6 strain controls. In each digest of liver

DNA, novel or altered bands evident in wst/wst DNA but not in controls are marked with arrows. Bands evident in controls were also detected in DNA from wst/wst mice, suggesting that wasted mice do not have a total deletion of the *Rag-1* gene. It is also interesting that the wst/• mouse used here expresses some of the abnormal bands evident in the wst/wst affected littermates, suggesting a heterozygosity in this wst/• mouse at this locus.

The results of a *Rag-1* gene alteration in wst/wst mice suggest that the *Rag-1* gene may map at or near the wst locus. While we and others have reported low expression of several genes in wasted mice relative to controls,[4] no reports have established an associated alteration in any gene at the DNA level.

The demonstration that mice expressing the wasted phenotype (i.e., sensitivity to ionizing radiation, mucosal immunodeficiency, and neurologic dysfunction) express abnormal *Rag-1* transcript suggests an important role for the this gene in normal development, since wst/wst mice do not live beyond 32 days of age.[1] The radiation sensitivity profiles of wasted mice implicate Rag-1 as a mammalian DNA repair enzyme required for complete repair of damage induced by ionizing radiation (double-strand breaks?), since failure to express the gene in this mouse system results in an extreme sensitivity to the killing effects of gamma rays. The importance of *Rag-1* expression in spinal cord and for CNS function suggests the presence of some as-yet-unidentified gene recombination event which is essential for maintenance and function of anterior motor neuron cells in the mouse cerebellar cortex and spinal cord. It is interesting that anterior motor neuron cells have been reported to be the most radiation-sensitive cells in mouse CNS tissues.[10]

The immunodeficiency of wasted mice is specifically directed toward defective IgA-mediated mucosal immunity, potentially relating *Rag-1* function with IgA responses. Several reports have suggested that IgA responses require multiple rounds of DNA synthesis and especially frequent recombination events in mice.[11,12] It has been hypothesized in the gut that the high antigen concentration is required to drive this IgA expression.[12] Defective IgA expression in wasted mice could be associated with a failure of normal recombination in gut-associated mucosal lymphocytes needed to drive an IgA response. This would explain the IgA deficiency at mucosal sites, a prominent feature of the wasted mouse immunodeficiency. On the other hand, a recent report by George and Cebra[13] has shown that a single B-cell can be driven to IgA expression in the absence of cell division, showing that at least some IgA-producing cells do not require prior cell division. Studies of the wasted mouse model may further resolve this issue.

Finally, several recent reports[14,15] have analyzed mice genetically altered at either the *Rag-1*[14] or the *Rag-2*[15] locus. These mice share some, but not all, features with wasted mice, especially the CNS abnormality which is not evident in either *Rag-1* or *Rag-2* mutated mice. We believe these differences could be attributed to several possible explanations: (1) wst/wst mice may produce some functional Rag-1 product, even through the gene is altered. (2) wst/wst mice may express a rearrangement of *Rag-1* gene such that another gene (into which it is rearranged) is altered. (3) wst/wst mice show abnormalities in expression of *Rag-1* and *Rag-2*, while the genetically engineered mutant mice have only one or the other gene affected. Further experiments will be required to examine these possibilities.

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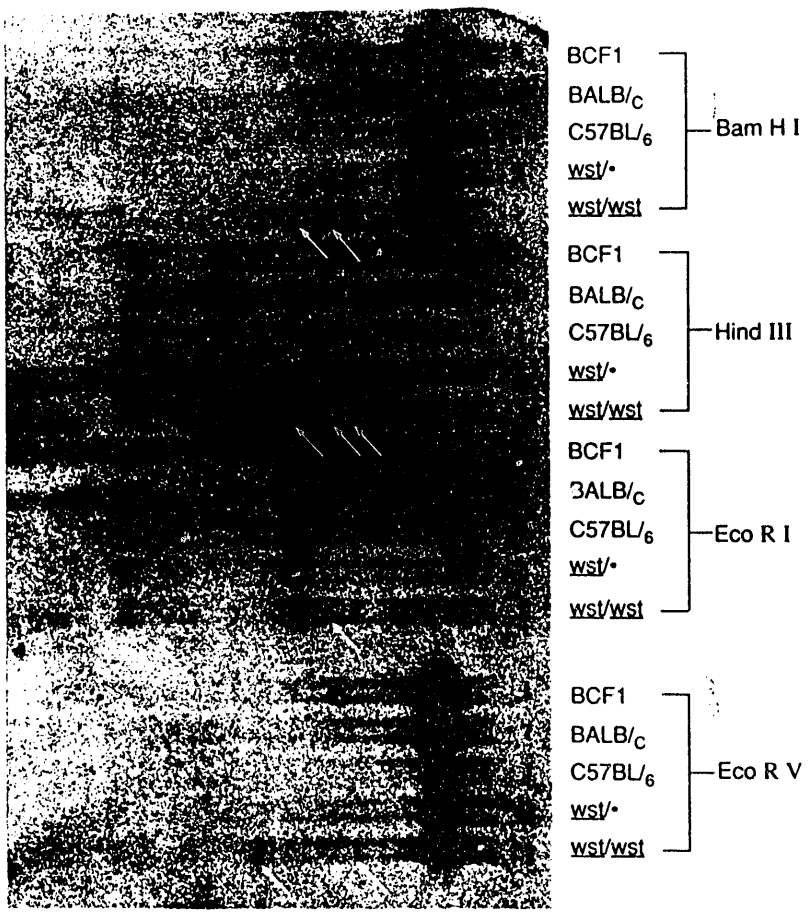
FIGURE LEGENDS

Figure 1. Southern blot of liver-derived DNA to *Rag-1* clone. Liver DNA from wst/wst (w/w), wst/• (w/•), BCF_1, BALB/C, and C57BL/6 mice was digested with the indicated enzyme prior to electrophoresis, blotting and hybridization to *Rag-1* cDNA clone.


BCF1
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