Retinal Degeneration in Motor Neuron Degeneration: A Mouse Model of Ceroid Lipofuscinosis

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Purpose. To evaluate the retinal degeneration of the motor neuron degeneration (mnd) mouse, and to confirm its inheritance pattern and gene location.

Methods. In screening the mnd/mnd mouse for ocular disease, a retinal degeneration was found that was evaluated by serial electroretinography, histology, electron microscopy, indirect ophthalmoscopy, and genetic and linkage analysis.

Results. In homozygous mnd mice, photoreceptor and outer nuclear layers show cell loss by 5 weeks after birth. By 2 months, the peripheral retina is preferentially thinner than central retina, and by 6 months the entire retina is reduced in thickness. The electroretinogram was extinguished by 6 months. Transmission electron microscopy at 3 and 6 months showed distinct cytoplasmic inclusions characteristic of the curvilinear profiles seen in human ceroid lipofuscinosis. Genetic analyses show that the retinal degeneration in mnd mice is inherited as a single autosomal gene with recessive expression, and a three-point cross placed the retinal degeneration at the mnd locus on the proximal end of mouse chromosome 8. Crosses with other known strains with retinal degeneration were normal.

Conclusions. The mnd mouse model is similar to the juvenile onset Spielmeyer-Vogt form of ceroid lipofuscinosis (Batten disease), and provides a good model for the retinal degeneration found in these patients. Invest Ophthalmol Vis Sci. 1994;35:1071-1076.
In this report, we describe a slow, progressive retinal degeneration that we found in the mnd/mnd mouse. The mnd mutant was originally discovered by Dr. Gene Rinchik in B6KB2/Rn, a congenic strain for markers on chromosome 17, and the disorder was subsequently studied by Messer and coworkers.\textsuperscript{15,16} It is characterized by gradual development of hindlimb paresis that begins at 5 months of age and progresses to paralysis by 14 months of age. Originally, Messer et al suggested that the mnd mouse had autosomal dominant inheritance and that it might be a good model for amyotrophic lateral sclerosis (ALS).\textsuperscript{15-17} An important recent discovery that helps to characterize mnd better, however, is that intracellular neuronal inclusions are present in homozygous mnd that are similar to those observed in neuronal ceroid-lipofuscinosis\textsuperscript{18} (Batten or Spielmeyer-Vogt disease).

Although neuronal degeneration in mnd has been known since 1985, this is the first report of retinal degeneration in the mnd/mnd mouse. Our studies suggest that mnd/mnd is a good model for Spielmeyer-Vogt disease because the inclusions and disease course are similar to those found in humans.\textsuperscript{19}

MATERIALS AND METHODS

Animals

The mice in this study were bred and maintained in standardized conditions in The Jackson Laboratory (Bar Harbor, ME), with light intensities ranging between 5 to 10 ft-c in the mouse room.\textsuperscript{20} All investigations adhered to the ARVO Resolution on the Use of Animals in Research. The C57BL/6J mnd/mnd mice came from the Mouse Mutant Resource at The Jackson Laboratory. Known retinal degeneration models, SWR/J rd/rd, C57H/rd-rd, C57BL/6J pcd/pcd, C3H/HeJ nr/nr, and RBF/DnJ rd-3/rd-3 and a normal control C57BL/6J were used to cross, intercross, and backcross with C57BL/6J mnd/mnd for genetic analysis and allele testing. The retinas from 125 mice of both sexes were evaluated for retinal degeneration for linkage analysis purposes by indirect ophthalmoscopy and electroretinography (ERG), from 1 to 6 months of age. For the natural history study, retinas of 20 C57BL/6J mnd/mnd and 20 C57BL/6J mnd/+ (control) mice of both sexes were examined by indirect ophthalmoscopy and ERG once a month for 6 months. Ten mice from each age group were killed for histologic examination at different stages of the disease process.

Retinal Examination

Indirect ophthalmoscopy with a 40- or 60-diopter aspheric lens and biomicroscopy with a 90-diopter lens were performed; retinas were examined through pupils dilated with 1% atropine solution.

Electroretinography

Pupils were dilated with 1% topical atropine, and the mice were dark adapted for at least 1 hour. Just before the ERG, the mice were anesthetized with a subdermal injection of a mixture of 0.0155 mg xylazine and 0.024 mg ketamine per gram-weight. Reference and ground were standard electroencephalographic subdermal needle electrodes placed over the mandibles of the anesthetized mouse, whereas the active electrode was a platinum wire (E2; Grass, Quincy, MA) moistened with balanced salt solution and placed gently on the superior corneal surface. Several intensity flash levels were used to stimulate the evoked response with the Grass photostimulator and neutral density filters (Kodak, Rochester, NY). Signals were recorded on a 386 personal computer system using an analogue-to-digital converter card and software designed for mouse ERG testing.

Histology

Light and electron microscopic examinations were performed; for light microscopic examination, eyes were immediately removed after the mice were killed with an anesthetic overdose, and immersed in cold fixative (1% paraformaldehyde, 2% glutaraldehyde, and 0.1 M cacodylate buffer) for 24 hours. The eyes were then transferred to a cold, 0.1-M cacodylate buffer solution for 24 hours. Tissues were embedded in hydroxyethylmethacrylate and anteroposterior sections were stained with hematoxylin and eosin.

For ultrastructural studies, eyes from two mnd/mnd mutants, 3 and 6 months of age, were fixed with one-half strength Karnovsky's fixative, postfixed in osmium tetroxide, embedded in epoxy resin, and sectioned. Thin sections were stained with lead citrate and uranyl acetate, and were examined on JEOL (Tokyo, Japan) 100cxII or 1200EX transmission electron microscopes.

RESULTS

Genetic Analysis and Allelic Testing

The retinas from F1 heterozygotes between C57BL/6J mnd/mnd and C57BL/6J were normal through 22 months of age, after which no further observations were made. There were five retinal degenerations and 21 normal mice in the F2 intercross (an approximate 1:3 ratio), and 25 retinal degenerations and 29 normal mice in the backcross (an approximate 1:1 ratio) between C57BL/6J mnd/mnd and mnd/SWR (Table 1). These results demonstrate that retinal degeneration in C57BL/6J mnd/mnd mice behaves as a single autosomal gene with recessive expression.

Allelic Testing. Crosses of mnd/mnd mice to strains with rd, rds, pcd, nr, and rd-3 resulted in normal off-
TABLE 1. The Phenotype and Segregating Ratio from Crosses, Backcross, and Intercross

<table>
<thead>
<tr>
<th>Crosses</th>
<th>No. of Mice</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B6mnd×B6)Fl</td>
<td>9</td>
<td>mnd/+</td>
<td>normal retina</td>
</tr>
<tr>
<td>(B6mnd×SWR-rd)Fl</td>
<td>7</td>
<td>mnd/+, +/rd</td>
<td>normal retina</td>
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<tr>
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<td>mnd/+, +/rds</td>
<td>normal retina</td>
</tr>
<tr>
<td>(B6mnd×B6-pcd)Fl</td>
<td>6</td>
<td>mnd/+, +/pcd</td>
<td>normal retina</td>
</tr>
<tr>
<td>(B6mnd×C3H-nr)Fl</td>
<td>8</td>
<td>mnd/+, +/nr</td>
<td>normal retina</td>
</tr>
<tr>
<td>(B6mnd×RBF-rd-3)Fl</td>
<td>9</td>
<td>mnd/+, +/rd-3</td>
<td>normal retina</td>
</tr>
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<table>
<thead>
<tr>
<th>Backcross</th>
<th>No. of Offspring</th>
<th>Expected</th>
<th>Observed</th>
<th>Genotype</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>mnd×(mnd/WR)</td>
<td>54</td>
<td>50% (27)</td>
<td>14f:11m (25)</td>
<td>mnd/mnd</td>
<td>ret degen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50% (27)</td>
<td>16f:13m (29)</td>
<td>mnd/+</td>
<td>normal</td>
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<table>
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<th>Intercross</th>
<th>No. of Offspring</th>
<th>Expected</th>
<th>Observed</th>
<th>Genotype</th>
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<tbody>
<tr>
<td>mnd/+×mnd/+</td>
<td>26</td>
<td>25% (6.5)</td>
<td>3f:2m (5)</td>
<td>mnd/mnd</td>
<td>ret degen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75% (19.5)</td>
<td>9f:12m (21)</td>
<td>mnd/+</td>
<td>normal</td>
</tr>
</tbody>
</table>

ret degen = retinal degeneration; f = female; m = male. RBF, C3H, and SWR are standard background strains maintained at the Jackson Laboratory; pcd, nr, rds, rd-3, and rd are gene symbols for known forms of retinal degeneration in mice. B6mnd is an abbreviation for C57BL/6J; mnd/WR for SWR-rd/rd, C3H-rds for C3H-rds/rds (rd free C3H strain), B6-pcd for C57BL/6J-pcd/pcd, C3Hnr for C3H-nr/nr, and RBF-rd-3 for RBF-rd-3/rd-3.

spring in the first generation (Table 1); this indicates that the retinal degeneration found in C57BL/6J mnd/mnd mice is new.

Linkage Studies

Multipoint recombination experiments with the known biochemical markers glutathione reductase-1, glutamate oxalate transaminase-2, and esterase-1 placed the retinal degeneration at the mnd locus on the proximal end of mouse chromosome 8. Furthermore, in all crosses, the retinal degeneration was always and only associated with the mnd/mnd genotype, suggesting that the retinal degeneration is a pleiotropic effect of mnd or is a very closely linked locus that simultaneously mutated with mnd.

Natural Course of Retinal Degeneration

Indirect Ophthalmoscopy. The initial abnormalities seen by 6 weeks were arteriolar attenuation, venous dilation, and a granular appearance to the retinal pigment epithelium. By 5 months, severe retinal vessel attenuation and sheathing, focal and diffuse loss of pigment epithelium, and patches of pigment deposits were obvious; these changes were used as linkage markers for identifying which mice had retinal degeneration. Between 3 to 7 months, biomicroscopy with a 90-diopter indirect lens showed tiny, scattered drusen throughout the level of the retinal pigment epithelium.

Histology. In C57BL/6J mnd/mnd mice, from birth through 4 weeks of age, the histologic and developmental features of the retina were similar to those of normal mice, including the photoreceptor cells (Fig. 1). By 5 weeks, the number of photoreceptors and the thickness of the outer nuclear layers were reduced. Photoreceptor cells were reduced in the peripheral retina by 2 months, and absent in the entire retina by 6 months (Fig. 1b).

Electroretinograms. The ERGs of C57BL/6J mnd/mnd mice (intensity, 73 fL-sec) showed normal b-wave amplitude at 575 μV at 1 month of age, but the implicit time was delayed to 112 ± 12 milliseconds (mean normal = 64 ± 6 milliseconds). Thereafter, the amplitudes of the a- and b-waves decreased with age (Fig. 2). By 5 months of age, the ERG was barely detectable only with the brightest stimulus, and undetectable with single-flash methods at 6 months. There was little variability in the ERG waveforms and values among inbred C57BL/6J mnd/mnd mice of the same age. The retinal appearance on indirect ophthalmoscopy paralleled the histologic status, with normal optic nerveheads and retinal vessels at 1 month that attenuated at 2 months and disappeared by 6 months.

Ultrastructural studies of eyes showed that cells in all layers of retina, including photoreceptor and ganglion cells, contained distinct cytoplasmic, membrane-bound, lysosome-like inclusions (Fig. 3a–c). These inclusions were characteristic of the curvilinear profiles observed in lysosomes of neurons from humans with ceroid lipofuscinosis. These consisted of stacked membranous material with a curving pattern. Some of the profiles had a heavy, thick central line bordered on
FIGURE 1. (Left) Serial histologic sections of retina from C57BL/6J mnd/mnd mice from 3 weeks to 6 months old compared to normal control C57BL/6J mnd/+ retinas at 1 and 6 months on right. At 4 weeks, the photoreceptor layer is normal, and by 5 weeks there is obvious disruption and loss of the outer nuclear layer and photoreceptors. At 5 months, only remnant photoreceptors are seen in the photoreceptor layer, and these are gone by 6 months. GCL = ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = outer nuclear layer; Ph = photoreceptors; RPE = retinal pigment epithelium; cc = choriocapillaris. Magnification X400. (Right) Histologic sections showing central and peripheral retina. 1: Normal control retina from C57BL/6J mnd/+ at 6 months of age. 2: At 2 months, C57BL/6J mnd/mnd shows reduction of the outer nuclear layer (ONL), and photoreceptors (PH) are evident throughout the retina, and are also marked in the periphery. Magnification X20.

DISCUSSION

The disease process seen in the homozygous mnd mouse is similar to Spielmeyer-Vogt disease in chil-

FIGURE 2. (Left) Electroretinogram at 1 to 5 months of age in the C57BL/6J mnd/mnd mouse demonstrating the maximal response from a 10-microsecond single-flash stimulation (intensity 75fl-sec). The waveform is normal at 1 month, and undetectable by 6 months. (Right) Normal control C57BL/6J mnd/+ at 1 and 6 months.
dren; in both mouse and human, retinal degeneration occurs before neuromuscular dysfunction. The time course of retinal disease and motor neuron disorder in C57Bl/6 j mnd/mnd mice is very different. The retinal degeneration was clearly distinguished by ERG and histology at 2 months of age, and by 5 months the ERG was barely recordable, whereas at 6 months the retina was atrophied and the photoreceptor layer missing (Fig. 1a). The motor neuron abnormalities began around 6 months of age, by which time the photoreceptors had disappeared.

Our investigations demonstrate that the gene responsible for the retinal degeneration is autosomal, recessive, and fully penetrant (Table 1). This result varies with past reports by Messer et al, who originally reported that mnd was autosomal dominant, and suggested that it was a model for amyotrophic lateral sclerosis. The finding of the characteristic curvilinear profiles of ceroid lipofuscinosis helps to clarify this discrepancy, and points to the true nature of this disorder.

The finding that retinal degeneration, which starts 4 months before the neuromotor disorder, links to the mnd locus is confirmatory evidence that the two dis-
orders are part of the same process; the early retinal dysfunction may reflect the high metabolic rate of the retina, and the fact that the inclusions were found in all layers of the retina supports the idea that there should be a panretinal effect. The reason why there is an early, preferential peripheral photoreceptor degeneration while central portions are relatively spared is not clear.

Although the primary genetic defect in neuronal ceroid lipofuscinosis is not known, the biochemical nature of the inclusions has been reported; direct protein sequencing of the storage material identified dicyclohexylcarbodiimide-reactive proteolipid, subunit c of mitochondrial adenosine triphosphate synthase, as the main component.19

Retinal degeneration in C57BL/6J mnd/mnd was not previously detected; with the presence of characteristic cytoplasmic inclusions in all layers of the retina, and in previous reports in the brain,18 this mouse disorder would appear to be a good model for ceroid lipofuscinosis in humans. In addition to the similar storage products, in both mouse and human the retinal degeneration occurs before the onset of neurologic signs. In addition, the disorder is inherited in the autosomal recessive mode in humans and mice. Homology mapping does not answer whether mnd is likely to be the same disorder as Spielmeyer-Vogt disease, where linkage studies tentatively have placed the Spielmeyer-Vogt gene on chromosome 16. The homologous region for mnd on the proximal end of mouse chromosome 8 is confusing, because human chromosomes 8, 13, 19, and 21 all have nearby homologous segments.21

Key Words
Batten disease, ceroid lipofuscinosis, linkage, neuromuscular disorder, retinal degeneration

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References
21. Hillyard AL, Davisson MT, Doolittle DP, et al. Locus and copy number of mice is a...