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Genetics

Purkinje cell degeneration, a new neurological mutation in the mouse

(cerebellar development/cellular pathology in brain/retinal degeneration/behavioral correlates/sperm)

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ABSTRACT **A new autosomal recessive mouse mutation, Purkinje cell degeneration (*pcd*), is described. Mutants exhibit a moderate ataxia beginning at 3 to 4 weeks of age. The ataxia results from postnatal degeneration of virtually all cerebellar Purkinje cells beginning around 15 to 18 days of age and progressing rapidly over the next 2 weeks. In addition to the cerebellar disease there is slow progressive degeneration in the retina (photoreceptor cells) and olfactory bulb. Also, adult males have abnormal sperm.**

Because of the uniform, stereotyped, and relatively simple organization of the cerebellar cortex many neuroscientists have chosen to study its functional organization (1), cytology and synaptic architecture (2), or development (3). Since the most obvious functions of the cerebellum involve posture and motor coordination, most mutations will affect behavior and are readily detected. In mice, a number of cerebellar mutants have been described (4) and have provided valuable insights into the normal and pathological development of the central nervous system (5).

The Purkinje cell is involved either directly or indirectly in several of the cerebellar mutants in mice. This cell is considered to be the central functional unit of the cerebellar cortex, for it is the direct or indirect target of all inputs and provides the sole pathway for the outflow of impulses from the cerebellar cortex. In reeler (*rl*) mutants, Purkinje cells are present but, along with many other cortical cells, are deranged in position, leading to abnormalities of cell volume and orientation (6, 7). In leaner (tg^{la}) mutants, there are focal losses of Purkinje cells (4, 8). In staggerer (*sg*) mutants, the granule cell neurons degenerate, but Purkinje cells are smaller than normal and show a marked reduction in number of dendritic spines (the sites of synaptic contact between granule cell axons and Purkinje cell dendrites) even prior to granule cell loss (9–11). The disorder of Purkinje cells is most severe in nervous (*nr*) mutants, where about 85% of these cells degenerate selectively (12, 13). In spite of this massive neuronal loss, the clinical signs are relatively mild compared to those of other cerebellar mutants.

The new autosomal recessive mutation described here also causes loss of Purkinje cells but to an even greater extent. With the exception of a very few cells in the nodulus, this new mutant loses all Purkinje cells postnatally, yet shows only a moderate ataxia. In addition, degenerations of slower progression occur in other areas of the central nervous system, including retina and olfactory bulb, and adult males have abnormal sperm. In spite of these pleiotropic effects, the loss of Purkinje cells is the most dramatic phenotypic expression and accounts for the behavioral abnormalities. We have, therefore, named the mutation Purkinje cell degeneration (gene symbol *pcd*).

Abbreviation: Chr, chromosome.

MATERIALS AND METHODS

The *pcd* mutation occurred in the C57BR/cdJ strain at The Jackson Laboratory. Because of the poor breeding performance of this strain, *pcd/pcd* females were mated to C3H/HeJ males and the F_1 animals were intercrossed to produce *pcd/pcd* F_2 females. These females were then crossed back to C57BR/cdJ males and the *pcd* mutation was maintained thereafter by brother–sister matings. The mutant gene is also being transferred to the C57BL/6J strain. The animals used in this study came from both lines.

For histological studies, the mice were fixed by perfusion through the heart with 1% formaldehyde and 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2. The brains were removed, dehydrated, and embedded in either paraffin or celloidin. Sections were cut at 7 or 20 μm and stained with cresyl violet. In addition, pieces of cerebellum and eyes were postfixed in OsO_4, dehydrated, embedded in an Epon–Araldite mixture, and sectioned at 1 μm, and the sections were stained with toluidine blue.

The various brain regions are designated according to the atlas of Sidman *et al.* (14). Day of birth is considered day 0.

To determine if there was any difference in body weights between mutants and controls, all young in 8 litters were earmarked on day 15 and weighed on days 15, 21, and 28.

RESULTS

Genetics. Breeding tests indicate that Purkinje cell degeneration (*pcd*) is a single autosomal recessive gene with full penetrance. Tests for allelism with *nr* (nervous) and *wv* (weaver) were negative. Linkage tests with the chromosome (Chr) 8 locus *Os* (oligosyndactylism), which is 25 recombination units from *nr* and less than 1 unit from tottering (*tg*), were negative, proving that *pcd* is not an allele of *tg* and not in the vicinity of either *tg* or *nr*. Linkage tests between *pcd* and the complex *T* locus on Chr 17 were negative, simultaneously ruling out allelism with the closely linked locus quaking, *qk*, a myelin-deficiency mutation; this negative test is important because mice with mutations at the *pcd*, *T*, and *qk* loci all show abnormal sperm (15, 16). In addition, linkage tests with the following chromosomally assigned loci were negative: Chr 1 *fz* (fuzzy), *ln* (leaden), *Dip-1* (dipeptidase-1); Chr 2 *Sd* (Danforth's short tail), *a* (nonagouti), *Ra* (ragged); Chr 3 *Car-2* (carbonic anhydrase-2); Chr 4 *b* (brown), *Pt* (pintail), *Gpd-1* (glucose-6-phosphate dehydrogenase); Chr 5 *Hm* (hammer-toe), *W* (dominant spotting); Chr 6 *wa-1* (waved-1), *mi* (microphthalmia); Chr 7 *p* (pink-eyed dilution), *Hbb* (hemoglobin β chain); Chr 9 *d* (dilute), *se* (short-ear); Chr 10 *Sl* (steel); Chr 11 *wa-2* (waved-2), *Re* (rex); Chr 12 *Va* (varitint-waddler); Chr 14 *s* (piebald spotting); Chr 15 *uw* (underwhite), *bt* (belted), *Ca* (caracul); and

Chr 19 *bm* (brachymorphic), *ep* (pale ear), and *ru* (ruby-eye).

Behavior. The major clinical sign is a moderate ataxia of gait. Some mutant animals can be detected behaviorally at 22 days after birth, whereas others cannot be detected until several days later. In the earliest stages, the aberrant behavior is subtle and, apart from the clue provided by their usually smaller size (see below), it is often necessary to challenge the animals by placing them on the rim of the cage rather than merely observing their behavior in the cage. During the next few weeks, their behavior becomes more obviously abnormal, and thereafter remains essentially constant. As with most other cerebellar mutants, there is some variation in severity of the ataxia among affected adults but it is slight and does not appear to be significant.

Body Weight. In the 8 litters studied, the average weights of mutants on days 15, 21, and 28 were approximately 91 ± 2, 84 ± 3, and 63 ± 5%, respectively, of the average weights of normal littermates. Some overlap with the weights of normal littermates existed, but in lieu of a linked marker gene, body weight is the most useful criterion for identifying homozygous *pcd* mice before behavioral signs appear.

Viability. Although *pcd/pcd* mice can survive to at least 17 months, generally the older mutants are in poorer health, being lighter in weight and less active than littermates.

Fertility. Affected adult females, although fertile, have difficulties in rearing the few litters they produce. Affected males are sterile, though capable of mating, as evidenced by vaginal plugs in estrus females.

Histopathology. The rate of Purkinje cell loss was measured in three cerebellar folia, the culmen, pyramis vermis, and nodulus. The number of cells counted per folium was divided by the measured length of the Purkinje cell lamina of that folium and the data were expressed as Purkinje cells per mm. The number of cells per mm normally changes as the cerebellum grows, so the number of Purkinje cells per mm at various ages in *pcd/pcd* mutants is expressed as a percentage of the values in the corresponding regions in littermate controls (Fig. 1).

At postnatal day 15 the Purkinje cells of *pcd/pcd* mice appear normal in 20 μm sections stained with cresyl violet, and their numbers in sagittal sections through the culmen near the midline are within the normal range. However, in 1 μm sections stained with toluidine blue, *pcd/pcd* homozygotes can be distinguished from heterozygotes and wild-type littermates by a homogeneous metachromatic region in the basal region of the Purkinje cell soma. This region corresponds to the basal polysomal mass characteristic of younger, normal Purkinje cells and of Purkinje cells in *nr/nr* mice on day 15 (13). The similarity between this new mutant and nervous does not extend to the mitochondria, however, for *pcd/pcd* Purkinje cells at day 15 do not show the spherical mitochondria characteristic of nervous mutant mice (13) at this age. Further, ultrastructural examination of *pcd/pcd* Purkinje cells discloses both an exceptional number of inclusion bodies and unusual configurations of cytoplasmic organelles not previously observed in other cerebellar mutants (S. Landis and R. J. Mullen, in preparation).

By the time the *pcd/pcd* mutants can be detected behaviorally, many Purkinje cells have degenerated. In 22- and 24-day-old mutants 25–50% of the Purkinje cells in the culmen and pyramis vermis had degenerated (Fig. 2B). Purkinje cell loss was less severe in more ventral areas; in the nodulus of the 24-day-old mutant, only 20% of the cells had degenerated.

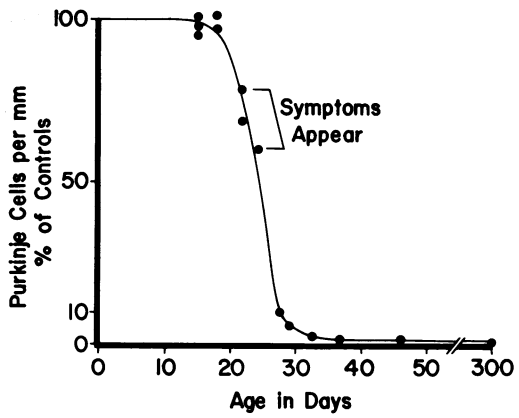

FIG. 1. Loss of Purkinje cells in *pcd/pcd* as a function of age. Purkinje cells per mm is expressed as percent of controls (see *Results*). Each point represents an individual mutant. The points between postnatal days 15 and 27 are based primarily on cell counts in the culmen but they have been adjusted slightly upwards to allow for the somewhat slower degeneration in the most ventral parts of the cerebellum (see *Results*). The points between days 29 and 300 represent cells almost exclusively in the ventral cerebellum, the last two points representing the few cells that persist in the nodulus.

At 4 weeks of age, the degeneration is nearly complete in most areas of the cerebellum. A 29-day-old *pcd/pcd* mouse which had been identified behaviorally as mutant on day 24 had only a few Purkinje cells in any folium except for the nodulus, flocculus, paraflocculus, and ventral side of the uvula vermis (i.e., the most ventral aspects of the cerebellum), where numerous Purkinje cells were still present. The thickness of the molecular layer was near normal at 4 weeks, but a microglia-macrophage reaction was evidenced by an obvious increase in the number of small pleomorphic cells. At 5 weeks, some Purkinje cells persisted in the nodulus, flocculus, and paraflocculus. By 7 weeks only a few remained, most of them in the nodulus (Fig. 2C).

The cerebella of adult mutants (3 months) are slightly reduced in size compared with littermate controls. They continue to display a few cells in the nodulus, the number ranging from zero to 10 per 20 μm sagittal section compared to 100 to 125 cells in corresponding areas of the normal nodulus. The rest of the cerebellar cortex shows only a few grossly abnormal and metachromatically staining remnants of Purkinje cells (Fig. 2D). The fact that the molecular layer in adults is noticeably thinner probably accounts for much of the reduction in the size of the cerebellum. The granule cell layer, which appears relatively normal in thickness, may incur a mild to moderate reduction in cell number per unit area, but this point is difficult to assess because the small and closely packed granule cell somas are difficult to count in 20 μm sections. Basket and stellate cells, the interneurons of the molecular layer, although present, are obscured by increased number of reactive glia and macrophages to preclude quantitation. There was no obvious reduction in the concentration of Golgi II cells of the granular layer or of neurons in the deep cerebellar nuclei.

Except for the cerebellum, the central nervous system of young adult mutants appeared relatively normal. Older mutants (10 months), however, revealed degeneration in the retina and olfactory bulb. In the retina, there is extensive loss of photoreceptor cells (Fig. 3) which begins early and continues slowly for more than a year (17).

The olfactory bulbs and lateral olfactory tracts of 10-

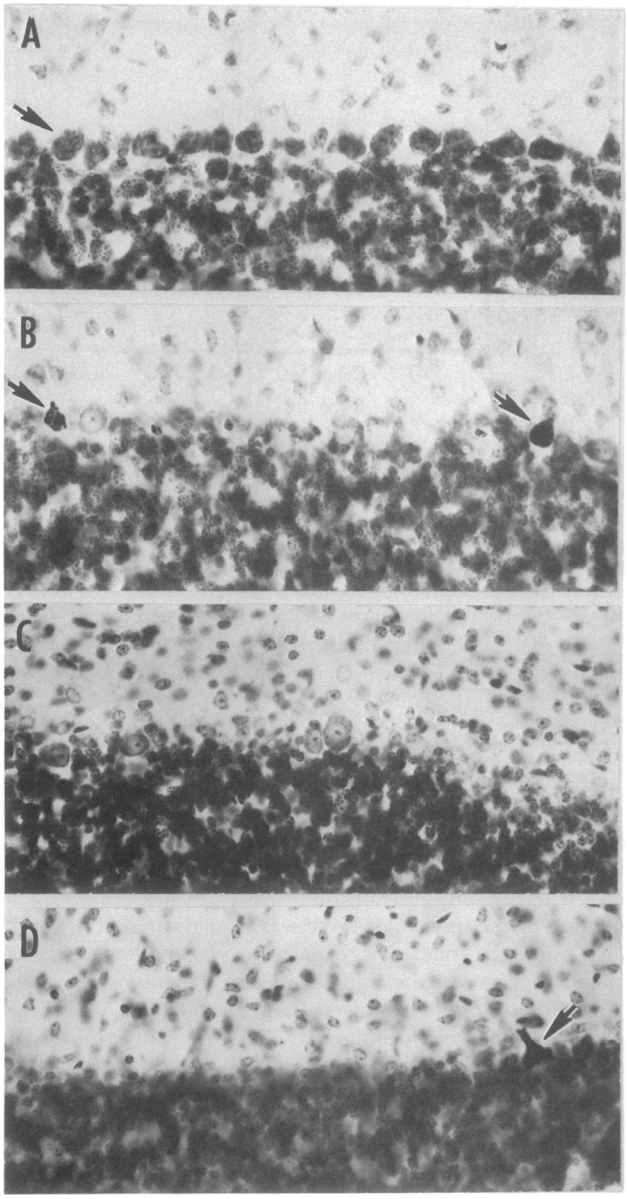

FIG. 2. Cerebellar cortex of mutants and control, 20 μm sections stained with cresyl violet, ×270. In each micrograph, the molecular layer is at the top, Purkinje cell layer in the middle (arrow in A) and granule cell layer at the bottom. (A) Sagittal section of culmen of day 24 control. (B) Corresponding region of culmen of day 24 *pcd/pcd;* many Purkinje cells have degenerated, others are in the process of degenerating (arrows). (C) Coronal section of nodulus of a day 33 *pcd/pcd* showing a few of the Purkinje cells that persist in this region. (D) Sagittal section of culmen of a day 113 *pcd/pcd;* a metachromatic "remnant" of a Purkinje cell is at the far right (arrow); all others have degenerated.

month-old mutants were smaller than normal on gross inspection. To pursue this observation, brains of mutants of various ages were stained by the Fink–Heimer silver method, which selectively stains degenerating axons. At 4 weeks, degeneration was obvious in the cerebellum, as expected, but was negligible elsewhere. At 13 weeks, a few degenerating fibers were present in the olfactory bulb, and at 38 and 45 weeks there was massive degeneration in the olfactory bulbs and tracts (Fig. 4) and some degeneration in other areas, including fibers of the crus cerebri and cingulum. In the older animals there was a loss of mitral cells in the olfactory bulb and preliminary observations on still older mutants suggest that the loss might become extensive.

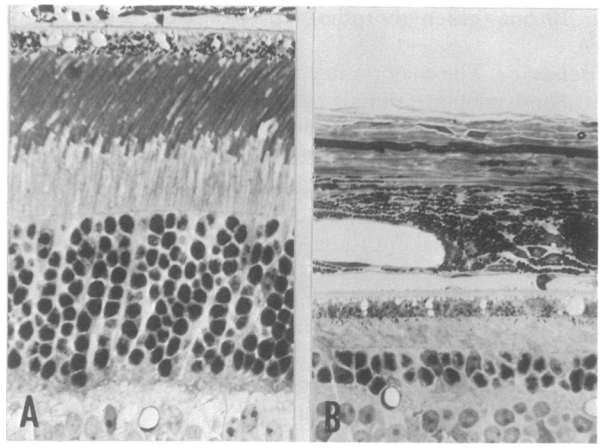

FIG. 3. Retinal degeneration in *pcd/pcd,* 1 μm sections stained with toluidine blue, ×425. (A) Retina of 10.5-month-old control with 8–10 rows of photoreceptor cell nuclei and dark-staining rod outer segments. (B) Retina of 10.5-month-old *pcd/pcd* with only 2 rows of photoreceptor cell nuclei remaining and no obvious outer segments.

To examine the cause of male sterility, smears were made of the fluid expressed from the cauda epididymis and vas deferens of adult mutant and control mice. Relatively few sperm were found in smears from mutants as compared to controls. Of the sperm cells that were present, only some were motile and all were grossly abnormal in shape, with defects including abnormal and vacuolated heads, no heads, and abnormal midpieces and tails (Fig. 5).

DISCUSSION

Although the position of the Purkinje cell degeneration (*pcd*) locus on the chromosome map is not yet known, the negative allelism and linkage tests indicate that it is probably a new locus.

The observation that *pcd* neither is an allele of *nr* nor maps close to it is particularly important because the cerebella of both mutants show selective and extensive loss of Purkinje cells. However, degeneration in the two mutants is different quantitatively, temporally, and in important cytological details. Purkinje cell degeneration mutants lose almost all Purkinje cells, the major loss occurring in the 4th week between postnatal days 22 and 28, whereas nervous mutants show mitochondrial abnormalities in all Purkinje cells at day 15 and then lose 85–90% of them in the 4th–7th weeks, while the surviving cells regain a normal cytological

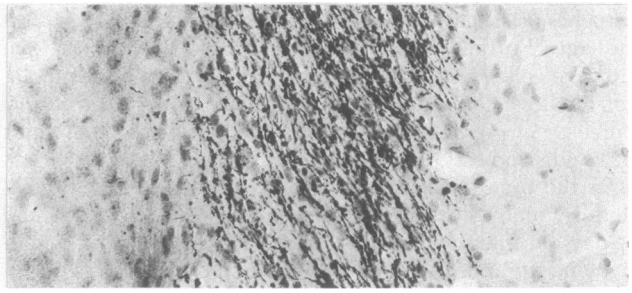

FIG. 4. Degenerating axons in olfactory tract of 38 week old *pcd/pcd.* Olfactory bulb is to the left. Sagittal section (20 μm), stained by Fink–Heimer method. ×240.

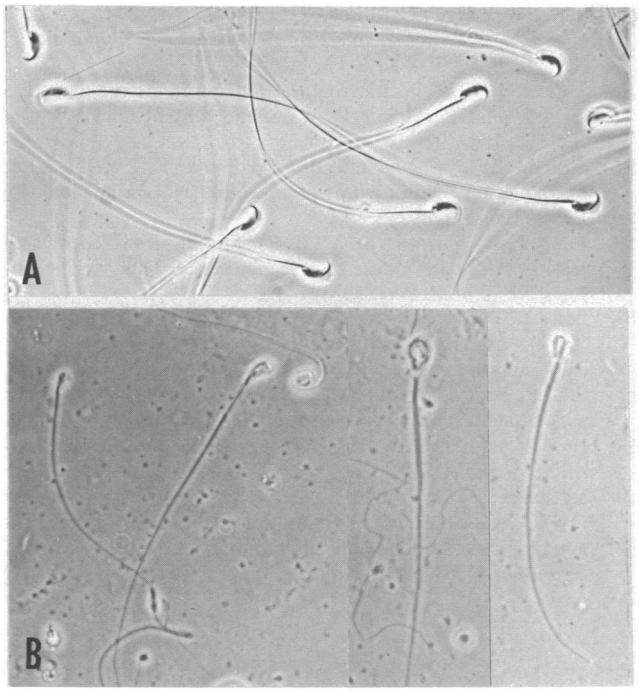

FIG. 5. Sperm collected from epididymis and vas deferens. Phase contrast, ×475. (A) Normal sperm from adult control. (B) Sperm from adult *pcd/pcd* mice showing some of the characteristic abnormalities. It is not known whether these were abnormal when produced, or represent stages of degeneration. The exceptional amount of debris suggests the latter.

appearance. In *pcd/pcd* mice nearly half the Purkinje cells have degenerated *before* behavioral signs appear between days 22 and 30; by contrast, in *nr/nr* mice, although abnormal behavioral signs appear at about day 14, there is little cell loss until *after* day 23 (13). Thus, at stages when many Purkinje cells are degenerating in *pcd/pcd*, the remaining ones would appear to be functioning quite normally and better than a complete complement of *nr/nr* Purkinje cells at comparable ages.

In *pcd/pcd* mice, the first Purkinje cells may degenerate between days 15 and 18, but the loss is quantitatively insignificant until about day 22. During the next week, approximately 95–99% degenerate, so that by day 29 significant numbers of Purkinje cells are found only in the ventral aspects of the cerebellum. Although a few cells apparently persist in the nodulus to at least 10 months, they represent no more than a small fraction of 1% of the normal total number of Purkinje cells in the cerebellum, and less than 5% of the normal number in the nodulus. Golgi and electron microscopic studies are needed to characterize these surviving cells and to establish that they are indeed Purkinje cells. Our present identifications are based only on their position, size, and shape at the light microscopic level.

The poor health of very old mutants might be secondary to the neurological deficit in some general sense, but in view of the slowly progressive neuronal degeneration in retina and olfactory bulb, the possibility that other late and as yet unrecognized neuronal degenerations might be a direct cause cannot be ruled out.

As mentioned above, the behavioral abnormality does not appear until significant numbers of Purkinje cells have degenerated. As the remaining Purkinje cells degenerate the ataxia becomes more severe but thereafter remains constant. It was most surprising to find so extensive a loss of Purkinje cell neurons in *pcd/pcd* mutants since the behavioral abnormality was only moderate. When a similarly mild locomotor defect was seen in *nr/nr* mice, the possibility was considered that persistence of 10–15% of the Purkinje cells could sustain relatively good motor coordination (12). This was considered particularly plausible since retention of Purkinje cells in *nr/nr* is the highest in the midline region of the cerebellum (the vermis) which is probably more concerned with stability of the trunk and with stance and gait than the lateral hemispheres (18). This argument is extremely difficult to sustain in the case of *pcd/pcd* mutants, since the adult affected animal retains fewer than 1% of its Purkinje cells and these are confined to the nodulus. Possible explanations for the discrepancy between the histopathological and behavioral features might be either extensive axonal sprouting with formation of functionally effective new synapses or utilization of alternate pathways in the nervous system.

Compared with the very rapid degeneration of Purkinje cells, the photoreceptor degeneration in the retina takes place slowly over the course of a year (17). Similarly, the mitral cells probably degenerate slowly but more data are needed to plot the rate. The sperm abnormality is a different problem because these cells are derived from a continuously proliferating population; it is not known whether they are abnormal when produced or normal when produced and then degenerate.

The significance of this curious pleiotropism is unknown. Purkinje, mitral, photoreceptor, and sperm cells would seem to have little in common. Purkinje and mitral cells are fairly similar in size and occupy somewhat analogous positions in the neuronal circuitry of cerebellum and olfactory bulb, respectively. Unlike Purkinje and mitral cells, photoreceptor and sperm cells have small nuclei with condensed chromatin. It is intriguing that photoreceptor cells degenerate in both *pcd/pcd* and *nr/nr* mice, the two known mutants that lose Purkinje cells selectively among all classes of cerebellar neurons, but do not degenerate in other mouse mutants with disorders of the cerebellum (17). It is also intriguing that NS-4, a new nervous system cell surface antigen, is present in high titer in mouse cerebellum, retina and sperm (19) (olfactory bulb was tested with the rest of the brain but was not tested separately). However, no common properties other than their participation in the *pcd/pcd* phenotype have yet been recognized for all four cell types either at adult or developmental stages. The relevance of a search for such a property will depend, at least in part, on whether *pcd* is a point mutation or some kind of chromosomal abnormality involving several genes.

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