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Expression of Glial Antigens C1 and M1 in Developing and Adult Neurologically Mutant Mice

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The distribution of two glial antigens (C1 and M1) has been studied by indirect immunofluorescence during postnatal development of the cerebella of normal and neurologically mutant mice (weaver, staggerer, reeler, Purkinje cell degeneration, and wobbler). During the first postnatal week of normal development, C1 antigen is expressed in ependyma, Bergmann glial fibers (BG), and astrocytes of the internal granular layer and white matter. After day 10, C1 antigen is restricted to BG and ependymal cells. During the second and third week, BG undergo a transient loss of C1 antigen that starts in medioventral areas and spreads in a gradient dorsally and laterally.

In reeler, weaver, and staggerer, C1 antigen expression is normal during the first postnatal week, and subsides in BG in a similar spatial gradient as described for the normal littermates. However, the loss of C1 antigen in BG occurs earlier (first in reeler, then in weaver, and last in staggerer) and is not reversible as it is in normal mice. In Purkinje cell degeneration, C1 antigen expression is diminished in BG after the onset of behavioral abnormalities. Wobbler is normal with respect to C1 antigen expression at adult ages.

M1 antigen is detectable in white matter astrocytes from postnatal day 7 on, and persists in these cells into adulthood. Astrocytes of the internal granular layer and BG express M1 antigen only transiently in normal mice during the second and third weeks. The appearance of M1 antigen in BG occurs in a spatiotemporal gradient, matching the one in which C1 antigen disappears. M1 antigen expression is abnormally maintained in BG of reeler, staggerer, and weaver. In Purkinje cell degeneration, M1 antigen is expressed abnormally at the onset of behavioral abnormalities first in astrocytes of the internal granular layer and, with growing age, increasingly also in BG. In wobbler, BG do not express M1 antigen. However, astrocytes of the granular layer are abnormally M1 antigen-positive.

Key words: development, neurological mouse mutants, cerebellum, glial antigens, monoclonal antibodies

Astroglia play an important role in development and maintenance of the nervous system [1, 2]. Their precise functional roles are, however, still poorly

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understood. During development, primitive astroglia and their radial processes have been postulated to serve as guides for migrating neurons in cerebellar and cerebral cortices [3, 4]. Knowledge of the functional roles of differentiated astrocytes is even more rudimentary, particularly with respect to their interactions with neurons and oligodendrocytes. Under pathological conditions, such as degeneration of neurons or oligodendrocytes, astrocytes are known to respond to their abnormal environment by cell division and hypertrophy [5–7]. Under normal conditions, proliferation of astrocytes in the white matter accompanies the formation of myelin, a process that has been called myelination gliosis [8].

To understand the particular functional states of astrocytes adequately, it seems important to describe the molecular repertoire characteristic of their differentiative stages. Recently, two antigens have been described that show a remarkable selectivity for nonoverlapping subclasses of astrocytes in the adult mouse cerebellum by immunohistological methods: M1 and C1 antigens [9, 10]. In contrast to glial fibrillary acidic (GFA) protein, a well-established marker for all types of astrocytes [11–13], M1 antigen is confined to astrocytes of the white matter, whereas C1 can be detected only in Bergmann glial fibers and ependymal cells. Despite their selective expression in glial subclasses, both antigens are not present only in glial cells: M1 antigen has additionally been found in Purkinje cell bodies, and C1 is also detectable in larger blood vessels of the brain.

During early postnatal cerebellar development, M1 and C1 antigens are less restricted to particular astrocytic subpopulations, but are present in all types of astrocytes: M1 antigen not only occurs in astrocytes of the white matter, but is also detectable in Bergmann glia and astrocytes of the developing internal granular layer. Likewise, C1 antigen is not only present in Bergmann glia, but is seen also in astrocytes of the developing white matter and granular layer.

A striking feature of M1 and C1 antigens is their abnormal expression in late postnatal and adult neurologically mutant mice weaver, staggerer, and reeler [9, 10; for review of mutants, see 14]. In adult weaver mice M1 antigen persists in Bergmann glia, thus showing a pattern of expression characteristic for developing animals. A similar abnormal distribution of M1 antigen has been found in the staggerer mutant. In addition, the detectability of M1 antigen is enhanced in all cerebellar astrocytes of this mutant. It was therefore speculated that M1 expression might be related to a gliotic reaction toward an abnormal environment, rather than reflecting a particular defect of Bergmann glia in the weaver mutant mouse [9]. In contrast, C1 antigen was not detectable in Bergmann fibers of any of these mutants, although ependyma of the fourth and other ventricles as well as retinal Müller cells were antigen-positive as in control littermates.

The consistent abnormal distribution of M1 antigen and the absence of C1 antigen in Bergmann fibers of all these mutants was surprising, because the phenotypic manifestation of each mutation is quite distinct. In the weaver mutant, granule cells are unable to undergo their early postnatal migration along Bergmann glial fibers [15–19]. The reeler mutant is characterized by a malposition of neurons during early embryogenesis of the cerebellum and in other brain

regions [14, 20–22]. In staggerer, Purkinje cells are abnormal from early postnatal ages on, and granule cells die after completing their migration from external to internal granular layers [23–25].

The aim of this study was to follow the expression of M1 and C1 antigens during abnormal early postnatal development of the cerebellar mutants, before and after the abnormalities become morphologically apparent. A more detailed analysis of normal development with respect to M1 and C1 expression antigen was undertaken in parallel. In order to obtain better insight into the regulation of M1 and C1 expression, two additional neurological mutants have been included in this study: Purkinje cell degeneration (pcd) and wobbler (wr).

In contrast to reeler, weaver and staggerer Purkinje cell degeneration undergoes an apparently normal cerebellar development during the first two postnatal weeks, but loses its Purkinje cells slowly thereafter [26–28]. The cerebellum of the wobbler mouse is apparently normal; however, this mutant shows similar motor abnormalities and a general weakness as the cerebellar mutants, probably owing to a defect in the spinal cord [29–31].

MATERIALS AND METHODS

Animals

Normal C57BL/6J mice and the neurological mutants [32] weaver (gene symbol wv), staggerer (sg), reeler (rl), Purkinje cell degeneration (pcd), and wobbler (wr) including their littermates have been used in this study. All mice were maintained in the animal facilities of the Theoretische Medizin Heidelberg University or in those of this department.

Weaver mice, originally obtained from the Jackson Laboratory (Bar Harbor, Maine), were bred on a C57BL/6J × CBA background. Homozygous mutants were obtained by mating either heterozygous or homozygous weaver animals. Weaver mice from 0 to 12 days of age were taken from matings of homozygous weaver mice. In these cases C57BL/6J mice of the same age served as controls. Homozygous mutants of older ages (up to 15 days of age) were taken from matings of heterozygous weaver animals, and were identified on the basis of their motor abnormalities and the reduced size of their cerebellum. For these older ages, behaviorally normal littermates that could be either heterozygous for the mutant gene or wild-type were used as controls.

Staggerer mice were bred from a C57BL/6J inbred stock carrying the two closely linked marker genes short ear (se) and dilute (d). Heterozygous breeding pairs (sg ++/+ d se × sg ++/+ d se) were a kind gift of Drs. S. Roffler-Tarlow and R.L. Sidman of Boston. Mutants between postnatal days 8 and 26 of age were studied, using gray littermates (+ d se/+ d se) as controls. Especially for younger staggerer mutants that do not show any behavioral abnormalities, the reduced size of the cerebellum served for unequivocal identification of homozygous mutants.

Reeler mice carrying the dominant marker gene hammertoe (Hm) were outbred on a C57BL/6J background ((Hm rl/++) × (Hm rl/++)). These animals were a kind gift of Dr. V. Caviness (Boston). Homozygous mutants were

studied between postnatal days 7 and 15 of age, using littermates with normal toes or heterozygotes (Hm rl/+ +) as controls. Heterozygotes are less affected by the dominant marker hammertoe than homozygous mutants owing to a gene dosage effect. Again, the reduced size of the cerebellum was used to identify homozygous reeler mutants.

Purkinje cell degeneration mutants (pcd), originally obtained from the Jackson Laboratory (Bar Harbor, Maine), were maintained on a C57BL/6J background. Homozygous mutants were obtained by mating heterozygous animals. They were used between 23 days and 2 months of age. Behaviorally normal littermates that were either of wild type or heterozygous genotype for pcd served as controls.

A 15-week-old wobbler mouse was a kind gift of Dr. H. Jockusch of this department. Genetic background and degree of inbreeding are not known to us. A 3-month-old C57BL/6J mouse served as control.

Antibodies

Monoclonal antibodies M1 and C1 have been obtained and partially characterized in this laboratory as described [9, 10]. M1 antibody was concentrated 30-fold from hybrydoma culture supernatants. Ascite fluid from mice bearing C1 antibody-producing tumors was diluted 1:50 in phosphate-buffered saline (PBS), pH 7.3, containing 10% horse serum (HS, from Seromed, Munich). Rabbit antisera to glial fibrillary acidic (GFA) protein were kind gifts of Drs. A. Bignami and L. Eng. They were used at a 1:300 dilution in PBS-HS. Fluorochrome labeled antiimmunoglobulin antibodies were obtained from Antibodies Inc. (through Paesel, Frankfurt) and Nordic Immunology (through Byk Mallinckrodt, Dietzenbach-Steinberg). Goat antimouse antibodies conjugated to fluorescein isothiocyanate (GAM-FITC) and goat antirabbit antibodies conjugated to tetramethyl rhodamine isothiocyanate (GAR-TRITC) were used at dilutions of 1:50 and 1:300 in PBS-HS, respectively. GAM-FITC antibodies were absorbed on rabbit antibodies covalently coupled to sepharose [33].

Immunohistological Procedures

Indirect immunofluorescence on histological sections was carried out as previously described [34]. In brief, fresh frozen mouse brain or eye was cut in 9-μm thick sections, dried onto glass coverslips, and incubated at room temperature with monoclonal antibodies for 30 minutes. The sections were then washed twice in PBS and fixed for 3 minutes at room temperature with 4% paraformaldehyde in PBS. They were again washed twice in PBS, incubated for 5 minutes in PBS-HS, and finally incubated with GAM-FITC. Sections were again washed twice in PBS, mounted on microscope slides with glycerin-PBS (1:1), and examined with a Zeiss fluorescence microscope with epiillumination and the appropriate filters. For double immunolabeling experiments rabbit anti-GFA protein antiserum was applied simultaneously with the monoclonal antibodies; similarly, GAR-TRITC was applied simultaneously with GAM-FITC.

Serial sections have been used to compare the distribution of C1 and M1 antigens.

RESULTS

Expression of C1 and M1 Antigens in Normal Mouse Cerebellum During Postnatal Development

The expression of C1 and M1 antigens in Bergmann fibers during normal postnatal cerebellar development is summarized in Tables I and II (upper lines), respectively. Only results obtained with C57BL/6J mice and normal homozygous littermates of the mutants have been included.

During the first postnatal week, C1 antigen is detectable in cerebellar Bergmann glial fibers by indirect immunofluorescence (for postnatal days 0 and 7, see Figs. 1a, b, and 2a, b, respectively). By day 8, detectability of C1 antigen starts to decrease in medioventral areas of the cerebellum. The decrease of C1 antigen detectability spreads from medioventral to mediodorsal and finally reaches lateral levels (for medioventral area at day 10, see Fig. 3a, b). At 3 weeks of age C1 antigen is weakly detectable in Bergmann fibers. However, at 2 months of age immunofluorescence intensities of Bergmann fibers have reached final levels (Figs. 4a, b, and 12b). Other types of cerebellar astrocytes are C1 antigen-positive at birth near the fourth ventricle. During the first postnatal week, astrocytes in the developing internal granular layer and presumptive white matter are C1 antigen-positive throughout all cerebellar folia. These astrocytes lose C1 antigen in a mediolateral gradient starting at day 10 in medial areas. In contrast to the Bergmann fibers, these astrocytes remain C1 antigen-negative throughout adulthood [10].

M1 antigen expression in astrocytes is subjected to an apparently related developmental gradient: It appears in areas where C1 antigen disappears during the second postnatal week and subsides in areas where C1 antigen reappears during the third and fourth postnatal weeks (for details, see Table II).

Expression of C1 and M1 Antigens in Cerebella of Neurologically Mutant Mice

Results concerning C1 and M1 expression in Bergmann glial fibers throughout mutant development are summarized in Tables I and II, respectively.

Weaver. In newborn weaver mice Bergmann fibers express C1 antigen as they do in normal control animals. At day 5, C1 antigen is strongly detectable in Bergmann glia and astrocytes of the internal granular layer and white matter of all cerebellar folia (Fig. 5a, b). By day 8, Bergmann glia of the medioventral part of the cerebellum have become C1 antigen-negative, whereas Bergmann glia of other parts of the cerebellum are positive, as they are in the controls. At day 10 immunostaining of Bergmann glia is generally weaker than in control animals. After day 12 Bergmann fibers are C1 antigen-negative in midsagittal sections. However, at more lateral levels, C1 antigen is still readily detectable. At 15 days few weakly C1 antigen-positive Bergmann fibers are observed in sections derived from very lateral areas (Fig. 6a, b). It may be noted that GFA protein does not show any mediolateral or ventrodorsal gradient of detectability. C1 antigen is never detectable in Bergmann fibers or other cerebellar astrocytes of adult weaver mutants. However, ependymal cells of all ventricles and

TABLE I. Detectability of C1 Antigen in Cerebellar Bergmann Glial Fibers by Indirect Immunohistology

		p0	p5	p7	p8	p10	p12	p15	p19	p23	p26	p30	p60	p108
+/+	Ventral	+	+	+	(+)	(+)	(+)	(+)	(+)	(+)	+	+	+	+
	Dorsal	+	+	+	+	+	(+)	(+)	(+)	(+)	+	+	+	+
	Lateral			+	+	+	+	(+)						
rl/rl	Ventral			+		−	−	−	−					
	Dorsal			+		−	−	−	−					
	Lateral			+		(+)	−	−	−					
wv/wv	Ventral	+	+	+	−	−	−	−					−	
	Dorsal	+	+	+	+	(+)	−	−					−	
	Lateral			+	+	+	+	(+)						
sg/sg	Ventral				−	−	−	−		−	−			
	Dorsal				+	+	+	−		−	−			
	Lateral				+	+	+	(+)			−			
pcd/pcd										(+)			(+)	
wr/wr														+

+ = Detectable.
(+) = Weakly detectable.
− = Not detectable.
Blank space = not done.
Ventral = medioventral.
Dorsal = mediodorsal.

TABLE II. Detectability of M1 Antigen in Cerebellar Bergmann Glial Fibers by Indirect Immunohistology

		p0	p5	p7	p8	p10	p12	p15	p19	p23	p26	p30	p60	p105
+/+	Ventral	–	–	–	(+)	+	+	+		–	–	–	–	–
	Dorsal	–	–	–	–	(+)	+	+		–	–	–	–	–
	Lateral			–	–	–	–	(+)						
rl/rl	Ventral					+	+		+					
	Dorsal					+	+		+					
	Lateral					+	+		+					
wv/wv	Ventral		–		+	+		+					+	
	Dorsal		–		(+)	+		+					+	
	Lateral				(+)	+		+						
sg/sg	Ventral				+		+	+			+			
	Dorsal				–		(+)	(+)			+			
	Lateral													
pcd/pcd										(+)			+	
wr/wr														–

\+ = Detectable.
(+) = Weakly detectable.
– = Not detectable.
Blank space = not done.
Ventral = medioventral.
Dorsal = mediodorsal.

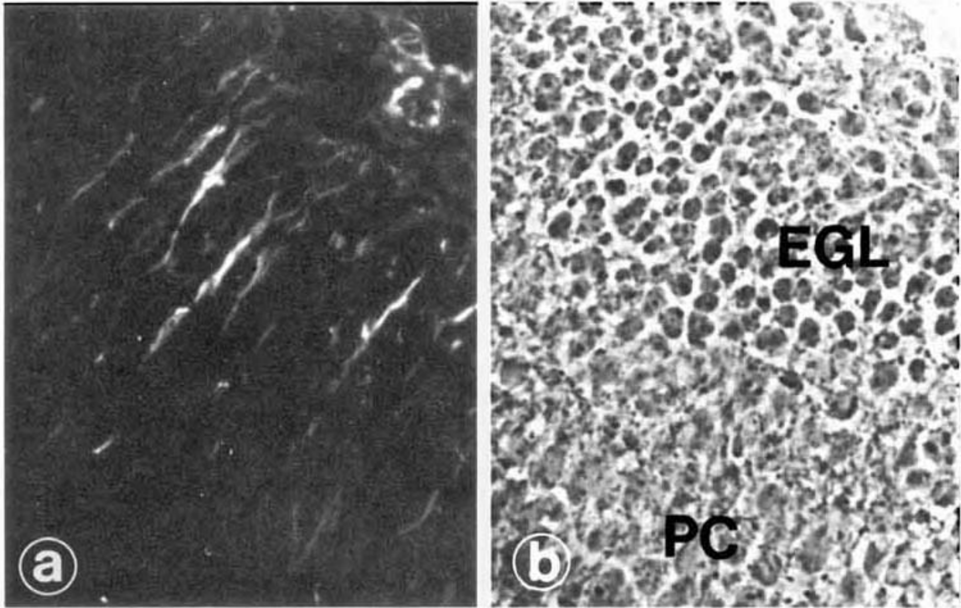
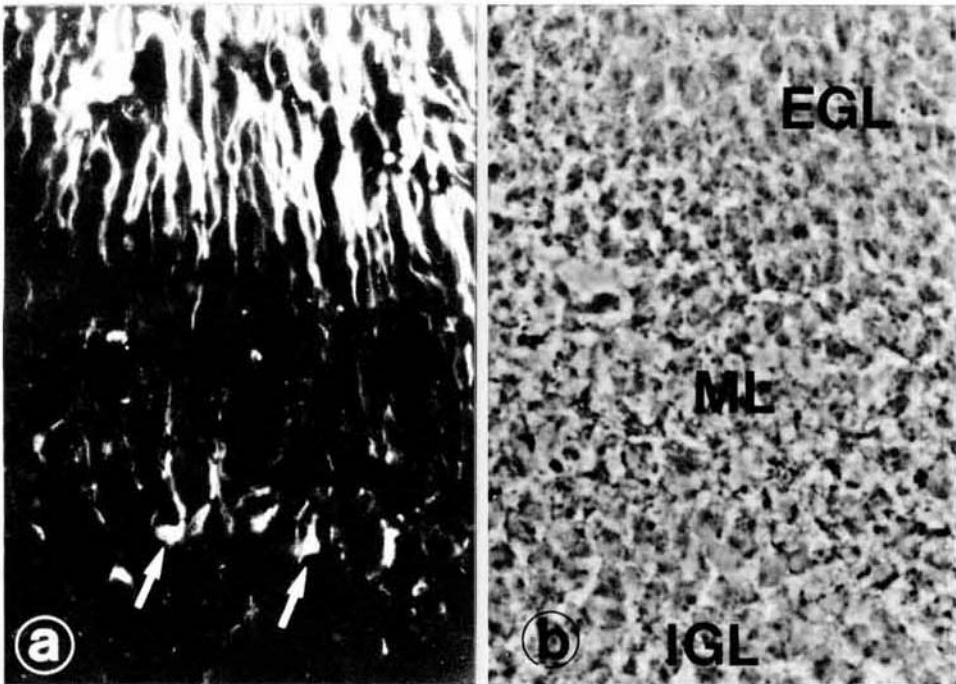


Fig. 1. Sagittal section of a newborn C57BL/6J mouse cerebellum stained with C1 antibody by indirect immunofluorescence (× 430). a) C1 antigen is detectable in Bergmann fibers that cross the external granular layer and extend into the Purkinje cell layer. b) Phase contrast micrograph of the same visual field as shown in (a). The external granular layer (EGL) is characterized by dark small cell bodies. Purkinje cells are visible in lower parts of the figure (PC).



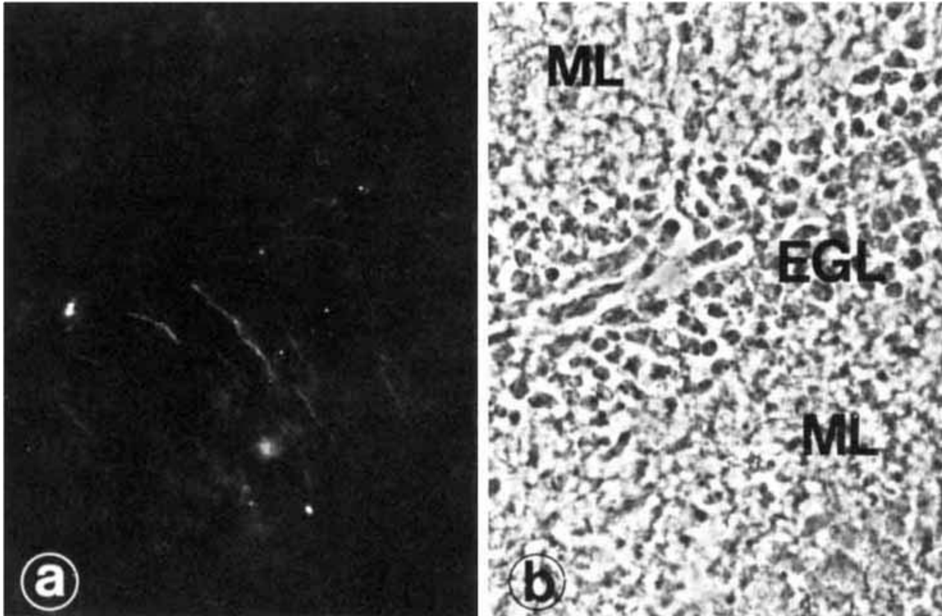


Fig. 3. Sagittal section of a 10-day-old mouse cerebellum (medioventral area) stained with C1 antibody by indirect immunofluorescence (× 430). a) C1 antigen is weakly detectable in some Bergmann fibers in the folium toward the right lower corner. C1 antigen is negative in the folium of the upper part. b) Phase contrast micrograph of the same visual field as shown in (a). External granular layer (EGL), molecular layer (ML).

retinal Müller cells stain in this mutant as they do in control littermates and C57BL/6J mice.

The expression of M1 antigen in Bergmann fibers of weaver mutants is generally more pronounced than in the normal controls. Already at day 8, M1 antigen is expressed strongly and in more Bergmann glial fibers than in control animals, particularly in more ventral areas. At day 10, M1 antigen is strongly detectable in all cerebellar folia, whereas in control animals it is restricted to some folia and expressed only at moderate levels of immunofluorescence staining. In contrast to the subsiding expression of M1 antigen in Bergmann fibers of normal animals, no decline of M1 antigen expression has been observed at late postnatal ages. Instead, M1 antigen persists in Bergmann fibers of the weaver mutant throughout adulthood [9].

Fig. 2. Sagittal section of a 7-day-old C57BL/6J mouse cerebellum stained with C1 antibody by indirect immunofluorescence (× 430). a) C1 antigen is detectable in Bergmann fibers that cross the external granular layer, developing molecular layer, and Purkinje cell layer. Note the reduced labeling intensity in the molecular layer. Knoblike structures near the internal granular layer possibly represent Bergmann glial cell bodies (arrows). b) Phase contrast micrograph of the same visual field as shown in (a). External granular layer (EGL), molecular layer (ML), internal granular layer (IGL).

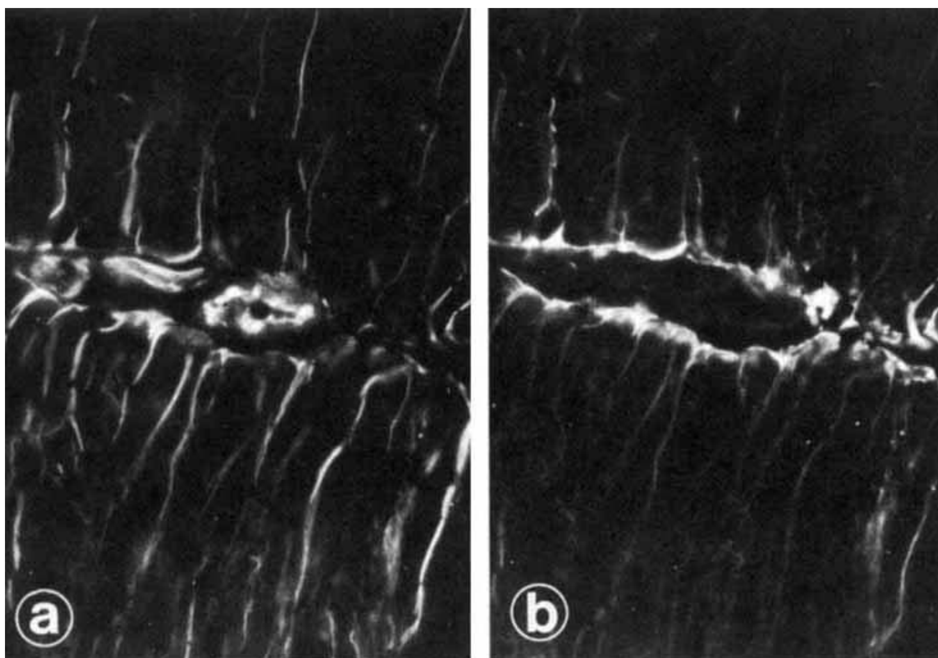
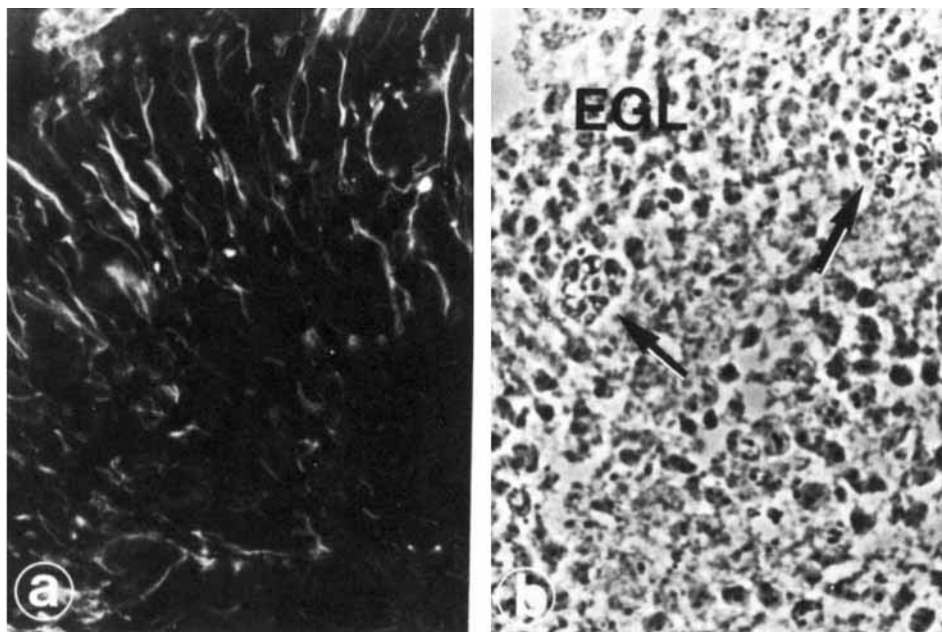


Fig. 4. Midsagittal section through the cerebellum of a normal 8-week-old littermate of Purkinje cell degeneration (genotype +/?) stained with C1 (a) and GFA protein (b) antibodies by double immunofluorescence (× 430). a) C1 antigen visualized by GAM-FITC is detected in Bergmann glial fibers in the molecular layer and in meningeal blood vessels. b) GFA protein, visualized by GAR-TRITC in Bergmann fibers, confirms the identity of C1 antigen-positive fibers in (a). Glial end feet are prominently labeled. Blood vessels are GFA protein-negative.



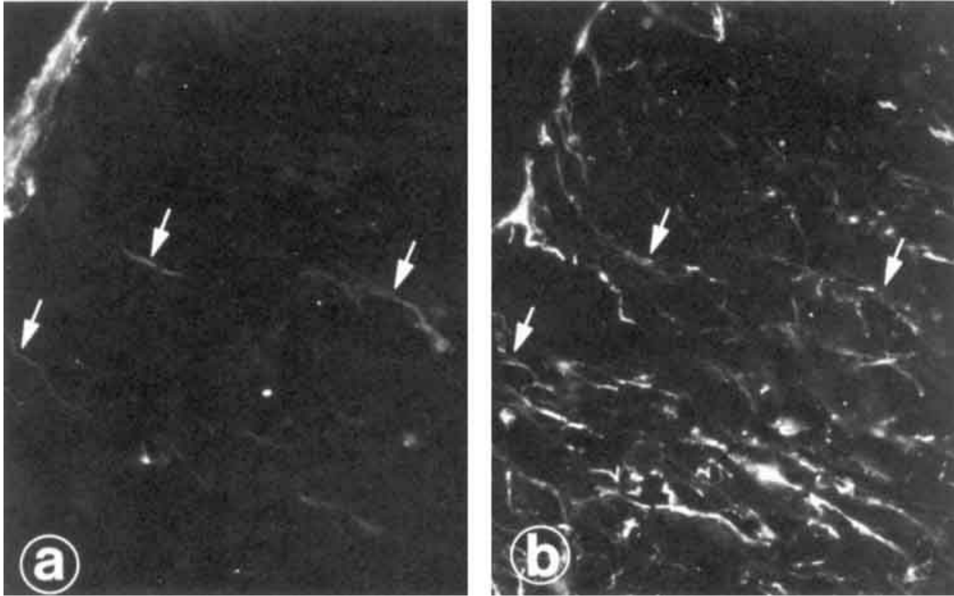


Fig. 6. Sagittal section of a 15-day-old homozygous weaver mutant cerebellum from very lateral levels stained with C1 (a) and GFA protein (b) antibodies by double immunofluorescence (× 430). a) C1 antigen is weakly expressed in few Bergmann glial fibers (arrows) identified by GFA protein in (b). Unidentified structures in the meningeal layer (upper left corner) are strongly C1 antigen-positive. (b) GFA protein staining of the same visual field as shown in (a). Bergmann fibers and their end feet (abutting to the meninges) are GFA protein-positive. Meninges are not stained. Arrows point toward GFA and C1 antigen-positive fibers.

Staggerer. C1 antigen is readily detectable in Bergmann fibers of 8-day-old staggerer mutants in mediodorsal areas of the cerebellum (Fig. 7a, b, c), whereas the ventral areas are only weakly C1 antigen-positve or negative. In contrast to the weaver mutant, C1 antigen remains detectable in Bergmann glia until postnatal day 13 in mediodorsal areas. By day 15, C1 antigen is detectable only in Bergmann fibers at the most lateral cerebellar regions. At older ages (3–4 weeks), C1 antigen is not detectable in any cerebellar astrocytes, although retinal Müller cells and ependymal cells are C1 antigen-positive as they are in normal control littermates.

In contrast to M1 antigen in normal littermates, M1 antigen is strongly detectable in Bergmann glia of 8-day-old staggerer mice and is more prominent at day 13 (Fig. 9a, b). In addition, other cerebellar astrocytes show a significant increase in M1 antigen expression when compared to normal control animals

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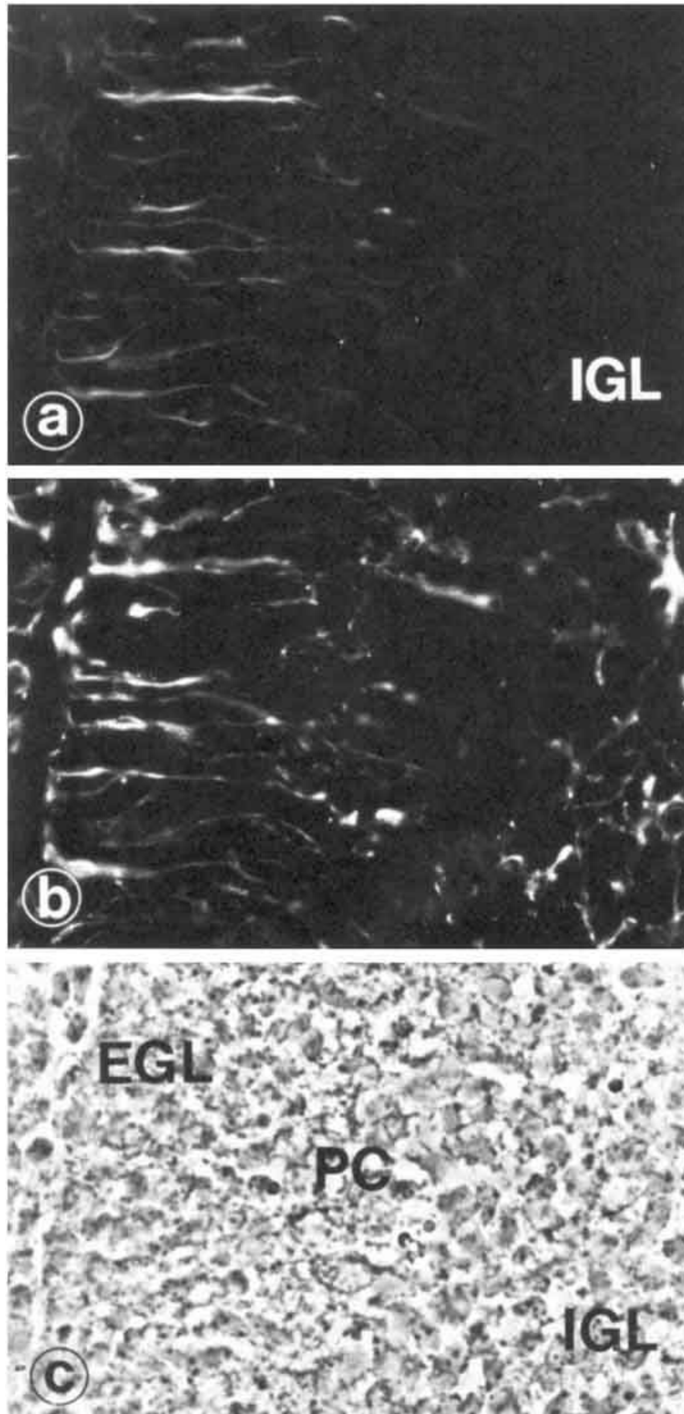
Fig. 5. Sagittal section of a 5-day-old homozygous weaver mutant cerebellum stained with C1 antibody by indirect immunofluorescence (× 430). a) C1 antigen is detectable in Bergmann fibers and in other types of astrocytes. b) Phase contrast micrograph of the same visual field as shown in (a). The external granular layer (EGL) curves from the upper right to the lower left. Clusters of degenerating granule cells at the interface of the external granular layer and the developing molecular layer are labeled by arrows.


IGL
a
b
EGL
PC
IGL
c

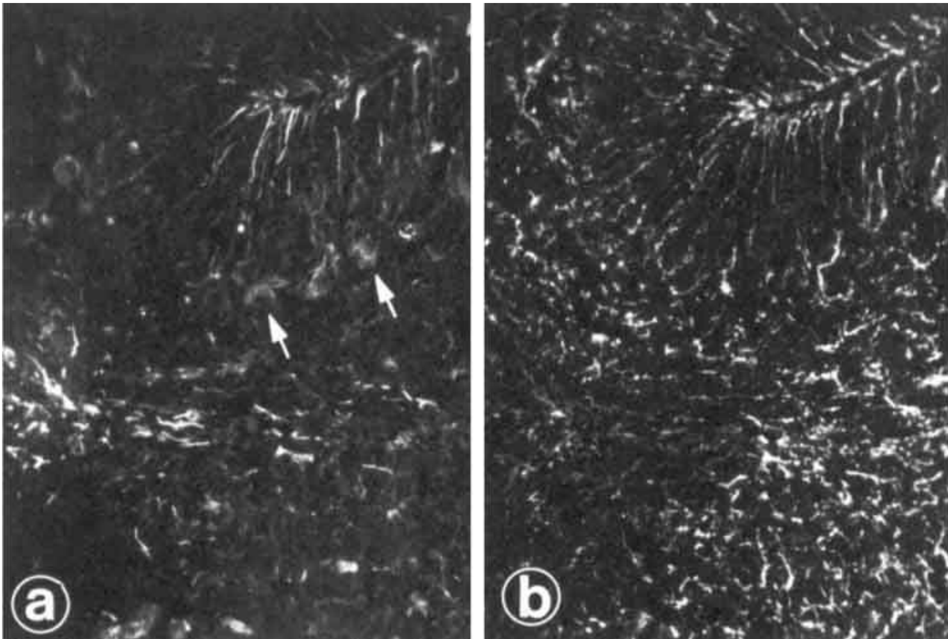


Fig. 8. Sagittal section of a 12-day-old cerebellum from a homozygous normal staggerer littermate (genotype: (+d se)/(+d se)) stained with M1 (a) and GFA protein (b) antibodies by double immunofluorescence (× 170). a) M1 antigen is detectable in some GFA protein-positive Bergmann fibers and in astrocytes of the developing white matter and internal granular layer. Purkinje cell bodies (arrows) are weakly labled. b) GFA protein staining of the same visual field as shown in (a).

(Figs. 8a, b, and 9a, b). Folia in dorsal areas are not as strongly stained by M1 antibody. When comparing C1 and M1 antigen expression in adjacent sections, these dorsal areas are congruent with those that are also C1 antigen-positive. Similar to the weaver mutant, M1 expression persists in Bergmann glia and in astrocytes of the internal granular layer until day 26, the oldest stage tested so far.

At all stages studied, Purkinje cells of the staggerer mutant fail to show any positive staining for M1 antigen (Fig. 9a) as observed in control littermates (Fig. 8a) and in some other cerebellar mutants (for normal and weaver mice, see [9]).

Fig. 7. Midsagittal section of an 8-day-old staggerer mutant cerebellum (dorsal part) stained with C1 (a) and GFA protein (b) antibodies by double immunofluorescence (× 430). a) C1 antigen is detectable in Bergmann fibers. Astrocytes in Purkinje cell and internal granular layers (IGL) that are easily detected by GFA antibodies, as shown in (b), are only weakly C1 antigen-positive or -negative. b) GFA protein staining of the same visual field as shown in (a). Bergmann fibers and astrocytes of the internal granular layer are GFA protein-positive. c) Phase contrast micrograph of the same visual field as shown in (a) and (b). External granular layer (EGL), internal granular layer (IGL), Purkinje cell layer (PC).

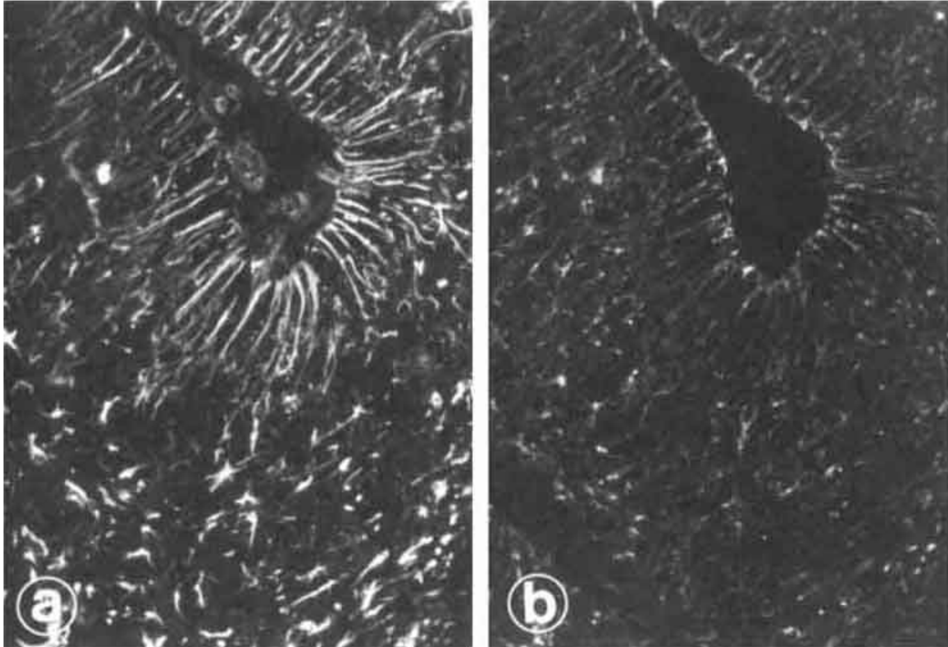
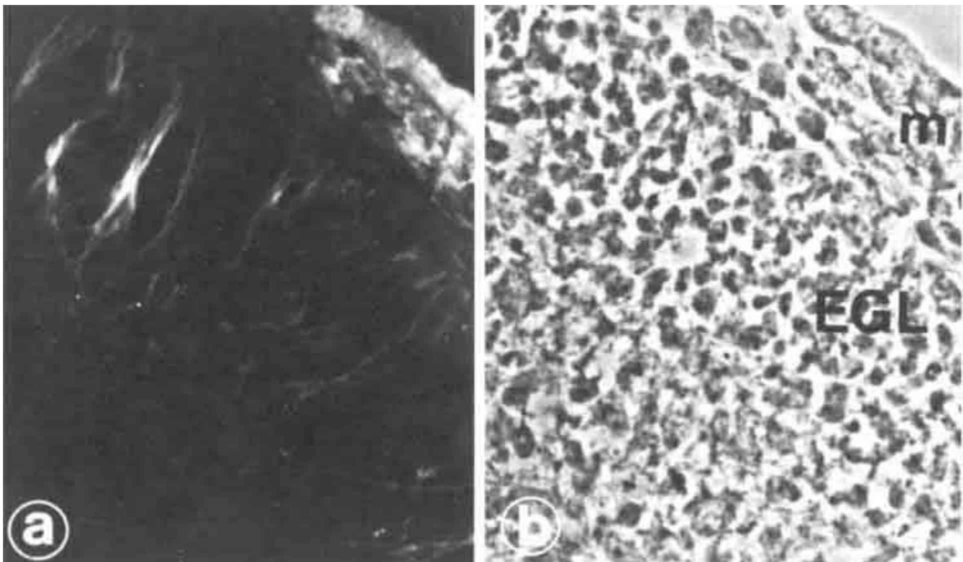


Fig. 9. Midsagittal section of a 13-day-old staggerer mutant cerebellum (ventral area) stained with M1 (a) and GFA protein (b) antibodies by double immunofluorescence (× 170). a) M1 antigen is strongly detectable in Bergmann glia and in other types of astrocytes. Purkinje cells do not stain with M1 antibodies in this mutant. (For staining of M1 antigen in normal animals, see Fig. 8.) b) GFA protein staining of the same visual field as shown in (a).



Reeler. At postnatal day 7 C1 antigen is detectable in Bergmann glial fibers of reeler mutants as it is in control littermates and in the cerebellar mutants mentioned before (Fig. 10a, b). In contrast to the weaver and staggerer mutants, however, C1 antigen is undetectable in reeler at day 10 in Bergmann fibers of midsagittal sections (Fig. 11a, b), and is only weakly detectable more laterally. Up to day 19 no positive staining of Bergmann glial fibers is observed although staining of ependyma and retinal Müller cells appears normal. Purkinje cells are M1 antigen-positive as in normal controls (not shown).

The early loss of C1 antigen detectability is accompanied by an early rise in detection of M1 antigen. At day 10, M1 antigen is strongly detectable in Bergmann fibers in medial (Fig. 11an b), and lateral regions. By day 13, strongly M1 antigen-positive fibers are detectable even more laterally.

Purkinje cell degeneration. Since Purkinje cell degeneration mutants are behaviorally normal during the first 3 postnatal weeks [27] and do not show any apparent reduction of brain size, early postnatal mutants could not be examined. In the youngest mutants studied (23 days of age), no obvious difference in C1 antigen expression in Bergmann fibers is apparent when compared to normal control littermates. At 2 months of age, however, a decrease in C1 antigen detectability in Bergmann fibers is seen (Fig. 12a, b). Ependyma (Fig. 12a, b) and retinal Müller cells (Fig. 13a, b) are C1 antigen-positive at this age as they are in normal controls.

In contrast to C1 antigen, expression of M1 antigen is already abnormal in the cerebellum at postnatal day 23. Astrocytes of the internal granular layer and predominantly those near the Purkinje cell layer are strongly M1 antigen-positive (Fig. 14a, b). Only few Bergmann glial fibers are M1 antigen-positive at this age (Fig. 14b); however, most of them show strong staining at 2 months of age (Fig. 15a, b). Interestingly Purkinje cell bodies are either M1 antigen-negative or only weakly positive in this mutant (Fig. 15a, b) even at 23 days postnatally, when at least half of the Purkinje cells are still alive [27] and can be visualized by other monoclonal antibodies (Weber, Sommer, and Schachner, in preparation).

Wobbler. In the cerebellum of the adult wobbler mutant mouse C1 antigen is detectable in Bergmann glia at levels comparable to those in normal control animals. Likewise, M1 antigen is rarely found in Bergmann glia, but is detectable in white matter astrocytes, thus showing a normal pattern of expression. However, astrocytes of the internal granular layer that are normally negative at this age are M1 antigen-positive in this mutant (not shown).

Fig. 10. Sagittal section of 7-day-old reeler mutant cerebellum labeled with C1 antibody by indirect immunofluorescence (× 430). a) C1 antigen is detectable in Bergmann fibers that cross the external granular layer. Meningeal structures in the upper right corner are strongly C1 antigen-positive. b) Phase contrast micrograph of the same visual field as shown in (a). The external granular layer (EGL) extends diagonally from the lower right to the upper left corner. Meninges (m).

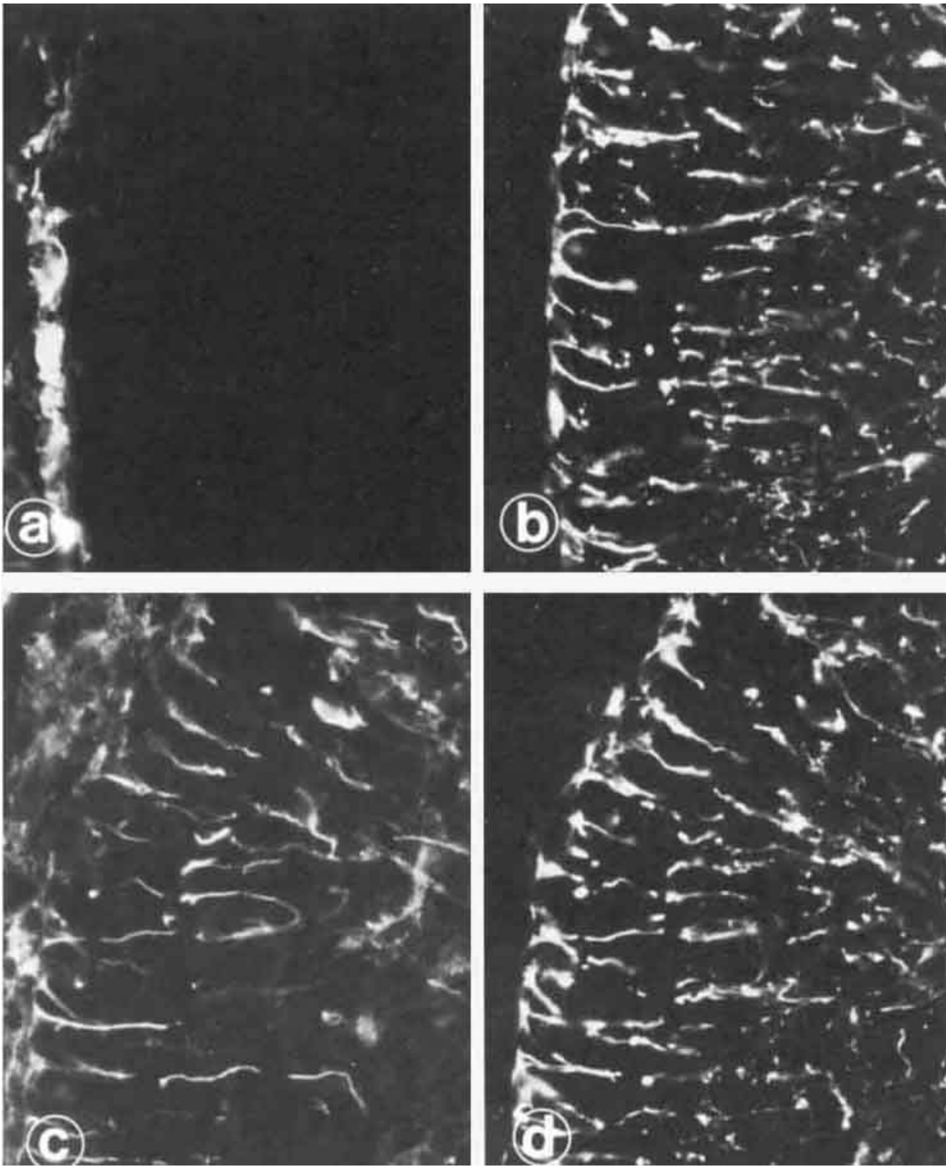


Fig. 11. Midsagittal section of a 10-day-old reeler mutant cerebellum stained with C1 (a), M1 (c), and GFA protein (b, d) antibodies by double immunofluorescence (× 430). C1 and M1 antigens are stained in adjacent sections. a) C1 antigen is not detectable in Bergmann fibers that are clearly stained in the same section by GFA protein antibodies as shown in (b). C1 antigen is, however, strongly positive in ependyma lining the fourth ventricle. b) GFA protein is prominently stained in Bergmann fibers, but not in ependyma. c) M1 antigen is positive in most Bergmann fibers that are GFA protein-positive in (d). d) GFA protein is prominent in Bergmann glial fibers.

Fig. 13. Section of Purkinje cell degeneration mutant retina taken from the same animal used for Figure 12a. Double immunolabeling was performed for C1 (a) and GFA protein (b) antibodies (× 170). a) Retinal Müller cell processes and somata are prominently stained by C1 antibody. Astrocytes of the ganglion cell layer are not C1 antigen-positive. b) GFA protein is detectable in a few retinal Müller cell processes in the lower right corner; cell bodies are weakly labeled. Astrocytes in the ganglion cell layer are strongly GFA protein-positive.

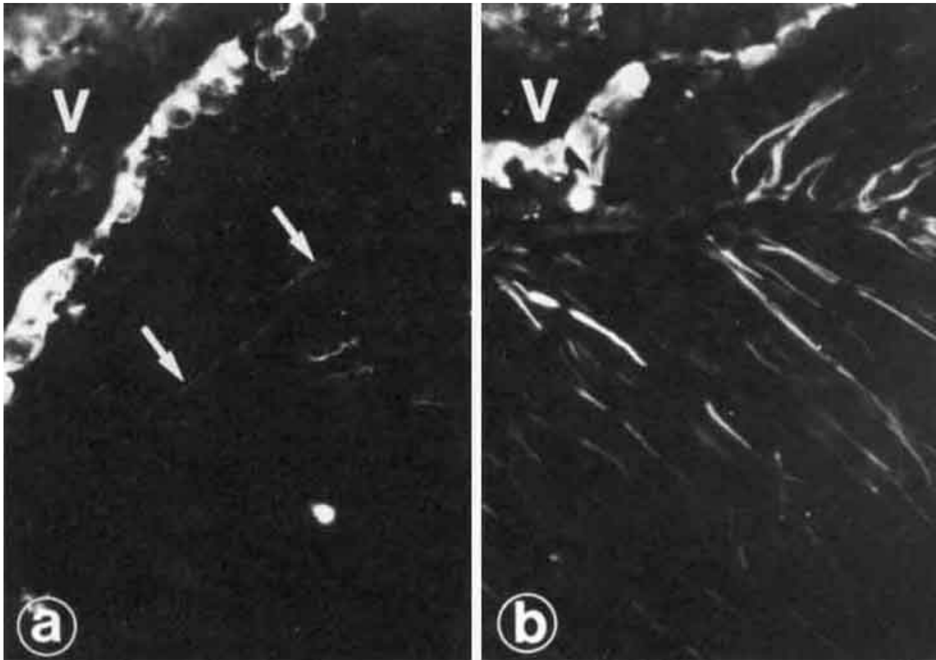
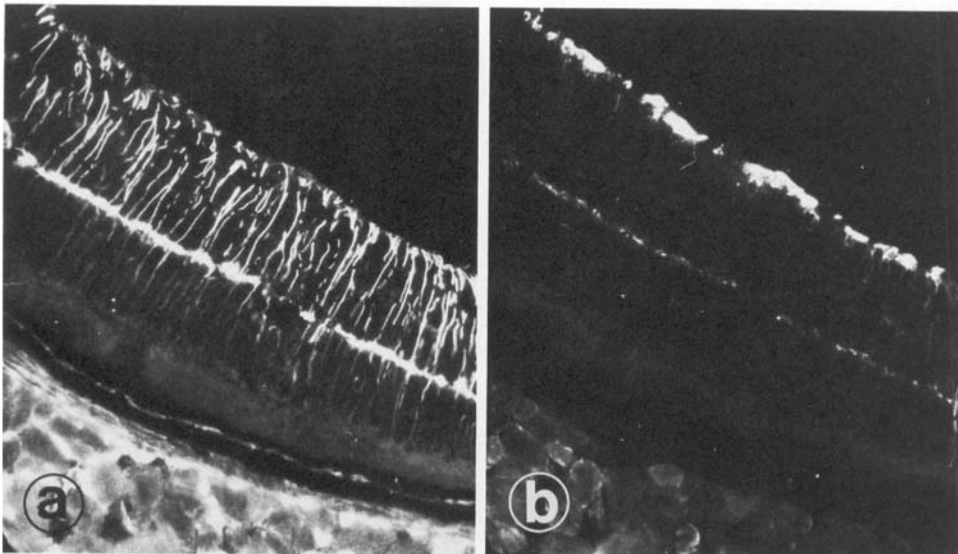


Fig. 12. Midsagittal sections through the cerebellum of an 8-week-old Purkinje cell degeneration mutant shown in (a) and its normal littermate (genotype +/?) shown in (b). Both sections were stained with C1 antibody by indirect immunofluorescence. Visual fields shown in (a) and (b) represent similar cerebellar areas near the fourth ventricle (× 430). a) C1 antigen is weakly detectable in few Bergmann fibers of the mutant (arrows), whereas ependymal cells lining the fourth ventricle (V) are stained as in the control animal shown in (b). b) C1 antigen is prominent in Bergmann fibers and in ependymal cells lining the fourth ventricle (V).



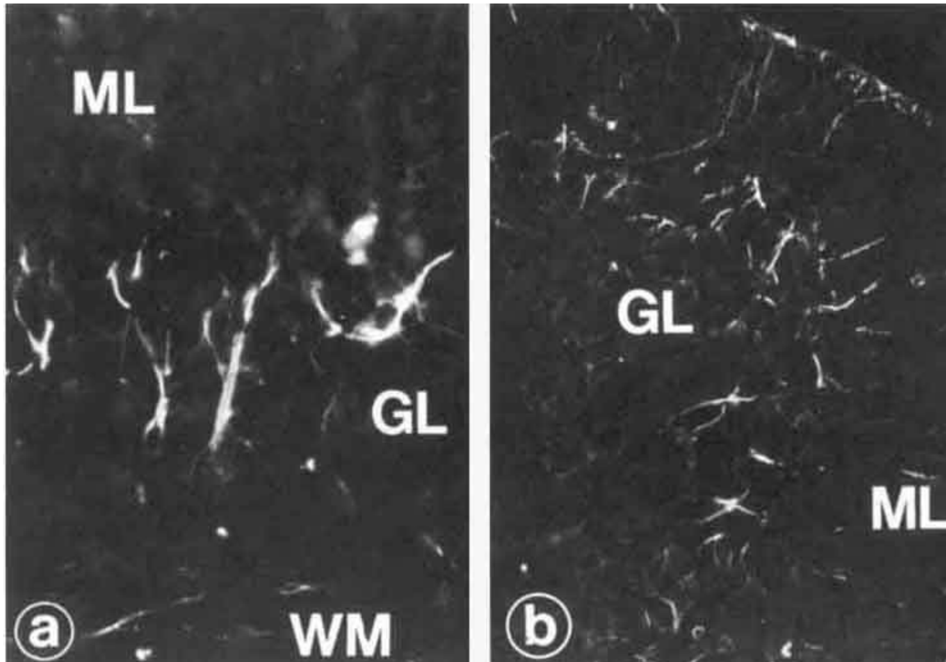
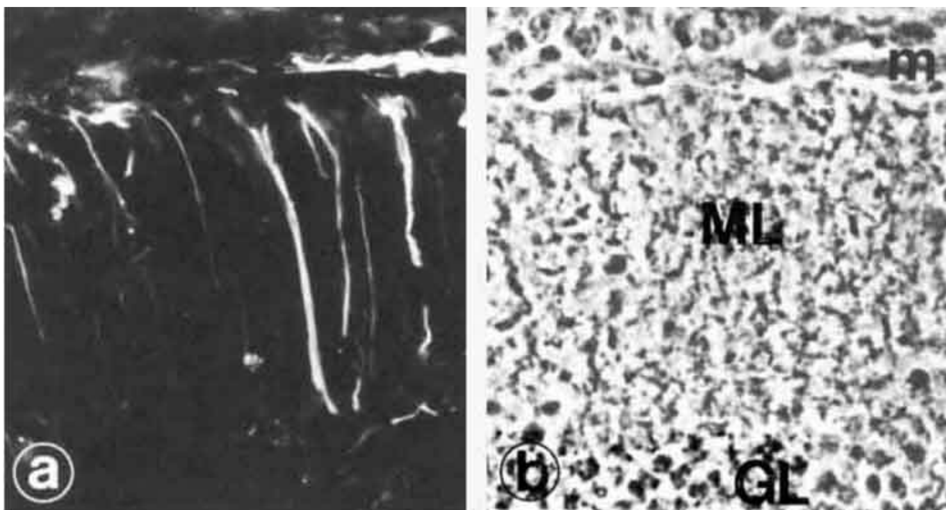


Fig. 14. Sagittal sections of a 23-day-old Purkinje cell degeneration mutant cerebellum stained with M1 antibody in (a) and (b) by indirect immunofluorescence. a) M1 antigen is strongly detectable in astrocytes of the upper part of the granular layer (GL) near the Purkinje cell layer and in astrocytes of the white matter (WM). Bergmann fibers in the molecular layer (ML) and Purkinje cells are not stained (× 430). b) M1 antigen is detectable in few Bergmann glial fibers and in many astrocytes of the granular layer near the Purkinje cell layer. Purkinje cells are not detectable by M1 antibody between the granular layer (GL) and the molecular layer (ML) (× 170).



DISCUSSION

During postnatal cerebellar development, C1 and M1 antigens are not restricted to Bergmann glia and astrocytes in the white matter, respectively, but are expressed in other types of cerebellar astrocytes that are antigen-negative in adulthood. At birth, C1 antigen is detectable by immunohistological methods in all types of astrocytes, but starts to become confined to Bergmann glia at day 10 of age. At day 8, C1 antigen starts to disappear transiently from Bergmann glial fibers, is almost not detectable between days 15 and 20, but resumes adult levels of expression at later ages.

M1 antigen is first seen in white matter astrocytes at approximately day 7 and in astrocytes of the internal granular layer, and in Bergmann glia at approximately day 8. It disappears from Bergmann glia and astrocytes in the internal granular layer at times, when C1 antigen expression becomes again fully detectable in Bergmann glia. The expression of the two antigens in Bergmann glia appears to occur in an almost complementary fashion, and is subjected to a spatiotemporal gradient from medioventral to mediodorsal and then to lateral levels within the external granular and developing molecular layers. C1 is lost at times in cerebellar regions, when and where M1 antigen starts to disappear. The loss of C1 antigen is first seen at day 8 in ventromedial areas and spreads along ventrodorsal to lateral regions, whereas M1 antigen appears first in ventromedial parts and spreads along the same gradient. It is impossible to conclude from the present data whether the two antigens are mutually exclusive at a single cell level or whether they can transiently be expressed concomitantly. The use of fluorochrome-labeled monoclonal antibodies or cross-adsorbed antiimmunoglobulin antibodies that are species-specific for rat and mouse, ie, for M1 and C1 antibodies, respectively, could resolve this question.

The spatiotemporal gradient of C1 and M1 antigen expression is comparable to the gradient of development of the external granular layer observed by Landis and Sidman [23]. Cessation of granule cell proliferation and migration causes a reduction in the thickness of the external granular layer that is seen first in ventromedial areas at approximately day 10, and spreads dorsally and laterally thereafter. It is impossible to determine from the present, purely descriptive data which type of differentiative signals may be involved in triggering the transient loss of C1 antigen and the appearance of M1 antigen.

A search for the signals that might regulate antigen expression becomes particularly pertinent when the development of normal and mutant cerebella is compared. In the cerebella of three neurological mutations (reeler, staggerer, and weaver), C1 antigen is normally expressed in Bergmann glia and other types of cerebellar astrocytes at early postnatal ages. The switch from C1 to

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Fig. 15. Midsagittal section through the cerebellum of an 8-week-old Purkinje cell degeneration stained with M1 antibody by indirect immunofluorescence (× 430). a) M1 antigen is strongly detectable in Bergmann fibers, some of which seem abnormally swollen. Unidentified structures in the meninges are antigen-positive. b) Phase contrast micrograph of the same visual field as shown in (a). Molecular layer (ML), granular layer (GL), meninges (m).

M1 antigen expression occurs in a similar spatial gradient as in the normal situation. However, the loss of C1 antigen and appearance of M1 antigen in Bergmann glia occur at slightly earlier ages in these mutants. The reeler mutant is the first to lose C1 antigen. It is followed by weaver and staggerer. In the mutant Purkinje cell degeneration, the mutation cannot be recognized before the third postnatal week, and antigen expression can therefore not be monitored at early postnatal ages. However, in contrast to the other cerebellar mutants, C1 antigen is detectable in Bergmann glial cells at day 23, and M1 antigen is only weakly detectable.

Interestingly, the onset of C1 antigen loss and M1 antigen appearance in the different mutants parallels the order of timing of the putative onset of mutant gene expression. The earliest defect is shown in reeler, which has been described to show prenatal defects in the development of the cerebellar cortex [14]. Weaver is characterized by an abnormal deployment of Bergmann glia during the first postnatal days, and degeneration of premigratory granule cell neurons on the external granular layer [15–17]. Staggerer shows postmigratory degeneration of granule cells, possibly due to an abnormality of their postsynaptic targets, the Purkinje cells [23]. Even later, during the third and fourth postnatal weeks, the abnormalities of Purkinje cell degeneration become manifest in the loss of Purkinje cells [26, 27].

Although the temporal sequence of antigen switch parallels the order of manifested mutant abnormality, a direct temporal correlation is difficult, for instance, in the case of the reeler mutation. Whereas prenatal abnormalities are documented by histological criteria, C1 antigen expression appears normal until the end of the first postnatal week. In Purkinje cell degeneration, however, the onset of the morphological abnormality coincides relatively accurately with the antigen switch. In addition, in the staggerer mutant, delayed disappearance of the external granular layer in dorsolateral regions of the cerebellum [23] is accompanied by a prolonged persistence of C1 antigen.

An interpretation that C1 and M1 antigen expression may be regulated by signals from an abnormal environment seems to be supported by recent evidence by Lagenaur, Masters, and Schachner (submitted for publication), who found a loss of C1 antigen and appearance of M1 antigen in Bergmann glia after infliction of a stab wound. In contrast to the continued suppression of C1 antigen and persistence of M1 antigen in the mutants, the wounded cerebellar cortex is only transiently afflicted by abnormal antigen expression. This difference might be explained by the capacity of the intact brain to restore normal conditions, whereas the mutant phenotype is irreversible. Both in the mutants and after wounding, the abnormality of antigen expression seems to be confined to the affected brain area, the cerebellum. In reeler, staggerer, and weaver whose retinae are not affected by the mutations, C1 and M1 expression in the retina is normal. Similarly the retina of Purkinje cell degeneration shows a normal distribution of C1 and M1 antigens at 8 weeks of age, although most of the photoreceptors eventually degenerate in this mutant [28]. Neuronal degeneration, however, does not occur until the fourth postnatal week, and proceeds very slowly with regional differences over a period of several months. Thus, at this age, retinal degeneration might be under the threshold of abnormality that is

needed to alter C1 and M1 antigen expression in Müller cells. We expect, however, that abnormal M1 and C1 antigen expression develops at older ages.

The fact that in the wobbler mutant M1 antigen expression is abnormal in the internal granular layer remains unexplained at present, since the cerebellar cortex in this mutant does not appear to be grossly altered. However, aberrant input from the spinal cord into the cerebellar cortex might be the basis for a limited reactive response of astrocytes that remains confined to the granular layer. A limited reactivity of astrocytes in the granular layer is also characteristic of a mild or subthreshold reaction to stab wounding. At sites far away from the wound, mostly astrocytes of the internal granular layer are abnormally M1 antigen-positive. Similarly, after most Bergmann glial fibers have returned to normal C1 and M1 antigen expression during recovery from stab wounding, astrocytes in the internal granular layer are still M1 antigen-positive. Thus, astrocytes in the granular layer seem to be more sensitive to changes in their environment than Bergmann glia and retinal Müller cells.

Despite our complete lack of insight into the cellular and molecular mechanisms underlying abnormal expression of glial antigens in mutants and after injury, certain features may correlate with the functional state of astrocytes during normal development. If in a pathological state a reactive response of astroglia is characterized by a loss of C1 antigen and an enhancement of M1 antigen, then the transient loss of C1 antigen and appearance of M1 antigen during normal development might result from an astrocytic response to the formation of myelin, the so-called myelination gliosis. Whether a causal relationship to developmental neuronal cell death can be established is also conjectural. Since the final cell division in Bergmann glia in the rat [35] coincides temporally with the switch from C1 to M1 antigen expression in these cells, it could be speculated that the onset of the transient loss of C1 and appearance of M1 antigen is linked to a particular differentiative state. It could also be speculated that susceptibility to abnormal environmental cues depends on the maturational state of Bergmann glia, since in the reeler mutant the antigen switch occurs approximately at the time of final division and well after the appearance of morphologically recognizable abnormalities.

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