Recognition of Bergmann Glial and Ependymal Cells in the Mouse Nervous System by Monoclonal Antibody

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ABSTRACT A monoclonal antibody designated anti-C1 was obtained from a hybridoma clone isolated from a fusion of NS1 myeloma with spleen cells from BALB/c mice injected with homogenate of white matter from bovine corpus callosum.

In the adult mouse neuroectoderm, C1 antigen is detectable by indirect immunohistology in the processes of Bergmann glial cells (also called Golgi epithelial cells) in the cerebellum and of Müller cells in the retina, whereas other astrocytes that express glial fibrillary acidic protein in these brain areas are negative for C1. In addition, C1 antigen is expressed in most, if not all, ependymal cells and in larger blood vessels, but not capillaries. In the developing, early postnatal cerebellum, C1 antigen is not confined to Bergmann glial and ependymal cells but is additionally present in astrocytes of presumptive white matter and Purkinje cell layer. In the embryonic neuroectoderm, C1 antigen is already expressed at day 10, the earliest stage tested so far. The antigen is distinguished in radially oriented structures in telencephalon, pons, pituitary anlage, and retina. Ventricular cells are not labeled by C1 antibody at this stage.

C1 antigen is not detectable in astrocytes of adult or nearly adult cerebella from the neurological mutant mice staggerer, reeler, and weaver, but is present in ependymal cells and larger blood vessels.

C1 antigen is expressed not only in the intact animal but also in cultured cerebellar astrocytes and fibroblastlike cells. It is localized intracellularly.

The ontogeny of the different types of neural cells and the developmental relationships between these cells pose a major problem in neurobiology and developmental biology in general. All classes of neurons and glial cells presumably arise from a common stem cell (31, 36, 37), which constitutes the epithelium of the neural tube. It is not known when the program of differential gene expression is initiated in these germinal cells, which is presumed to result in the divergent differentiation pathways and cell lineages of various types of neurons and glia.

The origins of glial cells seems particularly mysterious at present, because, in contrast to neurons, glial cells continue to proliferate and divide often throughout life (23, 48). Whereas different types of neurons originate in distinct temporal patterns by withdrawing from the cell cycle of their neuroblast precursors, an orderly sequence of growth and differentiation of glial cells and glial subpopulations has yet to become apparent.

Equally unsolved is the problem of differentiative pathways taken by the glial cell subclasses, particularly the astroglia and oligodendroglia. These two types of macroglial cells seem to arise from a common precursor, the glioblast (36, 37). Whether these are multipotential glial cells that are capable of giving rise to astroglia as well as oligodendroglia (55, 56) or whether they constitute distinct subsets of glial precursors is presently undecided. It is also an open question whether differentiated astrocytes and oligodendrocytes arise simultaneously or consecutively (50, 54), but it seems widely accepted that oligodendrocytes differentiate later than astrocytes.

The problem of origin and differentiation of glial cells has been hampered both by the lack of understanding of glial functions and by the fact that unequivocal criteria for identification of glial cells at early developmental stages have not been established. In addition to the existing ultrastructural description of developing glial cells, a search for biochemical markers detectable by appropriate immunological reagents seems imperative.

The present study and the one described in the companion paper (39) characterize two astroglial markers, a new antigen, C1, and vimentin, the protein subunit of a class of intermediate

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filaments (13). These two antigens can be detected in the adult as well as in the developing mouse nervous system. Both C1 antigen and vimentin are expressed at least as early as embryonic day 11, whereas glial fibrillary acidic (GFA) protein, the well-characterized astroglial marker, is expressed only postnatally in radial glial fibers of the rat (3), which shares many features in cerebellar development with the mouse. C1 is detectable in the adult neuroectoderm only in distinct subclasses of astroglial cells, the radial glial cells in the cerebellum (Bergmann or Golgi epithelial cells) and retina (Müller cells). Ependymal cells also express C1 antigen at early postnatal and adult stages.

MATERIALS AND METHODS

Animals

C57BL/6J mice were obtained from the Jackson Laboratories, Bar Harbor, Me., and maintained at the animal facility of Theoretische Medizin, University of Heidelberg. BALB/c mice were obtained from Zentrale Tierzuchtanlage, Hannover, W. Germany. Weaver mutant mice (gene symbol *wv*) were originally obtained from the Jackson Laboratory. They are outbred on a C57BL/6J × CBA background. Staggerer mutant mice (*sg*) were bred from a C57BL/6J inbred stock carrying two closely linked marker genes, *se* (short ear) and *d* (dilute). Heterozygous staggerer breeding pairs [(*sg* + +)/(+ *d se*)] × [(*sg* + +)/(+ *d se*)] were a kind gift of Drs. S. Roffler-Tarlov and R. L. Sidman (Boston, Mass.). Reeler mutant mice (*rl*) carrying the dominant marker gene Hammertoe (*Hm*) are outbred on a C57BL/6J × C3H background and were maintained by mating heterozygous breeding pairs [(*rl Hm*)/(+ +)] × [(*rl Hm*)/(+ +)]. These animals were a kind gift of Dr. V. Caviness (Boston, Mass.). For controls, behaviorally normal littermates have been used. In the case of the weaver mutant, the genotype of normal littermates may therefore have been wild type or heterozygous for the weaver gene. For reeler, animals without the dominant genetic marker *Hm* served as controls. For staggerer, heterozygous black mice or grey wild-type animals, carrying homozygously the closely linked, recessive marker alleles *d* and *se* were used. Embryos were taken from C57BL/6J mice. Embryonic day 0 (E0) was designated as the day a vaginal plug was found. Bovine brains were obtained from the local slaughterhouses in Boston, Mass., and Heidelberg, W. Germany.

Immunizations

BALB/c female mice (4–6 wk old) were injected with homogenate of white matter dissected from corpus callosum of bovine brain. Homogenates were prepared as described previously (34).

Mice received two subcutaneous injections at intervals of ~2 wk. Each injection contained 15 mg of protein of homogenate in 0.5 ml of phosphate-buffered saline (PBS), pH 7.3. The third and last injection was performed intravenously. 3 d after the last injection, animals were sacrificed and their spleen cells prepared for fusion with myeloma cells.

Cells and Cell Culture

Myeloma P3-NS1/1Ag4-1 (NS1) were obtained from Dr. G. Hämmerling, Cologne, W. Germany. Myeloma cells and myeloma-splenocyte hybrids were maintained in RPMI 1640 culture medium containing 10% fetal calf serum, as described by Lagenaur et al. (16).

Primary monolayer cultures of dissociated early postnatal mouse cerebellum were maintained on poly-L-lysine-coated cover slips as described by Schnitzer and Schachner (40) as modified from Willinger and Schachner (58) and Barkley et al. (2). Explants were obtained by cutting cerebella sagitally into small pieces (~0.5 mm thick), and culturing these on poly-L-lysine-coated glass cover slips.

Cell Fusion and Cloning Procedures

Cell fusion was carried out by a modification of the techniques of Lemke et al. (18) and Galfré et al. (14), as described by Lagenaur et al. (16). Hybridomas were cloned twice in 20-μl microtest plates (C. A. Greiner und Söhne, GmbH, Nürtingen, W. Germany) also as described previously (16).

Antibodies

Monoclonal antibody C1 was identified as an IgG1 class antibody by a double immunodiffusion test, using subclass specific antibodies (Meloy Laboratories Inc., Springfield, Va.). Supernates from C1 antibody-producing hybridoma cells were concentrated 10-fold as previously described (16) and used undiluted for immunofluorescence experiments. Ascites fluid obtained from C1-hybridoma tumor-bearing female BALB/c mice was diluted 1:50 or 1:100 for indirect immunofluorescence. Under the same conditions for indirect immunofluorescent staining as described for C1, other mouse monoclonal antibodies of the same or different Ig subclass gave completely different patterns of staining. For comparative studies monoclonal antibody M1 (16) and antineurofilament antiserum (34), originating from rat and mouse, respectively, were used. Double-labeling experiments were performed with rabbit antibodies to GFA protein and to fibronectin (kind gifts of Drs. L. Eng and R. Hynes, respectively).

Immunocytological Procedures

Indirect immunofluorescence on histological sections was carried out as previously described (15).

In brief, fresh frozen mouse brain was cut sagitally in 10-μm thick sections, dried onto cover slips, and incubated at room temperature with monoclonal antibody or antineurofilament antiserum for 20 min. The sections were then washed twice in phosphate-buffered saline (PBS) and incubated with fluorescein isothiocyanate–conjugated goat anti–mouse IgG (GAM-FITC) heavy and light chains (Antibodies Inc., obtained via Fa. Paesel, Frankfurt, W. Germany). Sections were again washed twice in PBS and examined with a Zeiss fluorescence microscope with epi-illumination and the appropriate filters. For double-labeling experiments, rabbit antibodies (anti-GFA or antifibronectin) were applied simultaneously with the monoclonal antibody; correspondingly, tetramethylrhodamine isothiocyanate–conjugated goat anti-rabbit IgG (GAR-TRITC; Nordic Immunology, obtained via Fa. Byk Mallinckrodt, Dietzenbach-Steinberg, W. Germany) was applied simultaneously with GAM-FITC. Sections of embryos were fixed in 4% paraformaldehyde after treatment with the first antibody, followed by two washes in PBS before the second antibody was applied. Cultures were washed twice in PBS containing 0.1% bovine serum albumin before application of the first antibody. For staining of intracellular structures cultures were treated for 30 s with 96% ethanol. Indirect immunofluorescence was then carried out as described for sections.

RESULTS

Histological Localization of C1 Antigen in Adult Mouse Cerebellum

Monoclonal anti-C1 antibody is secreted by a hybridoma clone isolated from a fusion of NS1 myeloma with spleen cells from BALB/c mice that had been injected with homogenate of white matter isolated from corpus callosum of adult bovine brains. The antibody was first detected by indirect immunofluorescence on histological sections of fresh frozen adult C57BL/6J mouse brain. A typical immunofluorescent staining pattern of the cerebellar cortex in sagittal sections is shown in Fig. 1*A*. Radially oriented processes of Golgi epithelial cells, the Bergmann fibers, are recognized by anti-C1 antibody, whereas astrocytes in white matter and granular layer of the cerebellar cortex are not detectable. The identity of astrocytes was confirmed in the same histological section by double immunolabeling using anti–GFA protein antiserum as a marker for astrocytes (Fig. 1*B*). In general, parts of Bergmann fibers proximal to the meninges are more heavily stained than inner portions near the cell bodies (Fig. 2*A*). In contrast, GFA protein is more uniformly distributed in these fibers, as can be shown by double immunolabeling of identical sections (Fig. 2*B*). Immunolabeling of the cell bodies of Bergmann glial cells has not been observed with anti-C1 antibodies. In addition to the staining of Bergmann fibers, Fig. 2*A* also shows prominent labeling of a larger blood vessel in the meninges that is not recognized by anti-GFA protein antiserum (Fig. 2*B*). These larger arteries are characteristically stained at their outer and inner boundaries towards the vessel lumen and the neuropile (Fig. 3*A* and *B*). Capillaries that are known to react strongly with antiserum to fibronectin (33) do not react with anti-C1 antibodies. Another striking feature of C1 antigen is its expression in ependymal cells, which overlaps with GFA protein expression in some cells and not in others (Figs. 4*A* and *B* and 5*A* and *B*).

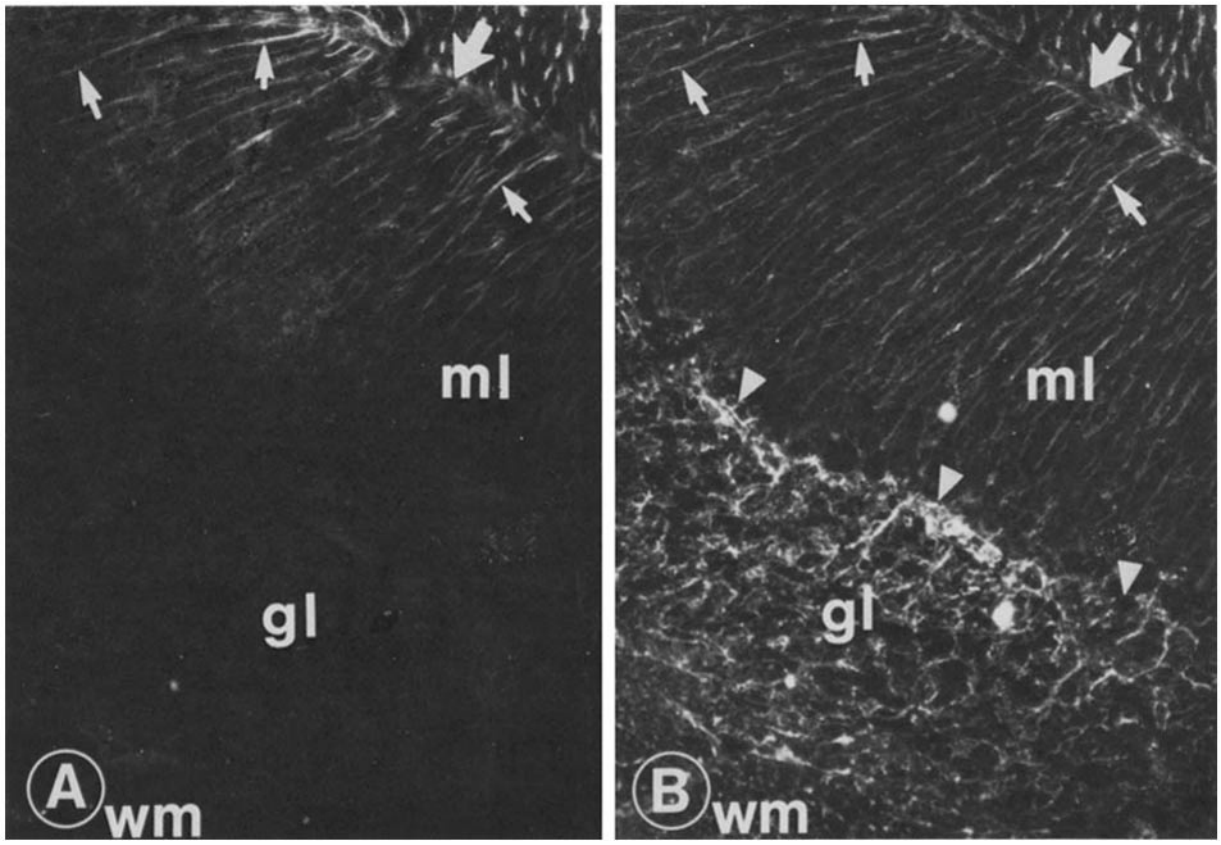

FIGURE 1 Immunohistological staining of C1 antigen and GFA protein in the cerebellar cortex of an adult C57BL/6J mouse. Simultaneous labeling of C1 antigen and GFA protein in one sagittal section is visualized using (*A*) fluorescein(FITC)- and (*B*) rhodamine(TRITC)-coupled second antibodies, respectively. C1 antigen-positive, radially oriented fibers are seen in the molecular layer (*ml*). These coincide with GFA protein-positive Bergmann fibers (*B*; small arrows). The meninges are negative with both antibodies (large arrow). Astrocytes in white matter (*wm*), Purkinje cell (arrowheads), and granular layers (*gl*) are distinctly stained for GFA protein (*B*), but not for C1 antigen (*A*). × 220.

Histological Localization of C1 Antigen in Adult Mouse Retina

Radial fibers in the adult mouse retina also express C1 antigen. Whereas GFA protein is most easily detectable in radial fibers at the ora serrata and at the exit of the optic nerve (7), C1 antigen is more uniformly expressed in all radial fibers. In contrast to GFA protein and M1 antigen (16), C1 antigen is not detectable in astrocytes of the ganglion cell layer. (Data given for adult retina are not shown.)

Developmental Expression of C1 Antigen

C1 antigen is already detectable in the developing neuroectoderm on embryonic day 10, the earliest stage tested so far. At this age the antigen is present in radially oriented structures in the anlage of the telencephalon and the developing pons. Radial fibers are shown in the retinal anlage at embryonic day 13 (Fig. 6*A* and *B*), in the telencephalic anlage at embryonic day 10 (Fig. 7*A* and *B*), and in the pituitary anlage at embryonic day 11 (Fig. 8*A* and *B*). At embryonic day 10, C1 antigen cannot be detected in cells lining the lumen of the neural tube and developing ventricles, the so-called ventricular cells, although its appears in ependyma postnatally. Double immunolabeling experiments with anti-GFA protein are not possible at embryonic days 10 and 13, because GFA protein is not expressed in the mouse at these early stages (4). However, comparison of staining patterns with antineurofilament antiserum (34) is possible at this age to substantiate the nonneuronal nature of these C1-positive fibers.

Radially oriented structures in retina (7) and pituitary anlage are not detectable with anti-neurofilament antiserum, and the distribution of neurofilament-positive fibers in the telencephalic anlage is clearly different from the one revealed by anti-C1 antibodies (data not shown). In addition, the staining pattern obtained by antifibronectin antibodies does not show any radially oriented fibers. At birth, C1 antigen is present in the cerebellum not only in Bergmann fibers but also in GFA protein–positive cells close to the peduncular portions of cerebellar white matter. At postnatal day 6, astrocytes are C1 antigen–positive in presumptive white matter and developing internal granular layer; Bergmann glial cells are, however, already more prominently stained than the other astrocytic cell types (Fig. 9*A* and *B*). Ependymal cells are also positive at this early postnatal age. The exact time of appearance of C1 antigen, however, in ependyma has not been determined. At adult ages, expression of C1 antigen has disappeared in granular layer and white matter astrocytes. The results concerning the developmental expression of C1 have been summarized in Table I.

Absence of C1 Antigen Expression in Bergmann Fibers of Neurologically Mutant Mice

Expression of C1 antigen in 18- to 21-d-old staggerer and reeler mice and 4- to 8-wk-old weaver mice was investigated

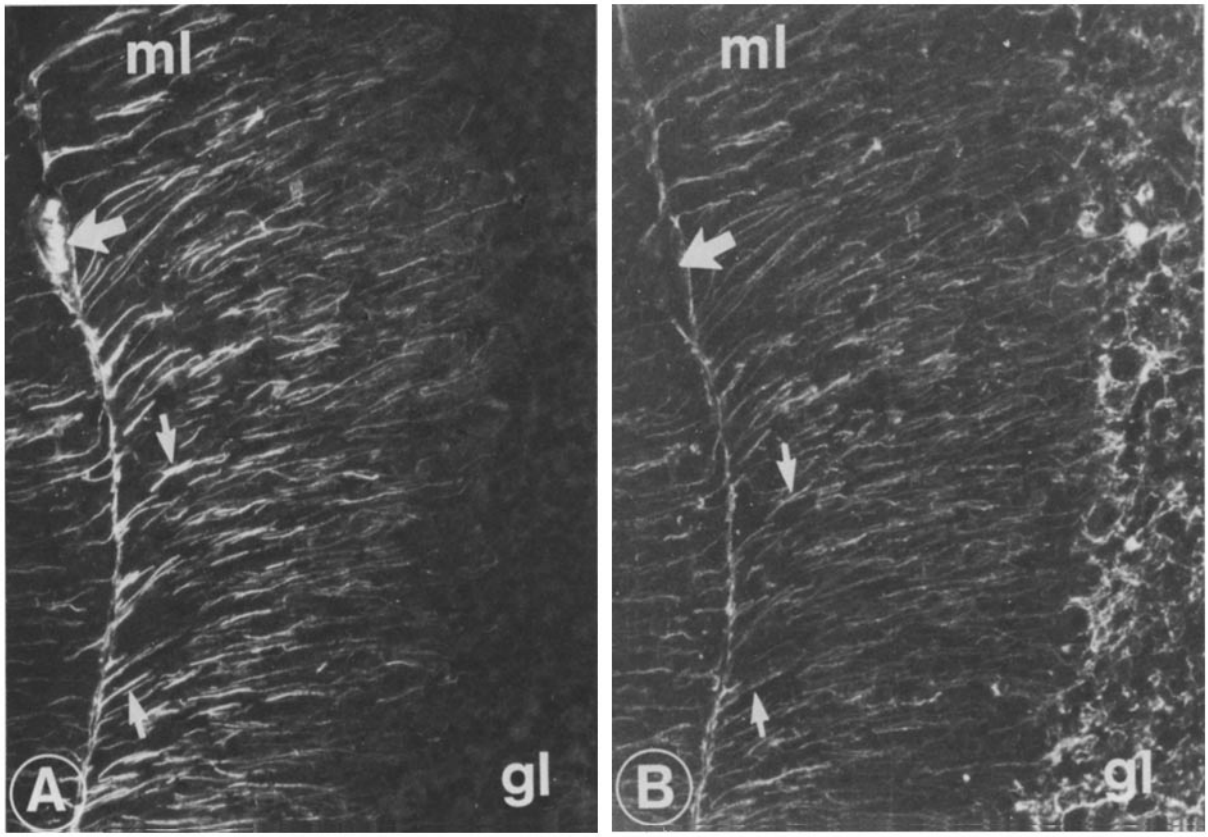

FIGURE 2 Immunohistological staining of C1 antigen and GFA protein in the cerebellar cortex of an adult C57BL/6J mouse. Double labeling of C1 antigen and GFA protein in a single sagittal section was performed as in Fig. 1. (*A*) C1 antigen is detected in radial Bergmann glial fibers in the molecular layer (*ml*). The meninges-proximal portions of these fibers are more heavily stained with C1 antibodies (*A*; small arrows) than with GFA protein antiserum (*B*), which labels Bergmann fibers more uniformly throughout the molecular layer (*ml*). The granular layer (*gl*) is negative for C1 antigen (*A*), but positive for GFA protein (*B*). A larger blood vessel in the meninges is stained by C1 antibodies (*A*; large arrow), but not with GFA protein antiserum (*B*). × 220.

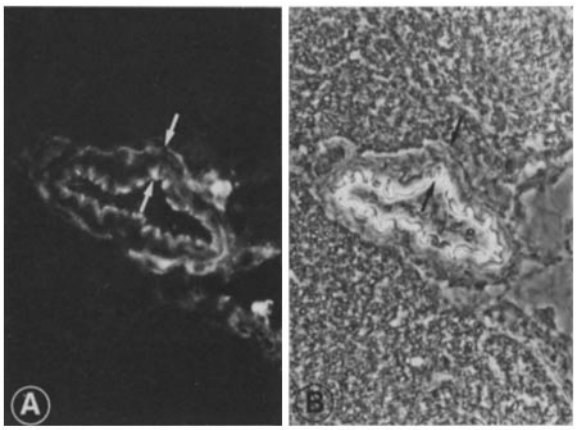

FIGURE 3 Immunohistological staining of C1 antigen of a large blood vessel in adult C57BL/6J mouse brain. (*A*) C1 antigen is detected at the inner and outer surfaces (arrows) of a large blood vessel. The lumen is filled with nonimmunofluorescent material. (*B*) Phase-contrast photomicrograph of the same visual field as shown in *A*. × 280.

using behaviorally normal littermates as controls as described in Materials and Methods. For all three mutants, C1 antigen is detectable neither in the more radially oriented fibers in the external parts of the cerebellar cortex nor in astrocytes of the internally located regions of the rudimentary cerebella. Ependymal cells and larger blood vessels are, however, as intensely stained with anti-C1 antibodies as they are in normal controls.

Expression of C1 Antigen in Cultured Cerebellar Cells

In monolayer cultures of dissociated cerebellar cells from early postnatal mice or explant cultures from embryonic cerebellum, C1 antigen is detectable only after the cells have been made permeable by treatment with 96% ethanol for 30 s at −20°C (Figs. 10 and 11). Fixation with 4% paraformaldehyde for 10 min at room temperature appears to destroy the antigenicity of C1. C1 antigen is expressed in GFA protein–positive astrocytes and in GFA protein–negative and fibronectin-positive fibroblasts or fibroblastlike cells.

Cerebellar cells from 7-d-old mice have to be maintained in vitro for ~5 d before C1 antigen becomes detectable in astroglial cells. Even after culture periods of up to 3 wk, not all GFA protein–positive cells express C1 antigen. However, in certain areas of the culture almost all GFA protein–positive cells are also C1 antigen–positive, whereas in other areas one out of ten GFA protein–positive cells may be C1 antigen–positive.

When 7-d-old cerebellar cultures from 7-d-old C57BL/6J mice are maintained in the presence of 10^{-6} M Colcemid (from 6 to 24 h), C1 antigen as well as GFA protein appear distributed as perinuclear rings (Fig. 11*A–C*). The percentage of C1

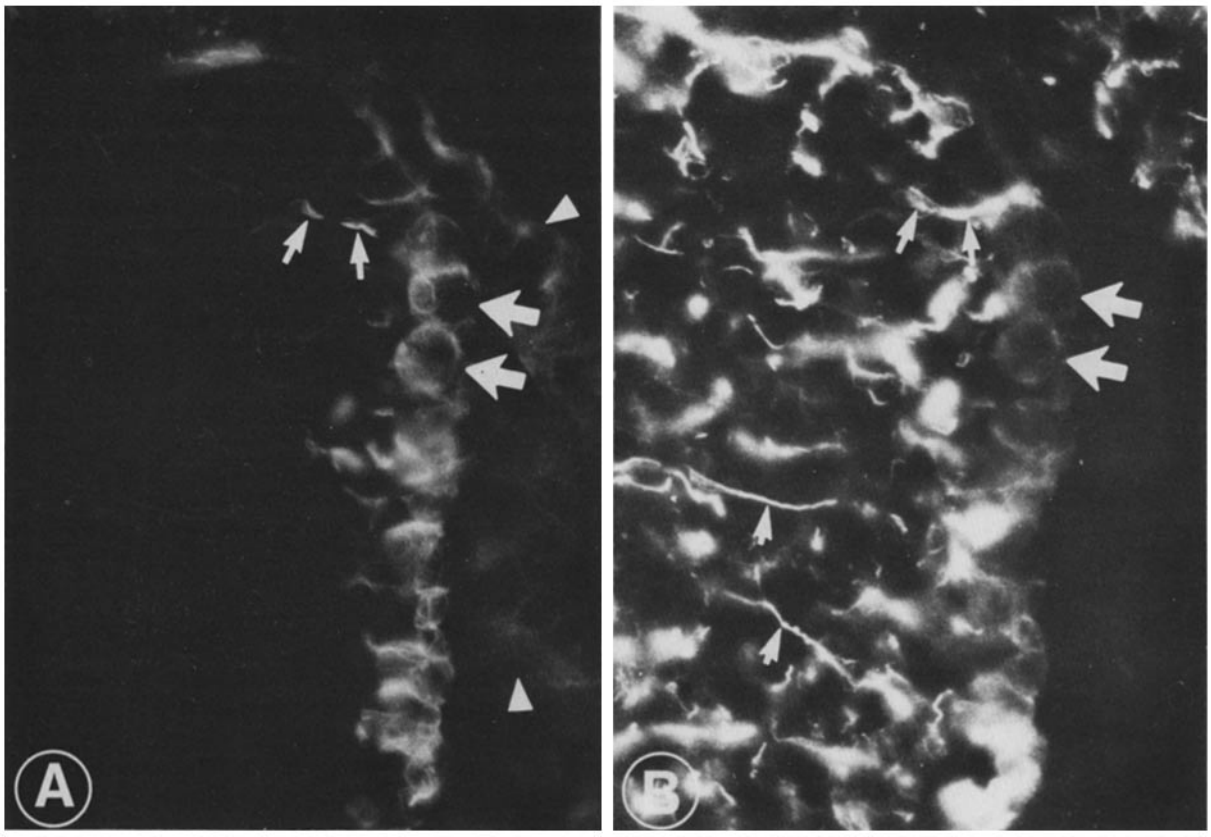

FIGURE 4 Immunohistological staining of C1 antigen and GFA protein of astroglial and ependymal cells in adult C57BL/6J mouse brain. (*A*) C1 antigen is detectable in ependymal cells (large arrows) and an occasional GFA protein-positive process (small arrows). A weak diffuse staining of choroid plexus (arrowheads) is visible. (*B*) Some ependymal cells are weakly GFA protein-positive (large arrows). Astrocytes are strongly GFA protein-positive (small arrows). × 560.

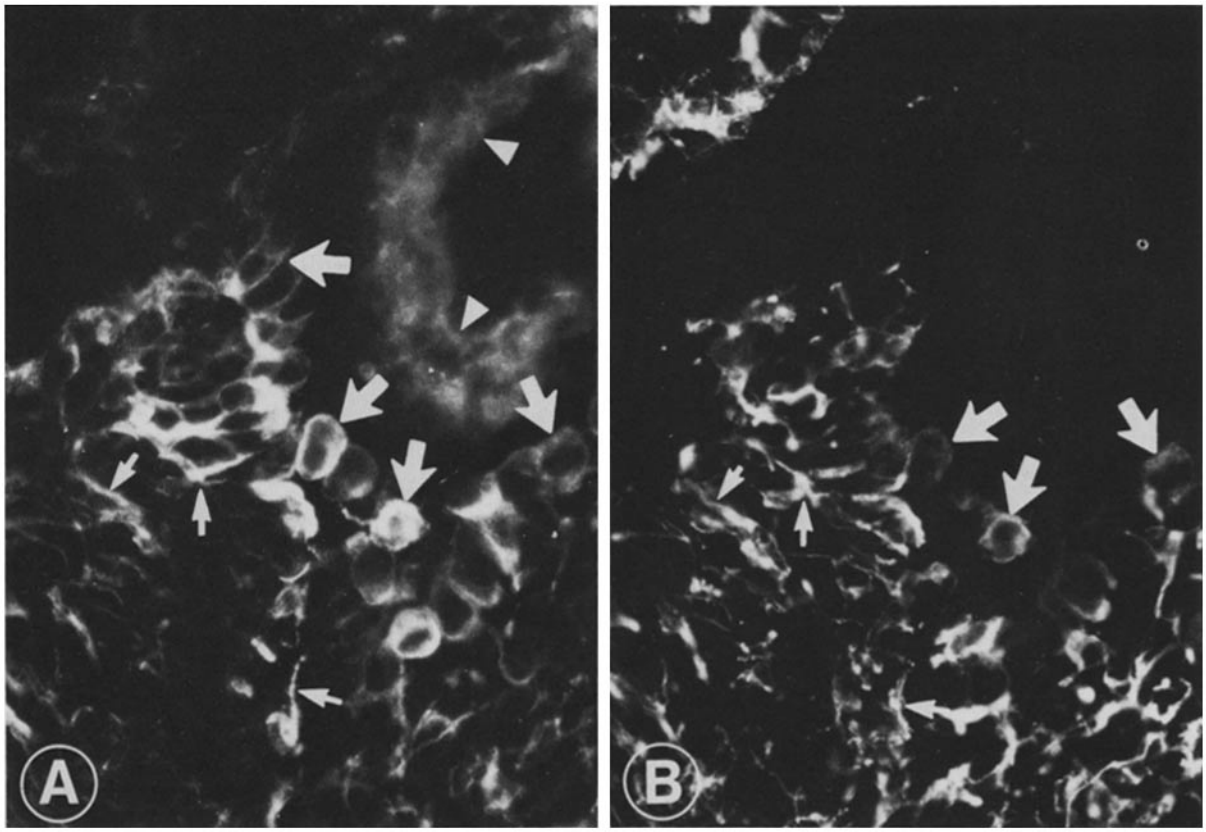

FIGURE 5 Immunohistological staining of C1 antigen and GFA protein in 6-d-old C57BL/6J mouse brain. (*A*) C1 antigen is present not only in ependymal cells (large arrows) but also in some GFA protein-positive processes (small arrows). A weak, diffuse staining of choroid plexus (arrowheads) is visible. (*B*) Some ependymal cells are GFA protein-positive (large arrows). Astrocytes are strongly fluorescent (small arrows). Choroid plexus is not stained. × 560.

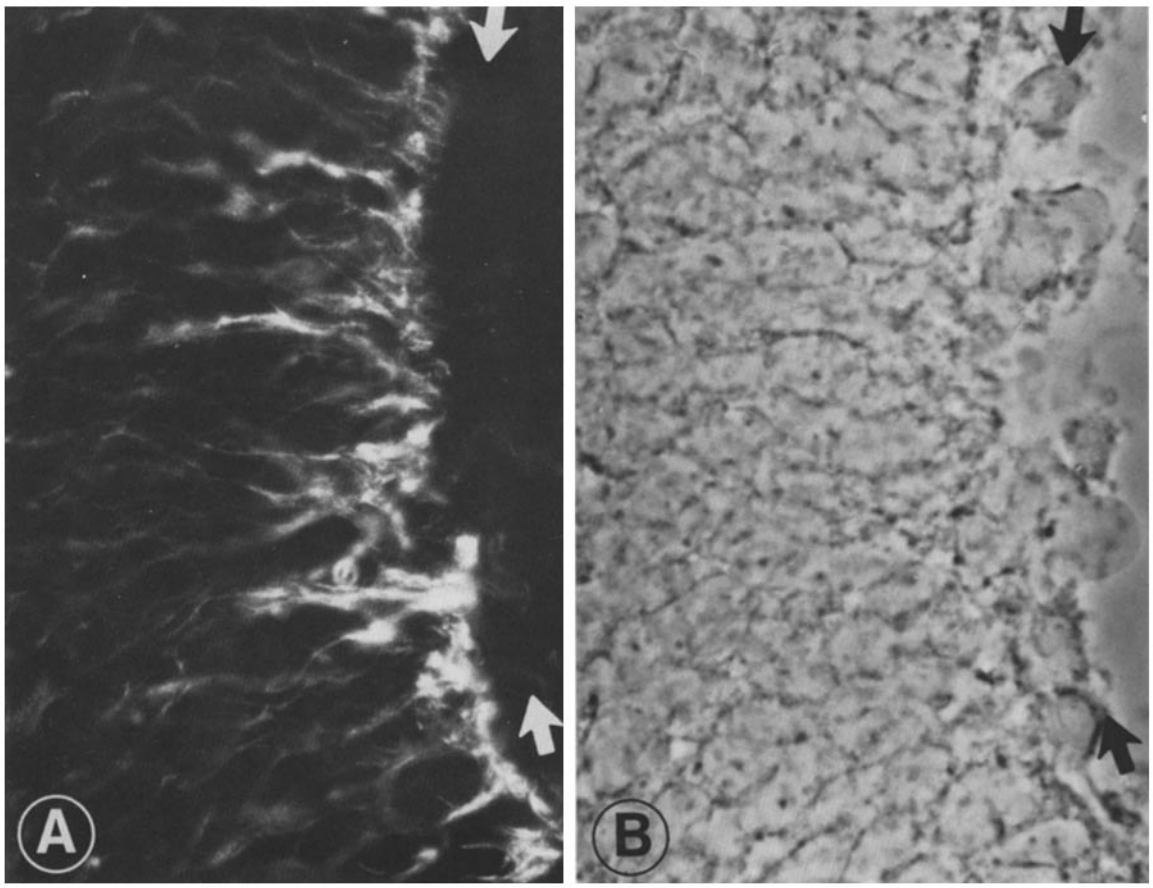

FIGURE 6 Immunohistological staining of C1 antigen in the anlage of neural retina of embryonic day 13 C57BL/6J mice. (*A*) C1 antigen is expressed in radially oriented fibers extending from the ganglion cell layer (large arrows) to the outer surface (not shown in picture) of the developing retinal neuroectoderm. Ganglion cell layer does not contain C1 antigen-positive structures. (*B*) Phase-contrast micrographs of the same visual field as shown in *A*. Arrows point to the ganglion cell layer. × 560.

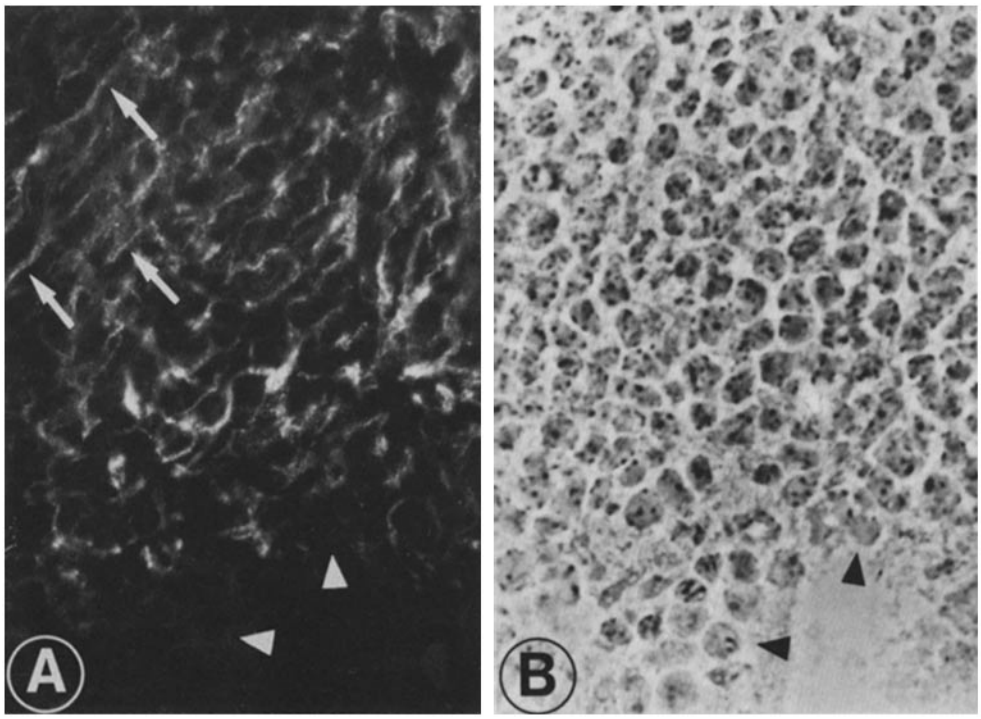

FIGURE 7 Immunohistological staining of C1 antigen in the telencephalic anlage of embryonic day 10 C57BL/6J mice. (*A*) C1 antigen is detectable in structures that show radial orientation in the upper left corner of the picture (arrows). Cell bodies lining the ventricular lumen are not stained (arrowheads). (*B*) Phase-contrast micrograph of the same visual field as shown in *A*. Arrows point to lumen-proximal cells. × 560.

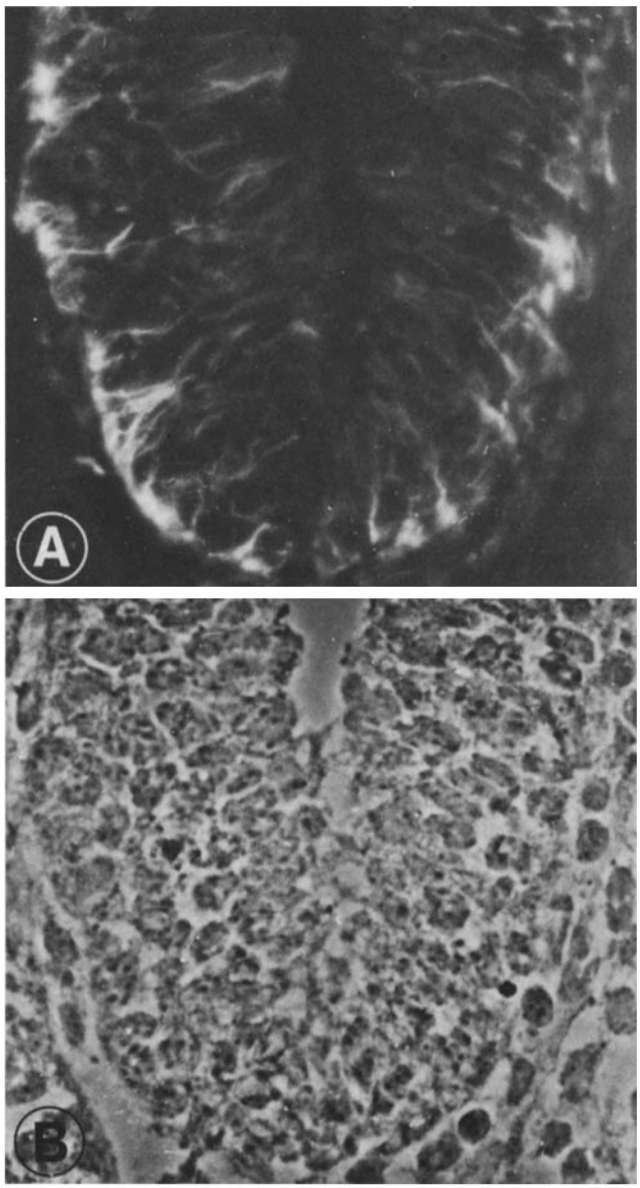

FIGURE 8 Immunohistological staining of C1 antigen in the pituitary anlage of late embryonal day 11 C57BL/6J mice. (*A*) C1 antigen is detectable in radial structures that are more strongly stained towards the periphery. (*B*) Phase-contrast micrograph of the same visual field as shown in *A*. × 600.

antigen-positive cells among all GFA protein-positive cells under Colcemid does not seem to differ greatly from that in the Colcemid-free control cultures.

DISCUSSION

Characterization of C1 Antigen-positive Cell Type

C1 antigen displays a remarkably selective expression in only a few cell types of the adult mouse central nervous system: ependymal cells and the radial glial cells in cerebellum (Bergmann glial cells, also called Golgi epithelial cells) and retina (Müller cells). Radially oriented processes of astrocytes in the dentate gyrus of the hippocampus, which are easily recognized by antiserum to GFA protein, do not express C1 antigen when tested by indirect immunohistological methods. Similarly, all other astrocytes that can be identified by their expression of GFA protein are negative for C1 in all white- and grey-matter-rich brain regions that have been investigated so far. Only a fraction of these C1-negative astrocytes express M1 antigen, a recently described marker for another subpopulation of astrocytes (16). At adult stages, M1 and C1 antigens have never been found expressed in the same cell type, as illustrated particularly in the retina, where Müller cells are negative for M1 but positive for C1 antigen, whereas astrocytes in the ganglion cell layer are C1 negative and M1 positive. Similarly, radial astrocytic processes in the area dentata of the hippocampus are C1 negative but M1 positive. However, a strict complementary expression of these two antigens has not been observed, because some types of astroglia, e.g., in the adult mouse cerebellar granular layer, are neither C1 nor M1 positive, although they express GFA protein.

A feature shared by Bergmann glial cells and Müller cells, which distinguishes them from the radially oriented glial cells of the area dentata, may be their developmental origin. Bergmann glial cells and Müller cells are direct descendants of ventricular cells, the processes of which span the neural tube from the ventricular lumen to the tube surface. Whereas Müller cells remain attached to the outer and inner surfaces of the neuroepithelium at adult stages, Bergmann glial cells lose their connection to the inner surface to retain solely their apposition to the pial surface. In contrast to the Bergmann glia and Müller cell processes, the radial fibers of astrocytes in the dentata gyrus arise after the disappearance of the primitive radial glial processes (A. Privat, personal communication). The loss of the primitive radial processes and subsequent elaboration of secondary processes has been described as an essential program in the formation of astrocytes in the occipital lobe of the monkey (38), and may be a common principle to the development of many fibrous and protoplasmic astrocytes. Thus, C1 antigen seems to reside primarily in processes descending directly from the primitive radial glia, whereas M1 is restricted to some but not all secondarily elaborated astrocytic processes.

Expression of C1 antigen in ependymal cells would indicate that these cells share common properties with astrocytes, as suggested by several authors using ultrastructural criteria (for review, see references 21 and 22). That ependymal cells retain some properties of primitive glial cells is evidenced by the existence of tanycytes of the third ventricle in vertebrates. These cells may span the neuroepithelium from ventricular to pial surface even at adult stages and are therefore presumed to unite glial and ependymal properties in one cell type (22). Glial cells of adult lower vertebrates are morphologically similar to primitive ependyma of higher vertebrates and seem to exert even similar functions. The so-called ependymal glial cells are able to re-form intercellular channels in the neighborhood of a lesion, as was shown in the spinal cord of the urodele (11, 20). These channels are reminiscent of those described for ependymal cells at embryonic stages of lower vertebrates as well as for primitive glial cells of higher vertebrates (44–46), and are presumed to function as mechanical and contact guideposts for the outgrowing axonal processes. In the mouse, these channels seem to originate as spaces between radially oriented primitive glial cells (46). The capacity to re-form these channels in ependymal cells during the regenerative phase appears to be lost or suppressed in mammals. It seems, however, that mature ependymal cells in the mouse retain at least one property that is reminiscent of a more primitive potential of this cell type, even at adult stages: the expression of C1 antigen.

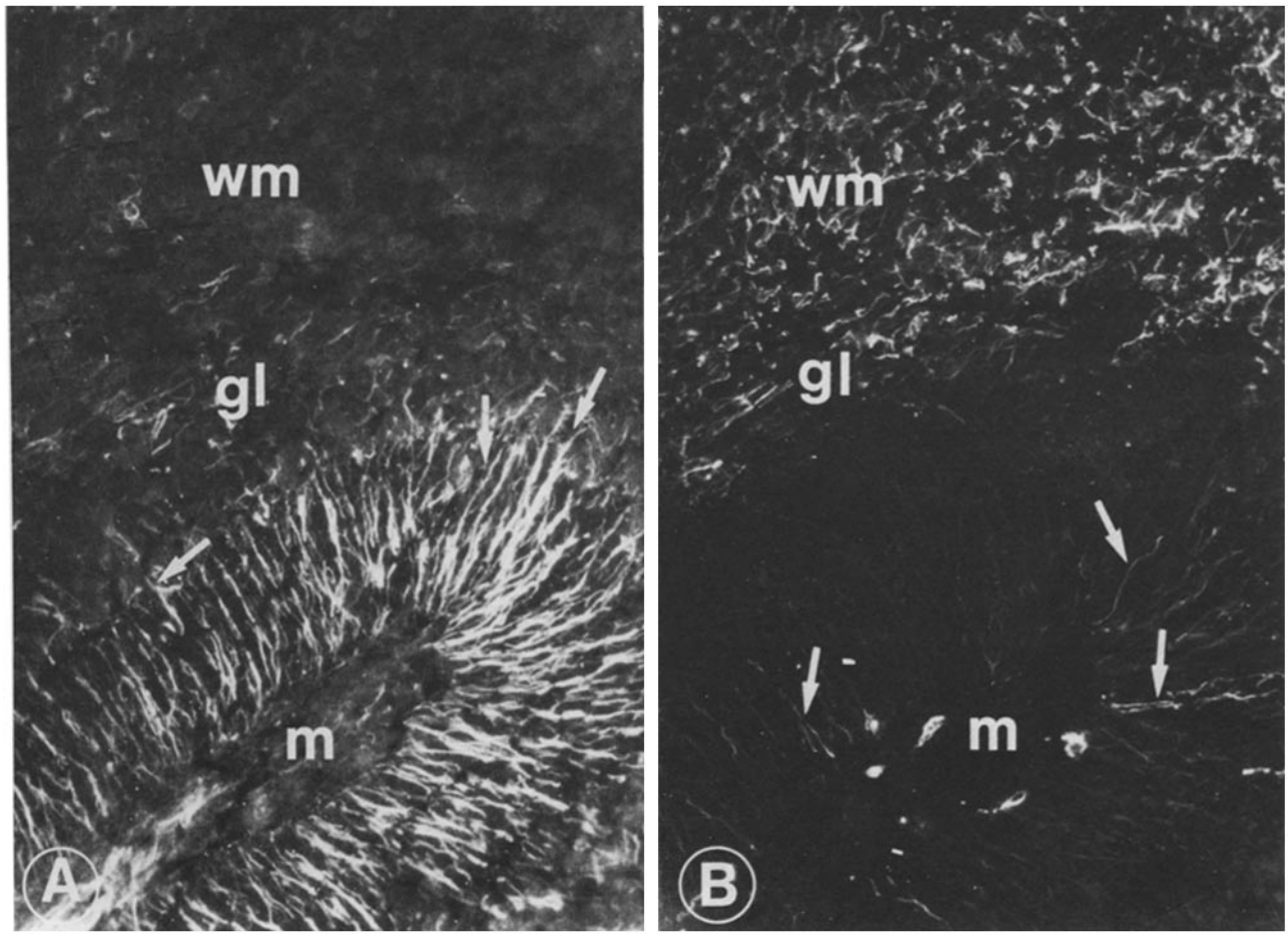

FIGURE 9 Immunohistological staining of the cerebellar cortex from 6-d-old C57BL/6J mice. (*A*) C1 antigen is detectable most prominently in radial Bergmann fibers (arrows). Astrocytes in the developing internal granular layer (*gl*) and presumptive white matter (*wm*) are less heavily stained. *m*, Meninges. (*B*) GFA protein-positive radial Bergmann fibers are weakly visible (arrows), whereas astrocytes in internal granular layer (*gl*) and presumptive white matter (*wm*) are distinctly labeled. *m*, Meninges. At higher magnifications coincidence of C1 antigen and GFA protein labeling of cellular structures is easily evident. × 220.

TABLE I

Developmental Expression of C1 Antigen in Selected Areas of the Central Nervous System *

	Embryonic age‡		
	E10	E11	E13
Radial fibers in anlage of			
Retina	nd (nd)	nd (nd)	+ (−)
Telencephalon	+ (−)	+ (−)	+ (−)
Ventricular cells	− (−)	− (−)	− (−)

	Postnatal age‡			
	P5	P7	P10	Adult
Radial fibers in retina				
Ependyma	nd (nd)	nd (nd)	nd (nd)	+ (+)
Cerebellum	+ (+)	+ (+)	+ (+)	+ (±)
(Prospective) white matter	+ (+)	+ (+)	− (+)	− (+)
Internal granular layer	+ (±)	+ (+)	− (+)	− (+)
Bergmann glia	+ (±)	+ (+)	+ (+)	+ (+)

* Signs in parentheses indicate expression of GFA protein.
‡ *E*, embryonic day; *P*, postnatal day; *nd*, not done.

The observation of C1 expression in larger blood vessels and fibroblasts or fibroblastlike cells warrants further investigations of nonneuroectodermally derived tissues. The absence of C1 antigen in capillaries, its presence in larger blood vessels and the relationship between C1-positive mesenchymal and neuroectodermal cells remain at present unexplainable.

Developmental Expression of C1 Antigen

The ontogenetic interrelationship between C1 antigen–positive cells (ependymal and radial glial cells) is emphasized by the observation that C1 antigen is found in radially oriented processes in the neural tube as early as embryonic day 10 in the mouse. Radially oriented fibers were also found to be C1 antigen–positive at embryonic day 11 in pituitary, mesencephalic, and telencephalic anlage and at embryonic day 13 in the anlage of the retina. These radial structures also express vimentin, a newly discovered marker for astroglial and ependymal cells in the adult central nervous system (see companion paper, reference 39).

It is, however, difficult to determine unequivocally at this stage, by light microscopic criteria alone, whether the radially oriented C1-positive processes belong to primitive glial cells, because established independent markers for these cells in the mouse are yet to be described. In contrast, embryonic radial

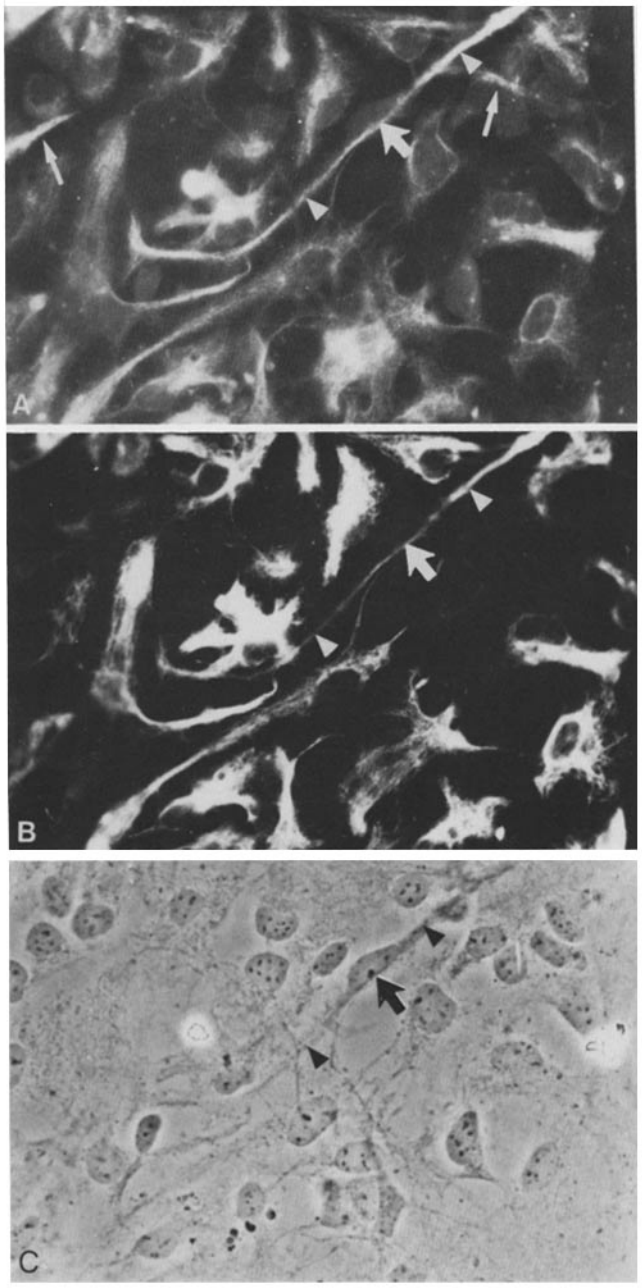

FIGURE 10 Immunocytological staining of 5-d-old explant culture from embryonic day 13 C57BL/6J mouse cerebellum. Cells were fixed by ethanol treatment (96%, 0.5 min, −20°C) (*A*) Culture stained with anti-C1 antibody. Most C1-positive cells are also GFA protein-positive (see *B*). Exceptional C1-positive, GFA protein-negative cells are indicated by small arrows. (*B*) Same culture stained with anti-GFA protein antibody. (*C*) Phase-contrast micrograph of the same visual field as images *A* and *B*. Small cell with oval-shaped cell body (large arrows), possibly a granule cell, attached to an astroglial process (arrowheads) is C1 antigen- and GFA protein-negative. × 400.

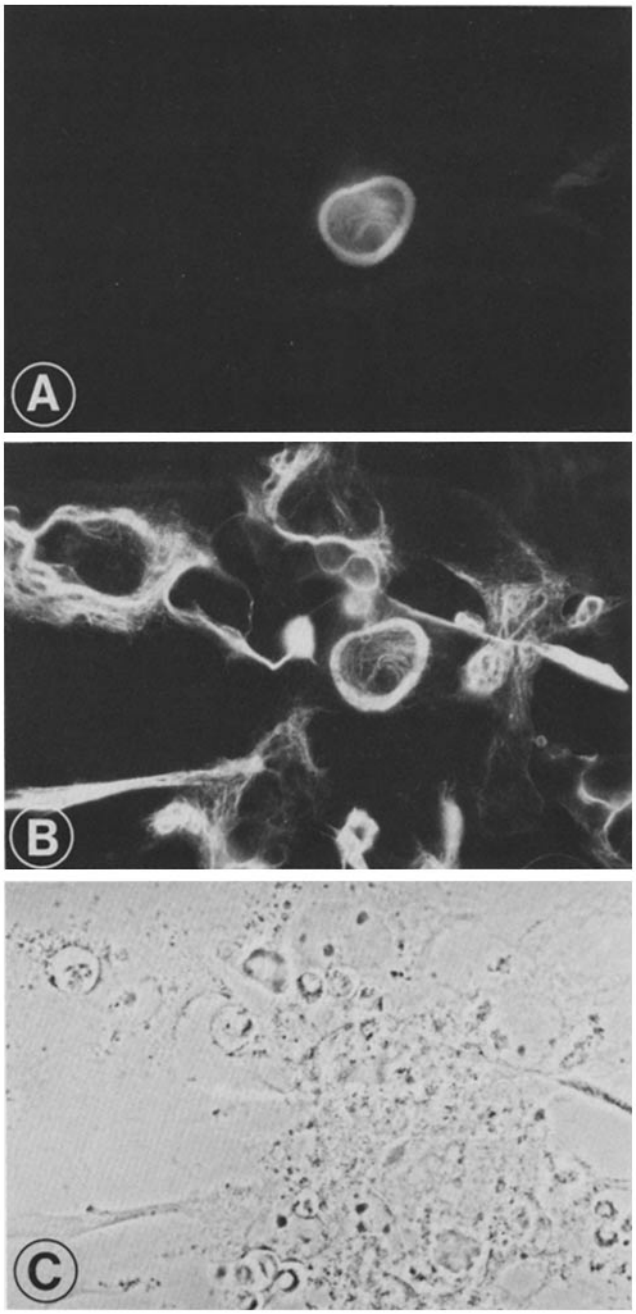

FIGURE 11 Immunocytological staining of a 7-d-old cerebellar culture from 7-d-old C57BL/6J mice, treated with Colcemid. Cultures were treated with 10^{-6} M Colcemid for 12 h and fixed immediately afterwards with ethanol as described in legend to Fig. 10. (*A*) C1 antigen is detectable in a cell with a perinuclear ring (arrow) coinciding with the GFA protein label (*B*). Other cells are weakly positive for C1 antigen or negative. (*B*) GFA protein is detectable in perinuclear rings in most cells remaining in culture after Colcemid treatment. (*C*) Phase-contrast micrograph of the same visual field as images *A* and *B*. GFA protein-positive astrocytes display an epithelioid morphology. Cytoplasmic vacuoles emphasize the unhealthy appearance of these cells. Tetanus toxin-positive cells with small cell bodies (mostly granule cells) have disappeared almost totally after Colcemid treatment. × 450.

fibers in primates have been identified as glial processes by immunocytological methods, using GFA protein as a marker (1, 9), and by ultrastructural criteria alone (24, 25). However, the stages of development in primates and rodents are not directly comparable.

The wide distribution of C1 antigen at fetal ages may indicate that, during development, C1 antigen is not restricted to a limited set of astroglial cells as in the adult but is present in most if not all astroglial cell precursors. Indeed, in the cerebellum of early postnatal mice, C1 antigen is found not only in Bergmann fibers but also in astrocytes of the prospective white matter and developing internal granular layer. Expression of

C1 in white matter and internal granular layer astrocytes, however, subsides during the second postnatal week. Whether cells precursor to other neural cell types, for instance the glial subclass of oligodendrocytes, express C1 antigen at early embryonic stages is unknown. Cells lining the ventricle, the ventricular cells (6), do not express C1 antigen at embryonic ages. It seems, therefore, that the differentiation of ventricular into ependymal cells in mammals (19, 32, 53, 57) is paralleled by the appearance of C1 antigen in these cells, at least in the mouse. This differentiation results in the loss of the ventricular cells' ability to generate subventricular cells, which give rise to both glioblasts and neuroblasts, and signifies a restriction in developmental potential. It is tempting to speculate, however, that with the restriction of developmental choices, ependymal cells acquire features of primitive astrocytes as indicated by their expression of C1 antigen. Recent data on transient expression of GFA protein in ependyma of the human embryo (30) support the view that ependymal cells share some properties with astrocytes.

C1 Antigen Expression in Neurological Mutants

C1 antigen is not only developmentally regulated but is also affected by the pleiotropic effects of gene mutations acting on the cerebellum. The three mutants investigated in this study, weaver (gene symbol *wv*), staggerer (*sg*), and reeler (*rl*), show a defective development of the cerebellar cortex (42). For each individual mutant, this results in a characteristic pattern of abnormal cytoarchitecture resulting from death of specific cell classes, defective cell migration, and/or segregation into cellular lamina. (For review, see reference 8.) None of these mutants shows immunohistologically detectable levels of C1 expression in the Bergmann glial fibers at the ages studied so far, whereas C1 is present in ependymal cells in amounts similar to those found in phenotypically normal control littermates. This observation may point to a disturbance of Bergmann glial cells themselves or their immediate cellular environment, leaving ependymal cells of the fourth and other ventricles unaffected.

The disturbance of Bergmann glial cells and other cell types in these mutants is not readily reconcilable on a common denominator of cellular abnormalities. Homozygous weaver animals show postnatal abnormalities of Bergmann glial cells and a reduced number of granule cells (26–29, 49, 51).

A different type of cellular abnormality is seen in the reeler mutant, which is defective in laminar cytoarchitecture in both cerebellar and cerebral cortices (for reviews, see references 8 and 41). Yet another type of abnormality is seen in the staggerer mutation, which carries abnormal Purkinje cells and also loses most of its granule cells (17, 42, 50, 52).

The mutant abnormalities are so diversified that it would appear difficult to reduce the causal relationships of the three mutations to an intrinsic abnormality of Bergmann glial cells as suggested by the absence of C1 antigen expression. It is conceivable, however, that Bergmann glial cells lose their "primitive" features under the influence of an abnormal environment and therefore become C1 antigen–negative. We would like to emphasize here that the term "primitive" is not used with the meaning of undifferentiated or immature, an expression that has been used to describe the abnormality in Bergmann glial cells in the adult weaver mouse (5, 51). Rather, we would like to point out that Bergmann glial cells might have lost their original features that characterize them as the direct descendants of the embryonic glial cells, the "primitive" radial glial cells.

Concluding Remarks

To test the hypothesis that suppression of C1 antigen in Bergmann glial cells is caused by an abnormal cellular environment, it seems pertinent to investigate C1 expression of radial glial cells in brain areas that are not noticeably affected by these mutations, as in, for example, the retinal Müller cells. It will also be of interest to study the regulation of C1 expression in these mutants during development, possibly even at embryonic ages, with the hope of gaining better insight into the primary targets of the mutation. Furthermore, it will be interesting to determine whether C1 expression can be suppressed in ependymal cells by traumatic influences such as injury, which seems to induce expression of GFA protein in these cells (10, 12).

Experiments are under way to characterize the distribution of C1 antigen in nonneural tissue and to determine its subcellular localization and molecular nature. Evidence from immunocytological studies on cultured monolayer cells of early postnatal mouse cerebellum indicates that C1 antigen is localized intracellularly in fibrillike structures that are localized in the cytoplasm in a pattern similar to the distribution of GFA protein. A redistribution of GFA protein–positive structures by Colcemid was found to parallel the rearrangement of C1 antigen–positive material, which could suggest a predominantly fibrillar arrangement of this antigen. The molecular identification of C1 should enable us to determine whether this antigen is in any way related to GFA protein or to vimentin, or whether C1 represents another antigen in addition to vimentin, with a remarkably early expression during neural development.

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