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Selective Staining of a Subset of Purkinje Cells in the Human Cerebellum with Monoclonal Antibody mabQ113

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SUMMARY

MabQ113 is a monoclonal antibody raised against rat cerebellum which selectively stains Purkinje cells. Likewise, in mabQ113-immunoperoxidase stained sections of human cerebellum, deposits of reaction product are found only in the Purkinje cells. The dendritic arborizations, cell body, and axonal processes are immunoreactive. In rat, mabQ113 reveals a series of parasagittal antigenic bands which run throughout the cerebellar cortex. The staining distribution in human cerebellar cortex likewise reveals heterogenous staining but the pattern is a complex one and seems to be unlike the parasagittal banding found in the rat. In a number of human diseases Purkinje cell degeneration is not uniform throughout the vermis and cerebellar hemispheres. It is possible that mabQ113⁺ and mabQ113⁻ subsets of Purkinje cells may respond differentially to various pathological conditions.

Key words: *Cerebellum – Monoclonal antibodies – Purkinje cells*

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INTRODUCTION

The sole efferent output from the mammalian cerebellar cortex are the Purkinje cell axons which extend to the deep cerebellar nuclei and to the lateral vestibular nucleus. Despite their morphological homogeneity there is much recent evidence that the Purkinje cells are divisible biochemically into several classes. For example, the studies of Chan-Palay and her colleagues in rat cerebellum (Chan-Palay et al. 1981) have shown sub-sets of Purkinje cells which are immunoreactive for glutamic acid decarboxylase and others, partly overlapping, which are immunoreactive for the peptide motilin. The most striking feature of this selective immunoreactivity is that the antigen-positive cells appear to be arranged into a series of parallel bands which extend sagittally throughout the cortex. Sagittal zonation of the mammalian cerebellar cortex has been known for many years (Klimoff 1897; Clarke and Horsley 1905) and has been shown to encompass the topographic arrangement of afferent inputs, both climbing fibers (via the inferior olive) and mossy fibers (via the lateral reticular nucleus) and the efferent Purkinje cell axon projections to the deep cerebellar nuclei (reviewed in Bloedel and Courville 1981). There are also various biochemical corollaries such as the histochemical distributions of acetylcholinesterase (Marani and Voogd 1977) and 5'-nucleotidase (Scott 1963a,b) as well as the mabB_{4,3} immunoreactivity (Marani et al. 1983). During cerebellar development, there is transient heterogeneity of guanosine 3',5'-phosphate-dependent protein kinase immunoreactivity in clusters of Purkinje cells (Wassef and Sotelo 1984). Perhaps the most dramatic demonstration of parasagittal zonation in the cerebellar cortex of rat is the pattern of immunoreactivity revealed by monoclonal antibody mabQ113. MabQ113 recognizes a 120 Kdalton polypeptide which, in the cerebellum, appears to be confined exclusively to the Purkinje cells. Not all Purkinje cells are mabQ113-immunoreactive. Immunoreactive cells are organized into prominent parasagittal bands which are disposed symmetrically about the midline and are separated by similar bands of non-immunoreactive cells. Therefore mabQ113 can be used to expose the underlying sagittal organization of the normal and pathological cerebellar cortex. Similar Purkinje cell heterogeneity has not been reported in human material although, by analogy with other mammals (e.g. Eager 1966; Voogd 1969; Haines 1976), it is highly probable that it is present. This is intriguing because it is possible that mabQ113⁺ and mabQ113⁻ subsets of Purkinje cells may respond differently to pathological conditions, such as toxin induced degenerations, or hereditary atrophies (Kark et al. 1978). We have therefore used mabQ113 to study the human cerebellar cortex in order to determine whether the selective staining of Purkinje cells in rat cerebellum extends to human tissue and to try to reveal a pattern of heterogeneous Purkinje cell immunoreactivity which might reflect the global organization of the human cerebellar cortex.

MATERIAL AND METHODS

Monoclonal antibodies

A panel of 6-week-old Balb/c female mice were immunized intraperitoneally with synaptosomal plasma membrane (SPM) isolated from 15-day-old (P15) Lewis rats

(Jones and Matus 1974). The first immunization was with 3 mg SPM in Freund's complete adjuvant; the second, 3 weeks later, was 3 mg SPM in Freund's incomplete adjuvant. Four days after the second injection, the mice were bled from the tail vein and the sera tested for anti-SPM antibodies with a dot-immunobinding assay (Hawkes et al. 1982). The two animals which gave the highest serum titers (1/16 000) were boosted 4 weeks later with 3 mg SPM in saline. Hybridomas were constructed 3 days later by the procedure of Galfrè et al. (1977). The fusion products were distributed into 480 × 1 ml culture wells in Iscove's medium (Iscove and Melchers 1978) supplemented with 15% fetal calf serum and the components of the HAT selection system (Littlefield 1964). Hybridomas grew in 89 wells. Culture media were tested for anti-cerebellar antibodies by immunoperoxidase staining of neonatal rat cerebellum. Interesting cell lines were subcloned by limiting dilution at 0.1–0.2 cells/well, expanded and frozen in liquid nitrogen. One of the antibodies obtained from this fusion, mabQ113, was used for this study.

Immunocytochemistry

Specimens of cerebellum were obtained at the time of autopsy from 6 individuals. Permission for the autopsy had been given by the closest relatives. There were 2 females aged 54 and 78 and 4 males aged 53, 53, 78, and 81 years. The premorbid diagnoses which necessitated custodial hospitalization included mental retardation in two individuals, schizophrenia in two, alcohol related psychosis in one, and senile dementia in one. Other premorbid neurological diagnoses included oculopharyngeal muscular dystrophy in one female, and microcephaly in one of the mentally retarded males. There were no premorbid signs or symptoms of cerebellar dysfunction in any of these individuals, and all were ambulatory prior to death. The cause of death was bronchopneumonia in three cases, bronchopneumonia secondary to acute food aspiration in two, and acute pulmonary edema in one. Autopsy was performed within 24 h of the time of death. Routine neuropathological investigation, both grossly and microscopically, did not reveal any other findings. In particular, the cerebellum was determined to be normal in all cases.

At the time of autopsy, sections of cerebellum and vermis were placed in ice-cold 4% paraformaldehyde 0.2% glutaraldehyde in phosphate buffer (0.1 M phosphate buffer, pH 7.4) for a period of 24 h. Tissue was stored subsequently in phosphate buffer containing 10^{-5} M sodium azide. Sections were cut horizontally or coronally at 75 μ m using a freezing microtome.

Adult Sprague–Dawley rats were deeply anesthetized with sodium pentobarbital. After surgical exposure of the heart, 75 units of heparin and 5 mg sodium nitrite were injected into the heart, and 1 min later the animal was perfused via the left ventricle with 200 ml of PBS (0.1 M phosphate buffer, pH 7.4, 0.15 M NaCl), followed by fixation with 200 ml of ice-cold 4% paraformaldehyde 0.2% glutaraldehyde in phosphate buffer. The brain was postfixed overnight in 4% paraformaldehyde alone. Sections were cut sagittally, horizontally or coronally at 50 μ m using a freezing microtome.

To detect specific immunoreactivity, sections were incubated overnight in the monoclonal antibody. In all examples of human material shown here, mabQ113 was used diluted 1/16 into 10% normal horse serum in PBS. The concentration of mabQ113

used for the rat sections was 1/32. To detect specific antibody binding, sections were incubated for 2 h in rabbit anti-mouse immunoglobulin conjugated to horseradish peroxidase diluted 1/100 in 10% normal horse serum (Dako Inc.). Antibody binding was revealed using 4-chloro-1-naphthol as substrate. The sections were washed for 10 min in each of three changes of PBS between the incubations. Sections in which the primary antibody was omitted gave no staining.

RESULTS

The distribution of mabQ113-immunoreactivity in rat cerebellar cortex is illustrated in Fig. 1. Bands of immunoreactive Purkinje cells extend parasagittally throughout the length of the cerebellum interspersed by similar antigen-negative bands. Within the individual Purkinje cells, deposits of reaction product are found throughout the cytoplasm including the cell body, the dendritic arborization and dendritic spines, the axons and the axon collaterals. No other cell types in the cerebellum are immunoreactive (Hawkes et al. 1985). Immunoreactive Purkinje cell axon terminals are found throughout the deep cerebellar nuclei but the nuclear neurons are themselves unstained.

With this pattern of staining in mind, we have examined the immunoreactivity of the human cerebellar cortex obtained at autopsy. Six separate cerebelli were examined. In all 6 cases mabQ113 staining of Purkinje cells were observed (Fig. 2A). In the better preserved material fine dendritic arborizations and axon collaterals of the Purkinje cells could be seen exactly as found in the rat (Figs. 2B and C). Purkinje cell axons were labeled both proximally, within the cerebellar white matter tracts (Fig. 2D), and distally

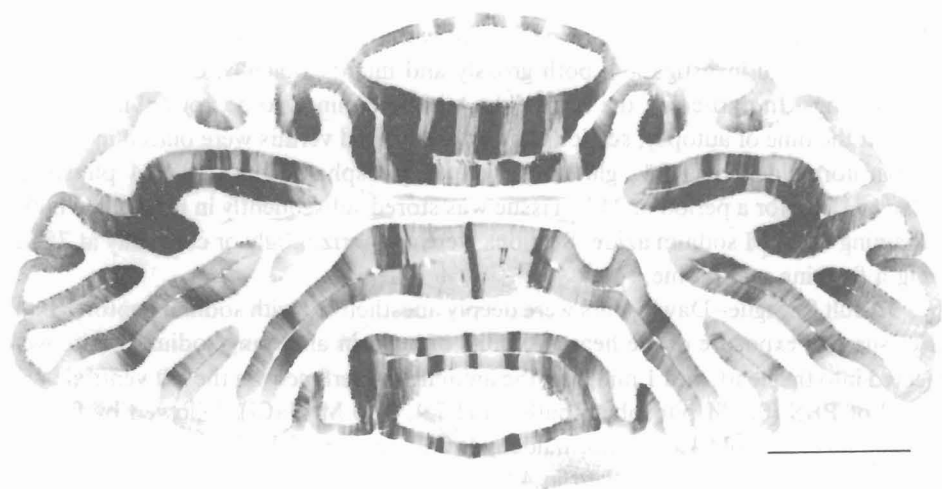


Fig. 1. Horizontal section through the adult rat cerebellum immunoperoxidase-stained with mabQ113. Subsets of Purkinje cells are stained by the antibody to reveal a parasagittal array of bands which occupy both the vermis and the hemispheres. Antigen-positive bands are interposed by bands of antigen-negative cells. The bar indicates 2 mm.

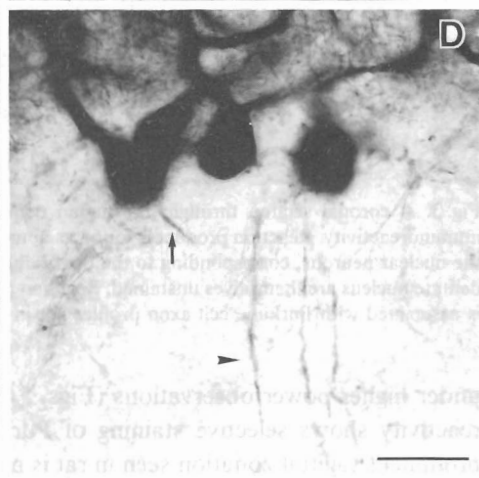
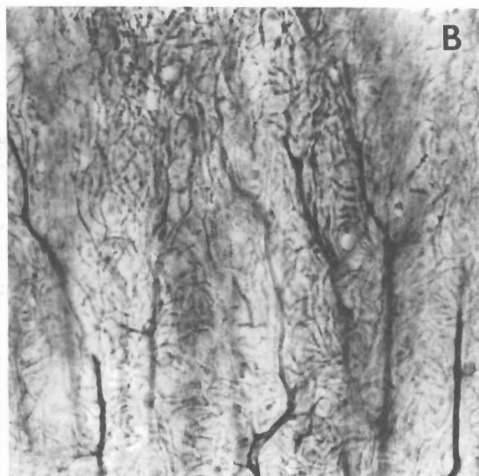
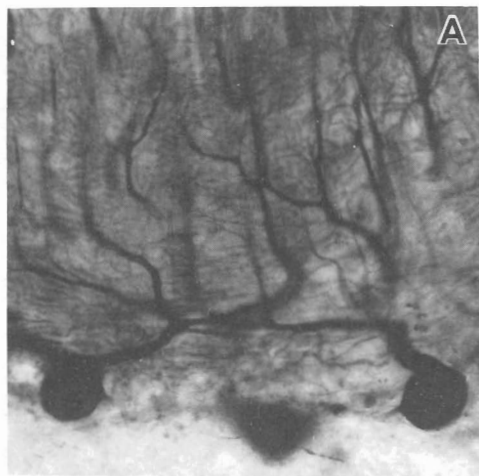


Fig. 2. Coronal sections through the human cerebellum stained with mabQ113. *A*: Purkinje cells are selectively stained. *B*: Fine dendritic arborizations of Purkinje cells are visible. *C*: Purkinje cell axon collaterals are indicated by the arrows. *D*: The proximal portion of the Purkinje cell axon is stained (arrow). Other Purkinje cell axons are coursing through the white matter (arrowhead). The bar indicates 50 μ m.

in the dentate nucleus where they gave a dense plexus of stained fibers and terminal boutons surrounding individual cell bodies (Fig. 3). As in rat, in no case were any cells labeled within the cerebellum other than Purkinje cells. When mabQ113 was replaced with any one of 10 different monoclonal antibodies, Purkinje cell staining was not observed.

Under lower power microscope observation, irregularities in the staining pattern were apparent (Figs. A and B). This results from the selective staining of some Purkinje cells while others are either weakly reactive or do not react at all. In some cases, the stained and unstained dendritic arborizations are closely interdigitated (e.g. Fig. 4A). In other cases, blocks of strongly immunoreactive cells alternate with lightly immunoreactive regions (e.g. Fig. 4B). This pattern was observed in all 6 cases and confirmed

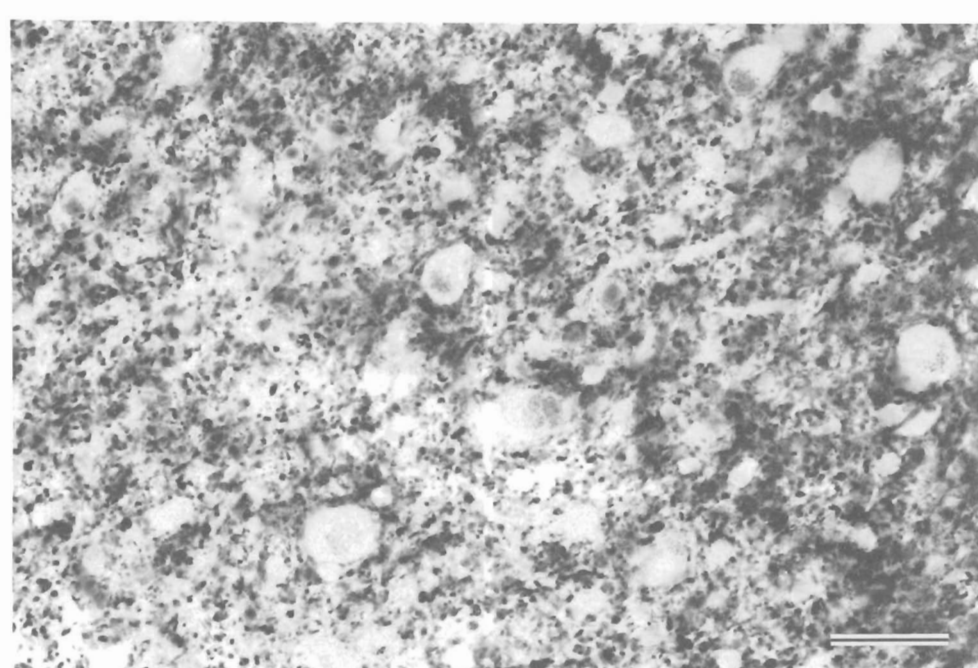
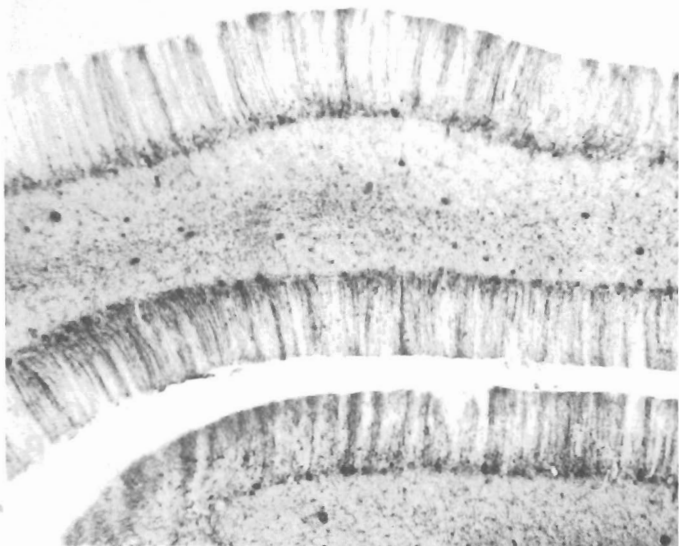
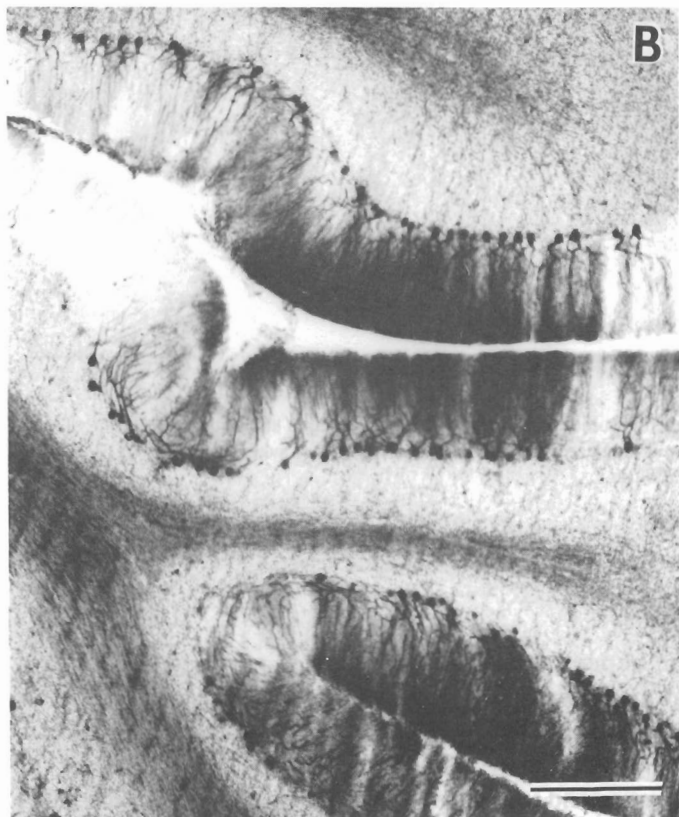


Fig. 3. A coronal section through the human dentate nucleus to show the distribution of mabQ113-immunoreactivity. Reaction product is found as abundant punctate deposits surrounding the cell bodies of the nuclear neurons, corresponding to the distribution of Purkinje cell axon boutons. The neurons of the dentate nucleus are themselves unstained. Reaction product in the interstices between the dentate neurons is associated with Purkinje cell axon profiles cut in cross-section. The bar indicates 50 μ m.

under higher power observations (Figs. 5A and B). Although the mabQ113 immunoreactivity shows selective staining of Purkinje cells in human cerebellar cortex, the prominent sagittal zonation seen in rat is not apparent. This is clearly illustrated in the human vermis (Fig. 6). In rat, there is an immunoreactive midline band with 3 lateral bands to either side (Fig. 1; Hawkes et al. 1985). Such a pattern is not apparent in human tissue. However there is some selective staining, and in particular some folia stain more intensely than others; in all cases the great majority of the Purkinje cells are mabQ113-positive (Fig. 6).

Fig. 4. Coronal sections through the human cerebellum stained with mabQ113 to demonstrate irregularities in the Purkinje cell staining pattern. Stretches of mabQ113⁺ cells are separated by weakly immunoreactive or negative cells. In some cases, we note that cell somata are stained in bands where dendritic staining is weak or absent. We do not feel that this represents differential staining within individual Purkinje cells but rather attribute it to the angle of sectioning such that the antigen-positive dendrites extend out of the plane of section and antigen-negative dendrites extend in. Staining of Purkinje cell axons is clearly seen in the white matter tract in B. The bar indicates 500 μ m.

A**B**

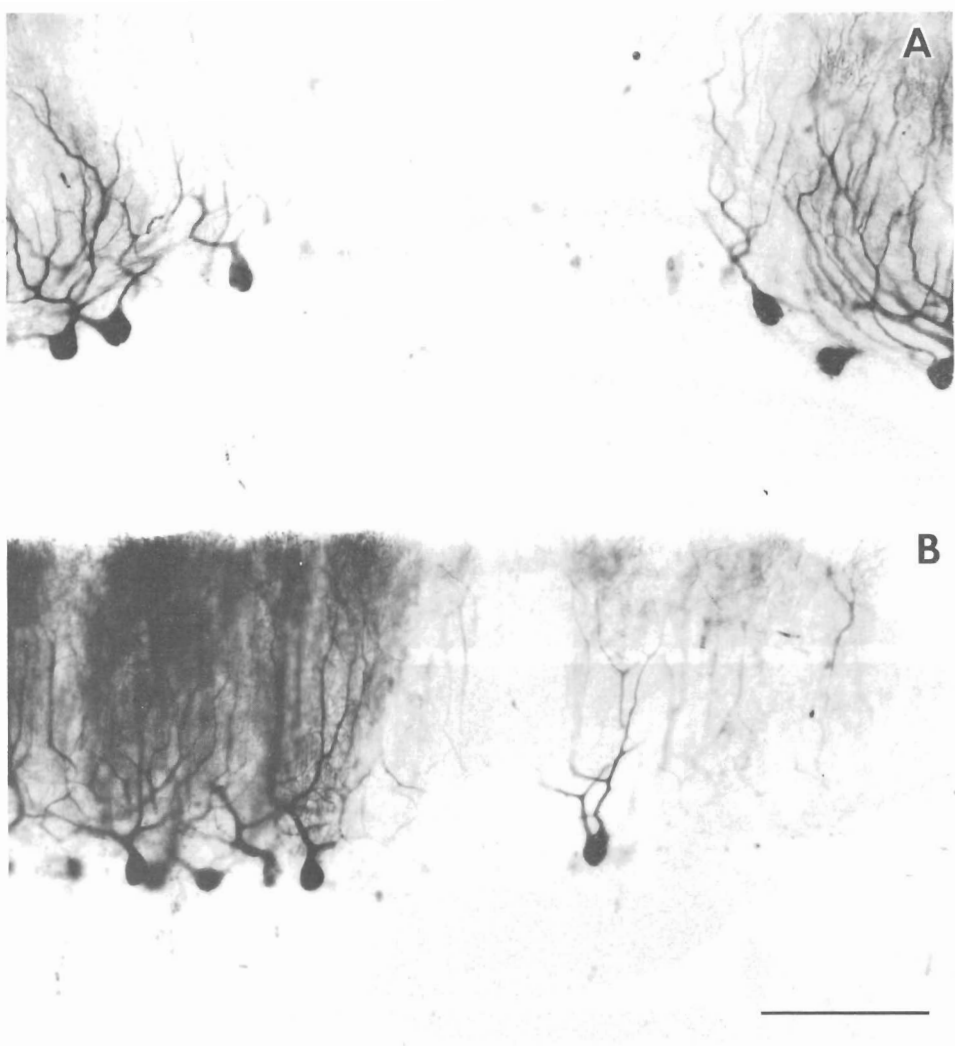


Fig. 5. Coronal sections through the human cerebellum showing selective staining of Purkinje cells with mabQ113. Both *A* and *B* show a single row of Purkinje cells some of which are mabQ113⁺, others mabQ113⁻. The bar indicates 200 μ m.

DISCUSSION

The dramatic pattern of parasagittal bands revealed in rat cerebellar cortex by mabQ113 emphasizes that the apparent uniformity of cerebellar architecture seen by conventional staining techniques is illusory. Numerous functional studies have likewise revealed an often complex cortical topography (e.g. Oscarsson 1979; Bloedel and Courville 1981). The organization and refinement of such complex cellular relationships could be a prime target for various cerebellar pathologies and Purkinje cell heterogeneity.

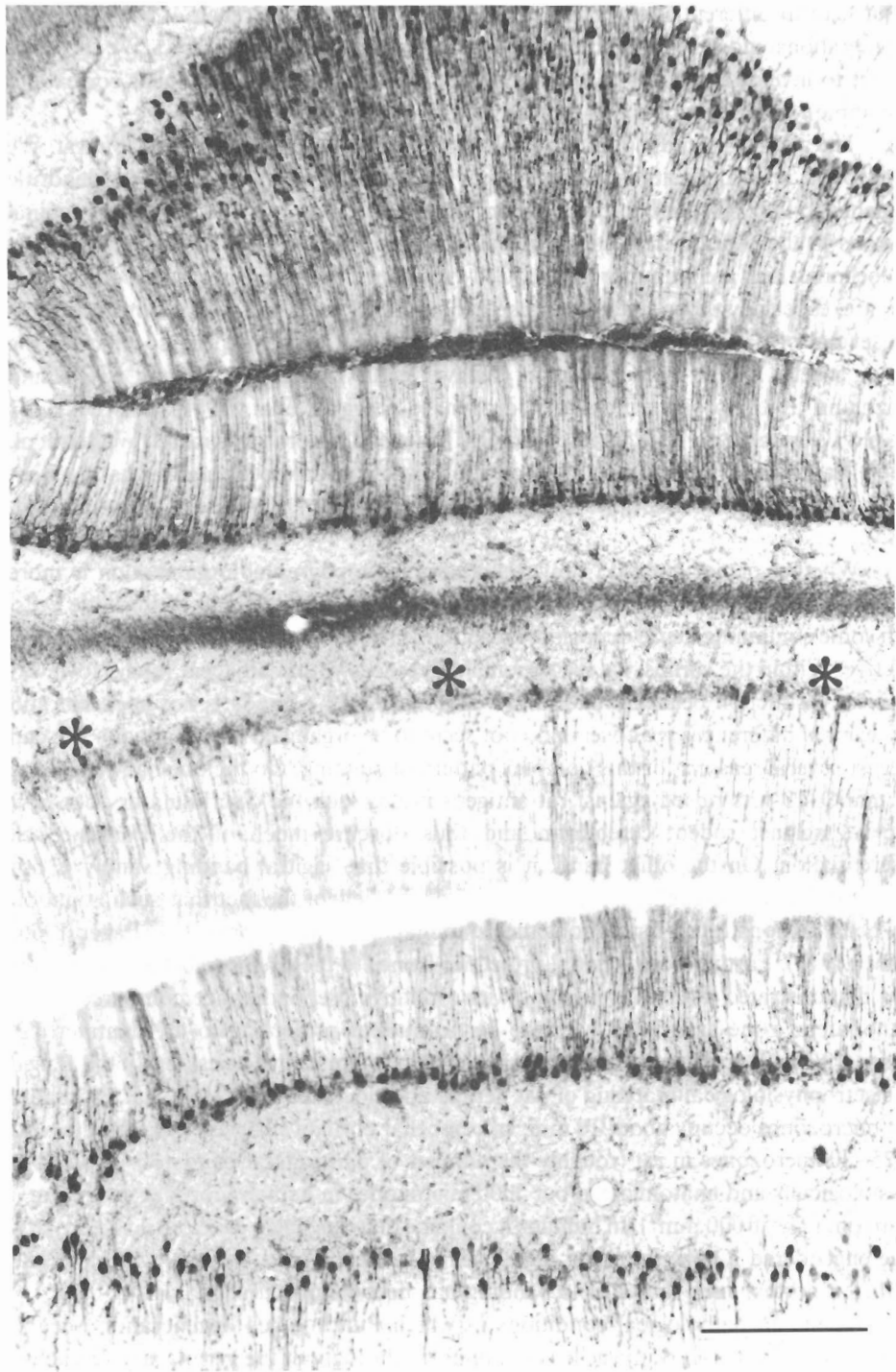


Fig. 6. A coronal section of human vermis stained with mabQ113. The asterisks mark a row of Purkinje cells which are only lightly immunoreactive. The adjacent rows of Purkinje cells are more intensely stained. As compared to staining in rodents, there is no evidence of clear parasagittal zonation. The bar indicates 500 μm .

could lead to differential responses to pathological conditions such as toxin-induced degenerations and hereditary familial cerebellar atrophies (Kark et al. 1978). We therefore set out to investigate mabQ113-immunoreactivity in the human cerebellar cortex and to compare its distribution with that in rat.

The staining of individual human Purkinje cells closely resembles that in rat. We have found stain deposits in all regions of the cell ranging from the finest dendritic branches to the cell soma, to the axon and recurrent collaterals and the axon terminal boutons in the dentate nucleus. As in rat, the Purkinje cells are the only cells in the cerebellum which are immunoreactive. Cell-type specific markers in the nervous system are rare, especially those suitable for use in human tissue. Remarkably, however, there are several other antigens which, in rodent cerebellum, appear to be specific for the Purkinje cells. These include motilin (Nilaver et al. 1982), parvalbumin (Celio and Heizmann 1981) prostaglandin dehydrogenase (Siggins et al. 1971), the UCHT-1 antigen (Garson et al. 1982) and cyclic GMP-dependent protein kinase (Walter et al. 1979). Based on its selective staining of a subset of Purkinje cells and on its apparent molecular weight, the mabQ113-antigen is different from any of these (Hawkes et al. 1985).

Whether or not mabQ113 reveals a complex topographic organization is more contentious. We have no doubt that there is heterogeneous staining of Purkinje cells, with some containing dense deposits of reaction product and others much less strongly reactive. Within the vermis, we see regional differences within adjacent folia. However, the clear distinction between mabQ113⁺ and mabQ113⁻ bands is not apparent and the regional differences we observe do not seem to be organized parasagittally. We can suggest several reasons for this complex pattern of staining. On the one hand, because the mabQ113 was raised against rat antigens it may lack the discriminatory power it displays against rodent cerebellum and thus obscure much of the Purkinje cell differentiation. On the other hand, it is possible that sagittal banding simply is not present in human cerebellum but this is unlikely in view of the fact that such zonation has been demonstrated throughout the Mammalia, including primates (Eager 1966; Haines 1976). Furthermore, sagittal projection zones to the deep cerebellar nuclei have been demonstrated directly in humans with a relative preponderance compared to rat of the lateral zone associated with the corticonuclear projection to the dentate (e.g. Jansen 1969). A possible explanation arises out of quantitative considerations. Based on electrophysiological mapping of cat vermis and flocculus, Ito (1984) has estimated that microzones occupy about 10 mm² of cerebellar cortical surface. Thus there would be 25–30 microzones in rat (roughly the number of parasagittal bands observed both physiologically and anatomically) but 5000 microzones in human (cortical surface area approximately 50 000 mm²). In humans it could be that the highly developed motor skills have engendered a corresponding high level of complexity in the organization of the cerebellar cortex, such that simple banding has become obscured.

In human pathological conditions in which Purkinje cell degeneration occurs, frequently the loss of Purkinje cells is not uniform throughout the vermis and cerebellar hemispheres. In type I olivopontocerebellar atrophy the vermian Purkinje cells are spared while the hemispheric ones degenerate, and in type III atrophy it is the Purkinje

cells over the superior surfaces of the hemispheres that are more severely affected (Konigsmark and Weiner 1970). Similarly, in Friedreich's ataxia the Purkinje cells in the superior portion of the vermis degenerate selectively (Netsky 1968), in alcohol-induced Purkinje cell degeneration the anterior and superior portions of the vermis are affected (Netsky 1968) and in hypothyroidism most of the Purkinje cell loss is in the vermis (Price and Netsky 1966). Thus, the anatomically restricted Purkinje cell degeneration in a variety of pathological conditions supports the notion of areas of Purkinje cell heterogeneity. However, it seems that these areas of involvement are macroscopic, not the microscopic heterogeneity as illustrated in Figs. 4–6.

This study has demonstrated selective and heterogeneous staining of human cerebellar Purkinje cells. It is possible that mabQ113⁺ and mabQ113⁻ subsets of Purkinje cells may respond differentially to pathological conditions such as cerebellar dysgenesis, congenital cerebellar hypoplasia, metabolic abnormalities, toxin exposures and hereditary cerebellar degenerations. This possibility will be pursued further using autopsy-derived pathological material.

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