Disorder of Cerebellar Foliation in Walker’s Lissencephaly and Neu-Laxova Syndrome

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Abstract. A diffuse disorder of cerebellar foliation was found in eight infants and one fetus with Walker’s lissencephaly. The cerebellar cortex consisted of fused and irregularly distorted folia. In the white matter, ill-siluated rings of cortex were concentrically arranged around blood vessels and mesenchymal tissue. The normal relative position of the different classes of cortical nerve cells was preserved. Cells of the external granular layer invaded the meninges and migrated along penetrating blood vessels. We believe that this foliation disorder is caused by a defect in the external basal lamina that allows adjacent folia to be fused and sulci obliterated by intrameningeal ectopies of external granule layer cells. Physical forces applied during development probably contribute to the distortion of the gyral pattern. There was a volumetric reduction of the neocerebellum, which might also be a consequence of the basal lamina defect. The cerebellum of a fetus with the Neu-Laxova syndrome showed the same abnormalities as in Walker’s lissencephaly. It is postulated that these two conditions belong to a class of prenatal developmental disorders that involves a defect of the extracellular matrix.

Key Words: Brain development; Cerebellar granule cells; Cerebellar malformation; External basal lamina; Extracellular matrix; Neu-Laxova syndrome; Walker’s lissencephaly.

INTRODUCTION

Walker’s lissencephaly (WL) (also called Walker- Warnerburg syndrome and type II lissencephaly) is a rapidly fatal familial syndrome, probably transmitted as an autosomal recessive trait and characterized by prenat al hydrocephalus, absence of the cerebellar vermis, disorganization of the cerebral and cerebellar cortex, retinal dysplasia and other ocular abnormalities and myopathy (1–6). As the cerebellar changes have received little attention, their analysis in nine cases of WL including a 25-week-old fetus was undertaken.

We incorporate in this report the case of a 32-week-old fetus with the Neu-Laxova syndrome (NLS), a rare autosomal recessive, rapidly fatal condition with severe intra-uterine growth retardation, typical facial features, multiple congenital abnormalities of limbs, skin, and external genitalia, and an extremely small lissencephalic brain (7–9). The changes in the cerebral hemispheres and cerebellum, which have not been described previously in detail, were found to be very similar to those in WL.

The cerebellar changes common to WL and NLS involve a widespread foliation disorder which is very different from other cortical dysplasias, particularly cerebellar microgyria and heterotaxia, both of which are focal defects. Cerebellar microgyria consists of segmental shrinkage of cortical lobules and is practically always associated with cerebellar microgyria. It is generally considered to be of ischemic origin (10, 11). Cerebellar heterotaxia, a term coined by Bruns (12), consists of circumscribed aggregates of disorganized cortical tissue located predominantly in the paraffoci or anterior vermis in an otherwise normal cerebellum or in association with other developmental defects.

Previous reports assign a role to lesions of the meninges and glial limiting membrane in the pathogenesis of the cerebral dysplasia in WL (6) and Fukuyama disease (13, 14), a closely related condition, but little has been said concerning the cerebellar defect.

Our neuropathological analysis of the cerebellum in WL and NLS suggests that these syndromes belong to a group of disorders related to a primary defect of the external basal lamina and the extracellular matrix.

MATERIALS AND METHODS

We studied the brains of eight infants aged 1 day to 4 months (four males and four females) with WL, a 25-week-old fetus most probably affected with WL and a 32-week-old fetus with extreme microcephaly (brain weight 16 g; normal 230 g), intra-uterine growth retardation, facial deformities, arthrogryposis, and other malformations typical of NLS. Six normal fetuses of 21 to 36 weeks gestational age were used as controls.

The formalin-fixed brains were embedded in paraffin, cut serially, and stained with hematoxylin-eosin and cresyl violet. Representative sections were stained with Masson’s and Mal-

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* In Dobyns’ classification, type II lissencephaly is synonymous with Walker’s lissencephaly, and type I lissencephaly refers to an entirely different condition caused by an abrupt arrest of migration in the cerebral neocortex at the end of the first prenental trimester with no significant cerebellar defect. This condition is often associated with the abnormal facial features of the Miller-Dieker syndrome (1).
lory’s trichrome and safran for connective tissue, Bielschowsky’s silver stain and Foot’s silver stain for reticular fibers, and orcein for the internal elastic lamina of vessels. Peroxidase-antiperoxidase immunohistochemical methods were used to mark laminin, vimentin, and glial fibrillary acidic protein (GFAP). Some sections were stained simultaneously with Oil Red O (ORO) and an antiserum to GFAP. Formalin-fixed sections were cut with a vibratome for immunostaining of macrophages with a monoclonal anti-human macrophage antibody (M 718 Dako; Glostrup, Denmark). Lectin UEA-1 (L 8146 Sigma; St. Louis, MO) was used to mark vessels. Epithelial membrane antibody (EMA), clone E29 (M 613 Dako), was used on paraffin-embedded sections.

RESULTS

Walker’s Lissencephaly: The cerebral hemispheres of the eight infants with WL showed a distinctive pattern of neocortical changes: markedly dilated ventricles, a partially fused interhemispheric fissure, and a smooth brain surface. Sulci were obliterated. The cortical architecture was completely disrupted and segmented by septae of mesenchymal tissue containing meningeal arteries. No radial or tangential organization of neurons was recognizable.

In the cerebellum, the vermis was absent. The volume of the cerebellar hemispheres was reduced (approximately 30%). The cerebellar surface was smooth with a few shallow indentations. Microscopic changes were generalized, with only small variations in the extent of folial distortion from case to case. Sulci were totally or partially occluded by apposition of the external granule layers (EGL) of adjacent folia (Fig. 1) or obliterated by the escape of EGL cells into the meninges (Figs. 2, 3). Narrow bands of fused EGL were irregularly arranged in a complex lattice (Fig. 1). In spite of considerable distortion of the folial pattern, the EGL, the molecular layer, the Purkinje cell layer, and the internal granule layer (IGL) could be recognized in their normal relative positions. In some areas, narrow partially occluded fissures separating strands of cortex with normal cytoarchitecture (Fig. 3) penetrated deeply into the white matter.

In the depth of the white matter, large cortical rings (Figs. 1, 6) were centered on one or several small vessels surrounded by a rich network of mesenchymal tissue re-
seeming that of the meninges. From a perivascular ring of EGL cells outward, the molecular layer, the Purkinje cell layer, and the IGL were arranged concentrically (Fig. 6). Although it appeared that many perivascular cortical rings represented transversely cut expansions of cortical digitations, such a connection could not always be established in serial sections.

The EGL showed areas of focal attenuation (Fig. 5), fragmentation (Fig. 3), and degeneration of nerve cells. A striking feature was the presence of ectopic EGL cells in the meninges filling the interfolial fissures (Figs. 2, 3) and the mesenchymal cores of the perivascular cortical rings (Fig. 6), or forming more limited clusters (Fig. 5) or bridges (Fig. 3). External granule layer cells also surrounded blood vessels in the molecular layer (Fig. 4).

In five cases, compact masses or convoluted bands of ectopic Purkinje cells were observed in the white matter, usually in close contact with the IGL of the perivascular cortical rings.

A moderate number of ORO-positive macrophages and GFAP-positive astrocytes were observed in the superficial part of the molecular layer. The meninges covering the surface of the cerebellum, marking the axis of the cortical digitations or present in the center of the perivascular rings were moderately thickened and contained a few ORO-positive macrophages; staining with Masson’s and Mallory’s trichrome, safranin and reticular fiber stains, as well as immunostaining with antibodies against laminin and vimentin, were comparable to controls. Epithelial membrane antibody did not mark the meninges. Meningeal and intraparenchymal vessels showed no obvious structural alterations. Some large meningeal arterioles were deeply engulfed as a consequence of the fusion and distortion of cerebellar folia. Although intraparenchymal vessels, marked with reticular fiber stains, orcein and lec-

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**Fig. 2.** WL, case 3. On the right, distal end of a sulcus occluded by granule cells invading the meninges (arrow). Aggregates of EGL cells in the molecular layer (arrowhead). Cresyl violet, ×100.

**Fig. 3.** WL, case 5. Segment of cortical digitation in deep white matter. Fragmentation of EGL (arrow). Aggregate of EGL cells in the interfolial space (arrowhead). Cresyl violet, ×195.

**Fig. 4.** WL, case 3. Granule cells surround vessels in molecular layer (arrow). m = meninges. Cresyl violet, ×125.

**Fig. 5.** WL, case 8. Attenuation of EGL layer. Small nests of EGL cells (arrow) in the meninges. Cresyl violet, ×180.

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Fig. 6. WL, case 4. Cortical ring in cerebellar white matter with concentric granule cells, molecular and Purkinje cell layers. Connective tissue and vessels in the center are obscured by ectopic EGL cells (arrow). Cresyl violet, ×100.

tin, were distorted, a normal vascular pattern could be recognized in less affected areas. In six cases, there were a few small, subcortical areas of atrophy and gliosis, occasionally containing macrophages with hemosiderin or calcifications.

The dentate nuclei were usually fragmented. The inferior olives showed no major structural abnormality and no evidence of cell destruction.

A 25-week-old Fetus with Walker’s Lissencephaly: This fetus was considered to have WL. The neuropathological changes in the cerebral hemispheres were similar to those observed in neonates and infants with WL and comparable to those of a previously reported fetal case of this disease (6). There was, however, no hydrocephalus. Eyes and muscle were not examined. According to Miller et al (6), hydrocephalus and ocular and muscular abnormalities may be absent in fetuses with WL. The vermis was lacking. A large portion of the surface of the cerebellar hemispheres was smooth, except for a few indentations marking shallow sulci. In age-matched controls, sulci were more numerous and deeper. The EGL, the molecular layer, and the Purkinje cell layer were easily identifiable and in normal relative positions. The EGL was sparse when compared to controls. The small sulci indenting the surface were either fused in their lower segment by apposition of the opposing EGL or filled with abundant meningeal tissue containing small clusters of ectopic EGL cells. In the central white matter there were small vessels encircled by a single ring of EGL cells. A few vessels surrounded by connective tissue were encircled by a trilaminar structure consisting of EGL, molecular layer, and Purkinje cells.

A 32-week-old Fetus with the Neu-Laxova Syndrome: The vermis was present and relatively unaffected. In the cerebellar hemispheres, the cortical abnormality was very similar to that observed in the previous fetus. In one area, sulci obliterated by apposition of adjacent molecular layers formed a complex network comparable to that seen in the neonatal brains with WL (Fig. 7). In other regions, only the proximal segment of the fissure was occluded, the distal part remaining open and filled with vessels and loose meningeal connective tissue (Fig. 8). Some partially obliterated fissures were relatively long and displayed multiple branching (Fig. 8). Deep-seated perivascular rings consisted either of EGL cells or, occasionally, of EGL cells and Purkinje cells (Fig. 8). In the latter case, the central vessels were surrounded by mesenchymal tissue. Perivascular EGL cells were observed in the white matter a great distance from the surface (Fig. 9). The meninges...
DISORDER OF CEREBELLAR FOLIATION

were thickened in some areas and contained a few ORO-positive macrophages, which were recognized by a monoclonal anti-human macrophage antibody. Laminin and vimentin were present in the meninges and in penetrating vessels and their connective tissue sheaths. Glial fibrillary acidic protein-positive astrocytes were seen in the molecular layer. The dentate nucleus was fragmented.

The lesions in the telencephalon were strikingly similar to those seen in Walker's lissencephaly. They will be described in detail elsewhere (K. Mogami et al., in preparation).

DISCUSSION

An identical developmental defect of the cerebellar hemispheres was found in eight cases of WL. It consisted of fusion of cortical fissures and considerable distortion of cerebellar folia. Trilaminated cortical rings centered on vessels and mesenchymal tissue were seen in the central white matter. Even in the most disorganized areas, the normal relative positions of the EGL, the molecular layer, the Purkinje cell layer and the IGL were maintained. Numerous EGL cells were present in the meninges and around penetrating blood vessels. A few aggregates of Purkinje cells in the white matter were in partial contact with the IGL of the perivascular cortical rings. The meninges were moderately thickened. There was no evidence in favor of a primary defect of neuronal migration, a vascular malformation, ischemia or infection.

Many of these features were present in the fetuses with WL and with NLS: widespread fusion of sulci, perivascular rings of trilaminated cortex in the deep white matter, and migration of EGL cells along blood vessels. There were no signs of necrosis or inflammation. Meninges were only moderately thickened.

Heterotopias of EGL cells and of Purkinje cells and the trilaminated perivascular cortical rings in the central white matter deserve comment. In many areas EGL cells (probably because of breaches in the external basal lamina [EBL]) invaded the meninges, obliterating the narrow spaces between folia. Other EGL cells migrated along penetrating blood vessels. In the two fetuses, perivascular EGL cells were particularly numerous in the white matter, even at a great distance from the surface. In this location, they apparently disappeared with age, since they were restricted to the molecular layer in the more mature brains. We are unaware of such extensive intrameningeal and perivascular EGL cell ectopias in other brain malformations. During normal development, perivascular EGL cells may be encountered transiently in restricted areas. Friede (15) states that they occur in the dentate and roof nuclei in 30% of normal infants before the age of 4 months. Also, EGL cells in the dentate nucleus are a common finding in trisomies 13 and 18, and occasionally in other chromosomal aberrations.

The ability of EGL cells to migrate along blood vessels in WL and NLS could be related to changes in the perivascular connective tissue. More probably, this is a mechanical consequence of insufficient foliation. Incapable of extending normally along the surface of the cerebellum, these cells could be forced to find a path along penetrating blood vessels. A similar situation is apparently created in cerebellar embryonic transplants in which EGL cells are also seen to ensheathe blood vessels (16–18). The impossibility of forming fissures in the confined portions of the transplant is considered to be the cause of this usually transitory phenomenon (16).

Ectopic masses of Purkinje cells in the white matter most probably do not play a significant role in the pathogenesis of the malformation. They occur as an isolated cerebellar abnormality in association with various developmental defects of the cerebral hemispheres and may also be seen in normal subjects.

In the white matter, normally laminated cortical rings were organized around blood vessels. As the axial vessels were surrounded by mesenchymal tissue whose histologic and immunostaining characteristics closely resembled those of the meninges, they probably represent transversely cut cortical invaginations. However, it was frequently impossible on serial sectioning to establish their connection with the overlying cortex. In the two fetuses, trilaminated cortical rings were observed around small blood vessels at a distance from a very poorly convoluted surface (Fig. 8). In a case of trisomy 21 in which the cerebellar cortex was normal, we observed typical perivascular cortical rings in the dentate nucleus, together with perivascular cuffs made only of EGL cells. The apparently isolated perivascular cortical rings could have been separated from the stem of a penetrating cortical digitation. One might also venture another explanation: Purkinje cells that have not reached their final destination (and some of which constitute subcortical aggregates) could gather around perivascular EGL cells to form small con-
centric cortical organizations. The ability of cerebellar neurons to rebuild normal cortical structures after their initial development has been considerably altered is remarkable, as is shown, for instance, in transplants of dissociated and reaggregated pellets of rat cerebellar primordia (17).

Attempts at interpreting disease on the basis of postmortem morphological studies necessarily have their limitations. We believe, however, that widespread fusion of normally laminated folia constitutes the basic mechanism of the cerebellar malformation in WL and NLS. Physical forces applied during growth probably contribute to the obliteration of sulci and to the very complex and characteristic distortion of the cerebellar cortex. Erasing of fissures by apposition of adjacent EGL and their obliteration by the outflux of EGL cells into the meninges implies an alteration of the EBL. Unfortunately, this could not be demonstrated, postmortem, by an ultrastructural analysis. The presence of a moderate and patchy thickening of the meninges and a few macrophages in the molecular layer and meninges cannot be considered a sufficient indication of a destructive process affecting mesenchymal structures at the surface of the cerebellum. Indirect evidence that an alteration of the EBL may play a role in the pathogenesis of a foliation disorder stems from experiments in newborn rats in which intracisternal injections of 6-hydroxydopamine (6-OHDA) resulted in a destruction of meningeal fibroblasts and the basal lamina, intrameningeal ectopies of EGL cells, loss of fissures, and fusion of adjacent folia (19, 20). Also, during normal development in the rat, small breaches in the EBL covering the fissura prima have been shown to cause restricted areas of fusion and meningeal ectopies of EGL cells (21).

A defect in the EBL could have other consequences. Hausmann and Sievers (22) have shown that EGL cells are attached to the basal lamina during their entire period of proliferation and suggest that the extracellular matrix at the surface of the cerebellum may be involved in the regulation of the mitotic activity of these cells. As an indirect confirmation of this hypothesis, lesions of the EBL following injection of 6-OHDA in the newborn rat result in a reduction in size of the cerebellum that correlates with a reduction in the number of granule cells (20). It is, therefore, plausible that a defect in the EBL could result in the degeneration of a portion of the EGL cells and account for the reduction of the size of the cerebellum found in our cases of WL. Furthermore, a relationship between the proliferative activity of EGL cells and the formation of sulci has been postulated (23), and meningeal cells have been said to be involved in foliation (20). One should, therefore, consider the possibility that a degree of inhibition of sulcal formation could play an additional role in the disorder of foliation in WL and NLS.

In conclusion, there is strong evidence that a defect of the extracellular matrix and more specifically the EBL is an essential factor in the pathogenesis of the abnormality of cerebellar development common to a group of conditions including WL, the closely related Fukuyama disease (24), and NLS. An alteration of the extracellular matrix is most probably also involved in the cerebral dysplasia in these disorders. Confirmation of this interpretation will require ultrastructural analysis of the EBL, meninges, and brain surface. A brain biopsy, however, is only very exceptionally justified in these circumstances.

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