

Retinopathy of Prematurity in Anencephaly: Pathological and Immunohistochemical Studies of Six Eyes from Three Female Stillborn Holoanencephalic Infants

Mikyung Kim¹ and Je G. Chi

*Department of Pathology, Seoul National University College of
Medicine, Seoul 110-799 and Department of Pathology¹, Dankook
University College of Medicine, Choongnam 330-714, Korea*

= Abstract = Retinopathy of prematurity (ROP), a disease of developing blood vessels, is seen predominantly in premature infants requiring oxygen administration. We have noted various degrees of ROP in 6 eyes from 3 female stillborn holoanencephalic babies. Their gestational ages ranged from 30 to 35 weeks. All 6 eyes showed marked hypoplasia of the ganglion cell layer, the nerve fiber layer, and the optic nerve head. At the junction of vascularized and avascular anterior retina, proliferation of vanguard spindle cells (in 4 eyes from 2 cases) and of rear guard angioblastic cells (in all 6 eyes) were noted. Intravitreal vascular proliferation (in 3 eyes from 2 cases) and partial retinal detachment with vitreous hemorrhage (in 1 eye) were also seen. Immunohistochemical studies revealed positive staining for S-100 protein and negative staining for laminin & Ulex europaeus in spindle cells (2/2 cases); the reversed pattern in angioblastic cells (3/3 cases). The reduced mass of retina generally lacking intact ganglion cells and their axons in our cases may induce local vasoconstriction in response to tissue hyperoxia, which result in the retinal hypoxia and consequently, provide the impetus for the establishment of neovascularization. The immunohistochemical findings lend support to the neuroglial origin of the vanguard spindle cells.

Key Words: *Anencephaly, Retina, Vasculogenesis, Spindle cell, Angioblastic cell, S-100 protein, Laminin, Retinopathy of prematurity, ROP*

INTRODUCTION

The retinopathy of prematurity (ROP) has been documented in anencephalic babies by several groups of authors since 1963 (Cogan 1963; Andersen *et al.* 1967; Addison *et al.* 1972; Lucey and Dangman 1984; Foos 1985; Cogan

et al. 1986; Bernardo *et al.* 1991). The incidence ranged from 4.8 to 12.3 % (Andersen *et al.* 1967; Addison *et al.* 1972; Bernardo *et al.* 1991). The retinal vessels in these cases had a wide range of neovascular changes, which consisted of two types of cell clusters in proliferation. Clusters of angioblastic cells were posteriorly located and underwent lumenization to form the definite vessels. Clusters of spindle cells were anteriorly located extending a variable distance toward the ora serrata, but were separated from it by a distinct boundary. These angioblastic and spindle cell clusters consist-

Received October 1992, and in final form December 1992.

서울대학교 의과대학 병리학교실: 지제근
단국대학교 의과대학 병리학교실: 김미경

ently observed in the paranatal retina have been generally believed to play a role in the developing vasculature (Ashton 1966; Foos 1985; Cogan and Kuwabara 1986).

The questions about the origin or role of the vanguard spindle cell clusters in normal developing or pathologically proliferating vasculature have not been solved. The source of the spindle cells has been widely believed to be mesenchyme migrating from the primitive hyaloid system (Ashton 1954; Manschot 1971; Foos and Kopelow 1973; Kretzer *et al.* 1984) but some authors have interpreted them as neuroglia on the basis of immunohistochemical study using glial fibrillary acidic protein (GFAP) (Hamada *et al.* 1984; Cogan and Kuwabara 1986).

The purpose of this article is to demonstrate the varying degrees of ROP found in the retinas of 3 anencephalic babies and to summarize the immunohistochemical properties of the two types of cell clusters in proliferation.

MATERIALS AND METHODS

The material for this study included 6 eyes from 3 anencephalic babies showing apparent ROP at the postmortem microscopic examination. All were stillborn females. Their birth weights and gestational ages, estimated from the available maternal menstrual history, were 750 gm/32 weeks (case 1), 1860 gm/35 weeks (case 2), and 910 gm/30 weeks (case 3).

The maternal ages of case 1 and case 2 were not stated; that of case 3 was 30 years. Maternal obstetrical history for case 1 was not available; in case 2, it was the second pregnancy; in case 3, the fifth pregnancy. None had antecedent disease or documented exposure to medications or environmental toxins during the pregnancy except for a history of unidentified drug injection at 5 months of gestational age in case 1. The previous pregnancies had given birth to a healthy child (case 2) and 2 healthy children and 2 stillborn infants with no specific anomalies (case 3). None had complications during pregnancies.

When compared with weight after correc-

tion (8-10% addition) for the absence of the calvarium, case 1 and 3 are small for the gestational age estimated from the available maternal menstrual history; case 2, appropriate for gestational age. In all cases external and internal examinations revealed typical features of dysraphic anencephaly including macroglossia(1/3), exophthalmos(2/3) or adrenal hypoplasia(3/3). Incomplete lobation of the right lung(2/3) and complex cardiovascular anomaly (1/3) were also found.

The eyes were removed at autopsy. Preparation, gross-sectioning, macroexamination and recording of findings (the shape, consistency, anteroposterior, horizontal and vertical dimensions of the globe, horizontal and vertical diameters of the cornea, the length of the attached segment of optic nerve) were performed according to our routine methods. Microsections were prepared from paraffin-embedded specimens and routine staining was supplemented with periodic-acid Schiff (PAS), Masson's trichrome (MT), Luxol-fast blue (LFB), and van Gieson preparations. For immunohistochemical studies, immunoperoxidase staining method was applied for S-100 protein, GFAP, laminin, and *Ulex europaeus*.

RESULTS

1. General ocular pathological findings

The anteroposterior diameter of the globes were 10, 14 and 17 mm, respectively (mean 13.7 mm). Persistent hyaloid vessels projecting from optic disc and persistent pupillary membrane, all indicating ocular immaturity were noted in case 1; the remainder of the globes were grossly unremarkable.

The histopathological examinations revealed marked hypoplasia of the ganglion cell layer, nerve fiber layer, and the optic nerve head in all cases. The ganglion cells were markedly reduced in number, with pyknosis of the nuclei. The optic disk showed an absence of most of the prelaminar tissue with encroaching sensory retina on each side. The optic nerve was markedly diminished in diam-

eter and consisted of the surrounding meninges and the connective tissue septa and showed varying degrees of astrocytic gliosis.

2. Retinal vascular changes

All six globes showed varying degrees of ROP in the background of the immature peripheral retina.

In case 1, there were extensive proliferations of both spindle cells and angioblastic cells in both eyes. In the superficial layer, the spindle cell clusters formed distinctive masses anterior to the angioblastic clusters and separated from them by a relatively cell-sparse intermediate zone (Fig. 1).

The spindle cell lamina arranged predominantly horizontal to the retinal surface and extended forward almost to the ora serrata. The nuclei of these spindle cells stained heavily with hematoxylin and were often departmentalized by the vertically coursing Müller fibers (Fig. 2). The PAS stainings revealed no detectable positive granules in spindle cell clusters, probably dissolved in the tissue processing. MT, LFB and van Gieson preparations revealed no additional findings.

The angioblastic cell clusters also formed



Fig 1. Angioblastic and spindle cell clusters in postequatorial retina. Angioblastic cluster (A) is mass on left side of mound in contact with developing capillaries, and spindle cell cluster (S) is on right. Masses are separated by narrow, cell-sparse interval (arrows). (x 100)

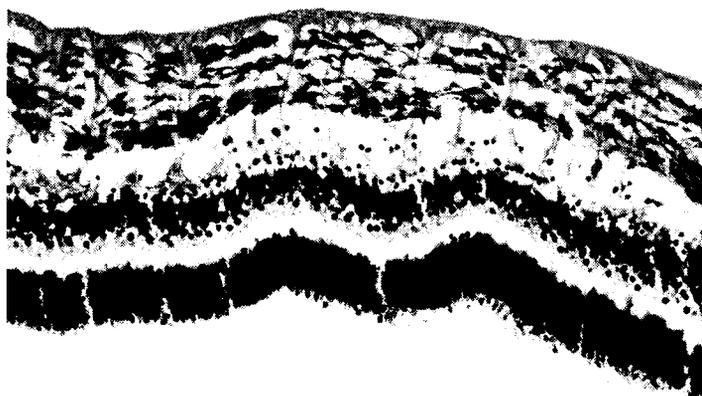


Fig 2. Prominent spindle cell lamina extending toward peripheral retina. (x 200)

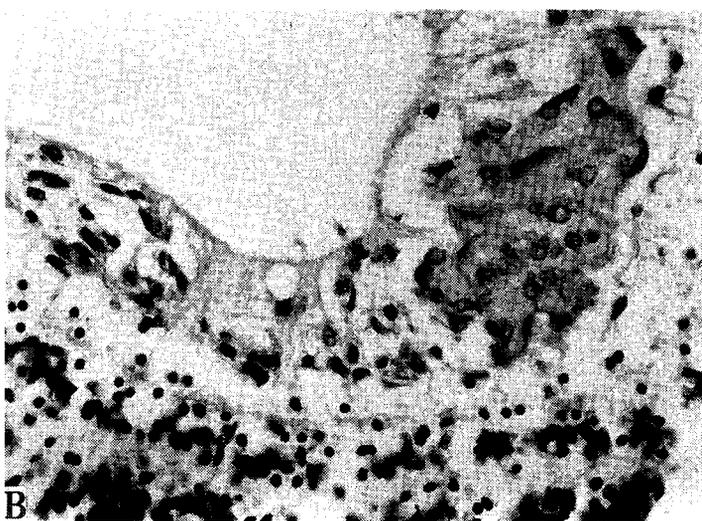
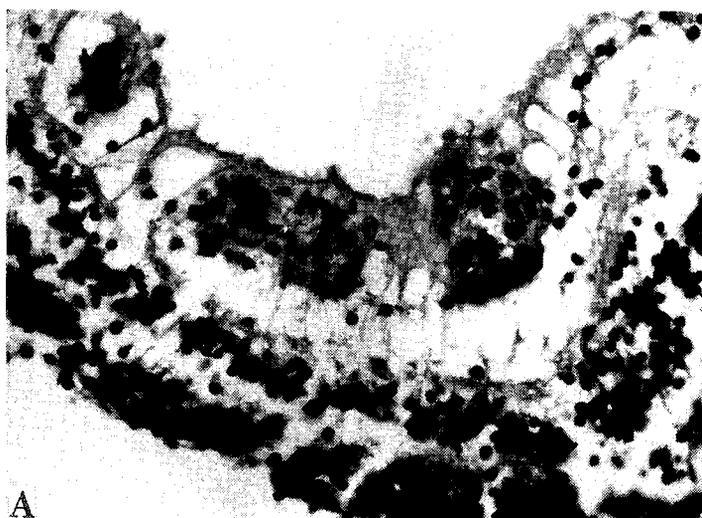


Fig 3. Proliferated capillary endothelium forms multifocal discrete whorls resembling a glomerulus (a&b). (x 400) A small cluster of vanguard spindle cells (S) is present (b).

hyperplastic masses in the posterior fundus with extraretinal extensions. Buckling and par-

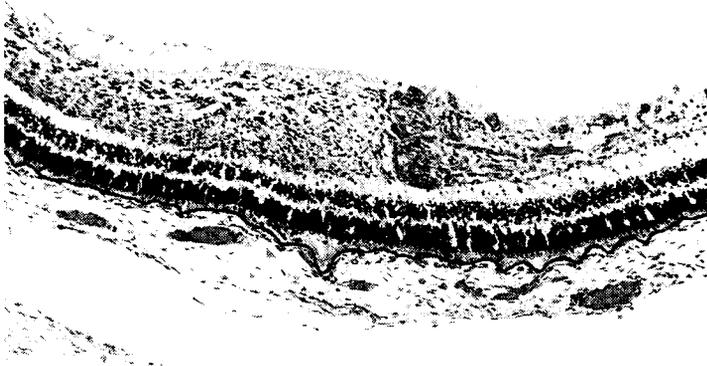


Fig 4. Angioblastic cell cluster (A) is seen with spindle cells (S) extending toward periphery. (x 100)

tial detachment of the retina with vitreous hemorrhage were also noted in one eye. Focal aggregates of immature erythrocytes were found in extraretinal vasoproliferative sites as well as in the choroid. The cystoid spaces in the inner retinal layers were noted on the vitreous side of the clusters.

In case 2, both retinas showed relatively well-vascularized, undulating superficial layer in which multifocal, glomeruloid, angioblastic cell proliferations were noted. Vanguard spindle cell clusters were barely seen (Fig. 4).

In case 3, both retinas showed bilateral angioblastic clusters, with spindle cells ex-

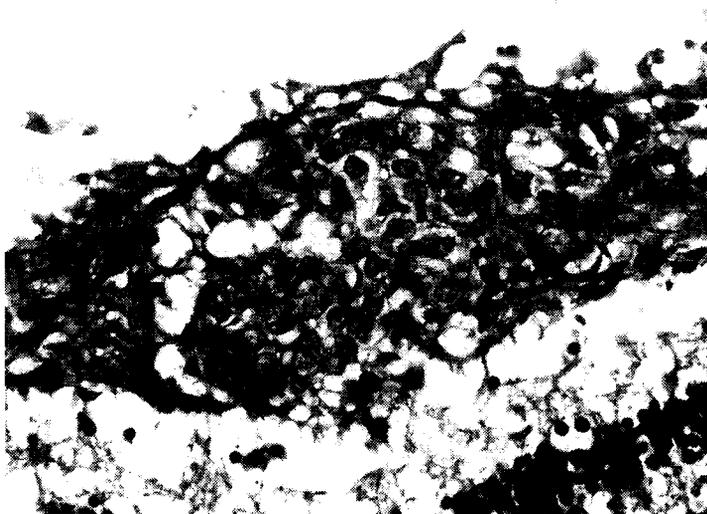


Fig 5. Internal limiting membrane is disrupted by proliferating capillary endothelium. (x 400)

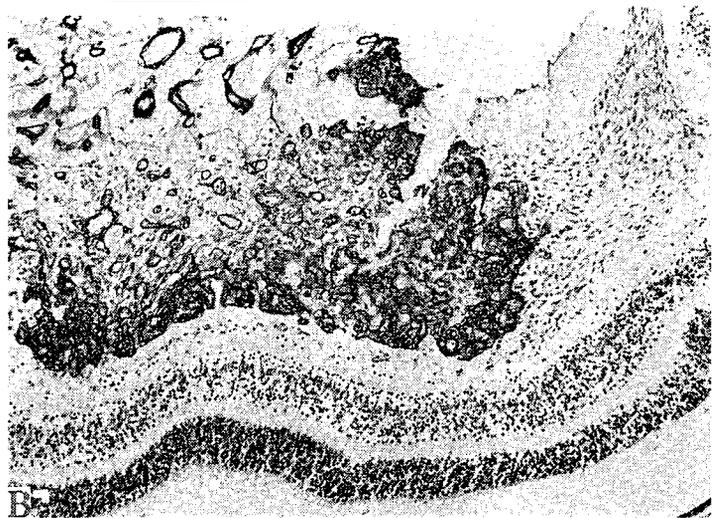
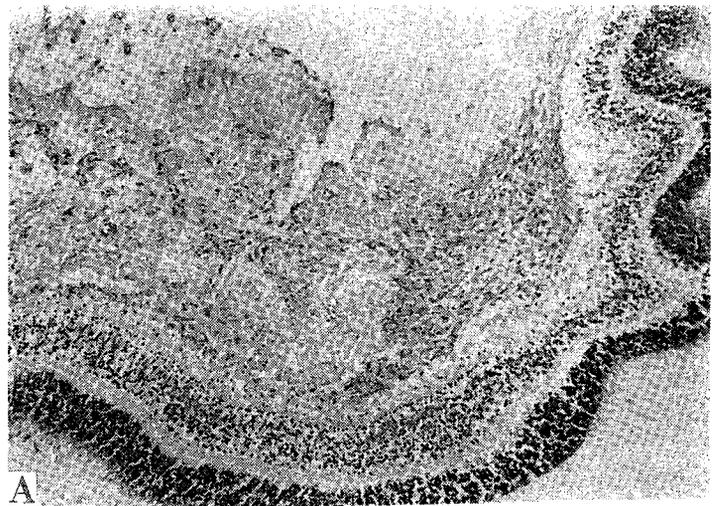


Fig 6. The vanguard spindle cells (S) show positive reaction to S-100 protein; the angioblastic cells (A), negative reaction (a). The angioblastic cells (A) show positive reaction to laminin; the spindle cells (S), negative reaction. Extraretinal angioblastic proliferations are also seen (a&b). (immunoperoxidase stain, x 100)

tending toward the periphery, such as might be interpreted as an exaggeration of the normal vasculogenesis (Fig. 5). A focus of impending intravitreal extension from the angioblastic cell mass was noted in one eye (Fig. 6).

According to the international classification for ROP (Committee for the classification of retinopathy of prematurity 1984 & 1987), the pathologic features of retinal vasculature of case 1 may correspond to stage 4; those of case 2, stage 2; those of case 3, early stage 3.

3. Immunohistochemical findings

Mutual exclusiveness between the vanguard and the rear guard cells was noted: positive reaction to S-100 protein and negative reaction to laminin & Ulex, in the vanguard cells (2/2 cases); negative reaction to S-100 protein and positive reaction to laminin and Ulex, in the rear guard cells (3/3 cases)(Fig. 7).

DISCUSSION

The neovascular responses of the retinal vessels noted in various pathologic states were not separate morbid processes, but exaggerations of normal behavior, *i. e.* retinas of the paranatal period uniformly exhibit angioblastic and spindle cell clusters in peripheral temporal portions, though varying in degrees (Ashton 1957 & 1966; Cogan and Kuwabara 1986).

Thus, before attempting to explain the ROP seen in present anencephalic cases, it might help to understand normal retinal vasculogenesis and determine common stimulating factors for vasoproliferation, operating in normal development as well as in pathologic states including oxygen-induced ROP.

Addison *et al.* (1972) have postulated that the oxygen requirements of the anencephalic retina are lower than normal, due to the lack of intact ganglion cells and their axons. In response to the relative tissue hyperoxia such as in the oxygen-induced ROP, local vasoconstriction might occur, leading to retinal hypoxia and consequently, provide the impetus for the establishment of neovascularization. Another hypothesis is that the retinal hypoxia might simply result from the abnormal vascular supply to the malformed brain.

Though we can not determine the exact conditions which seriously disturbed the retinal circulation, resulting in hypoperfusion and ischemia in our 3 cases, it is probable that the immature retinal vasculoformation, atrophic ganglion cells and nerve fiber layers result in the reduced mass of retina in a state of relative tissue hyperoxia. Thereafter, either local

vasoconstriction in response to tissue hyperoxia or abnormal development of the ophthalmic artery related to the anencephalic malformation may result in retinal hypoxia and, consequently, provide the impetus for the establishment of neovascularization.

And the very low-birth-weight, premature infants such as in the present cases may have many risk factors of ROP described by Lucey *et al.* (1984). Complex interplay of these factors may result in ROP in the developing retina, which is highly sensitive to any disturbance in its oxygen supply, either hyperoxemic or hypoxemic, and is subject to the same wide fluctuations in its circulation as the brain.

The results of immunohistochemical studies suggest that though the negative result for GFAP and the limited diagnostic utility of the S-100 protein due to its wide expression, repeated positive reactions for S-100 protein in the vanguard cells prefer the neuroglial origin of these cells to the origin from the mesenchyme migrating from the primitive hyaloid system on the papilla in the present three cases.

REFERENCES

- Addison DJ, Font RL, Manshot WA. Proliferative retinopathy in anencephalic babies. *Am J Ophthalmol* 1972; 74:967-76
- Andersen SR, Bro-Rasmussen F, Tygstrup I. Anencephaly related to ocular development and malformation. *Am J Ophthalmol* 1967; 64:559-66
- Ashton N. Pathologic basis of retrolental fibroplasia. *Br J Ophthalmol* 1954; 38:385-96
- Ashton N. Retinal vascularization in health and disease. *Am J Ophthalmol* 1957; 44:7-17
- Ashton N. Oxygen and the growth and development of retinal vessels: In vivo and in vitro studies. *Am J Ophthalmol* 1966; 62:412-35
- Bernardo AI, Kirsch LS, Brownstein S. Ocular anomalies in anencephaly: a clinicopathological study of 11 globes. *Can J Ophthalmol* 1991; 26:257-63
- Cogan DG. Development and senescence of the human retinal vasculature. *Trans*

- Ophthalmol Soc UK 1963; 83:465-89
- Cogan DG, Kuwabara T. Accessory cells in vessels of the paranatal human retina. Arch Ophthalmol 1986; 104:747-52
- Committee for the Classification of Retinopathy of Prematurity. The International Classification of Retinopathy of Prematurity. Arch Ophthalmol 1984; 102:1130-4
- Committee for the Classification of Retinopathy of Prematurity. The International Classification of Retinopathy of Prematurity. Arch Ophthalmol 1987; 105:906-12
- Foos RY, Kopelow SM. Development of retinal vasculature in paranatal infants. Surv Ophthalmol 1973; 18:117-27
- Foos RY. Chronic retinopathy of prematurity. Ophthalmology 1985; 92:563-74
- Hamada Y, Hamada M, Yonemoto H, Nakao Y, Otori T. Histopathological studies of demarcation line and avascular area in very low birth weight infants. Folia Ophthalmol Vis Sci 1984; 35:2414-9
- Kretzer FL, Mehta RS, Johnson AT, Hunter DG, Brown ES, Hittner HM. Vitamin E protects against retinopathy of prematurity through action on spindle cell. Nature 1984; 309:793-5
- Lucey JF, Dangman B. A reexamination of the role of oxygen in retrolental fibroplasia. Pediatrics 1984; 73:82-96
- Manschot WA. Eye findings in hydranencephaly. Ophthalmologica 1971; 162:151-9