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RESEARCH NOTE

A New Cell Surface Relationship between Neuroepithelial Cells during Rat Neural Tube Development

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Electron microscopic examination of the developing neural tube in 11th to 13th day rat fetuses revealed a new cell surface relationship between differentiating neuroepithelial cells. Cytoplasmic projections possessing terminal dilations were observed extending from neuroepithelial cells through cytoplasmic furrows into large coated pits at the surface of adjacent cells. This cell-to-cell relationship provides a mechanism for the internalization of surface molecules and possibly even cytoplasmic constituents. Communication between donor and recipient cells mediated in this way suggests a route for the sharing of macromolecules, including cytoplasmic fragments, which could function as regulators in embryonic development and differentiation.

In the course of studying successful and failed regulative regenerative recovery in rat fetuses after extensive primitive cell-killing by X-irradiation (3–5) and in a mutant rat with a genetically determined inability to regulate its nervous system development at a certain stage (2, 6, 8), a new cell surface relationship between differentiating neuroepithelial cells was discovered.

The experiments that led to the identification of this relationship have been described (2–6, 8) and, briefly stated, were as follows. In rats exposed to 150R on the 11th fetal day, one-half to two-thirds of the primitive neuroepithelial and mesenchymal cells were destroyed. However, these animals recovered rapidly through regenerative growth of residual primitive cells, except for some eye defects. When 11th day fetuses were exposed to 175R

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to 225R, the regenerative process failed at certain sites with the midbrain–thalamic junction appearing to be especially vulnerable. Overgrowth of this region was the consequence and led to narrowing of the aqueduct to occlusion with resulting hydrocephalus of the lateral and third ventricles late in the prenatal period. Some 175R hydrocephalics survived to adulthood whereas 225R fetuses did not live after birth (3, 5).

A similar narrowing of the aqueduct with prenatal hydrocephalus was one of the principal expressions, and the most serious one, that resulted from a recessive mutation that arose spontaneously in a line of albino Wistar rats. The affected animals survive to adulthood and reproduce. Light and electron microscopic studies were directed to see whether or not there were common features of development around the 11th fetal day in the mutant and 225R fetuses, and how the 150R animals might be different.

The mutant was found to have focal discontinuities in the basement membrane that separated the neuroepithelium of the midbrain–thalamic junction from its underlying mesenchyme. There was no destruction of primitive cells. In the 225R fetuses, animals in which numerous neuroepithelial cells were destroyed, there were comparable defects in the basement membrane of the midbrain–thalamic junction region of the neural tube whereas in the 150R fetuses, whose regulative regeneration is highly successful, no disturbances in the basement membrane were observed.

Examination of various kinds of junctions and contacts between neuroepithelial cells and other cells has failed to show any qualitative differences between the rats irradiated at 150R or 225R, the mutants and normal control fetuses. During these observations the neuroepithelial cell surface relationship that we describe in this paper was found to be present in all four categories of fetuses just mentioned. Efforts are now focused on a study of consequences that focal damage of the basement membrane might have on the developmental relationship between the neuroepithelial and mesenchymal cell populations. As a result, qualitative as well as quantitative studies detailing the morphology of cell contacts, including information about the cell surface relationship described in this paper, will form an important part of this work.

The rat fetuses examined were surgically removed by sequential caesarian section from normal and experimental mothers between the 11th and 13th fetal day and immediately fixed by immersion 24 h in 3% glutaraldehyde in 0.1 *M* cacodylate buffer. Tissues were then rinsed in cacodylate buffer, post-fixed 1 h in 2% aqueous osmium tetroxide, and block stained 30 min in 0.5% uranyl acetate in maleate buffer. The tissues were dehydrated, first with a brief rinse in cold 50% ethanol and then by passing successively through one 10-min change in cold 70% ethanol, two 10-min changes in cold 95% ethanol, and then three 15-min changes in 100% ethanol at room temperature. After two 10-min changes in propylene oxide, the tissues were infiltrated

with Epon resin and polymerized 2 days at 60°F. The tissues were cut on a Huxley ultramicrotome, stained 30 min in uranyl acetate and 10 min in lead citrate, and examined and photographed with a Philips 400 electron microscope.

Figure 1 illustrates the surface relationship observed in all control and experimental tissues examined. Short processes extending from the surface of primitive neuroepithelial cells were observed projecting into large coated pits at the surface of adjacent cells. These processes frequently ended in small dilations which were covered with an extracellular surface coat. They also contained a core of radially oriented microtubules and filaments, cytoskeletal elements whose presence suggested that these surface processes were actively growing, extending out from the cell surface much like filopodia or growth cones.

Figure 1 indicates how growth at the cell surface might result in the structures just described. The arrow identifies a focal coated region of the plasma membrane of a neuroepithelial cell. Active surface growth of the opposed noncoated segment of cell membrane could result in the type of neuroepithelial surface feature seen in lower center of Fig. 1 and the inset. Subsequent pinching off of the coated pit with endocytosis of surface macromolecules and small pieces of cell cytoplasm would explain the morphology of the coated vesicles and their contents indicated by the arrowheads.

Since coated pits were first described by Roth and Porter (10) in 1962, their postulated role in specific protein transport has been demonstrated in a variety of systems in which an assortment of polypeptides have been demonstrated to be bound and moved by this organelle. Their presence at the cell surface certainly indicates a site where endocytosis of extracellular material is taking place even though the intracellular targets to which the material is subsequently delivered can vary, apparently being dictated by the cell type and particular material ingested (7).

Although small molecules can exert their influence on cellular growth and embryogenesis by entering cells via intercellular gap junctions, macromolecular species such as proteins and polysaccharides cannot move into cells in this fashion. Yet data are available that indicate that such molecules have significant effects on embryonic differentiation and development (9). Pathways for communication essential for the development of recognition mechanisms in orderly patterns of differentiation should, therefore, include processes that allow for the internalization of these molecules. The neuroepithelial surface feature we describe could provide such an avenue for internalization utilizing a mechanism of endocytotic uptake by coated pits and vesicles of surface macromolecules and, more interestingly, cytoplasmic constituents.

Although the internalization of fragments of cell cytoplasm has, to the authors' knowledge, not been previously implicated as a possible source of

FIG. 1. Electron micrograph of the neuroepithelium from a 12-day genetic mutant rat fetus. In the lower center note the cytoplasmic process extending from the surface of one cell through a narrow furrow into a coated pit at the surface of an adjacent cell (×10,000). A similar structure from a 12-day fetus irradiated with 150R is seen at higher magnification in the inset (×21,500). The arrow indicates the site where another surface feature might be forming and the arrowheads identify internalized coated vesicles and their contents.

material regulating embryonic differentiation and development, our observations certainly lead us to seriously consider this hypothesis. The cell surface features described in the literature which most closely resemble those presented in this paper occur in the rat testis. Christensen and Gillim (1) described microvilli extending from the surface of interstitial cells into coated pits at the surface of adjacent macrophages. Although not considered in detail, they did observe that these structures perhaps indicate "some predatory activity on the latter's part."

Knowing all these facts leads us to extrapolate that the surface features described in this paper might have important functional ramifications. Further study of this potential mechanism of cell-to-cell recognition in embryonic systems is necessary to further implicate and understand its potential role in normal developmental mechanisms and also, appreciate its importance in cases where defective morphogenesis can lead to malformations.

REFERENCES

1. CHRISTENSEN, A. K., AND S. W. GILLIM. 1969. The correlation of fine structure and function in steroid-secreting cells, with emphasis on those of the gonads. Pages 415–488 in K. W. MCKERNS, Ed., *The Gonads.* Appleton–Century–Crofts, New York.
2. D'AMATO, C. J., AND S. P. HICKS. 1981. Prenatal genetic aqueduct stenosis causing hydrocephalus. *J. Neuropathol. Exp. Neurol.* **40:** 358.
3. D'AMATO, C. J., AND S. P. HICKS. 1981. Hydrocephalus produced by prenatal irradiation. *Soc. Neurosci. Abstr.* **7,** 467.
4. D'AMATO, C. J. 1982. Regeneration and recovery in the fetal nervous system after radiation injury. *Exp. Neurol.* **76:** 457–467.
5. GLOVER, R. A., C. J. D'AMATO, AND S. P. HICKS. 1983. Recovery or malformation (hydrocephalus) following X-irradiation: neuroepithelial–mesenchymal basal lamina relations. *Soc. Neurosci. Abstr.* **9:** 593.
6. GLOVER, R. A., C. J. D'AMATO, AND S. P. HICKS. 1983. Basement membrane–neuroepithelial interrelations in the etiology of genetic aqueductal stenosis with hydrocephalus. *Neurology* **33:** 221.
7. GOLDSTEIN, J. L., R. G. W. ANDERSON, AND M. S. BROWN. 1979. Coated pits, coated vesicles, and receptor mediated endocytosis. *Nature (London)* **279:** 679–685.
8. HICKS, S. P., C. J. D'AMATO, AND R. A. GLOVER. 1983. Recovery or malformation after fetal radiation and other injuries. In C. R. ALMLI AND S. FINGER, Eds., *The Behavioral Biology of Early Brain Damage.* Academic Press, New York, in press.
9. MOSCONA, A. A. 1976. The cell surface and cell recognition in embryonic morphogenesis. Pages 151–168 in J. W. LASH AND M. M. BURGER, Eds., *Cell and Tissue Interactions.* Raven Press, New York.
10. ROTH, T. F., AND K. R. PORTER. 1962. Specialized sites on the cell surface for protein uptake. Pages LL–4 in S. S. BREESE, JR., Ed., *5th International Congress for Electron Microscopy.* Vol. 2. Academic Press, New York.