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Regulation of Gamma-Glutamyl Transpeptidase and Alkaline Phosphatase Activities in Immortalized Rat Brain Microvessel Endothelial Cells

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Rat brain microvessel endothelial cells were immortalized by transfection with a plasmid containing the E1A adenovirus gene. One clone, called RBE4, was further characterized. These cells display a nontransformed phenotype and express typical endothelial markers, Factor VIII-related antigen and *Bandeiraea simplicifolia* binding sites. When RBE4 cells were grown in the presence of bFGF and on collagen-coated dishes, confluent cultures developed sprouts that extend above the monolayer and organized into three-dimensional structures. The activity of the blood-brain barrier-associated enzyme, gamma-glutamyl transpeptidase (γGTP), was expressed in these structures, not in the surrounding monolayer. Similar results were obtained with the microvessel-related enzyme alkaline phosphatase (ALP). Addition of agents that elevate intracellular cAMP reduced the formation of three-dimensional structures, but every cell inside the aggregates still expressed γGTP and ALP activities. Such structures, associated with high levels of γGTP and ALP activities, were also induced by astroglial factors, including (1) plasma membranes from newborn rat primary astrocytes or rat glioma C6 cells, (2) C6 conditioned media, or (3) diffusible factors produced by primary astrocytes grown in the presence of, but not in contact with RBE4 cells. RBE4 cells thus remain sensitive to angiogenic and astroglial factors for the expression of the blood-brain barrier-related γGTP activity, as well as for ALP activity, and could constitute the basis of a valuable in vitro model of the blood-brain barrier.

Cerebral capillary endothelial cells, pericytes, and astrocytes are the main cellular components of the microvasculature of the central nervous system. These endothelial cells form a selective permeability barrier between blood and brain and display an unique phenotype, characterized by the presence of a continuous network of complex tight junctions, the lack of fenestrations and vesicular transcytosis, and the expression of asymetric transport systems and specific enzymes, such as gamma-glutamyl transpeptidase (γGTP) and alkaline phosphatase (ALP) (DeBault and Cancilla, 1980; Goldstein and Betz, 1983; Pardridge, 1988; Brightman, 1989).

The signals that induce endothelial cells to express the blood-brain barrier phenotype are believed to result from specific interactions between capillary endothelial cells and the ensheathing perivascular astrocytes (Stewart and Wiley, 1981; Janzer and Raff, 1987). The in vitro study of the molecular mechanisms of this induction has been dependent so far on the availability of pure primary cultures of brain microvessel endothelial cells (Abbott et al., 1992; Joo, 1992). Considering the large variability in the starting material, the rapid senescence observed after passages and/or the paucity of pure endothelial cells available in primary cultures, we aimed at the production of an immortalized cellular clone displaying a stable, non-transformed phenotype.

In the present report, we describe the production of an immortalized cellular clone (RBE4) by transfection of rat brain microvessel endothelial cells with a plasmid containing the E1A adenovirus gene. These cells display a nontransformed endothelial phenotype and retain the sensitivity to astroglial factors for the expression of three-dimensional structures expressing the brain microvessel-associated enzymes γGTP and ALP.

MATERIALS AND METHODS

Collagenase/dispase, fetal calf serum, fibronectin, and basic fibroblast growth factor were obtained from Boehringer (Mannheim, Germany), and media from

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