A Unique Fibrillar Coat on the Surface of Migrating Human Primordial Germ Cells

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Summary. Primordial germ cells (PGCs) were studied by electron microscopy in human embryos at 27 and 30 days of gestation.

PGCs were mainly found in the hindgut epithelium, some at the stage of separation from the endoderm, and others in migration through the mesenchyme of the dorsal mesentery between the primitive intestinal wall and the coelomic epithelium, including the coelomic angle. A few PGCs were still located in the endodermal epithelium. Before the process of separation and migration, PGCs appeared clearly different from neighboring somatic cells (endodermal, mesenchymal and epithelial cells of the hindgut and the coelomic layer). PGCs located in the endodermal epithelium were large and showed an irregular cell body often provided with filopodia-like processes that contacted somatic cells to form small areas of focal junctions. As a rule, PGCs possessed a large, round nucleus containing one or two conspicuous nucleoli. Lipid droplets, abundant glycogen particles, ribosomes and mitochondria were often observed in their cytoplasm.

A detailed ultrastructural analysis revealed a delicate fibrillar coat frequently present on the free surface of PGCs during their migratory phase through the mesenchyme of the dorsal mesentery. This surface coat, likely corresponding to a glycocalyx, appeared as a particular, conspicuous filamentous layer of 30 nm thickness, mainly in the leading pseudopodial projections of PGCs migrating toward their target.

The surface coat of PGCs can be associated with the binding sites of specific macromolecular components of the extracellular matrix, including fibronectin, and hence plays a role in PGCs recognition and migration during this special embryonic phase.

Therefore, it is suggested that PGCs, with their fibrillar surface coat, might be able to provide their own substrate-adhesion or cell recognition molecules which are a necessary prerequisite for cell movement through the migratory pathway in the mesentery toward the genital anlage.

The primordial germ cells (PGCs) were first identified in humans extragonadally, near the root of the allantois and close to the vitelline sac. These cells become incorporated into the developing gut, from which they migrate, through the dorsal mesentery, to the gonadal anlage (WITSCHI, 1948; FUJIMOTO et al., 1977).

Active locomotion of human PGCs was demonstrated *in vitro* (KUWANA and FUJIMOTO, 1983). These observations seem to indicate that, for the mesenchymal interstitial migration of human PGCs to take place *in vivo*, active movement of the PGCs is required. During the migratory phase, the PGCs undergo changes in shape. Further studies of human PGCs, employing scanning and transmission electron microscopy, showed membrane extensions and pseudopodia-like processes, often containing microfilaments and microtubules (FUKUDA, 1976; MIYAYAMA et al., 1977; MOTTA and MAKABE, 1982; MAKABE et al., 1989). A characteristic, amoeboid shape was noted ultrastructurally by FUJIMOTO et al. (1977) in human PGCs. These authors suggested that, after separation from the primitive intestinal epithelium, human PGCs can migrate *in vivo* by the active elongation of their cell body in the direction of their target. Thus, considering the morphological features of the PGCs *in vivo*, these authors speculated that amoeboid movement is at least one important mechanism for the migration of human PGCs.

More recently, researcher interest has focused on the close associations between the PGCs and the extracellular matrix on the PGCs' migratory route, and on the mechanism that controls PGCs' adhesion and migration to their target. After separation from the endoderm, human PGCs encounter an extracellular milieu that influences their progression and final settlement in the genital ridge. It has been shown at

least in mice that fibronectin, a large extracellular glycoprotein associated with the cell surface, is among the components of the pathways of PGC's migration (FUJIMOTO et al., 1985).

Fibronectin has been shown to mediate cell migration both *in vitro* and *in vivo* (YAMADA, 1983). In particular, it induces the migration of previously immobile cells (ALI and HYNES, 1978). It seems that the primary role of fibronectin is to promote cell-to-substrate adhesion and migration (YAMADA and OLDEN, 1978). The mode of interaction of fibronectin with the cell membrane of PGCs in mammals is unknown. This study provides cytological evidence of the interactions occurring between specific domains on the PGCs and embryonic mesenchymal cells. Furthermore, our results provide strong evidence for the existence of a characteristic surface coat likely corresponding to a glycocalyx (BENNETT, 1963; ITO, 1974) formed of a network of fibrillary components at the surface of PGCs.

Fig. 1. Semithin section of the hindgut (G) and mesentery (M) of a 27-day-old human embryo, as seen in a transverse section. The endodermal epithelium (arrows) and mesenchyme (m) are bulging into the coelomic cavity (CC). A aorta. $\times 100$

MATERIALS AND METHODS

In this study two human embryos obtained from tubal pregnancies and estimated to at 27 and 30 days gestation were analyzed. The whole embryos were fixed for electron microscopy in 3% glutaraldehyde in 0.2 M phosphate buffer at pH 7.2, and then post-fixed in 1% osmium tetroxide in 0.2 M phosphate buffer at pH 7.4, for approximately 1 h. After dehydration with gradual concentrations of ethanol, the specimens were embedded in Epon 812. Serial sections 1 μ m thick were cut and stained with toluidine blue for light microscopic observations. Serial thin sections were made on selected areas of each embryo. The sections were stained with uranyl acetate and lead citrate (REYNOLDS, 1963), and examined with a Philips 301 electron microscope.

RESULTS

In both embryos, before separation (FUJIMOTO et al., 1977) and thereafter, PGCs clearly differed in appearance from neighboring endodermal epithelial and mesenchymal cells.

In the 27-day-old embryo (Fig. 1), numerous PGCs were found in the hindgut epithelium (Fig. 2a), some escaping from the epithelium (Fig. 2b), and a few migrating through the mesenchyme of the dorsal mesentery (Fig. 2c).

Under the electron microscope, the PGCs lodged in the endodermal epithelium (Fig. 3) were large, irregular in shape, and showed round and extremely large nuclei with conspicuous nucleoli. Numerous filopodia-like processes were also present. Their cytoplasm contained numerous lipid droplets and glycogen granules, and the cytoplasmic matrix showed a low electron density when compared with the matrix of neighboring somatic cells. Small areas of focal junctions were observed where the filopodia-like processes contacted somatic cells (Fig. 3).

In the 30-day-old embryo, most of the PGCs were found migrating through the mesenchyme of the dorsal mesentery (Fig. 2d), between the primitive intestinal wall and coelomic epithelium. Some PGCs were found at the coelomic angle. A few PGCs still persisted at the endodermal epithelium, while none at all were found in the gonadal primordium.

The basal lamina of the endodermal epithelium was continuous, except where PGCs in the course of separation protruded from it. Irregular cytoplasmic processes, similar to pseudopodia, were frequently seen escaping through the breaks in the basal lamina of

Fig. 2 a-c. Twenty-seven-day-old human embryo. Semithin sections stained with toluidine blue, showing the relationship between primordial germ cells and their surrounding mesenchymal cells (*m*). **a.** Primordial germ cells (*arrows*) before separation from the endodermal cell (*EC*) of the hindgut epithelium (*G*). $\times 640$. **b.** Primordial germ cell at the time of separation from the endodermal cell (*EC*). Note that a break in the thin basal lamina (*arrow*) is produced at the site of the cell projection pole. $\times 640$. **c.** Migrating primordial germ cell (*arrows*) located between mesenchymal cells of the dorsal mesentery. *E* endoderm. $\times 640$. **d.** Thirty-day-old embryo. Numerous primordial germ cells (*arrows*) are seen migrating in groups throughout the dorsal mesentery. $\times 640$

the gut epithelium into the outer mesenchyme (Fig. 2b).

The features of PGCs located in the dorsal mesentery (Fig. 4) were quite similar to those of PGCs observed in the endodermal epithelium. Mesenchymal PGCs were usually surrounded by adjacent somatic cells possessing elongated irregular cytoplasmic processes, reflecting the close association between them. These migrating PGCs were characterized by their large size (15–20 μm in diameter) and by a big nucleus containing one or two conspicuous nucleoli which occupied the greater part of the cell. The nucleus was generally round, irrespective of the cell shape, except when the PGCs were escaping from the gut epithelium. The cytoplasm of the migrating PGCs contained abundant glycogen particles which often formed large groups (Fig. 4). In addition, the cytoplasm possessed numerous lipid droplets, ribosomes and mitochondria, among other cytoplasmic organelles. No intravascular PGCs were observed in the blood vessels located in the mesenchyme of the two embryos analyzed.

A detailed subcellular analysis of the PGCs revealed that a characteristic surface coat was frequently present on the plasmalemma of migrating

PGCs (Fig. 5). This cell coat appeared as a filamentous lamina having a very constant thickness of 30 nm (Fig. 6). As a rule, this coat was observed in the leading projection of PGCs (Fig. 7) migrating through the mesenchyme toward their target. The surface coat consisted of a smooth layer of fine filaments arranged to form a very delicate net, and attached to the outer surface of the plasma membrane (Fig. 8). The smooth outer limits of the surface coat were likely the consequence of a partial loss of the glycocalyx during specimen preparation.

DISCUSSION

Our results, obtained by means of electron microscopy, clearly demonstrated that a surface coat likely corresponding to the glycocalyx originally described by BENNETT (1963) was only present on the surface of human PGCs that were migrating through the mesentery. Extraneous coats having features similar to those observed in this work have also been demonstrated in other tissues, where they were considered to be a cell product, and were directly associated

Fig. 3. Thin section showing part of the endodermal epithelium of a 27-day-old embryo. The primordial germ cell (PGC) is surrounded by somatic cells (SC). Note the close contact between the PGC and somatic cell membranes (*arrows*). L Lipid glycogen, *gl* granules. $\times 11,400$

Fig. 4. Low magnification of two primordial germ cells (PGC) during the migratory phase through the somatic cells (SC) of the dorsal mesentery. Close contact between the two PGCs is shown (*small arrows*). Note that the cytoplasm contains glycogen granules and lipid droplets (L). Numerous cytoplasmic projections of PGCs are observed (*big arrows*). *nl* Nucleolus. $\times 6,700$

Fig. 5. High magnification of the projecting pseudopodium of a migrating primordial germ cell (PGC). *Arrows* indicate a delicate cell coat. *N* nucleus. $\times 35,000$

Fig. 6. Electron micrograph of a portion of a migrating primordial germ cell. A very regular and constant cell coat is observed (*arrows*). *N* nucleus. $\times 30,000$

Fig. 7. Electron micrograph of a thin section through a migrating primordial germ cell. At the cell surface, a cell coat appears as a filamentous structure with a very regular thickness (*arrows*). *N* nucleus, *L* lipid, *gl* glycogen granules. $\times 45,000$

Fig. 8. Higher magnification of the rectangle shown in Figure 7. The delicate net of fine filaments attached to the outer surface of the plasma membrane (*arrows*) is observed. *L* lipid, *gl* glycogen granules. $\times 90,000$

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Figs. 5-8. Legends on the opposite page.

with the outer leaflet of the cell membrane (ITO, 1974). These coats normally represent glycosaminoglycans associated with protein, and are involved especially in cell recognition, cell adhesion and cell migration. From their glycoprotein nature, this cell surface differentiation has been associated with the binding sites of specific macromolecular components of the extracellular matrix (McCLAY and ETTENSOHN, 1987).

The occurrence of a unique fibrillar coat on the surface of mammalian or human PGCs having the same ultrastructural features of a typical glycocalyx (FAWCETT, 1981) has never been reported before. Therefore, this surface differentiation occurring in migrating human PGCs — even though solely detected by using electron microscopic techniques — may nevertheless be relevant to explain the mechanisms of PGCs adhesion and migration. In the last few years, the interest of investigators has been focused especially on the factors that control the adhesion and guidance of PGCs to the gonadal anlage. This migration process, which in humans occurs through the mesenchyme of the dorsal mesentery and throughout the area of the developing gonadal ridge, is achieved by active movements of the PGCs (FUJIMOTO et al., 1988; MAKABE et al., 1989; PEREDA and POZO, 1989). In mice, this migratory activity of PGCs has been clearly shown to be controlled by the extracellular matrix (HYNES, 1981; DONOVAN et al., 1987).

Immunocytochemical studies have demonstrated that fibronectin may be involved in the control of cell migration (McCLAY and ETTENSOHN, 1987). Fibronectin is a glycoprotein which is associated with the cell surface or related to the extracellular matrix, where it plays a significant role in cell adhesion and movement. PGCs, as well as other mesenchymal cell types, also utilize fibronectin as a preferred substratum for migration (HYNES, 1981; HEASMAN et al., 1981; BOUCAUT and DARRIBERE, 1983). The work by MAYER et al. (1981), who studied chick embryogenesis by immunocytochemistry, described the presence of fibronectin on the surface of moving cells, and also at the extracellular matrix. FUJIMOTO et al. (1985), using similar techniques, showed that fibronectin was in the pathway of the migration of PGCs and was localized on leading pseudopodia of the mice PGCs. They suggested that fibronectin and other components of the extracellular matrix probably contribute significantly to the migration of PGCs in the human. Fibronectin is probably used as an adhesive substrate by the migrating cells. However, the mechanism involved in the interaction between fibronectin and the cell surface has yet to be defined.

Our results clearly demonstrated the presence of a

cell coat organized in a fibrillary network at the surface of embryonic PGCs and which greatly resembled a glycocalyx (FAWCETT, 1981). Therefore, also in this case, it is likely that the fibrillary protein components of the surface coat could be the sites for fibronectin activity, which in turn may play a role in cell recognition and migration of PGCs toward the genital anlage. Further immunocytochemical methods will certainly characterize in detail the chemical surface components and the possible involvement of fibronectin in the cell coat of PGCs detected in this study.

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