Isolation and Culture of Arterial Smooth Muscle Cells From Human Placenta

Courtney E. Leik, Amy Willey, Martin F. Graham, Scott W. Walsh

Abstract—A simple and economical technique was developed to isolate and culture human arterial smooth muscle cells from chorionic plate vessels. Placentas from healthy women were collected at the time of term delivery. Chorionic plate arteries were identified, excised, and cut into small pieces. An explant technique was used to grow cultures of placental arterial smooth muscle (PASM) cells. Small pieces of vessel with lumens down were placed in 100-mm culture plates and grown in Dulbecco modified eagle medium and 10% fetal bovine serum. Cells appeared from explants within 1 week and grew to confluence in approximately 4 weeks. At confluence, PASM cell cultures had a uniform cell morphology that was characterized by elongated cells in parallel rows, typical of smooth muscle cells. Smooth muscle cell phenotype was evaluated by morphology and by immunoblotting and immunofluorescence of smooth muscle myofilament proteins. All PASM cell cultures expressed α-smooth muscle actin, β-tropomyosin, and h-caldesmon. Expression was similar to that of human aortic smooth muscle cells, but not to endothelial cells or fibroblasts. PASM cells stained uniformly for α-smooth muscle actin and lacked staining for a fibroblast-specific antigen. PASM cells were evaluated for their response to inflammatory mediators, tumor necrosis factor-α, and interleukin-1β by measurement of interleukin-8 production. Cells cultured for 18 hours showed a progressive increase in interleukin-8 production with time. Treatment with inflammatory mediators increased interleukin-8 production by 3-fold as compared with media control. This technique provides a simple method to obtain normal human arterial smooth muscle cells for in vitro studies of physiology and pathophysiology. (Hypertension. 2004;43:837-840.)

Key Words: vascular smooth muscle ■ interleukins ■ tissue culture

The availability of normal human arterial smooth muscle cells for experimental studies is limited. Primary cultures of vascular smooth muscle cells can be difficult to obtain and grow, and commercially available cells are expensive. Vascular tissue can be obtained from various organs during organ transplant procedures,1–3 but access to this tissue is limited. Furthermore, the age of the donor is often advanced and health problems or death of the donor may have altered the phenotype of the cells. There is always a question as to whether donor cells are truly normal. Consequently, many investigators use arterial smooth muscle cells obtained from animal vessels.2–4–6 A readily available source of normal human arterial smooth muscle cells for in vitro studies would be useful.

Recently, we demonstrated increased vascular smooth muscle expression of interleukin-8 (IL-8), a potent chemotactic agent for neutrophils, coincident with neutrophil transendothelial migration into the systemic vasculature of women with preeclampsia, a hypertensive disorder of pregnancy.7 Increased vascular smooth muscle expression of IL-8 indicated inflammation and dysfunction of vascular smooth muscle in preeclampsia, but the cause of this dysfunction is unknown.

The purpose of this study was to develop a simple and economical technique for the isolation and culture of human arterial smooth muscle cells and to characterize the phenotype of these cells. Such cells would be of value for in vitro experiments to explore mechanisms for vascular smooth muscle cell dysfunction. This has been performed for endothelial cells, which are commonly used for cardiovascular studies, by isolating endothelial cells from human umbilical veins. We applied a similar rationale and developed a simple and economical technique to isolate and culture arterial smooth muscle cells from placental chorionic plate arteries. Smooth muscle cell phenotype was verified by surveying expression of smooth muscle cell myofilamentous proteins and functionality of the cells by responsiveness to inflammatory mediators.

Methods

Placental Arterial Smooth Muscle Cell Isolation and Culture

Placentas were collected at term deliveries from normal pregnant women at MCV Hospitals of Virginia Commonwealth University Medical Center. Informed consent was obtained before delivery.

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This study was approved by the Virginia Commonwealth University Office of Research Subjects Protection. Procedures were in accordance with institutional guidelines.

Under sterile conditions, chorionic plate artery explants were treated with a solution of 1% BSA in PBS without serum for 24 hours. This was performed to eliminate any contaminating cells, such as fibroblasts and endothelial cells, because these cells do not survive without serum. After 24 hours, placental arterial smooth muscle (PASM) cells were placed in Dulbecco modified eagle medium with 10% fetal bovine serum for 4 days and grown to confluence in 100-mm culture plates with 10 mL medium. PASM cells and fibroblasts were seeded on sterile coverslips in 24-well plates. Primary cultures of PASM cells and fibroblasts were cultured according to the supplier’s instructions, and HUVECs were cultured according to standard procedures for endothelial cells. PASM cells were seeded and grown to confluence in 100-mm culture plates with 10 mL medium (M199) and 10% fetal bovine serum. PASM cells were harvested 6 days after the last addition of fresh media and serum. At confluence, cells were removed and replaced with fresh media twice per week. Culture growth and cell morphology were examined daily using an inverted light microscope. Cells started growing from explants within 1 week. At 2 weeks, the volume of media per 100-mm culture plate was increased to 10 mL because of increased numbers of cells.

Cell cultures became confluent in approximately 4 weeks. At confluence, cells were placed in Dulbecco modified eagle medium without serum for 24 hours. This was performed to eliminate any contaminating cells, such as fibroblasts and endothelial cells, because these cells do not survive without serum. After 24 hours, placental arterial smooth muscle (PASM) cells were placed in Dulbecco modified eagle medium with 10% fetal bovine serum for trypsinization, passage, or freezing, according to standard protocols. PASM cells were used between passages 2 and 7.

Verification of Vascular Smooth Muscle Cell Phenotype

Western Blot

Vascular smooth muscle cell phenotype for all PASM cells was verified by immunoblotting for smooth muscle myofilament proteins: α-smooth muscle actin, β-tropomyosin, and h-caldesmon. Human aortic smooth muscle (HASM) cells (Cambrex, East Rutherford, NJ), adult dermal fibroblasts (Cambrex), and human umbilical vein endothelial cells (HUVEC) were evaluated for comparison. HUVECs were isolated from umbilical veins as previously described and verified as endothelial cells by cobblestone morphology that was characterized by elongated cells in parallel rows, typical of smooth muscle cells. PASM cell morphology was characterized by uniform round, parallel rows, typical of smooth muscle cells.

Results

Cells were observed growing from chorionic plate vessel explants within 1 week (Figure 1a). Primary cultures of PASM cells grew to confluence in approximately 4 weeks (Figure 1b). At confluence, PASM cell cultures had uniform cell morphology that was characterized by elongated cells in parallel rows, typical of smooth muscle cells. PASM cell cultures also showed evidence of overlapping layers forming ridges, which is characteristic of smooth muscle cell cultures. PASM cell morphology was retained after several passages.
All PASM cell cultures expressed smooth muscle myofilament proteins: α-smooth muscle actin (MW: 42 kDa), β-tropomyosin (MW: 36 and 39 kDa), and h-caldesmon (MW: 150 kDa) (Figure 2). PASM cultures showed similar protein expression to that of HASM cells. HUVECs and fibroblasts did not express α-smooth muscle actin or h-caldesmon.

PASM cell cultures showed uniform immunostaining for α-smooth muscle actin (Figure 3a). PASM cell cultures did not stain for a fibroblast-specific protein (Figure 3c), which strongly stained fibroblast cultures (Figure 3e). Panels b, d, and f of Figure 3 show staining for f-actin to identify cells in panels a, c, and e, respectively. Negative controls for α-smooth muscle actin and fibroblast-specific protein had no staining (data not shown).

PASM cells produced IL-8. A time-course for PASM cells cultured overnight in M199 showed a progressive increase in IL-8 production (Figure 4). Stimulation with TNF-α or IL-1β significantly increased IL-8 production by PASM cells and HASM cells as compared with media control (Figure 5a and 5b, respectively).

Discussion

In this study, we describe a simple and economical method to obtain human arterial smooth muscle cells for in vitro studies using chorionic plate arterial explants. PASM cells started growing from explants within 1 week and cultures reached confluence in approximately 4 weeks. At confluence, primary cultures of PASM cells had uniform cell morphology that was characterized by elongated cells in parallel rows.

Immunoblotting and immunostaining indicated that the established primary PASM cell cultures were vascular...
smooth muscle cells and were not contaminated with endothelial cells or fibroblasts. All PASM cell cultures expressed smooth muscle contractile proteins: α-smooth muscle actin, β-tropomyosin, and h-caldesmon. HASM cells showed similar expression to PASM cells, while HUVECs and fibroblasts did not express α-smooth muscle actin or h-caldesmon. PASM cells showed uniform immunostaining of α-smooth muscle actin and lacked immunostaining of a fibroblast-specific antigen.

PASM cells were responsive to inflammatory mediators as evidenced by production of IL-8. A time-course for PASM cells cultured overnight showed a progressive increase in IL-8 production. Stimulation with TNF-α or IL-1β increased IL-8 production by PASM cells and HASM cells, demonstrating that PASM cells respond similar to HASM cells and that they were functionally active and responsive to inflammatory mediators.

Perspectives
These data suggest that PASM cells are an alternative to donor or commercially available arterial smooth muscle cells. Placentas are readily available and an inexpensive source of arterial smooth muscle. Donor age and questionable health problems are not a concern because medical information regarding the health of the mother and sex of the baby are easily obtained. In conclusion, we have established a PASM cell isolation technique, which is a simple and economical method to obtain normal human arterial smooth muscle cells for in vitro studies. PASM cells, like HUVECs, may prove useful for the study of cardiovascular disease.

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References
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