19. A Migration Stimulating Factor for Vascular Endothelial Cells is released by Cultured Astrocytes

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Introduction. In the brain, the abluminal surface of microvessels is almost entirely covered by the foot processes of astrocytes,1,2 and this is supposed to be the structural basis for the induction and the maintenance of the blood-brain barrier by astrocytes.3 However, the mechanism by which the cerebral microvessels are surrounded by the processes of astrocytes has been completely unknown up to now.

In the present study, we demonstrate that cultured astrocytes from cerebrum of rat embryos secrete a factor (or factors) which has potent migration stimulating activity for vascular endothelial cells.

Materials and methods. Primary culture of astrocytes. Astrocytes were prepared from cerebra of 19-day-old rat embryos as previously described.4 They were grown in tissue culture flasks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) until confluence was achieved. Ninety-two percent of these cells were labelled by an antiserum against glial fibrillary acidic protein (GFAP), a useful marker for astrocytes.5 To obtain conditioned medium, confluent astrocytes were washed twice with sterile phosphate-buffered saline (PBS), and incubated in a medium containing 0.5% bovine serum albumin (BSA) for 4 days. The resultant conditioned medium was then collected and passed through a 0.22 µm filter to remove cellular debris and was stored at −80°C until required.

Assay of endothelial cell migration. Endothelial cells were isolated from bovine carotid arteries as described,6 and maintained in DMEM supplemented with 10% FBS. Migration of endothelial cells was determined in a 48-well microchemotaxis chamber (Neuroprobe, Bethesda, MD). Nucleopore membrane filters of 10 µm thickness and 8 µm pore size were used. The lower wells were filled with conditioned medium (diluted or undiluted) or DMEM containing 0.5% BSA (control) and the upper wells were filled with cell suspensions (2×10⁴ cells/50 µl of DMEM containing 0.5% BSA/well). Incubation was for 4–6 hours at 37°C in a humidified incubator gassed with 5% CO₂/95% air. The number of cells that migrated to the opposite side of the filter was counted after hematoxylin staining.

Results. As shown in Fig. 1, conditioned medium of cultured astrocytes stimulated the migration of bovine arterial endothelial cells in a dose-dependent way. Fig. 2 shows the difference between the migration of endothelial cells in a gradient, with conditioned medium present only in the bottom well (b), and in the absence of a gradient, when conditioned medium was present above and below the cells (c). It will be noticed that although migration was highest in a gradient, implying a chemotactic effect, there was also some stimulation in the absence
Effect of conditioned medium of cultured astrocytes on migration of vascular endothelial cells. Values are the mean number of migrated cells ± s.e.m.; **, p<0.01; (significantly different from control); n=4-6. 0, control (Dulbecco’s modified Eagle’s medium (DMEM) containing 0.5% BSA). 1/5, conditioned medium five-fold diluted with DMEM containing 0.5% BSA. 1/2, two-fold diluted conditioned medium. 1, undiluted conditioned medium.

Migration stimulating factor in conditioned medium of astrocytes is both chemotactic and chemokinetic to endothelial cells. Values are the mean number of migrated cells ± s.e.m.; **, p<0.01 (significantly different); n=6. a, control. b, conditioned medium in lower wells only. c, conditioned medium in both upper and lower wells.

Discussion. Our results demonstrate that astrocytes secrete a factor (or factors) that has potent migration stimulating activity for vascular endothelial

of a gradient. Presumably this was due to stimulation of random movement (chemokinesis). This factor was heat-labile (100°C, 10 min) and susceptible to trypsin digestion (0.05%, 37°C for 30 min) (data not shown). The molecular weight of this factor was estimated to be greater than 30,000, as the migration stimulating activity was recovered in the retentate, not in the filtrate, after ultrafiltration through the anisotropic membrane with a molecular mass cut off of >30,000 (data not shown).

Discussion. Our results demonstrate that astrocytes secrete a factor (or factors) that has potent migration stimulating activity for vascular endothelial
cells. Although physiological roles of this factor in brain are unknown at present, there is a possibility that astrocytes attract endothelial cells by releasing this factor and the peculiar structure of cerebral microvessels that they are wrapped by the foot processes of astrocytes is consequently formed during development and maintained thereafter. Recently, a factor that stimulates the motility of fibroblasts has been discovered and isolated from conditioned medium of fetal and breast cancer patient fibroblasts.\textsuperscript{7} A factor was also purified from conditioned medium of a human melanoma cell line which increases the motility of producer and responsive fibroblast lines.\textsuperscript{8} It has not been examined yet whether these factors affect the motility of endothelial cells or not. A factor which enhances the motility of epithelial cells has also been isolated from conditioned medium of fibroblasts.\textsuperscript{9} This factor was reported to have no effect on the motility of endothelial cells.\textsuperscript{10} Although identity of the migration stimulating factor in conditioned medium of cultured astrocytes is unknown at present, there is a possibility that this factor belongs to this group of proteins that stimulate the motility (both chemotactic and chemokinetic) of cells.

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**References**