Semliki forest virus 감염에 의한 뉴우런의 탈수초

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Demyelination of Neuron by Infection of Semliki Forest Virus

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요 약

쥐의 배아의 척수신경절로 부터 슈반세포와 뉴런 세포가 각각 배양되었다. in vitro 시스템에서 배양되었다. 항 유사분열제로 처리한 정제 뉴런 세포와 정제 슈반세포들이 공동배양 되었고 그 때 수초화 과정이 진행되었다. 수초화된 공동 배양에 Semliki forest virus를 감염시켜 탈수초화를 진행 시켰다. 우리는 수초화의 형성을 의미하는 neuropeptide Y의 항체를 이용하여 수초화와 탈수초화를 확인하였다.

ABSTRACT

Schwann cells and neuron cells from dorsal root ganglion (DRG) in embryos of rat were cultured in vitro respectively. The purified neronal cells added with anti-mitotic agents and purified Schwann cells were cocultured and then accomplished myelination processing. Infection of Semliki forest virus into this myelinated co-culture system was performed and then accomplished demyelination. We identified myelination and demyelination processing using antibody of neuropeptide Y meaning presence of myelinated neuron.

Keyword

demyelination, myelination, neuropeptide Y, Semliki forest virus

Ⅰ. INTRODUCTION

Many viruses such as Theiler's virus, mouse hepatitis virus (MHV), corona, measles, herpes viruses, and Semliki Forest virus are known as cause of inducing demyelination (meaning destruction of myelination) in nervous system of mice. Especially Semliki Forest virus infection induces a demyelinating encephalomyelitis in the central nervous system of mice. Mice and rats are use d asa nimportant model for the study of myelination and demyelination research *in vitro* and *in vivo*. Generally adult mammalian DRG neuron cells can survive and regenerate in culture.

In vitro myelination had been established by co-culturing with pure populations of primary Schwann cells and primary neuronal cells. These pure populations of primary Schwann cells and primary neuronal cells were driven from DRG of rat embryos. This method produced highly purified populations of Schwann cells and neuronal cells faster than any other conventional method.

From this research, we constructed a

population of myelinated cells with co-culture of neuronal cells and Schwann cells from DRG. After this myelinated cells were infected with Semliki Forest virus and processing of demyelination was progressed. We could identify and distinguish myelination and demyelination processing using antibody of neuropeptide Y which represented as myelinated cells.

II. MATERIALS AND METHODS

A. preparation of embryonic DRG

Embryos of mouse were decapitated with spring scissors and transfered the head to a pre-labeled 1.5 mL Eppendorf tube prepared. The tips of spring scissors vertically were inserted into the rostral end, just anterior to the vertebral column and begin making 3–4 sequential cuts from rostral to caudal (from head to tail) direction. The ganglia from the dorsal root starting from one end of the spinal cord were pinched off. All the ganglia (approximately 37–42 ganglia/spinal cord/embryo) were removed and repeated with the step for all the spinal cords. The 1.5 mL of 0.25% trypsin were added to each dish and placed the dishes into a 37℃ air incubator for 15 –20 min. It were added with 10 mL of L-15/10% FBS into each tube and centrifuged the ganglia at 50 *g* for 5 min. The pellet was added and resuspended with 10 mL of L-15/10% FBS. After centrifugation for 5 min as above, it was added with 1 mL of DRG plating medium to each tube. Using narrow-bored glass Pasteur pipettes by flaming the tips, the pellet was triturated 10–15 times and added with additional 1 mL of DRG plating medium to the cell suspension. This dissociated cells in an uncoated 35 mm dish were incubated.

B. suspension of the embryonic dorsal root ganglion cells

After 18 hr, plating of the dissociated DRG was replaced the medium with 2 mL of N2/NGF. Under the serum-free condition, fibroblast growth is suppressed while axon-associated Schwann cell precursors continue to proliferate. There is no need to change the medium. After 6-7 days, Schwann cell-neuron network forming axon bundles was transferred into culture medium.

C. purification of Schwann cells.

After collecting Schwann cell–neuron networks, the cells were pelleted by centrifugation at 200 *g* for 5 min.

The pellet was resuspend in trypsin-collagenase solution and incubated at 37 ℃ for 30 min. The reaction was stopped by adding Schwann cell medium and the cells were pelleted as above. The pellet was resupended in 1 ml of anti-Thy 1.2 antibody solution and incubated 30 min at 37 ℃. After centrifugation, pellet was resupended in 1 ml of rabbit complement solution and incubated 30 min at 37℃. Cells were plated onto a poly-L-lysine coated 100 mm culture dish in Schwann cell medium and incubated 30 min at 3 7℃. Next day, bipolar, spindle-shaped Schwann cells were changed the medium to N2-Schwann cell growth medium. Neuregulin and Forskolin contained in the growth medium start to stimulate Schwann cell proliferation while growth of any contaminating fibroblasts is suppressed under the serum-free condition. Neurons should die off within 2 days in the absence of NGF in the medium. After 6-7 days, the cells were replated onto new poly-L-lysine-coated plates at a density of 1×10^6 cells/100 mm plate.

D. addition of anti-mitotic cocktail

Cultures were incubated at 37 ℃, with 5% CO_2. 24 h later, 150 mL of NGF stock solution (40 mM of 5-fluorodeoxyuridine and uridine, 1 mM Arabinofur-anosyl Cytidine (Ara C, Sigma-Aldich, Saint Louis, MO) in NG medium) was added into each well to make the final concentration of 5-fluorodeoxyuridine/uridine 20 mM. Cultures were incubated at 37 ℃ in a 5% CO_2 incubator. After 72 hours, 2/3 of the NGF stock solution was changed to NG medium and the cultures were re-fed every 2 days with NG medium. After three medium changes, the neurons were ready for the Schwann cell addition.

E. addition of Schwann cells into DRG neuronal cell culture

Schwann cells were digested and washed once with DMEM (Invitrogen, Carlsbad, CA) containing 10% FBS, and then resuspended in C medium (MEM, 10% FBS, 2mM l-glutamine, 0.4% glucose, and 50 ng/mL 2.5 S NGF). 100 mL (approximately 200,000 cells) of Schwann cells was added to each of the DRG cultures in C media. After 3

days, the co-cultures were supplemented with 50 mg/mL of ascorbic acid to initiate basal lamina formation and myelination. Myelination was allowed to proceed for up to 21 days. The process of myelination was observed and recorded under phase contrast microscope.

F. Immunocytochemistry

To observed the formation of the myelin, the DRG neuron/SC co-cultures (14 days after initiation myelin formation) were then labeled with primary antibodies, which included monoclonal antibody (mAb) against neuropeptide Y. After three washes with PBS, the coverslips were further incubated with Alexa Fluor 488 mouse anti-rabbit IgG and Alexa Fluor 594 goat anti-mouse IgG (Invitrogen, Carlsbad, CA) for 60 min at room temperature. After a final wash in PBS, the slides were mounted with mounting fluid (DAKO Ltd., Carpenteria, CA) and visualized under a fluorescence microscope (Olympus, Tokyo, Japan). The images were digitally recorded and processed with Image-Pro Plus (Media Cybernetics, Atlanta, GA).

III. RESULTS AND CONCLUSIONS

This procedure contains following four steps: first step of suspension of the embryonic dorsal root ganglion cells, secondstep of addition of anti-mitoticcocktail, third step of purification of dorsal root cells, and fourth step of addition of Schwann cells to dorsal root ganglion cells.

As a result of this study, for formation of myelination, Schwann cells and neuronal cells, respectively, were prepared and cultured from DRG of rat embryos (E 16 day) (Figure 1).

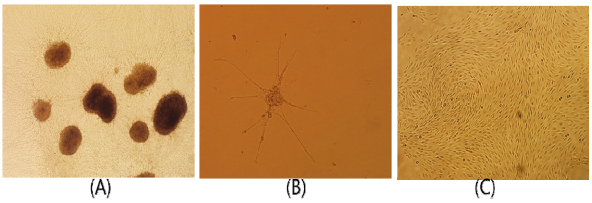

Figure 1. Purfication of populations of Schwann cells and neuronal cells, respectively, from DRG of rat embryo (E 16 day) (A: DRG cells; B: neuronal cells; C: Schwann cells).

To identify and distinguish myelination and demyelination processing, population of cells were labeled with monoclonal antibody against neuropeptide Y and observed by fluorescent microscope. Population of myelinated cells represent fluorescent spots due to monoclonal antibody against neuropeptide Y which binds myelinated proteins. On the other hand, population of demyelinated cells did not because of absence of monoclonal antibody against neuropeptide Y (Figure 2).

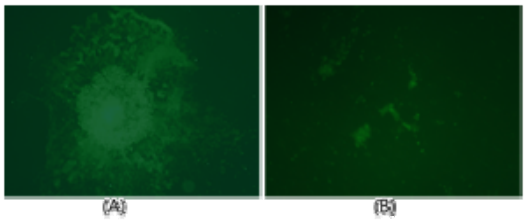

Figure 2. Identification and distinction of myelination and demyelination processing with monoclonal antibody against neuropeptide Y (A: population of myelinated cells, B: population of demyelinated cells).

In this study, myelination had been constructed by co-culturing with pure populations of primary Schwann cells and primary neuronal cells, respectively. These pure populations of primary Schwann cells and primary neuronal cells, respectively, were driven from DRG of rat embryos. Semliki Forest viruses infected and broke down myelination processing through acting to nerve cells. Presence of monoclonal antibody against neuropeptide Y labelled with fluorescent dye indicates presence of myelinated cells. Moreover, we recognize that infection with Semliki Forest virus induces demyelination processing.

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