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Communication

Induction of Demyelination by Infection of Semliki Forest Virus

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ABSTRACT: Schwann cells and neuronal cells from dorsal root ganglion (DRG) in embryos of rat were cultured *in vitro* respectively. The purified neuronal cells with anti-mitotic agents and purified Schwann cells were co-cultured 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.

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.[1] Mice and rats are used as an important 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.[3,4,5]

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.

Cultures were incubated at 37 ℃, with 5% CO_2. 24 h later, [2]50 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.[6] 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.

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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.

To observe the formation of the myelin, the DRG neuron/Schwann co-cultures (14 days after initiation myelin formation) were then labeled with primary antibodies, which included monoclonal antibody 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).

Processing for co-culture of Schwann cells and neuronal cells for myelination from DRG of rat embryos was described.[2]

This procedure contains following four steps: first step of suspension of the embryonic dorsal root ganglion cells[5], second step of addition of anti-mitotic cocktail[2], third step of purification of dorsal root cells[5], and fourth step of addition of Schwann cells to dorsal root ganglion cells[2].

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).

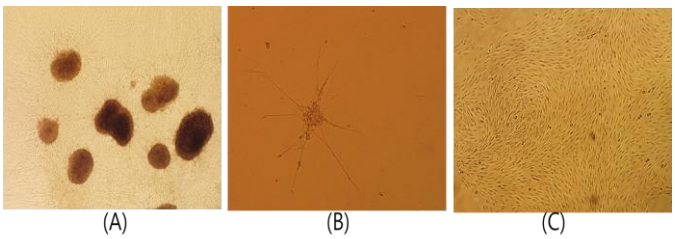

Figure 1. Purification of populations of Schwann cells and neuronal cells, respectively, from DRG of rat embryo (E 16 day) (A: DRG cells; B: neuronal cells; C: Schwann cells).

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 represents 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).

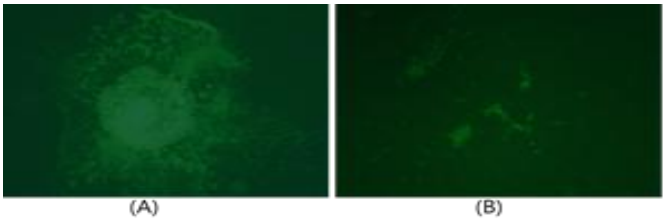

Figure 2. Identification and distinction of myelination and demyelination processing with monoclonal antibody against neuropeptide Y (A: population of myelinated cells, B: population of demyelinated cells).

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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KEYWORDS: demyelination, myelination neuropeptide Y, Semliki Forest virus

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