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# *M. leprae*의 특이 phenolic glycolipid-1 (PGL-1)를 이용한 탈수초화의 검정

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## Identification of Demyelination using *M. leprae*-specific phenolic glycolipid-1 (PGL-1)

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### 요 약

탈수초화를 위해 쥐의 배아(임신 16일)의 슈반세포와 뉴런 세포가 척수신경절로부터 *in vitro* 시스템에서 배양되었다. 항 유사분열제로 첨가된 분리 정제된 척수신경절 세포와 분리 정제된 슈반세포가 공동배양 배양되었고 배양되었고 수초화 과정이 구축되었다. 이렇게 형성된 공동 배양에 *M. leprae*-specific phenolic glycolipid-1 (PGL-1)을 처리하고 myelin basic protein (MBP)의 항체를 이용하여 탈수초화가 형성되었음을 확인하였다.

### ABSTRACT

For myelination, Schwann cells and neuron cells from dorsal root ganglion (DRG) of rat embryos (E16) were cultured *in vitro* system. The purified DRG cells with anti-mitotic agents and purified Schwann cells were cocultured and then accomplished myelination processing. Treatment of *M. leprae*-specific phenolic glycolipid-1 (PGL-1) into this coculture system was performed and then accomplished demyelination. Therefore, we identified demyelination processing using antibody of myelin basic protein (MBP).

### Keyword

demyelination, phenolic glycolipid-1, *M. leprae*, myelin basic protein

### I . INTRODUCTION

The study of Schwann cell, Neuronal cell, and myelination has been facilitated by the availability to isolate and establish pure population of primary Schwann cells. More over, mice serve as an important model for the study of Schwann cell research. The specialized source of neurons from nonneuronal cells were provided in Dorsal root

ganglia [1]. Adult mammalian DRG neuron cells can survive and regenerate in culture [2, 3, 4]. There are several researches on purified populations of these neurons. Coculture of purified DRG neurons and Schwann cells can be used in myelin formation.

The most widely used method for preparing primary Schwann cell culture uses DRG as the primary source of Schwann cells. The procedure

is very simple and produces a highly purified population of Schwann cells in a short time. The method has also been used to prepare Schwann cells from mouse embryos.

In this study, we performed a purified population of myelination by coculture of DRG neuronal cells and Schwann cells. The purified DRG cells with anti-mitotic agents and purified Schwann cells were cocultured and then accomplished myelination processing. Treatment of *M. leprae*-specific phenolic glycolipid-1 (PGL-1) into this coculture system was performed and then accomplished demyelination. Therefore, we identified demyelination processing using antibody of myelin basic protein (MBP).

## II. MATERIALS AND METHODS

### A. preparation of embryonic DRG

Embryos of mouse were decapitated with spring scissors and transferred 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°C 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 °C for 30 min. The reaction was stopped by adding Schwann cell medium and the cells were pelleted as above. The pellet was resuspended in 1 ml of anti-Thy 1.2 antibody solution and incubated 30 min at 37 °C. After centrifugation, pellet was resuspended in 1 ml of rabbit complement solution and incubated 30 min at 37°C. Cells were plated onto a poly-L-lysine coated 100 mm culture dish in Schwann cell medium and incubated 30 min at 37°C. 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  $1 \times 10^6$  cells/100 mm plate.

### D. addition of anti-mitotic cocktail

Cultures were incubated at 37 °C, with 5% CO<sub>2</sub>. 24 h later, 150 mL of NGF stock solution (40 mM of 5-fluorodeoxyuridine and uridine, 1 mM Arabinofur-anosyl Cytidine (Ara C, Sigma-Aldrich, 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 °C in a 5% CO<sub>2</sub> 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 observe 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 myelin basic protein (MBP). 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., Carpinteria, 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

Processing for coculture of Schwann cells and neuronal cells for myelination from DRG of rat embryos was described in Fig 1.

This procedure contains following four steps: first step of suspension of the embryonic dorsal root ganglion cells [4], second step of addition of anti-mitotic cocktail [1], third step of purification of dorsal root cells [4], and fourth step of addition of Schwann cells to dorsal root ganglion cells [1].

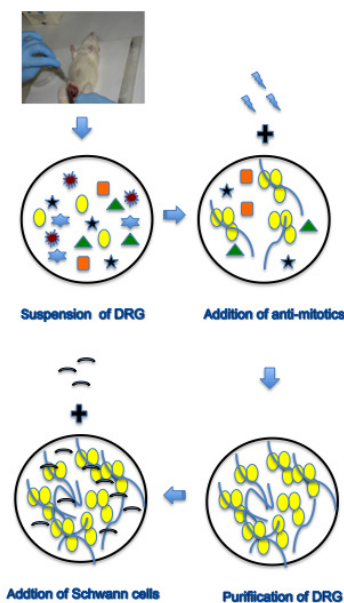


Fig. 1. Procedure for coculture of Schwann cells and neuronal cells for myelination from rat embryos.

For formation of myelination, cultured DRG cells and neuronal cells, respectively, were prepared from rat embryo (E 16 day) (Fig. 2).

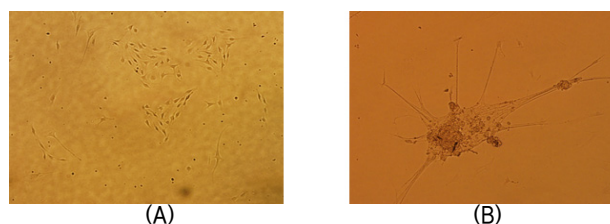


Fig. 2. Purification of cultured Schwann cells and DRG neuronal cells, respectively, from rat embryo (E 16 day). (A: Schwann cells; B: DRG neuronal cells, )

To identify the myelination formed by coculture of DRG cells and neuronal cells, cultured cells were labeled with monoclonal antibody (mAb) against myelin basic protein (MBP) and were observed by fluorescent microscope. The green-fluorescent regions represent myelinated cells (Fig. 3 A).

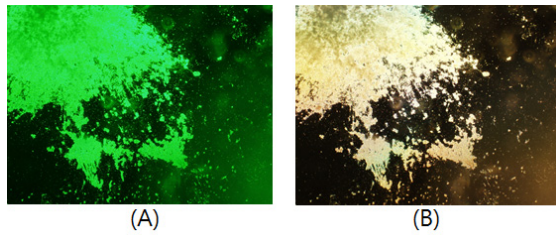


Fig. 3. Identification of myelination which is formed by coculture of DRG neuronal cells and Schwann cells (A: fluorescent microscop, B: optical microscope)

Through this study, we accomplished a purified population of myelination through this procedure and identified myelination basic protein using antibody of myelin basic protein. After treatment of *M. leprae*-specific phenolic glycolipid-1 (PGL-1) into this coculture system were also identified demyelination of this coculture in vitro system because of absence of monoclonal antibody (mAb) against myelin basic protein (MBP).

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