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Communication

Generation of Demyelination through Use of *M. leprae*-specific phenolic glycolipid-1 (PGL-1)

Ji-Young Kim,[1] Chang-Shik Choi,[2*] and Seong-Karp Hong[3*]

[1]Department of Anesthesiology and Pain Medicine, Yonsei University College of Medicine, Seoul, South Korea.
[2]Department of Food and Fermentation, Far East University, Eumseong, Chungbuk 369-851, Korea.
[3]Division of Bio and Health Sciences, Mokwon University, Daejeon, South Korea.

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).

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. Moreover, 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).

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.[5] 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.

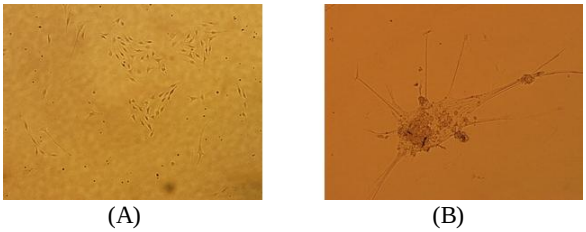
Schwann cells were digested and washed once with DMEM (Invitrogen, Carlsbad, CA) containing 10% FBS, and then resuspended in C medium (MEM, 10% FBS, 2mM l-glutamine, 0.4% glucose, and 50 ng/mL 2.5 S NGF). 100 mL (approximately 200,000 cells) of Schwann cells was added to each of the DRG cultures in C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

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].

For formation of myelination, cultured DRG cells and neuronal cells, respectively, were prepared from rat embryo (E 16 day) (Figure 1).



(A) (B)

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

***To whom correspondence should be addressed.**
E-mail: cschoi@kdu.ac.kr, karp@mokwon.ac.kr

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 (Figure 2 A).

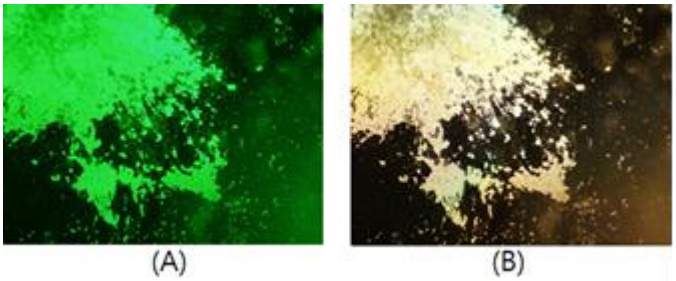


Figure 2. Identification of myelination which is formed by coculture of DRG neuronal cells and Schwann cells (A: fluorescent microscope, B: optical microscope).

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, generation of demyelination in this *in vitro* coculture system was also identified because of absence of monoclonal antibody (mAb) against myelin basic protein (MBP).

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

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