Short communication

Differential Schwann cell migration in adult and old mice: an in vitro study

Arnaldo R. Santos Jr\textsuperscript{a}, Maria Lucia F. Wada\textsuperscript{a}, Francesco Langone\textsuperscript{b}, Alexandre L.R. Oliveira\textsuperscript{c,*}

\textsuperscript{a}Department of Cell Biology, State University of Campinas–UNICAMP, CP 6109, CEP 13089-970, Campinas, SP, Brazil
\textsuperscript{b}Department of Physiology and Biophysics, State University of Campinas–UNICAMP, Campinas, SP, Brazil
\textsuperscript{c}Department of Anatomy, State University of Campinas–UNICAMP, Campinas, SP, Brazil

Accepted 8 August 2000

Abstract

The influence of aging on Schwann cell (SC) proliferation, migration and viability was studied in vitro. SCs were cultured in Ham F-10 medium enriched with 20\% fetal calf serum (FCS), 40\% FCS or collagen I gel plus 20\% FCS. The migration of adult mice derived SCs was stimulated with FCS and collagen. With aging, SC migration, multiplication and viability decreased, indicating that ideal culturing conditions should be adjusted.

Ó 2000 Elsevier Science B.V. All rights reserved.

Theme: Development and regeneration

Topic: Glia and non-neuronal cells; Aging process

Keywords: Schwann cell; Cell culture; Collagen; Cell migration; Aging; Mouse

Peripheral nerve regeneration occurs as a result of a series of events, which involve axonal regeneration and reorganization of the extracellular microenvironment [2,4,5,11]. After nerve lesion, Wallerian degeneration takes place distally to the lesion. This process is characterized by macrophage invasion and Schwann cell (SC) multiplication, mainly into the distal stump [1,2,8]. Such newly produced SCs organize themselves within the basal lamina left by degenerated axons, originating the so-called ‘bands of Büngner’.

Taking into account that with aging, nerve regeneration is less successful [6,10] and considering the importance of the SC for the development of this process, the aim of this study was to investigate in vitro its multiplication and migration capacity with aging. Also, we have investigated the importance of collagen and serum factors for SC migration and multiplication.

For this study, sciatic nerves from adult (8 months old, \( n = 5 \)) and old (2 years old, \( n = 5 \)) C57BL/6J male mice were used. After dissection, the nerves were reduced into fragments about 1 cm long and washed in Ham F-10 medium (Sigma) supplemented with 20\% fetal calf serum (FCS) and 100 \( \mu \text{g/ml of gentamicin (Schering-Plough)} \).

The fragments were then cut in smaller, 2 mm long pieces and cultured in culture plates with six wells (Corning / Costar) at 37\°C for 20 days. Three different experimental conditions were used: (1) Ham F-10 medium supplemented with 100 \( \mu\text{g/ml of gentamicin and 20\% FCS} \), (2) Ham F-10 medium supplemented with 100 \( \mu\text{g/ml of gentamicin and 40\% FCS} \), or (3) Ham F-10 medium supplemented with 100 \( \mu\text{g/ml of gentamicin and 20\% FCS} \) in well culture plates coated with 1 ml of collagen I gel. The collagen was extracted according to the method described by Schor [9] and was prepared with 0.9 ml of collagen solution, 0.05 ml of 4\% NaHCO\(_3\) and 0.05 ml of 10 times concentrated Ham F-10 medium (Sigma). Within the incubation period, the total number of migrant cells was evaluated on days 1, 2, 4, 6, 8 and 10 in all experimental conditions. On the 20th day, the culture medium was collected and the non-adherent cells were counted with an Olympus IX-50 inverted microscope with a phase contrast system.

\*Corresponding author. Tel.: +55-19-788-7391; fax. +55-19-289-3124.
E-mail address: alroliv@unicamp.br (A.L.R. Oliveira).
After 15 or 20 days of culture, the samples were fixed in 10% formalin for 1 h and washed in phosphate buffered saline (PBS) 0.1 M in pH 7.2 at 37°C. In order to block nonspecific staining, the specimens were incubated for 1 h with 1% bovine serum albumin (BSA, Sigma) in PBS, washed and the monoclonal anti-S-100 antibody (dilution 1:300) was applied. The samples were rinsed and the anti-rabbit CY-3 secondary antibody was added.

In all experimental conditions, cells from adult or old animals were able to migrate from the explants. The cell migration pattern from adult or old animals was very similar except that in general, the cells from adult animals showed a more intense migration rate than those from old animals. Also, an increase of migrating cells was proportional to the increase of FCS concentration. The cells that grew on collagen I gel showed the highest migration rate (Fig. 1).

After 15 days of culture, we observed SCs migrating from explants to the culture plate or to collagen I gels in all experimental conditions. The Schwann cells labeled with anti-S-100 antibody showed a bipolar morphology with thin- and long-cell prolongations, except in the nuclear region (Fig. 2). SC counting revealed that explants from adult animals cultured with 20% FCS plus collagen I gel or 40% FCS displayed an increase of SCs when compared to samples cultured only with 20% FCS. On the other hand, the explants from old animals showed a decreased SC number in relation to adult animals. An important finding was that the number of SCs derived from old animals did not increase substantially when the medium was enriched with FCS or collagen I (Fig. 1A).

Schwann cell autograft produced in vitro has been reported as a novel method for repairing long gaps [3,5] following extensive peripheral nerve lesions. However, with aging, there is evidence that SCs decrease in their capacity to multiply and produce neurotrophic factors, basal lamina components and myelin [12]. Considering the relative importance of SCs for nerve regeneration and taking into account that a percentage of peripheral nerve lesions occur in middle age or elderly individuals, we have investigated the behavior of SCs obtained from old mice, submitted to different experimental conditions. Under these experimental conditions, it was possible to determine if extracellular stimuli would be able to increase the survival, migration and proliferation rates of the SCs.

With regard to the migration of SCs, both adult and aged cells started migrating around the fourth day, but with different rates. Basically, migration in the old mouse-derived cells (OMDC) and adult mouse-derived cells (AMDC), when cultured with FCS 20 and 40%, was similar up to the sixth day. On the other hand, culturing with collagen stimulated SC migration only in the AMDC. These findings show that the SCs retain its migratory ability with aging but the capacity to respond to extracellular stimuli may be reduced. This fact can be related with the capacity to synthesize and express receptors for matrix components, such as collagen, fibronectin and basal lamina elements, which are essential for cell migration [7,11].

In this context, we observed that the viability of the SCs in culture was greatly increased when the medium was supplemented with FCS 40% or with collagen plus FCS 20% (Fig. 1B). Such results reinforce the hypothesis that the absence of extracellular stimuli as well as the relation with other cell types which synthesize trophic substances, as well as cytokines, may be a strong factor in changing the behavior of SCs when in vitro. With regard to the SCs from old mice, the viability assay showed that these cells are even more sensitive and almost all of them detached from the plate after 20 days of culture. Interestingly, when the medium was supplemented with FCS 40%, cell detachment was considerably reduced. The results were even better when collagen was added. Taken together, our results reinforce the fact that cultured SC behavior is altered compared to that shown during in vivo Wallerian degeneration. On the other hand, enriching the medium...
with extracellular matrix components as well as trophic substances may increase cellular viability and make the cell behavior closer to the in vivo conditions. Also, with aging, all the behavioral alterations are sharper and cellular migration as well as multiplication and viability in culture are greatly reduced. These facts should be taken into account and studied further in order to define ideal culturing conditions for SCs at different donor ages, which will be crucial for the development of nerve repair techniques employing cultured SCs.

Acknowledgements

We are grateful to Prof. Dr. Áureo T. Yamada for providing the secondary antibody used in this study. The authors are also thankful to Prof. Dr. Mary Anne H. Dolder for the careful reviewing of the writing style.

References

[8] L. Lubinska, Patterns of Wallerian degeneration of myelinated fibers in short and long peripheral stumps and in isolated segments of rat...


