

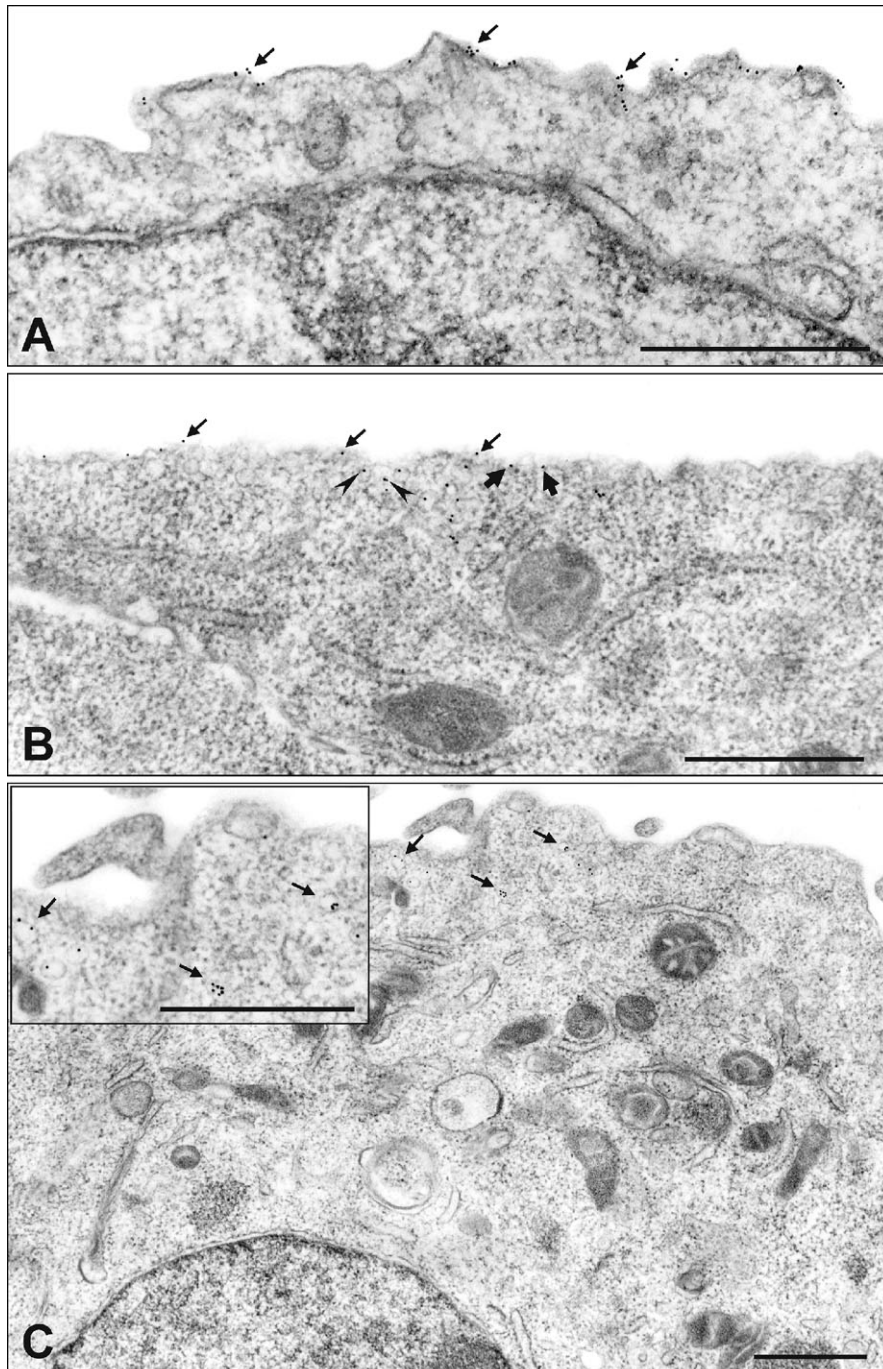
IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF THE MANNOSE RECEPTOR IN SCHWANN CELLS.

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The mannose-fucose receptor (MR) is a transmembrane glycoprotein recently included into a group of molecules termed pattern recognition receptors. These receptors act as pivotal molecules of innate immune response and homeostasis, being important for the recognition and internalization of infectious agents, and also of self-antigens [1-2]. MR is expressed in several cell types but little information is available on its expression in Schwann cells. In the present work we showed, through the binding to horseradish peroxidase (HRP) or to the neoglycoprotein mannosyl/bovine serum albumin-fluorescein isothiocyanate conjugate (mannosyl/BSA-FITC), that Schwann cells express the MR in a functional state. We have tested the occurrence and functionality of MR in a human Schwann cell line (ST88-14) isolated from patients with type 1 neurofibromatosis or in Schwann cells from explants of rat peripheral nerves (SEPN). Flow cytometry of living ST88-14 cells incubated with mannosyl/BSA-FITC demonstrated a dose-dependent increase in the number of tagged receptors on the cellular surface. Addition of D-mannose or HRP to ST88-14 cultures impaired mannosyl/BSA-FITC binding. In order to detect MR on SEPN and ST88-14, a cytochemistry assay with neoglycoprotein mannosyl/BSA-FITC (Sigma) binding was performed. The cells adhered on coverslips were fixed in 1% paraformaldehyde (Sigma) for 15 min at 4C. Afterward, the samples were washed three times in Ringer solution and incubated with 50µg/ml mannosyl/BSA-FITC diluted in labeling solution (5mM CaCl₂ diluted in Ringer solution plus 1% BSA) plus 0.1% of saponin for 1 h at 37C. Controls were obtained by incubation of the cells with 250mM D-mannose or 50µg/ml HRP diluted in labeling solution plus 0.1% of saponin, followed by wash and incubation with 50µg/ml mannosyl/BSA-FITC as described above. For unequivocal characterization of the Schwann cells, the SEPN and ST88-14 cells were double labeled with S100 and mannosyl/BSA-FITC, fixed and then examined by confocal microscopy. The MR was found widely distributed on the cell surface and, also in the intracellular domain close to the nucleus. At the optical plane of the maximal nuclear diameter, there was intense labeling for MR in regions compatible with the endoplasmic reticulum. For ultrastructural cytochemistry assay, the living ST88-14 cells adhered to 35mm Petri dishes were incubated in HRP-colloidal gold conjugate (HRP-Au) diluted 1:5 (v/v) in labeling solution. After 40 min of incubation with HRP-Au at 4C, the cultures were rinsed in Ringer solution to remove nonadhered particles. Some samples were fixed in 2.5% (v/v) glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2) at 4C for 1 h while others were maintained in labeling solution at 37C for 1 and 3 h. Negative controls were obtained by preincubating the cells with 250 mM D-mannose at 4C for 40min. Cells were scraped out of dishes on ice, centrifuged at 900 × g for 10min, and post-fixed in 1% osmium tetroxide (Sigma) in 0.1 M cacodylate buffer containing 3.5% sucrose for 30 min at 4C. Cells were then washed in the same buffer, dehydrated in acetone, and embedded in PolyBed 812. Thin sections were obtained in an OmU3 Reichert ultramicrotome, stained with aqueous 2% uranyl acetate, and examined in a Zeiss EM 10C transmission electron microscope. Ultrastructural analyses showed the distribution of large number of HRP-colloidal gold conjugate (HRP-Au) on the cell surface of ST88-14 cells after incubation at 4^oC for 40 min (Fig. 1A). Incubation at 37^oC for 1h resulted in internalization of the probe, although some particles still remained adhered to the cell surfaces (Fig. 1B). After longer incubation at 37^oC, virtually all particles were seen within small vesicles (Fig. 1C). Our current study documents for the first time the occurrence and functionality of MR in Schwann cells. These results open the possibility that MR could participate in multiple physiologic and pathologic conditions in the peripheral nervous system. Moreover, our data support the notion that Schwann cells may have a role in the immune response.

References:

- [1] Linehan et al. *Eur J Immunol* 31 (2001) 1857-1866.
- [2] Taylor et al. *Trends. Immunol.* 26 (2005) 104-110.



Ultrastructural analysis showing the localization of MR in ST88-14 cells under different experimental conditions. A. Incubation of cells with HRP-Au at 4°C for 40 min reveals numerous gold particles restricted to the cell membrane (arrows). B. The gradual increase of the temperature to 37°C for 1 hr results in gold particles being still found at the cell surface (arrows), in membrane invaginations (thick arrow) as well as within small vesicles (arrowheads) next to the internal face of the plasma membrane. C. After 3 hr at 37°C, virtually all gold particles are found in small vesicles (arrows), indicating MR internalization. The inset is a high magnification view to show some (arrows) of the numerous HRP-Au-containing vesicles. Bars = 0.5µm.