12

Isolectin IB4-Mediated Cytotoxic Targeting of Sensory Neurons

Lucy Vulchanova and Christopher N. Honda

INTRODUCTION

Lectins are carbohydrate-binding proteins that recognize distinct oligosaccharide moieties of glycoproteins and glycolipids. In vertebrates, endogenous lectins serve a variety of functions, including cell adhesion, cell recognition, signal transduction, and endocytosis, in both neuronal and nonneuronal cells *(1–5)*. A role for cell surface carbohydrate recognition has been described in processes, such as fertilization, development, leukocyte homing, and the innate immune response *(1,2)*. Among the known classes of vertebrate carbohydrate-binding proteins are some cytokines (e.g., interleukin 1β [IL-1β]) and growth factors, which possess a receptor-binding domain and a carbohydrate recognition domain *(4,5)*.

A number of exogenous lectins that recognize cell surface carbohydrates in vertebrates are used as research tools. Among these is isolectin I-B4 from *Bandaireae simplicifolia* (IB4), which recognizes terminal α-D-galactose residues of glycosphingolipids and glycoproteins. In rodents, IB4 binds to a number of cell types, including sensory neurons, activated microglia, endothelial cells, and macrophages *(6–9)*. In most cases, the identity or function of the IB4-binding site is unknown. For example, in sensory neurons, there is evidence for the existence of an IB4-binding glycolipid *(10)*, as well as IB4-binding glycoproteins *(11)*, whereas in macrophages, IB4 binds to a glycoprotein, eliciting signal transduction that leads to modulation of gene transcription *(7)*.

One of the most common research applications of IB4 is its use as a histochemical marker and neuronal tracer in dorsal root ganglia (DRG). We and

From: *Molecular Neurosurgery With Targeted Toxins*
Edited by: R. G. Wiley and D. A. Lappi © Humana Press Inc., Totowa, NJ

others have extended these applications to cytotoxic targeting of IB4-binding neurons in DRG using a conjugate of IB4 and saporin (IB4-sap) *(12–14)*. These studies were designed as a "loss-of-function" approach exploring the role of IB4-binding sensory neurons in acute and persistent pain. This chapter presents a discussion of the application of IB4-sap in pain studies.

OVERVIEW OF IB4-BINDING SENSORY NEURONS

The subset of IB4-binding DRG neurons is neurochemically, and most likely functionally, heterogeneous. The majority (85%) of these neurons also contain the enzyme fluoride-resistant acid phosphatase (FRAP) *(9,15,16)*. Although FRAP neurons are often referred to as nonpeptidergic, it has been shown indirectly that many of them contain calcitonin gene-related peptide *(16)*. A detailed characterization of the subpopulation of IB4-binding neurons showed that it includes nearly all (95%) FRAP-positive DRG neurons, all somatostatin-positive neurons, and 40% of the substance P (SP)-positive neurons *(16)*.

It has been shown that a large proportion of IB4-binding neurons express other proteins linked to nociception or chronic pain, such as the adenosine triphosphate-gated ion channel P2X3, the capsaicin receptor VR1, cyclooxygenase-1, and tetrodotoxin-resistant sodium channels *(17–22)*. Based on double-labeling experiments reported in some of these studies, it can be deduced that the presence or absence of FRAP, SP, somatostatin, P2X3, and VR1 can define as many as six neurochemical subtypes of IB4-binding neurons; the largest is FRAP/IB4/VR1/P2X3 positive.

It should be noted that histological characterization of IB4-binding neurons has been done almost exclusively in the rat, and reports have pointed out that the neurochemical signature of IB4-binding neurons in the mouse is different. For example, mouse IB4-binding neurons do not appear to express VR1 *(23)*.

Based on studies in culture, the functional properties of IB4-binding neurons are consistent with the idea that they represent a heterogeneous population, which includes nociceptors *(24–26)*. Finally, IB4-binding neurons are glial cell line-derived neurotrophic factor-dependent in adulthood, but there is functional and histochemical evidence that a fraction is also nerve growth factor responsive *(26,27)*.

When injected into the sciatic nerve, IB4 recognizes binding sites on axons and undergoes endocytosis, followed by retrograde transport to the cell body and central terminals of IB4-binding sensory neurons *(16,28–30)*. It has been