Targeting Talk: *Time Course of Targeted Toxins*

by Dr. Ronald G. Wiley

Q: How long does it take to see the cell death occurring from the use of targeted toxins using saporin? Is there a time course of hours or days?

A: Details of the time course of early events have not been extensively studied. After ricin injections into the cervical vagus nerve, the proximal nerve becomes unresponsive to electrical stimulation between 36 and 48 hours. After septal injection of 192-Saporin, hippocampal theta rhythm begins to diminish on the third postoperative day and reaches a minimum by 7 days which is maintained indefinitely. Anatomical disintegration is complete within 10-14 days after injection of most toxins.

Q: Will this time course be the same regardless of the targeted toxin used or the method of administration?

A: Presumably, injection of toxin into the vicinity of target cell bodies and dendrites should produce effects somewhat sooner than toxin injections into axonal terminal fields where retrograde axonal transport must first deliver toxin to the perikarya. In the cervical vagus, based on transport times for ricin and inhibition of toxin transport by vincristine, we concluded that fast axonal transport is involved. Colchicine co-injected intraventricularly with 192-Saporin prevents destruction of cholinergic basal forebrain neurons suggesting that fast axonal transport also is involved with i.c.v. toxin injections. Consequently, the delay introduced by injecting toxin into axon terminal fields is usually a few hours at most.

Q: What are some assays/methods to use to be able to graphically demonstrate cell death?

A: Toxin-induced cell death can be observed and documented with a variety of techniques. Often the easiest is simple Nissl staining because all of the RIP toxins (ricin, volkensin, saporin) produce profound chromatolysis that is readily apparent in Nissl stains (*i.e.* cresyl violet).

Electron microscopy can demonstrate details of neuron degeneration including loss of axon terminals at a distance from the cell body which can be useful in anatomic tracing studies.

Typically, target neurons express proteins that can be visualized with immunocytochemical techniques. Thus, immunofluorescence or peroxidase immunohistochemistry can be useful in detecting loss of staining for target molecules and co-expressed molecules in the neurons being targeted. The use of multiple markers is recommended to insure that cell loss occurred rather than down regulation of marker expression.

Advanced Targeting Systems offers these controls:

Mouse IgG-SAP (Cat. # IT-18)
serves as a control for immunotoxins that use a mouse monoclonal: 192-Saporin, anti-DBH-SAP, ME20.4-SAP, OX7-SAP

Rat IgG-SAP (Cat. # IT-17)
serves as a control for immunotoxins that use a rat monoclonal: Mac-1-SAP, mu p75-SAP

Goat IgG-SAP (Cat. # IT-19)
serves as a control for "second" immunotoxins: Mab-ZAP, Rab-ZAP

Saporin (Cat. # PR-01)
serves as an unconjugated control for targeted toxins
anti-Saporin (Cat. # AB-15) goat polyclonal
anti-Saporin (Cat. # AB-17AP) affinity purified chicken polyclonal

Mac-1, Monoclonal IgG_1 (Cat. # AB-N06)
serves as a control for Mac-1-SAP

NGFR, Rat Monoclonal IgG_1 (Cat. # AB-N02)
serves as a control for mu p75-SAP

NGFR, Mouse Monoclonal ME20.4 IgG (Cat. # AB-N07)
serves as a control for ME20.4-SAP