Targeting Talk: *Retrograde Transport*

by Drs. Douglas Lappi and Ronald G. Wiley

Q: I have a question about the issues raised in the last edition of Targeting Trends *(Volume 7, Issue 2). There is a comment "In fact, we recommend that you wait two weeks at least to see immunohistological evidence of a toxic effect after injection of a saporin toxin in vivo." Are there data to support this recommendation?*

As a researcher who utilizes your toxin products I often get asked about the time course of toxin action. It's difficult to answer because the literature is currently limited with regard to in vivo *toxin application. Any citations, advice, or comments would be greatly appreciated.*

A: Actually there's quite a bit of *in vivo* use. Or is it just that I think the glass is half full? If you search PubMed for the use of the immunotoxin 192-IgG-SAP using the terms '192' and 'saporin,' you'll get 223 hits, and all of these describe *in vivo* use.

As far as the two-week idea, you're right, it's a bit more challenging to pin that down in the literature. In our book, *Suicide Transport and Immunolesioning* (1), Ron Wiley discusses at several points the microglial infiltration that occurs and subsides by 14 days. That is what cleans out the antigens that you probably would use to demonstrate cell death--that is, they aren't there any more. You might want to see if it's in your library; it's a good basic source of info. To get a full list of articles, select the References button on the home page of the ATS website and click on some of the toxins.

As far as the process, Waite *et al*. (2) show the appearance of behavioral effects associated with neuronal loss at day four and plateauing at day 7. This coincides with the time course seen *in vitro* (3). At this point, microglia will infiltrate; this is nicely described in Seeger *et al*. (4). However, they stop at 7 days, which is probably the peak day for infiltration. Once there is complete removal of the detritus, microglia down-regulate and at 14 days, you don't see them, or the antigens that belonged to the cells that were eliminated. So that's the idea behind waiting.

Q: Do you have a product which can be used to produce retrograde lesions WITHOUT killing cells at the site of injection? What I'd like to do is to kill neurons that project to an efferent nucleus without damaging neurons in the efferent nucleus itself.

A: Making a selective retrograde neural lesion based only on the criterion that the cells to be lesioned are afferent to a particular nucleus or population of neurons, is a formidable challenge at present. Conceptually, this would seem to require a targeted toxin that was taken up only by afferent terminals and not by dendrites, cell bodies and/or axonal membranes of the neurons that are to be de-afferented.

There are some instances in which you can avoid local killing, but only in the case in which there are no receptors in that area, except from projections. So for instance, cholinergic neurons will project to the cortex. You can inject 192-IgG-SAP there; it will be taken up and eliminate basal forebrain neurons with little harm to other cortical neurons. Or you can inject anti-DBH-SAP into the spinal cord; it will eliminate brainstem neurons that project to there. Currently, this task is best suited to immunotoxins since there is little data on using neuropeptide toxin conjugates to produce retrograde lesions. If armed to kill, a growth factor such as NGF might also work, if toxin conjugation did not damage binding and intracellular trafficking of the NGF. But these are special cases (which you can find in our reference lists for these products).

Targeting presynaptic antigens that are common to all types of axon terminals would seem a dubious undertaking since success with an immunotoxin requires the target molecule be displayed on the external surface of the terminal and not be present at all on cell bodies or dendrites. I do not know of a suitable target molecule for this purpose.

Send your questions to: ats@atsbio.com

References

1. Wiley RG, Lappi DA (1994) *Suicide Transport and Immunolesioning*. R.G. Landes, Houston.
2. Waite JJ, Wardlow ML, Chen AC, Lappi DA, Wiley RG, Thal LJ (1994) Time course of cholinergic and monoaminergic changes in rat brain after immunolesioning with 192 IgG-saporin. *Neurosci Lett* 169:154-158.
3. Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, Daughters RS, Lappi DA, Wiley RG, Simone DA (1997) Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. *Science* 278:275-279.
4. Seeger G, Hartig W, Rossner S, Scliebs R, Bruckner G, Bigl V, Brauer K (1997) Electron microscopic evidence for microglial phagocytotic activity and cholinergic cell death after administration of the immunotoxin 192IgG-saporin in rat. J *Neurosci Res* 48:465-476.