January 2001
Volume 2, Issue 1

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- Merging of technologies makes everyone a winner
- IB4-Saporin, Neuroscience Antibodies
- Targeted Toxins: Safe, effective *in vivo* administration

ADVANCED TARGETING SYSTEMS

Immunolesioning: From Spinal Cord to Brain

Dr. Ann Schreihofer, University of Virginia, contributes this issue's article from the laboratories of ATS customers. Dr. Schreihofer summarizes her research with anti-DBH-SAP (Cat. #IT-03) to immunolesion specific rostral ventral medulla neurons that project to the spinal cord by injection of anti-DBH-SAP into the rat spinal cord. The toxin is taken up and retrogradely transported to the cell bodies, eliminates protein synthesis and causes cell death. She examines the effect of neuronal loss on sympathetic nerve activity and arterial pressure.

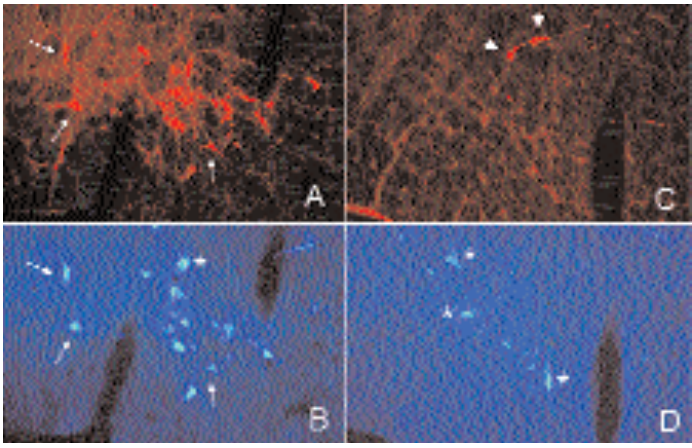


Figure Legend: Depletion of bulbospinal C1 adrenergic neurons with intraspinal injection of anti-DBH-SAP. (A) Phenylethanolamine-N-methyltransferase-immunoreactive (PNMT-ir) neurons in the RVLM from a rat after intraspinal injection of a control toxin, saporin conjugated to a mouse IgG, showing an abundance of C1 cells. (B) Same area of section in A showing many C1 neurons retrogradely labeled from intraspinal injection of Fast Blue (Arrows). (C) PNMT-ir neurons in the RVLM from a rat treated with anti-DBH-SAP showing a massive depletion of bulbospinal C1 neurons. Arrowheads indicate C1 cells with no Fast Blue, suggesting these are not bulbospinal. (D) Same area of section in C showing neurons retrogradely labeled with Fast Blue. Asterisks indicate bulbospinal neurons that are not C1 cells, which are spared by treatment with anti-DBH-SAP. The ventral surface of the medulla is at the bottom left of each photomicrograph.

The rostral ventrolateral medulla (RVLM) is an essential structure for the generation of the sympathetic tone that maintains arterial pressure (AP) and for the generation of many sympathetic reflexes. Spinally-projecting C1 neurons, whose firing characteristics resemble those of recorded sympathetic nerves, are located in the RVLM. These cells have been speculated to be the critical presympathetic neurons. However, the RVLM also contains non-catecholaminergic neurons whose properties suggest they may have an important role in the generation of sympathetic vasomotor tone. The relative roles of the C1 and non-C1 bulbospinal RVLM neurons have been difficult to determine. Until recently the selective

(continued on page 6)

ATS Joins Forces with Cytometry Research

Early in 2000, Douglas Lappi and Leo Fernandez began a series of discussions that soon led to the merging of resources, technologies and scientific expertise of their two companies: Advanced Targeting Systems, Inc. (ATS) and Cytometry Research, LLC. The two scientists quickly realized there were advantages that each company possessed that would greatly complement the other. The two companies now share laboratory and office space in the booming biotechnology community of San Diego.

Cytometry Research offers a wide range of services in flow cytometric analysis as well as consultation on current flow cytometry applications. Some of these include assays in apoptosis, cell cycle, intracellular cytokine expression, and tetramer peptides. Cytometry Research brings over 15 years of experience in flow cytometry analysis and FACS sell sorting. ATS enhances these services by providing expertise in cell culture and manipulation, use and production of fluorescent cell-binding materials, cell transfections, and cell labeling.

(continued on page 2)

ATS Joins Forces with Cytometry Research

(continued from page 1)

Leo Fernandez and Doug Lappi in their facilities in San Diego, California

ATS develops products for targeting cells based on what is presented on their cell surfaces. Cytometry Research provides the capabilities to monitor product successes. One example is the quality control of ATS antibodies for activity that is most important – binding to live cells. In addition, neuronal stem cells are important research tools and can be identified easily and effectively through FACS analysis.

Clinical trial applications are an important part of the GLP/GMP services provided by Cytometry Research. This is another of the considerations that led to the two companies joining forces. ATS is developing one of its targeting reagents, Substance P-Saporin (SP-SAP), as a therapeutic for the treatment of chronic pain. One of the most important assays under development is the binding of SP-SAP to the target SP receptor-expressing cells which can then be seen through FACS analysis. SP-SAP is added to a cell population, the cells are fixed, and then fluorescent-labeled anti-Saporin is used to see fluorescent-labeled cells. The demonstration of the presence of both active SP and immunoreactive SAP is necessary. The results show the integrity of the molecule, a vital step in pharmaceutical development.

It's clear that the two companies are strengthened by combining resources. But the scientific community benefits as well. High-quality targeting reagents and GMP/GLP services provide important tools for research and pharmaceutical development.

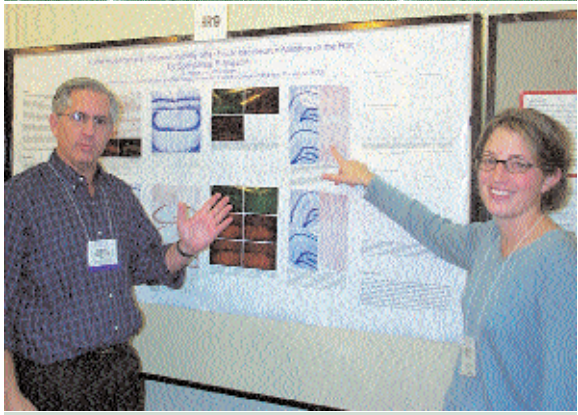
Upcoming Events

Experimental Biology
Orlando FL • March 31 - April 4, 2001
Booth #192

Jennifer Martin wins "ATS Abstract of the Year Award"

Jennifer L. Martin, a graduate student at the University of Arizona, won the annual award presented by Advanced Targeting Systems to the first author of the best poster using an ATS product. Ms. Martin's poster, produced under the supervision of Dr. Robert S. Sloviter, demonstrated the effect of the elimination of SP receptor-expressing interneurons in the rat hippocampus. The scientists used SSP-SAP (Cat# IT-11), the conjugate of saporin with the Sar^9 analog of substance P (SP), for the elimination of these neurons. They had previously attempted use of SP-SAP (Cat# IT-07), which has given excellent results for elimination of SPR+ neurons when delivered intrathecally into spinal fluid. However, it is known that the half-life of the SP moiety is quite brief upon entry into tissue. Martin and Sloviter wanted to inject directly into tissue, but found that SP-SAP gave very little effect, presumably due to the lability of SP. They switched to the more stable SSP-SAP ("stable" SP-SAP) and found extensive and specific elimination of SPR+ neurons in the region of injection. Epileptic pathophysiology was observed in the area of cell loss, indicating that a very focal loss of neurons is sufficient for replicating epileptic disinhibition and hyperexcitability.

Congratulations to this team for an excellent poster!



Dr. Robert S. Sloviter and Jennifer L. Martin University of Arizona SFN 2000, New Orleans LA

Targeting Topics: Recent Scientific References

Summarized by Matthew Kohls

Loss of nerve: a molecular approach to better treatment of chronic pain

Friedrich MJ
JAMA 283(2):187-188, 2000.

The use of SP-SAP (Cat. #IT-07) as a promising new method for chronic pain relief is discussed in this review article. Chronic pain has classically been treated in ways that frequently have adverse effects on the patient's quality of life. Friedrich touches on recently developed toxins that are useful in techniques of molecular neurosurgery. These techniques allow the dissection of pain pathways in the brain and spinal cord which will provide not only a greater understanding of these pathways but also potential therapies for chronic pain and other pain conditions.

Cat. #IT-07 SP-SAP

Species: mammalian

Mab-ZAP: A tool for evaluating antibody efficacy for use in an immunotoxin

Kohls MD, Lappi DA
Biotechniques 28(1):162-165, 2000.

Immunotoxins are useful tools for elimination of specific cell populations *in vitro* and *in vivo* for research and therapeutic applications. One of the factors limiting the use of immunotoxins is the selection of an appropriate antibody. Advanced Targeting Systems has created a reagent that allows researchers to select antibodies with the desired characteristics before an immuntoxin is made, purified, and assayed. Using a goat anti-murine IgG coupled to the ribosome-inactivating protein saporin, researchers can screen hundreds of antibodies in a time and cost-effective manner.

Cat. #IT-04 Mab-ZAP

Species: target of primary antibody

Selective impairment of corticotropin-releasing factor 1 (CRF_1) receptor-mediated function using CRF coupled to saporin

Maciejewski-Lenoir D, Heinrichs SC, Liu XJ, Ling N, Tucker A, Xie Q, Lappi DA, Grigoriadis DE
Endocrinology 141(2):498-504, 2000.

Corticotropin-releasing factor 1 (CRF_1) is a 41-amino acid peptide which mediates many of the body's behavioral, autonomic, immune, and endocrine responses to stress. Reduced activation of the CRF systems plays a role in a variety of psychiatric and metabolic disease states. Maciejewski-Lenoir *et al*. have developed a CRF-SAP targeted toxin that can eliminate cells expressing the CRF_1 but not $CRF_{2\alpha}$ receptors. These data indicate that CRF-SAP may be useful as a tool to examine receptor-selective impairment of CRF system function.

Cat. #IT-13 CRF-SAP

Species: mammalian

Antibody for human p75 LNTR identifies cholinergic basal forebrain of non-primate species

Tremere LA, Pinaud R, Grosche J, Hartig W, Rasmusson DD
NeuroReport 11(10):2177-2183, 2000.

192-SAP (Cat. #IT-01) is a highly successful reagent for eliminating cholinergic neurons in rats. Because the targeting antibody only recognizes rat p75, it is unable to be used in other species. Tremere *et al*. have stained basal forebrain sections with ME20.4, a monoclonal antibody to human p75 (Cat. #AB-N07) and found excellent cross-reactivity in dog, raccoon, cat, pig and rabbit. The authors state that an ME20.4-saporin conjugate could be used to produce cholinergic basal forebrain lesions in several species. Last quarter, ATS highlighted the use of ME20.4-SAP in the rabbit (*Targeting Trends* 1:1, 2000).

Cat. #AB-N07 αhuman p75 Mab

Species: human, primate, rabbit, sheep, dog, cat, pig, raccoon

Combined lesions of cholinergic and serotonergic neurons in the rat brain using 192 IgG-saporin and 5,7-dihydroxytryptamine: neurochemical and behavioural characterization

Lehmann O, Jeltsch H, Lehnhardt O, Pain L, Lazarus C, Cassel JC
Eur J Neurosci 12(1):67-79, 2000.

Lesioning of septohippocampal pathways has often been used as a model for Alzheimer's disease because these lesions alter cognitive capabilities such as spatial memory. Recent work in the behavioral neurosciences has shown that other neurotransmitter systems such as GABAergic, noradrenergic, and serotonergic systems also play a role in learning and memory. Lehmann *et al*. combined the effects of the cholinergic immunotoxin 192-SAP and the serotonergic toxin 5,7-dihydroxytryptamine to examine interactions between these two pathways. The effects of lesioning these two pathways in concert indicate that they both play roles in cognitive functions related to working memory.

Cat. #IT-01 192-SAP 2 μg/lateral ventricle

Species: rat

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Effects of cholinergic depletion on neural activity in different laminae of the rat barrel cortex
Herron P, Schweitzer JB
Brain Res 872(1-2):71-76, 2000.

Cat. #IT-01 192-SAP 8.0 μg/300 g body weight, nucleus basalis of Meynert

Species: rat

Sustained effect of metrifonate on cerebral glucose metabolism after immunolesion of basal forebrain cholinergic neurons in rats
Bassant MH, Poindessous-Jazat F, Schmidt BH
Eur J Pharmacol 387(2):151-162, 2000.

Cat. #IT-01 192-SAP 134 ng in 0.2 μl, basal forebrain

Species: rat

Development of a method for intraparenchymal infusions of 192 IgG-saporin: a comment on Pizzo *et al.* (1999)
Sarter M, Bruno JP, Miner LH, McGaughy J
J Neurosci Meth 96(2):169-170, 1999.

letter pertaining to use of 192-SAP

Cat. #IT-01 192-SAP

Species: rat

Nerve growth factor (NGF) augments cortical and hippocampal cholinergic functioning after p75NGF receptor-mediated deafferentation but impairs inhibitory avoidance and induces fear-related behaviors
Winkler J, Ramirez GA, Thal LJ, Waite JJ
J Neurosci 20(2):834-844, 2000.

Cat. #IT-01 192-SAP 1.0 or 2.7 μg in 10 μl, intracerebroventricular

Species: rat

Preserved olfactory short-term memory after combined cholinergic and serotonergic lesions using 192 IgG-saporin and 5,7-dihydroxytryptamine in rats
Wirth S, Lehmann O, Bertrand F, Lazarus C, Jeltsch H, Cassel JC
NeuroReport 11:347-350, 2000.

Cat. #IT-01 92-SAP 2 μg, intracerebroventricular

Species: rat

Pain control: breaking the circuit
Hunt SP
Trends Pharmacol Sci 21(8):284-287, 2000.

Cat. #IT-07 SP-SAP Review and analysis of the value of SP-SAP in research and as a therapeutic.

Species: mammalian

***In Vivo* [^{125}I]-Iodobenzovesamicol binding reflects cortical cholinergic deficiency induced by specific immunolesion of rat basal forebrain cholinergic system**
Sorger D, Schliebs R, Kampfer I, Rossner S, Heinicke J, Dannenberg C, Georgi P
Nucl Med Bio 27:23-31, 2000.

Cat. #IT-01 192-SAP, 2 μg into each lateral ventricle

Species: rat

The role of cortical cholinergic afferent projections in cognition: impact of new selective immunotoxins
McGaughy J, Everitt BJ, Robbins TW, Sarter M
Behav Brain Res 115(2):251-263, 2000.

Cat. #IT-01 192-SAP and IT-15 ME20.4-SAP, Review and comparison of immunolesioning techniques

Species: multiple

Brainstem noradrenergic control of nociception is abnormal in the spontaneously hypertensive rat
Taylor BK, Roderick RE, Basbaum AI
Neurosci Lett 291:139-142, 2000.

Cat. #IT-03 anti-DBH-SAP, 5 μg

Species: rat

Increased susceptibility to generalized seizures after immunolesions of the basal forebrain cholinergic neurons in rats
Silveira DC, Holmes GL, Schachter SC, Geula C, Schomer DL
Brain Res 878:223-227, 2000.

Cat. #IT-01 192-SAP, 4 μg intracerebroventricular injection

Species: rat

Please visit our website (www.ATSbio.com) to see a complete list of references.

Targeting Talk: *In vivo* Use of Targeted Toxins

Dr. Ronald G. Wiley is a founder and scientific advisor to Advanced Targeting Systems. His expertise in the use of 192-Saporin and other targeted toxins is invaluable to targeted toxin users.

Q: Can you use targeted toxins in vivo?

A: Yes, Molecular Neurosurgery is designed as a tool for *in vivo* use.

Q: How do you recommend administration of the targeted toxin?

A: There are several ways to administer the toxins depending on the cells being targeted:

1. Direct intraparenchymal pressure microinjection can be used to deliver the targeted toxin directly to target cells. This approach has been used successfully with several toxins, including SP-Saporin (Cat. # IT-07), in the striatum to kill striatal interneurons that express the NK-1 receptor. Long, slow infusions (0.1 μl/min) are probably the best way to do intraparenchymal injections.

   Wiley RG and Lappi DA. Neurosci Lett 230:97-100, 1997.

2. Targeted toxins can also be injected into terminal fields and retrogradely transported to the cell bodies. This approach has been used successfully to selectively destroy locus coeruleus noradrenergic neurons that project to the olfactory bulb by injecting anti-DBH-saporin into the olfactory bulb.

   Blessing WW, Lappi DA, Wiley RG. Neurosci Lett 243:85-88, 1998.

   Intracortical injections of 192-Saporin (Cat. #IT-01) also have been used to destroy cholinergic basal forebrain neurons projecting to the injected patch of cortex.

   Sachdev R, Lu SM, Wiley RG, Ebner FJ. Neurophysiol, 79:3216-3228, 1998.

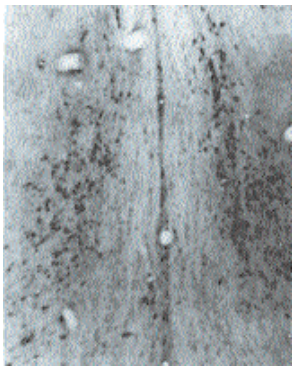
3. Intrathecal injections, either intraventricular or subarachnoid, have been used with great success. Intraventricular injections of 192-Saporin (Cat. #IT-01) in rats can reliably destroy the cholinergic basal forebrain.

   Wiley RG, Oeltmann TN, Lappi DA. Brain Res 562:149-53, 1991.

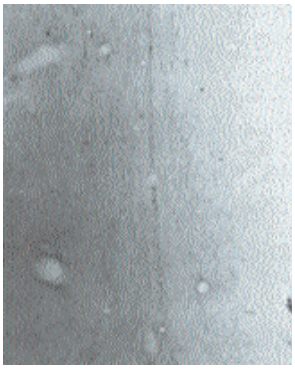
   Lumbar subarachnoid injections of SP-Saporin (Cat. #IT-07) can destroy lamina I neurons in the dorsal horn that express the NK-1 receptor.

   Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, Daughters RS, Lappi DA, Wiley RG, Simone DA. Science 278:239-40, 1997.

4. Lastly, SP-Saporin (Cat. #IT-07) has also been applied directly to the surface of the spinal cord to kill lamina I neurons expressing NK-1 receptor. In all cases, pilot studies to determine optimal toxin dose and injection parameters are recommended.



Control



Treated

LNGFR+ neurons of rat cholinergic forebrain. Photo on the right shows neurons after icv injection of 192-Saporin. The result is >95% elimination of LNGFR (p75)-positive neurons.

Photos supplied by C. Wrenn and R.G. Wiley

Immunolesioning: From Spinal Cord to Brain

(continued from page 1)

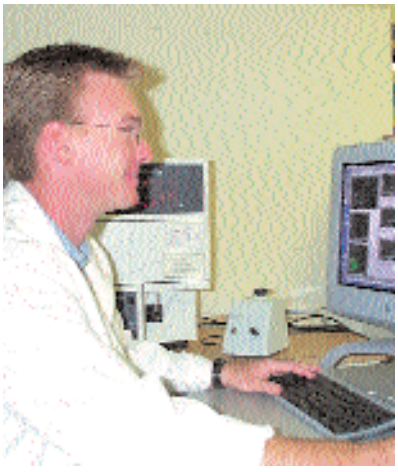
depletion of either class of presympathetic neuron was not possible because the C1 cells are insensitive to classic catecholaminergic neurotoxins, such as 6-hydroxydopamine, and a marker for the non-C1 cells has not been identified. The recent development of the immunolesioning tool, saporin conjugated to an antibody for dopamine beta hydroxylase (anti-DBH-SAP), has provided an effective tool for examining the effects of the selective elimination of the catecholaminergic bulbospinal RVLM neurons.

We depleted bulbospinal C1 cells in rats by microinjection of anti-DBH-SAP bilaterally into two levels of the upper thoracic spinal cord (21 ng/100 nl/injection; 4 injections/rat). To directly examine the depletion of the bulbospinal RVLM neurons we also injected the retrograde tracer Fast Blue into two alternate levels of the spinal cord in some rats. This protocol produced an average depletion of >74% of bulbospinal C1 cells (range, 50-95%) and several other bulbospinal catecholaminergic cell groups (84% of C3 cells and 98% of A5 cells).[1,2,3] After 3-5 weeks these rats have a normal mean AP, and a sympathetic nerve activity (SNA) that continues to be modulated by baroreceptor inputs,[1,3] although the range of this reflex is reduced.[1] Stimuli that inhibit SNA and decrease AP, such as intravenous phenyl biguanide[1] or clonidine,[2] appear to be unaffected by treatment with anti-DBH-SAP. In contrast, stimuli that increase SNA and AP, such as intravenous cyanide[1] or electrical stimulation of the RVLM itself,[3] appear to be markedly reduced or absent after treatment with anti-DBH-SAP. These data suggest that the non-C1 bulbospinal RVLM neurons may be sufficient to maintain the basal SNA that maintains resting AP; however the C1 cells may be critical for the full expression of sympatho-excitatory responses mediated by the RVLM.

Anti-DBH-SAP is an effective tool for examining the effects of catecholaminergic bulbospinal RVLM neurons.

References

1. Schreihofer AM and Guyenet PG. *Am J Physiol Regulatory Integrative Comp Physiol* 279:R729-R742, 2000.
2. Schreihofer AM and Guyenet PG. *Am J Physiol Regulatory Integrative Comp Physiol* 279:R1753-R1762, 2000.
3. Schreihofer AM, Stornetta RL and Guyenet PG. *J Physiol* (Lond.), in press, 2000.



Brian Russell, Research Assistant for Cytometry Research, assists customers in FACS analysis (see cover story)

Targeting Ticklers

Lab Rats

The National Institutes of Health have announced that they will no longer be using rats for medical experimentation. In their place, they will use attorneys. They have given three reasons for this decision:

1. There are now more attorneys than there are rats.
2. The medical researchers don't become as emotionally attached to the attorneys as they did to the rats.
3. No matter how hard you try, there are some things that rats won't do.

Targeting Teaser Winners

Congratulations to the puzzle solvers from our last newsletter. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

The solution to the puzzle was:

Jumbles: SAPORIN CHOLERA FRONTAL KNOCKOUT

Answer: Why the scientists couldn't finish their experiment --- THEY WERE OUT OF CONTROL

WINNERS: Mark DeSantis, *Dept Biol Sci, Univ of Idaho* * Michelle Edwards, *MRB 10134, UTMB* * Dr. Wen Sheng, *Univ of Minnesota* * Ken Giuliano, *Cellomics, Inc* * James Doll, *Castro Valley, CA* * Dr. Gail Johnson, *Univ of Alabama* * Barbara Ferbel, *Univ Rochester Med Ctr*

Targeting Tools: New Products

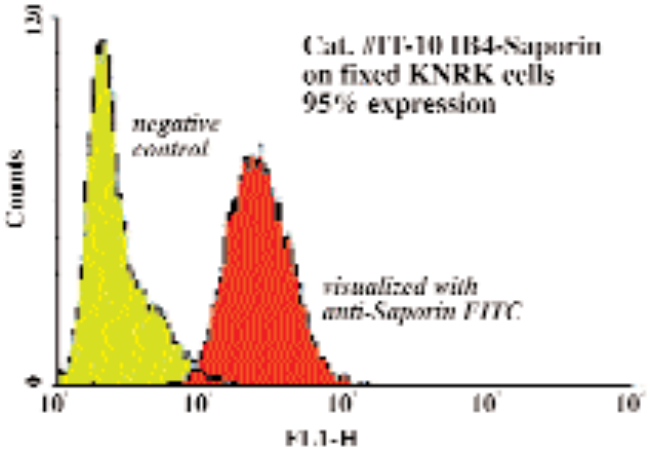
IB4-Saporin

IB4, or the B4 lectin isoform from *Bandeiraea simplicifolia* (*Griffonia simplicifolia*), has played an important role in the delineation of the pathways of pain transmission. One of the two major groups of primary afferents that target the spinal cord dorsal horn neurons are labeled by IB4; the other group by TrkA and peptides such as CGRP and substance P and TrkA (Snider and McMachon, Tackling pain at the source: New ideas about nociceptors. *Neuron* 20:629-632, 1998).

ATS has designed a reagent that can eliminate *in vivo* the IB4-labeled neurons by conjugating the lectin to saporin. This reagent has begun to yield important information about pain pathways, .

According to work presented at the Society for Neuroscience meeting, IB4-SAP specifically eliminates the IB4-positive neurons, while sparing the peptidergic neurons (see Vulchanova *et al*. Role of IB4-binding sensory neurons in the effects of intradermal capsaicin injection. *Soc Neurosci Mtg, New Orleans LA*, 2000 Abstract #212.7 and Tarpley *et al*. Contribution of IB-4-positive sensory neurons to NGF-induced hyperalgesia in the rat. *Soc Neurosci Mtg, New Orleans LA*, 2000 Abstract #633.18).

Upon binding to the alpha-glactosyl group expressed on the cell surface, IB4-SAP becomes internalized and saporin inhibits protein synthesis, resulting in the elimination of the neurons. The cytotoxin is extremely potent, with an ED_{50} of 80 pM for alpha-galactosyl-expressing cells *in vitro*. For an excellent discussion of these two classes of primary afferents, see Basbaum AI. Distinct neurochemical features of acute and persistent pain. *Proc Natl Acad Sci USA* 96:7739-7743, 1999.



More Products for Pain Research

Substance P-Saporin
Stable Substance P-Saporin
Dermorphin-Saporin

NK-1 Receptor Antibody
TrkA Antibody

Coming Soon! **TrkA-Saporin**

Featured Neuroscience Antibodies: Nerve Growth Factor (p75) receptor

AB-N01 Anti-p75 monoclonal

Species Reactivity:	mouse (low affinity nerve growth factor receptor)
Applications:	immunohistochemistry (cells, tissue); immunoprecipitation; immunoblotting; blocks function of nerve growth factor
Reference:	Huber and Chao. *Devel Biol* 167:227-238, 1995.

AB-N02 Anti-p75 monoclonal

Species Reactivity:	mouse (low affinity nerve growth factor receptor)
Applications:	immunohistochemistry; immunocytochemistry; immunoprecipitation
Reference:	Rao MS and Anderson DJ. *J Neurobiol* 32(7):722-746, 1997.

AB-N07 Anti-p75 monoclonal

Species Reactivity:	multiple: human, primate, rabbit, sheep, dog, cat, hamster, pig
Applications:	immunohistochemistry; Western blot; electron microscopy; immunoprecipitation
Reference:	Ross AH *et al*. *Proc Natl Acad Sci USA* 81:6681-6685, 1984.

Visit the ATS website for a complete list of antibodies.

Kristina Majer, an ATS Researcher, works on antibody development and quality control assays.

Targeting Technology

Advanced Targeting Systems' technology - Molecular Neurosurgery™ - is a modification of one of the most widely used techniques in the neurosciences: lesioning of a region by surgical means and observation of the effect.

Choose an ANTIBODY[§] specific to your cell type.

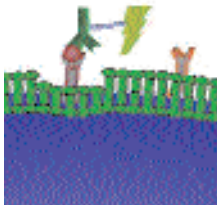
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

ATS binds SAPORIN with your ANTIBODY to make a powerful targeting agent.

[§]or growth factor, peptide, ligand, or cytokine

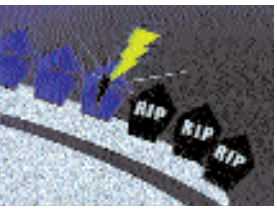
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.



Cells which do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

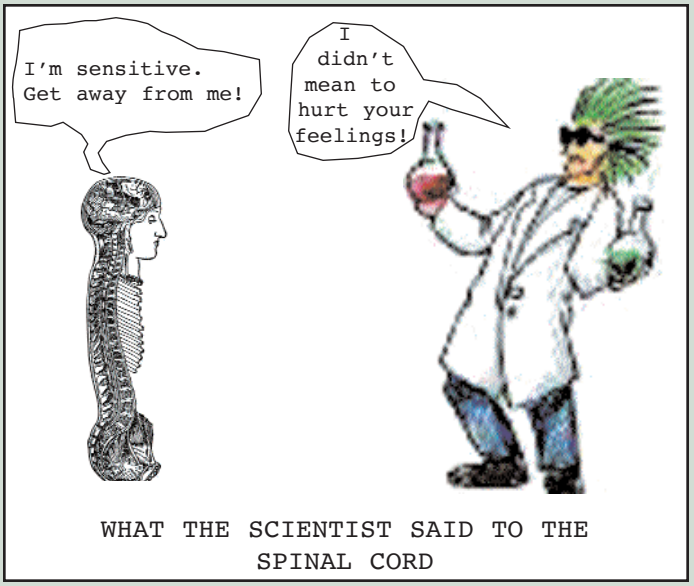
Unscramble these four Jumbles, one letter to each block, to form four words used in science.

DOTBINAY

PETCRORE

MOVELU

FETCEF



WHAT THE SCIENTIST SAID TO THE SPINAL CORD

Arrange the circled letters to form the surprise answer, as suggested by the above cartoon.

YOU'VE GOT...

Answer:

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by February 28, 2001.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

Please correct the address information below and provide the following:

Your Name:
Phone:
Fax:
Email:

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

11175-A Flintkote Avenue
San Diego, CA 92121

BULK RATE
UNITED STATES
POSTAGE PAID
PERMIT #1088
LA JOLLA, CA

April 2001
Volume 2, Issue 2

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- Cytometry Research partners with Cytomation *(page 2)*
- New Controls for Immunotoxins *(page 7)*
- Suicide Transport Explained *(page 5)*

ADVANCED TARGETING SYSTEMS

Safety Studies Begin for Chronic Pain Therapeutic

Early last month (March 2001), Advanced Targeting Systems (ATS) received funding from the National Institute of Mental Health (NIMH) to begin toxicology/safety studies of Substance P-Saporin (SP-SAP), a potential therapeutic for the treatment of chronic pain. The studies will be completed under the direction of three scientists who are experts in their respective fields.

Dr. Douglas Lappi (President and Chief Scientific Officer of ATS) is principal investigator for the project and will oversee the various aspects of the studies. He is an expert in the design, construction and testing of targeted toxins. His laboratory will be producing the drug and performing quality control assays throughout the project.

Dr. Tony Yaksh (Professor of Anesthesiology and Pharmacology at the University of California, San Diego School of Medicine) will direct the administration of the drug. He is the leading expert in spinal cord delivery of experimental agents. The dog is one of the species routinely used to satisfy most regulatory requirements for drug safety evaluation. The studies will assess safety from four points: 1) intrathecal dose ranging to determine the maximum tolerated dose, 2) kinetics of cerebral spinal fluid to determine how the drug penetrates spinal tissue, is redistributed and eliminated, 3) histopathology to determine impact of drug on organs and tissue, and 4) spinal GLP safety studies to determine physiological (heart and respiratory rate, blood pressure) and behavioral (arousal, muscle tone, coordination) impacts of drug administration (4).

Dr. Patrick Mantyh (Professor, University of Minnesota, Minneapolis) has established the efficacy of SP-SAP in rats and is internationally acclaimed for his immunohistochemical analysis. His laboratory will measure parameters involving the efficacy and specificity of the SP-SAP treatment. Immunohistochemistry will help in determining where the drug travels and what impact, if any, it has on spinal cord neurons *(See Figure)*.

The development of SP-SAP was first published in 1997 (1) by Drs. Ronald G. Wiley and Douglas Lappi, two of the founders of ATS. Their collaboration with Dr. Patrick Mantyh led to two publications in the journal *Science* (2, 3). These three articles describe the results of experiments with SP-SAP in the rat.

SP-SAP is a targeted toxin that permanently eliminates cells that bear the Substance P

(continued on page 6)

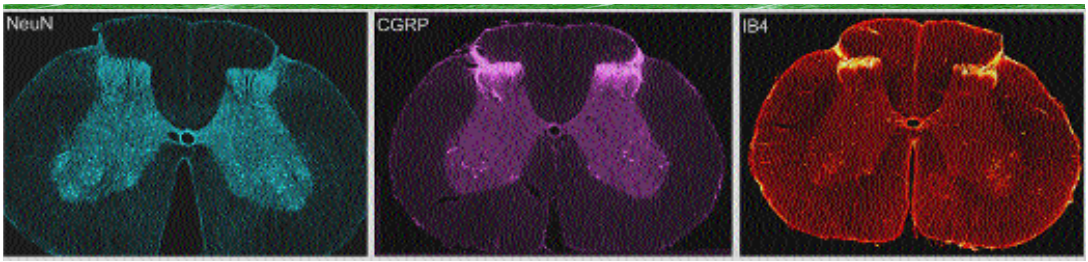


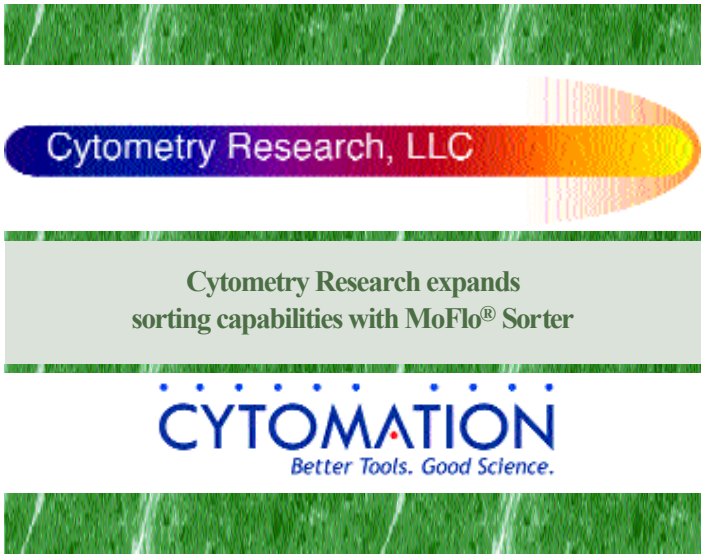
Figure Legend: This figure shows the staining of NeuN, CGRPand IB4 in the canine spinal cord. Immunostaining is one of the tests that will be used for determination of specificity and evaluation of bystander effects by SP-SAP. Confocal photomicrographs show the pattern of immunohistochemical labeling of the neuronal nuclear marker, NeuN, a peptidergic sensory nerve fiber marker, CGRP, and the non-peptidergic sensory nerve fiber marker, IB4, in the dog spinal cord. The NeuN staining is distributed throughout the entire gray matter, while the staining for the sensory fibers (CGRP, IB4) is localized to the dorsal horn (Photo supplied by Dr. Patrick Mantyh).

San Diego Biotechnology Center Opens

On March 12, Cytometry Research, LLC and Cytomation, Inc. announced their new partnership to establish the San Diego Biotechnology Center. This new center will make Cytomation's MoFlo® ultra high-speed cell sorter available to the region's biotech companies, which now rely on Cytometry Research for flow cytometry services.

Flow cytometry is the science of examining physical and chemical properties of live cells, beads, or other biological particles as they pass in a fluid stream through a measuring apparatus. This apparatus, known as a flow cytometer, uses laser excitation and fluorescence signal detection to measure parameters such as size, shape, DNA content, surface receptors, enzyme activity, membrane permeability and calcium flux.

The MoFlo® is equipped to separate and collect fluorescence-labeled single cells or beads from a sample. With this instrument, the flow cytometer nozzle is vibrated at a high frequency by a piezoelectric transducer that causes the microscopic fluid stream exiting the flow chamber to break into discrete droplets. As a cell or bead of interest reaches the droplet break-off point, it receives a positive or negative charge. As the droplets pass individually through two vertical deflection plates, the electric field created by those plates directs them toward the appropriate, user-specified collection receptacles (e.g. 96-well plate). Uncharged droplets flow into a waste receptacle. The MoFlo® can collect four samples of interest, in addition to the waste stream, by charging cells or beads with a range of electrical charges. Cytometry Research is pleased to be able to offer its customers the expanded, state-of-the-art capabilities that the MoFlo® provides.



Cytometry Research, LLC is a private company, that provides GLP flow cytometry services for clinical and basic research applications in San Diego and throughout the U.S. Headquartered in Fort Collins, Colorado, Cytomation, Inc. is a private company with offices in Freiburg, Germany and Melbourne, Australia.

Upcoming Events

Society for Neuroscience
San Diego CA • November 10 - 15, 2001

Featured Neuroscience Antibodies: Mac-1 and TrkA receptor

AB-N05 Mac-1 monoclonal, IgG_{2b}

Species Reactivity:	mouse, human Mac-1 α-chain (M1/70)
Applications:	immunoprecipitation; FACS analysis
Reference:	Springer T *et al*. (1978) *Eur J Immunol* 8:539-551.

AB-N06 Mac-1 monoclonal, IgG_1

Species Reactivity:	rat CD11b (1B6)
Applications:	immunoprecipitation
Reference:	Mulligan MS *et al*. (1993) *J Immunol* 150:2407-2417.

AB-N03 TrkA Receptor polyclonal

Species Reactivity:	rat TrkA receptor (high affinity nerve growth factor receptor)
Applications:	immunohistochemistry (cells, tissue); immunoprecipitation; immunoblotting
Reference:	Clary DO *et al*. (1994) *Mol Biol Cell* 5:549-563.

Visit the ATS website for a complete list of antibodies.

Targeting Topics: Recent Scientific References

Summarized by Matthew Kohls

Up-regulation of growth-associated protein 43 mRNA in rat medial septum neurons axotomized by fimbria-fornix transection

Haas CA, Hollerbach E, Deller T, Naumann T, Frotscher M
Eur J Neurosci 12:4233-4242, 2000.

Axonal growth and regeneration is limited in adult mammals, however if injured CNS neurons are in an environment permissive for growth, they can regenerate. Transection of septohippocampal fibers is a widely used method for studying CNS neuron response to injury. These fibers are composed of both cholinergic and GABAergic neurons. Haas *et al.* used a combination of cholinergic lesioning by 192-Saporin (Cat. #IT-01) and double staining to investigate whether both cell types were involved in neuron regeneration. The findings show that both transmitter phenotypes up-regulate mRNA levels of a protein associated with growth and synaptogenesis in developing neurons, and plasticity in adult neurons.

Baroreceptor sensitivity of rat supraoptic vasopressin neurons involves noncholinergic neurons in the DBB

Grindstaff RJ, Grindstaff RR, Cunningham JT
Am J Physiol Regul Integrative Comp Physiol 279:R1934-R1943, 2000.

Baroreceptors are one component of the system that buffers acute changes in blood pressure. Part of this control stems from the baroreceptor ability to regulate vasopressin release from the neurohypophysis. Using 192-Saporin (Cat. # IT-01) to specifically eliminate cholinergic neurons in the diagonal band of Broca, Grindstaff *et al.* demonstrated that these neurons are not utilized in the pathway that relays baroreceptor information to the brain.

Dissociation of memory and anxiety in a repeated elevated plus maze paradigm: Forebrain cholinergic mechanisms

Lamprea MR, Cardenas FP, Silveira R, Morato S, Walsh TJ
Behav Brain Res 117:97-105, 2000.

The septo-hippocampal pathway has been implicated in many behavioral processes such as learning, anxiety, and motivation. Using 192-Saporin (Cat. #IT-01) to lesion the cholinergic neurons of the medial septum of rats, the authors demonstrate changes in exploratory behavior associated with learning, but no changes in anxiety-associated behavior in their elevated plus maze paradigm.*

** Dr. Thomas J. Walsh, who recently passed away, will be remembered, among many other things, for his contributions to science. The next issue of Targeting Trends will feature Dr. Walsh's contributions to the field of targeting.*

Early migratory rat neural crest cells express functional gap junctions: Evidence that neural crest cell survival requires gap junction function

Bannerman P, Nichols W, Puhalla S, Oliver T, Berman M, Pleasure D
J Neurosci Res 61:605-615, 2000.

Gap junctions are vital for intercellular communication, especially during development. Neural crest cells develop into several types of neural cells, often migrating as a mass of cells to their final destinations. Bannerman *et al.* use the anti-p75 antibody (catalog #AB-N01) to confirm the presence of p75 in neural crest cells. The authors examine how crucial survival signals are communicated during migration and demonstrate that interfering with gap junction formation causes death of neural crest cells.

The molecular dynamics of pain control

Hunt SP, Mantyh PW
Nature Rev/Neurosci 2:83-91, 2000.

Over the last twenty years a great deal of progress has been made in the understanding of how pain is processed and transmitted by the CNS. The authors of this review highlight advances in systems neurobiology, behavioral analysis, genetics, and cell and molecular techniques. One method discussed is the use of the targeted toxin substance P-saporin (SP-SAP, Cat. # IT-07, also available with a more stable analog of substance P, SSP-SAP, Cat. # IT-11). This targeted toxin selectively lesions neurons expressing the NK1 receptor. Injection of SP-SAP into the spinal cord of rats dramatically attenuates the response to chronic pain stimuli, yet leaves acute pain response intact.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Regulation of sympathetic tone and arterial pressure by rostral ventrolateral medulla after depletion of C1 cells in rat

Schreihofer AM, Stornetta RL, Guyenet PG
J Physiol 529(1):221-236, 2000.

The rostral ventrolateral medulla (RVLM) controls and maintains basal sympathetic vasomotor tone, and is also vital to many sympathetic reflexes. Sympathetic nerve activity and arterial pressure correlate with the C1 adrenergic neurons in the RVLM, but there are also non-catecholaminergic neurons present. Schreihofer *et al.* used anti-DBH-SAP (Cat. # IT-03) to eliminate the C1 cells of the RVLM to investigate the non-catecholaminergic neuron contribution to vasomotor tone. Their data indicate C1 cells are necessary for full expression of sympathoexcitatory responses generated by the RVLM.

Neuronal lesioning with axonally transported toxins

Wiley RG, Kline IV RH
J Neurosci Meth 103:73-82, 2000.

Functional neuroanatomy studies have long utilized lesioning. Given the complexity of heterogeneous neuron populations, conventional lesioning methods have proved relatively crude and have provided limited information. Wiley and Kline detail some of the immunotoxins utilizing saporin as well as neuropeptide-saporin conjugates that have found use in recent neurological research. These products include SP-SAP (Cat. #IT-07), which eliminates neurons expressing the neurokinin 1 receptor, 192-Saporin (Cat. #IT-01), which eliminates neurons expressing the p75 receptor in rats, anti-DBH-SAP (Cat #IT-03), which destroys noradrenergic and adrenergic neurons, and OX7-SAP (Cat. #IT-02), which is a suicide transport agent targeting all rat neurons. The authors also discuss some of the protocols and methods utilized with these compounds.

Non-linear cortico-cortical interactions modulated by cholinergic afferences from the rat basal forebrain

Villa AEP, Tetko IV, Dutoit P, Vantini G
BioSystems 58:219-228, 2000.

Elimination of the cholinergic neurons of the basal forebrain (BF) is an excellent model for some aspects of Alzheimer's Disease (AD). 192-Saporin (Cat. # IT-01) is a very effective tool for elimination of cholinergic neurons in the BF. Villa *et al.* investigate whether field potential changes in the brains of lesioned animals mimic changes observed in the brains of human AD patients. The data presented indicate depletion of cholinergic neurons from the BF of both rats and humans produces similar changes in field potential.

Antinociceptive action of nitrous oxide is mediated by stimulation of noradrenergic neurons in the brainstem and activation of α_{2B} adrenoceptors

Sawamura S, Kingery WS, Davies MF, Agashe GS, Clark JD, Kobilka BK, Hashimoto T, Maze M
J Neurosci 20(24):9242-9251, 2000.

Nitrous oxide has been used extensively in surgical anesthesia for more than 150 years, but the molecular mechanism of action has not yet been defined. Sawamura *et al.* investigate whether noradrenergic neurons in the brainstem are involved in the analgesic action of nitrous oxide. The authors injected rats with anti-DBH-SAP (Cat. #IT-03) to destroy pontine noradrenergic neurons. The treated rats demonstrated the usual sedative effects of nitrous oxide, but the analgesic effects were reduced or blocked. Coupled with data from null mice for the α_{2B} adrenoceptor, the data indicates that α_2 adrenoceptor subtypes and ligands are involved in the analgesic but not sedative effects of nitrous oxide.

Cortical cholinergic inputs mediate processing capacity: Effects of 192 IgG-saporin-induced lesions on olfactory span performance

Turchi J, Sarter M
Eur J Neurosci 12:4505-4514, 2000.

Many experiments support the theory that the basal forebrain (BF) is involved in major aspects of attention that influence learning and memory. Elimination of cholinergic neurons in the BF by 192-Saporin (Cat. #IT-01) has been shown to reduce the ability of rats to perform a task while paying attention to more than one thing. The authors tested the treated rat's ability to identify one olfactory stimuli from an increasing number of such stimuli. While the performance of the treated rats returned to control levels within four weeks post-lesion, their performance reflected increased time between tests. These data indicate that cholinergic neurons of the BF play a role in attentional capacities.

Please visit our website (www.ATSbio.com) to see a complete list of references.

Targeting Talk: Suicide Transport and Immunolesioning

By Dr. Ronald G. Wiley

Q: What is immunolesioning?

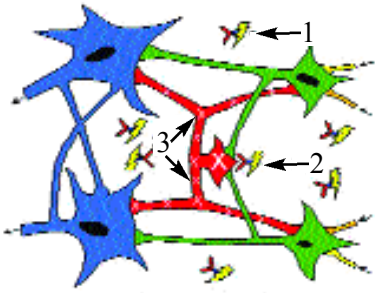
A: Immunolesioning is a technique for making highly selective cellular lesions using immunotoxins. The immunotoxins consist of a monoclonal antibody to a cell surface molecule and a toxic effector moiety such as saporin, a ribosome-inactivating protein. The selectivity of the lesion made with this technique depends on the selective expression of the target surface molecule on the cells of interest. Immunotoxins may be applied in a projection field where the toxin is taken up by axon terminals and retrogradely transported to the cell bodies resulting in destruction of an entire neuron. Other routes of application include: directly in vicinity of cell bodies, into CSF, and into culture supernatant.

Q: What is suicide transport?

A: Suicide transport is an anatomically selective neural lesioning technique that relies on axonal uptake of a toxin that is retrogradely transported to the cell body resulting in destruction of the entire neuron (1). Examples include the toxic lectins [ricin (2), volkensin (3, 4)] and immunotoxins [192-Saporin: Cat. #IT-01 (5), OX7-SAP: Cat. #IT-02 (6, 7), Anti-DBH-SAP: Cat. #IT-03 (8)]. The goal of using this technique is to selectively destroy a group of neurons based on where the corresponding axons project.

Q: How do I administer a targeted toxin to achieve suicide transport?

A: Generally, precise control of dose and location of injection is important in suicide transport experiments. Consequently, pressure microinjection is the preferred method of toxin delivery. In the peripheral nervous system, subepineurial injection (inside the connective tissue sheath of a peripheral nerve) works well. Within the CNS, stereotactic techniques are typically used to deliver toxin to the desired target.



1. Saporin is conjugated to specific targeting agent.
2. Conjugate is bound to receptor/antigen and internalized.
3. Target cells are eliminated; other cells are left untouched.

References

1. Wiley RG, Lappi DA (1994) *Suicide Transport and Immunolesioning*. Houston, R.G. Landes.
2. Helke CJ, Charlton CG, Wiley RG (1985) Suicide transport of ricin demonstrates the presence of substance P receptors on medullary somatic and autonomic motor neurons. *Brain Res* 328:190-195.
3. Contestabile A, Fasolo A, Virgili M, Migani P, Villani L, Stirpe F (1990) Anatomical and neurochemical evidence for suicide transport of a toxic lectin, volkensin, injected in the rat dorsal hippocampus. *Brain Res* 537:279-286.
4. Panaglos MN, Francis PT, Pearson RCA, Middlemiss DN, Bowen DM (1991) Destruction of a sub-population of cortical neurons by suicide transport of volkensin, a lectin from *Adenia volkensii*. *J Neurosci Meth* 40:17-29.
5. Wiley RG (1992) Neural lesioning with ribosome-inactivating proteins: suicide transport and immunolesioning. *Trends in Neurosci* 15:285-290.
6. Roberts RC, Harrison MB, Francis SMN, Wiley RG (1993) Differential effects of suicide transport lesions of the striatonigral or striatopallidal pathways on subsets of striatal neurons. *Exp Neurol* 124:242-252.
7. Wiley RG, Stirpe F, Thorpe P, Oeltmann TN (1989) Neuronotoxic effects of monoclonal anti-Thy 1 antibody (OX7) coupled to the ribosome inactivating protein, saporin, as studied by suicide transport experiments in the rat. *Brain Res* 505:44-54.
8. Wiley RG, Kline IR (2000) Neuronal lesioning with axonally transported toxins. *J Neurosci Meth* 103(1):73-82.

Safety Studies Begin for Chronic Pain Therapeutic

(continued from page 1)

receptor. This receptor is one of many involved in the transmission of pain signals to the brain. There are two general categories of pain to be considered in this process: 1) acute pain, a physiologically important survival tool (rose thorn pricking finger, cat claws scratching cheek), and 2) chronic or noxious pain (pain that persists beyond normal healing time), often the cause of severe pathological states. The scientists used standard models of chronic pain to determine that the perception of noxious pain in the models was greatly reduced in those animals who received injections of SP-SAP. But just as important, the perception of acute pain was left intact. This was an extraordinarily important finding and led ATS to the decision to begin the process of developing SP-SAP as a drug.

Over the next few months, ATS will be interacting with the Center for Drug Evaluation and Research. This organization is part of the U.S. Food and Drug Administration and will evaluate the drug development plan and make determinations about the composition and guidelines for the initial clinical trials in humans. Their preliminary feedback has been a recommendation to begin clinical trials in patients with terminal cancer whose pain is no longer treatable with opioid-based drugs such as morphine. The size of this patient population may qualify SP-SAP for development as an orphan drug.

ATS is optimistic about the therapeutic possibilities of SP-SAP. The funding from NIMH is an important first step in getting the drug development process under way. The process toobtain the additional funding needed to complete the toxicology tests required by the FDA has already begun. The present goal is to be able to begin the first clinical trials in humans before the end of 2002. Progress reports will be printed in this newsletter and on the ATS website at: www.ATSbio.com.

Watch future newsletters and the ATS website for drug development updates.

References

1. Wiley RG, Lappi DA (1997) Destruction of neurokinin-1 receptor expressing cells *in vitro* and *in vivo* using substance P-saporin. *Neurosci Lett* 230:97-100.
2. Mantyh PW, Rogers S, Honore P, Allen B, Ghilardi JR, Li J, Daughters RS, Vigna SR, Lappi DA, Wiley RG, Simone DA(1997) Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance Preceptor. *Science* 278:275-279.
3. Nichols ML, Allen BJ, Rogers SD, Ghilardi JR, Honore P, Li J, Lappi DA, Simone DA, Mantyh PW (1999) Transmission of chronic nociception by spinal neurons expressing the substance P receptor. *Science* 286:1558-1561.
4. Yaksh TL, Rathbun ML, Provencher JC. (1999) Preclinical safety evaluation for spinal drugs. In: *Spinal Drug Delivery*, Yaksh TL (ed.), Elsevier Science B.V., Amsterdam, pp. 417-437.

Targeting Ticklers

Lab Personals

I don't always express myself on the surface, but I'm looking for a signal that you appreciate my complexity. Send me the right message that will penetrate my membranes, turn on my protein expression and release my potential energy.

Mature cell seeks same who still enjoys cycling and won't go apoptotic on me. Let's fight senescence together!

Some dates have called me a promotor. Others have referred to me as a real operator. Personally, I think I'm just a cute piece of DNA who is still looking for that special transcription factor to help me unwind.

I'm a prolific progenitor with great potential for growth and self-renewal. Call me if you're a potent hematopoietic factor who still believes in endless nights of colony stimulation.

Targeting Teaser Winners

Congratulations to the puzzle solvers from our last newsletter. Each winner receives $100 credit towards research product purchases from ATS.

The solution to the puzzle was:

Jumbles: ANTIBODY RECEPTOR VOLUME EFFECT

Answer: What the scientist said to the spinal cord --- YOU'VE GOT A LOT OF NERVE!

WINNERS: Dr. Reema Shafi, *Ohio State Univ* * Petra Moessner, *Baylor College Dentistry* * Dr. Judith Ball, *Texas A&M Univ* * Anne Goldman, *Northwestern Univ* * Dr. Michelle Edwards, *Univ Texas, Med Branch* * Theresa Sarkeda, *Univ Minnesota* * Mandy Lucas, *Univ Arkansas* * Dr. Michael Olszewshi, *Univ Michigan* * Dr. Sanjay Danthi, *Ohio State Univ* * Maxim Cheeran, *MMRF* * Dr. Erik Yu, *Naval Med Res Ctr* * Dr. Gretchen Kohls, *Sacramento, CA* * Dr. Bruce Pappas, *Carleton Univ* * Emily Topacio, *Oridigm* * Kristen Phend, *Univ North Carolina* * Dr. Pierre Vaysse, *Synaptic Pharm Corp* * Dr. Alexander Murashov, *E. Carolina Univ School of Med* * Dr. Lara Hutson, *Univ Utah* * Linda Lan, *Stanford Univ* * Shannon Shields, *UCSF* * Dr. Joseph McGivern, *Amgen Inc* * Dr. Grace Li, *UCLA* * Dr. Lisa Banner, *CSU Northridge* * Dr. Wendy Smith, *Northeastern Univ* * Deborah McCarty, *Lilly Res Lab* * Dr. Mathieu Lesort, *Univ Alabama at Birmingham* * James Doll, *Castro Valley, CA*

Targeting Tools: New Products

Controls for Immunotoxins

Advanced Targeting Systems announces two new control molecules for use with immunotoxins. We now offer mouse IgG or rat IgG conjugated to saporin. These new controls are the same molecular weight, consist of similar, comparable materials — saporin and a rat or mouse IgG — and are synthesized with the same protocols as the targeted immunotoxins. The difference is the cell-specific antibodies are replaced with "blanks," antibodies that have no specificity, and no ability to target cells. In short, they are the perfect control molecules for behavioral experiments with Advanced Targeting Systems' immunotoxins.

Controls are a vital part of the scientific procedure; without them it is difficult to isolate the specific effects from the non-specific or artifactual. With targeted toxin research, the same is true, and Advanced Targeting Systems often receives questions as to what makes the best control for a targeted toxin.

In the past, the response has been given according to what has been available. For an immunotoxin (a conjugate between an antibody and saporin), the suggested control is a mixture of the two components in their non-conjugated form. Of course, the lack of the conjugation process may detract from using this as a control, and there is always the question of how much antibody to mix with how much saporin.

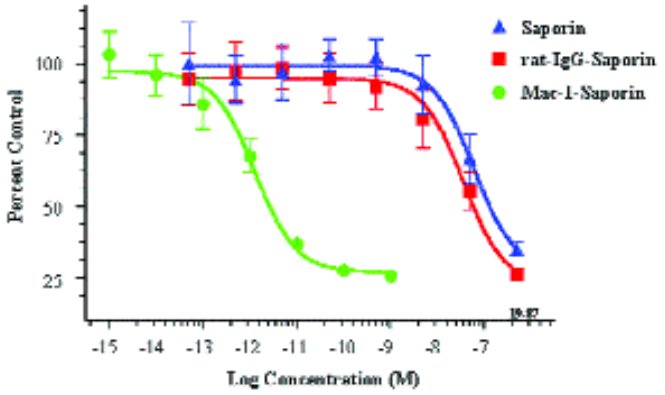
Another suggestion for a control, since often saporin is connected to its targeting agent via a disulfide bond, is to reduce the disulfide bond. This method has some difficulties: 1) the reducing agent, if not removed, can have its own effect, and 2) usually the process is incomplete (unless carried out under drastic conditions), leaving a percentage of active material in the control. Finally, it's expensive.

The new control immunotoxins avoid all of these difficulties. First of all, they are synthesized using the identical procedures that are used to synthesize the targeted immunotoxins, so there is no difference from the chemical point-of-view. They are very easy to use: they have the same molecular weight, you just use equal amounts of the control immunotoxin and the targeted immunotoxin. There are no complicated calculations to make. They are cost-effective. They are reasonably priced and time-saving because of the ease of preparation. As with all of the targeted immunotoxins, they are sterile-filtered and ready to go in phosphate-buffered saline at physiological pH.

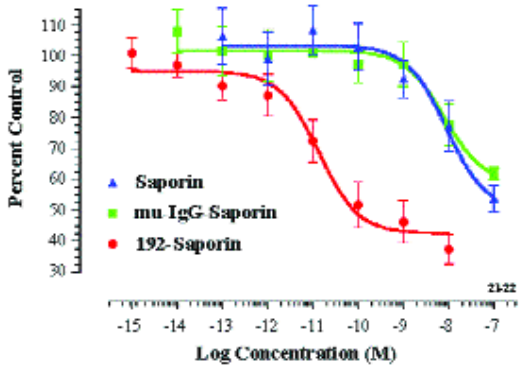
Mouse IgG-SAP(Cat# IT-18) serves as a control for immunotoxins that use a mouse monoclonal
192-Saporin (Cat# IT-01) Anti-DBH-SAP (Cat# IT-03) ME20.4-SAP (Cat# IT-15) OX-7-SAP (Cat# IT-02)
Rat IgG-SAP (Cat# IT-17) serves as a control for immunotoxins that use a rat monoclonal
Mac-1-SAP (Cat# IT-06) murine p75-SAP (Cat# IT-16)

In vitro data in the displayed graphs show that the control immunotoxins have orders of magnitude less cytotoxicity than the targeted immunotoxins. Their low toxicities are similar to that of saporin (on a molar basis), which is only taken into cells by bulk phase endocytosis, as opposed to antibody-mediated or receptor-mediated endocytosis of the targeted immunotoxins. These new molecules will make getting definitive data much easier.

Coming Soon: a new control peptide-toxin, that will use a randomly-generated, nonsense peptide conjugated with saporin. It will be the perfect control for SP-SAP, dermorphin-SAP and SSP-SAP.



WEHI-274.1, a murine monocytic cell line
ED_{50} for Mac-1-SAP: 1.3 pM; saporin: 60 nM; rat IgG-SAP: 36 nM.



C6 glioma, a rat tumor cell line that expresses p75
ED_{50} for 192-Saporin: 13 pM; saporin: 8.8 nM; mouse IgG-SAP: 7.9 nM
(The latter two calculated ED_{50}'s are skewed because the dosages are not sufficiently high to give the bottom plateau)

In both figures, cells were plated at 2500 cells per well in 90 microliters of medium.
After allowing acclimatization overnight, the cells were exposed to the various reagents in 10 μl at the indicated concentrations for 72 hours.
MTS (according to supplier's protocol: Promega) was added and after two hours, plates were read at 492 nM on a Molecular Diagnostics Spectramax 340 plate reader with SoftMax software.

Data analyzed by Prism 3.0 software.

Targeting Technology

Advanced Targeting Systems' technology - Molecular Neurosurgery™ - is a modification of one of the most widely used techniques in the neurosciences: lesioning of a region by surgical means and observation of the effect.

Choose an ANTIBODY[§] specific to your cell type.

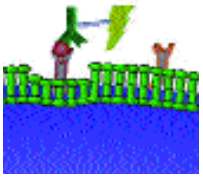
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

ATS binds SAPORIN with your ANTIBODY to make a powerful targeting agent.

[§]or growth factor, peptide, ligand, or cytokine

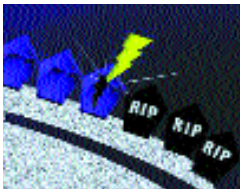
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.



Cells which do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these four Jumbles, one letter to each block, to form five words used in science.

GYRRUSE

ROOTPLOC

THESMAR

PLOEDNIET

MULEHI

HOW THE SCIENTIST PREPARED THE COLD FORMULA

Arrange the circled letters to form the surprise answer, as suggested by the above cartoon.

Answer: HE...

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by May 31, 2001.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

Please correct the address information below and provide the following:

Your Name:
Phone:
Fax:
Email:

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

11175-A Flintkote Avenue
San Diego, CA 92121

BULK RATE
UNITED STATES
POSTAGE PAID
PERMIT #1088
LAJOLLA, CA

July 2001
Volume 2, Issue 3

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- A Field Trip to Torrey Pines State Reserve *(page 2)*
- Targeted Toxin Delivery *(page 5)*
- Saporin, a Ribosome-Inactivating Protein *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

Dermorphin-SAP Kills MOR-Positive Cells

Advanced Targeting Systems announces the release of its new, very exciting targeted toxin, dermorphin-SAP. It is a conjugate of the mu opioid receptor (MOR) agonist dermorphin and the ribosome-inactivating protein, saporin. Its cytotoxicity to cells that express the MOR promise to make it an important tool in the discovery and definition of the role of these cells in many biological processes.

In the latest issue of the *Journal of Neuroscience*, Porreca *et al*. (1) use this molecule for an important characterization of the descending pain pathways and the possible role of "ON" cells, the MOR-expressing cells of the rostroventromedial medulla (RVM), in the processes of chronic pain models. They injected dermorphin-SAP into the RVM and demonstrated loss of MOR-expressing cells near the injection site (Fig. 1). These neurons project to the spinal cord and it has been suggested by Howard Fields that they are responsible for a tonic discharge that mediates descending facilitation of nerve injury-induced pain. In fact, Porreca *et al*. demonstrate that with the loss of these cells, the expression of experimental neuropathic pain is ablated. This striking demonstration of supraspinal neurons having such a powerful effect on spinal cord properties is, well, sensational.

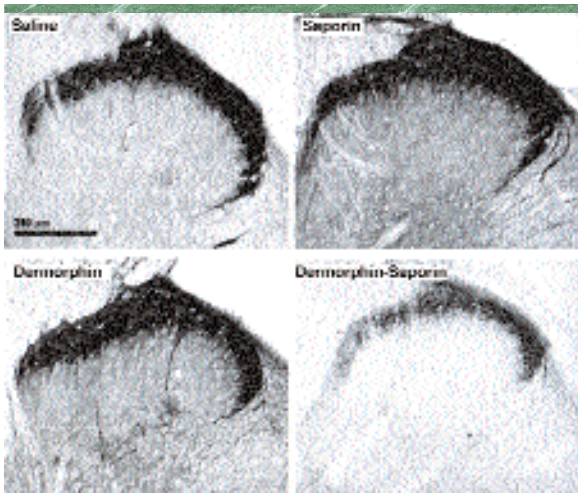


Figure 1. MOR staining in the dorsal horn of rats treated as indicated. Loss of staining in dermorphin-SAP-treated animals is evident.
Figures supplied by Drs. Frank Porreca and Josephine Lai

(continued on page 6)

A Tribute to Thomas J. Walsh, Ph.D.

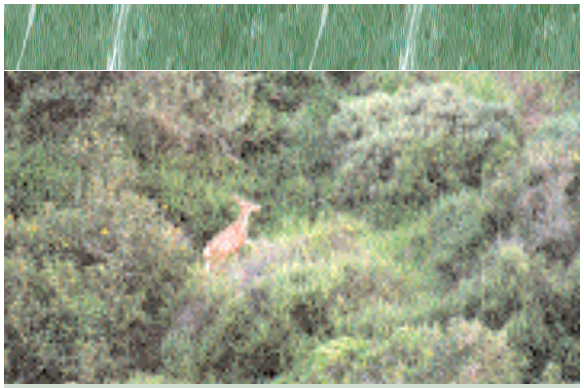
Tom Walsh passed away on May 12, 2000. He will be missed. His contribution in the area of targeted toxins has helped to advance the field in many ways.

1. Lamprea MR, Cardenas FP, Silveira R, Morato S, **Walsh TJ** (2000) Dissociation of memory and anxiety in a repeated elevated plus maze paradigm: forebrain cholinergic mechanisms. *Behav Brain Res* 117(1-2):97-105.
2. Angner RT, Kelly RM, Wiley RG, **Walsh TJ**, Reuhl KR (2000) Preferential destruction of cerebellar Purkinje cells by OX7-saporin. *Neurotoxicology* 21(3):395-403.
3. Gandhi CC, Kelly RM, Wiley RG, **Walsh TJ** (2000) Impaired acquisition of a Morris water maze task following selective destruction of cerebellar purkinje cells with OX7-saporin. *Behav Brain Res* 109(1):37-47.
4. Dougherty KD, Turchin PI, **Walsh TJ** (1998) Septocingulate and septohippocampal cholinergic pathways: involvement in working/episodic memory. *Brain Res* 810(1-2):59-71.
5. Dougherty KD, Salat D, **Walsh TJ** (1996) Intraseptal injection of the cholinergic immunotoxin 192-IgG saporin fails to disrupt latent inhibition in a conditioned taste aversion paradigm. *Brain Res* 736(1-2):260-269.
6. **Walsh TJ**, Herzog CD, Gandhi C, Stackman RW, Wiley RG (1996) Injection of IgG 192-saporin into the medial septum produces cholinergic hypofunction and dose-dependent working memory deficits. *Brain Res* 726(1-2):69-79.
7. **Walsh TJ**, Kelly RM, Dougherty KD, Stackman RW, Wiley RG, Kutscher CL (1995) Behavioral and neurobiological alterations induced by the immunotoxin 192-IgG-saporin: cholinergic and non-cholinergic effects following i.c.v. injection. *Brain Res* 702(1-2):233-45.

Going to Work is Like a Walk in the Park

Advanced Targeting Systems (ATS) is located in San Diego, California just a couple of miles from the Pacific Ocean. That alone makes the location desirable, but what makes it ideal is the company's proximity to Torrey Pines State Reserve.

The Reserve offers a trail that starts just 100 yards out the ATS front door and ends at Torrey Pines State Beach. There is a variety of plant life and flowers, and at least 209 species of birds. If you're quiet and walking the trail before or after work, you might even spot some deer. It's important to watch where you're stepping though, because there is also the occasional snake crossing the path!



ATS is located at the edge of the beautiful Torrey Pines State Reserve in San Diego, California

Occasionally, the ATS employees will go for a field trip to the Reserve to see the changing scene. Although San Diego does not experience the radical change of seasons like some other locations, spring and early summer offer delightful displays of new color and life.

In the picture shown here, the group was on a mission to see the newly-bloomed fields of flowers. Left to right are: Brian Russell *(Research Assistant)*, Kristina Majer *(Research Assistant)*, Matthew Kohls *(Sr. Research Associate)*, Lori Bradley *(Administrative Assistant)*, and Dr. Douglas Lappi *(President/Chief Scientific Officer)*. All of the scientists are graduates of University of California, San Diego.

Upcoming Events

Society for Neuroscience (SFN)
San Diego CA • November 10 - 15, 2001

3rd Forum of European Neuroscience (FENS)
Paris France • July 13 - 17, 2002

Featured Neuroscience Antibodies: Nerve Growth Factor (p75) Receptor

AB-N01 Rabbit Polyclonal
AB-N01AP Affinity Purified Rabbit Polyclonal

Species Reactivity: mouse (low affinity NGFR)
Applications: immunohistochemistry (cells, tissue); immunoprecipitation; immunoblotting; blocks function of nerve growth factor; FACS analysis
Reference: Huber LJ *et al*. (1995) *Devel Biol* 167:227-238.

AB-N02 Rat Monoclonal, IgG_1

Species Reactivity: mouse (low affinity NGFR)
Applications: immunohistochemistry (unfixed tissue); immunoprecipitation; immunoblotting
Reference: Rao MS *et al*. (1997) *J Neurobiol* 32(7):722-746.

AB-N07 Mouse Monoclonal (ME20.4 IgG)

Species Reactivity: multi-species: human, primate, rabbit, sheep NTR
Applications: immunohistochemistry; Western blot; electron microscopy; immunoprecipitation
Reference: Ross AH *et al*. (1984) *Proc Natl Acad Sci USA* 81:6681-6685.

Visit the ATS website for a complete list of antibodies.

Targeting Topics: Recent Scientific References

Summarized by Matthew Kohls

Selective Destruction of Medial Septal Cholinergic Neurons Attenuates Pyramidal Cell Suppression, but not Excitation in Dorsal Hippocampus Field CA1 Induced by Subcutaneous Injection of Formalin

Zheng F and Khanna S
Neurosci 103(4):985-998, 2001.

Previously, the authors have shown that an injection of formalin in the hindpaw of rats will excite a select population of CA1 pyramidal cells within a larger suppressed population. This response is accompanied by increased theta activation. The authors selectively eliminated medial septal cholinergic neurons using 192-Saporin (0.4 μl; Cat. # IT-01) to investigate the role of these neurons in response to a persistent noxious stimulus such as a formalin injection. The data indicate a CA1 network modulated by cholinergic neurons in the medial septal region may influence pyramidal cell theta and pyramidal cell suppression.

Sequential Upregulation of Cell Adhesion Molecules in Degenerating Rat Basal Forebrain Cholinergic Neurons and in Phagocytotic Microglial Cells

Hartlage-Rübsamen M, Schliebs R
Brain Res 897(1-2):20-26, 2001.

Neurodegeneration, found in brain disorders such as Alzheimer's, Parkinson's, and Huntington's diseases, is marked by a significant microglial response. This microglial activation is characterized by increased migratory activity and potential cytotoxic action on injured neurons. The interaction of microglial cells with degenerating axons and neural somata is known to be mediated by expression of cell adhesion molecules. The authors use a single intracerebroventricular injection of 192-Saporin (4 μg; Cat. # IT-01) to initiate neurodegeneration of choline acetyltransferase-immunoreactive neurons and follow the expression of two cell adhesion molecules, ICAM-1 and LFA-1, using immunohistochemistry. The results indicate that these adhesion molecules may function as intercellular recognition signals through which degenerating cholinergic neurons actively participate in their own targeting and removal by microglia.

Immunotoxic Destruction of Distinct Catecholamine Subgroups Produces Selective Impairment of Glucoregulatory Responses and Neuronal Activation

Ritter S, Bugarith K, Dinh TT
J Comp Neurol 432(2):197-216, 2001.

Control of regulatory responses to low glucose levels in the brain have been linked to catecholaminergic neurons. Studies of these neurons have been hindered by the lack of a selective and precise lesioning agent. Ritter *et al*. use anti-DBH-SAP (Cat. # IT-03) to create very precise lesions of catecholamine neurons in the paraventricular nucleus of the hypothalamus and spinal cord. Injection of anti-DBH-SAP into the spinal cord eliminates cells with caudal projections while injection into the paraventricular nucleus of the hypothalamus eliminated cells with rostral projections. This ability to selectively eliminate very specific subpopulations of cells is a valuable characteristic in dissecting neuronal function.

p75-Expressing Elements are Necessary for Anti-Allodynic Effects of Spinal Clonidine and Neostigmine

Paqueron X, Li X, Eisenach JC
Neurosci 102(3):681-686, 2001.

It has been suggested that α2-adrenergic agonists produce analgesia by activating spinal cholinergic neurons. The authors reason that since spinal cholinergic neurons in the ventral horn express p75 following peripheral nerve trauma, cholinergic dorsal horn neurons might also. Instead, they find that dorsal horn neurons express little or no p75 under normal conditions or following spinal nerve ligation. Since dorsal horn neurons do not express p75 they are not eliminated by 192-Saporin (0.1-0.6 μg; Cat. # IT-01), but the data indicate that p75-expressing elements do play a role in pain transmission in the dorsal horn. The authors note that when afferents that express p75 are eliminated, mechanical hypersensitivity is unaffected, but the reduction of hypersensitivity by α2-adrenergic agonists or cholinergic agents is blocked.

Neuropeptide-Toxin Conjugates in Pain Research and Treatment (Review)

Wiley RG
Reg Anesth Pain Med 25(5):546-548, 2000.

Several lines of evidence indicate dorsal horn neurons that respond to substance P (SP) play a role in nociception. Wiley discusses the attributes of SP-SAP (Cat. # IT-07), a targeted toxin that eliminates cells expressing the neurokinin-1 receptor. Animals treated with this material using a lumbar intrathecal injection show a decrease in both hyperalgesia and allodynia in several pain models. The success of SP-SAP indicates that other neuropeptides, hormones, and growth factors would be useful as targeted toxins.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Rat Basal Forebrain Cholinergic Lesion Affects Neuronal Nitric Oxide Synthase Activity in Hippocampal and Neocortical Target Regions

Hartlage-Rübsamen M, Schliebs R
Brain Res 889(1-2):155-164, 2001.

Nitric oxide (NO) mediates a variety of mechanisms in the brain including cortical perfusion, learning and memory, and neuronal plasticity. Cholinergic dysfunction has been associated with some of these same processes, notably reduced cortical cerebral blood flow and impaired performance in learning and memory tasks. The authors use a single intracerebroventricular injection of 192-Saporin (2.8 µg; Cat. # IT-01) to deplete the cholinergic neurons of the basal forebrain. Although total cortical neuronal NO synthase levels are not affected, the activity levels in select neocortical hippocampal neurons are reduced. The data suggest the ratio of catalytically active and inactive cortical NO synthase may be driven in part by basal cholinergic forebrain input.

Behavioural, Histological and Immunocytochemical Consequences Following 192 IgG-Saporin Immunolesions of the Basal Forebrain Cholinergic System

Perry T, Hodges H, Gray JA
Brain Res Bull 54(1):29-48, 2001.

192-Saporin (Cat. # IT-01) has been used extensively as a model for Alzheimer's Disease. The neuronal deficits caused by intraparenchymal forebrain injections (0.3-0.51 µg/µl) are apparent during tasks demanding attentional processing, but not standard tasks of learning and memory. Perry *et al*. compare the testing strategies for each deficit. They find that the water maze may not demand enough attentional processing to demonstrate deficits caused by this lesion. The authors also study long-term effects of 192-Saporin in rats. Although the authors produced very useful data at five to six months, they found evidence of an inflammatory response and non-specific cell death eleven months post treatment, indicating 192-Saporin may be problematic for very long-term experiments.

Septal Cholinergic Neurons Suppress Seizure Development in Hippocampal Kindling in Rats: Comparison with Noradrenergic Neurons

Ferencz I, Leanza G, Nonobashvili A, Kokaia Z, Kokaia M, Lindvall O
Neurosci 102(4): 819-832, 2001.

Kindling can be caused in rats by lesioning forebrain cholinergic or noradrenergic projections. Ferencz *et al*. utilize 192-Saporin (2.5 µg; Cat. # IT-01) to lesion forebrain cholinergic neurons and 6-hydroxydopamine to lesion noradrenergic neurons, administering both compounds by intraventricular injection. Upon comparing various aspects of hippocampal kindling, the authors determine that while both noradrenergic and cholinergic projections to the forebrain exert inhibitory effects, the cholinergic effect is less pronounced and occurs prior to seizure generalization.

Toxin-Induced Death of Neurotrophin-Sensitive Neurons (Review)

Wiley RG
Meth Mol Biol 169(1-2):217-222, 2001.

Wiley discusses some of the specifics of using 192-Saporin (Cat. #IT-01) to eliminate cells expressing the rat p75 low-affinity nerve growth factor receptor. Wiley also describes the sequence of events following treatment with 192-Saporin from binding of the immunotoxin through ribosomal inactivation and cell death. Methods of handling the immunotoxin and injection are also addressed.

Model for Aging in the Basal Forebrain Cholinergic System (Review)

Gu Z, Wortwein G, Yu J, Perez-Polo JR
Antiox Redox Signal 2(3):437-447, 2000.

A wide range of evidence indicates that cholinergic neurons play a role in memory and learning. Loss of these neurons is seen both in aged subjects and Alzheimer's Disease patients. The authors discuss the use of 192-Saporin (Cat. #IT-01) to model this phenomenon. Many lesioning methods have been developed, including fimbria-fornix transections, mechanical lesions with radiofrequency or electrolysis, and intracerebral injections of excitotoxins. Information obtained through these methods suffers because non-cholinergic neurons are depleted as well as the desired cholinergic neurons. 192-Saporin provides a solution by specifically targeting and eliminating cholinergic neurons expressing p75 in the basal forebrain, closely mimicking a key component of aging.

Please visit our website (www.ATSbio.com) to see a complete list of references.

Targeting Talk: *In Vivo* Delivery of Targeted Toxins

Q: What are the options for delivery of targeted toxins?

A: The options for toxin delivery are varied and limited only by investigator ingenuity. Generally, injection has been the route of choice. Some toxins can be given intravenously, such as 192-Saporin (Cat. # IT-01) or anti-DBH-SAP (Cat. # IT-03), in which case all cells expressing p75 or dopamine beta-hydroxylase and exposed to the systemic circulation are potential targets. Intravenous injections will not deliver toxins to the CNS.

Subarachnoid injections have been used successfully for immunotoxins and peptide toxins such as SP-SAP (Cat. # IT-07).

Direct intraparenchymal injections have been used to restrict toxin application to just a few target cells. However, intraparenchymal injections require careful attention to injection technique and are impractical for large target structures.

Q: When injecting directly into tissue, are there any special techniques that should be used?

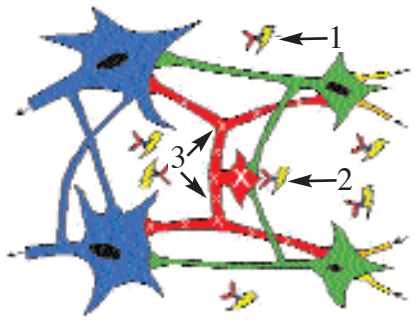
A: Direct injections into brain or spinal cord have been used successfully by some investigators. Specifics of toxin dose, concentration, injection volume and speed of injection have varied considerably. If a high concentration of toxin is deposited locally, lesion specificity is often lost. Presumably, if toxin concentration is too high, cellular uptake by non-specific bulk fluid-phase endocytosis (pinocytosis) can internalize enough saporin to be lethal.

There is currently interest in "convective" delivery techniques developed in the laboratory of Dr. Edward Oldfield at the NIH. The basic principle is to deliver a relatively large concentration slowly over an extended period, often using a rather dilute solution. The parameters for any given species and injection site need to be determined by pilot experiments.

Q: What sort of special care should be given to the animal after administration of the targeted toxin?

A: The toxins generally bind and internalize within minutes, although some immunotoxins circulate for longer periods if injected intravenously. However, no significant amount of active toxin is excreted. So, animals can be returned to group housing immediately after toxin injection. The only special requirements may derive from the specific target being studied. For example, rats given intraventricular 192-Saporin (Cat. # IT-01) develop decreased fluid and food intake for several days after injection. Since the adipsia is significant, providing the animals with fresh, juicy vegetables, such as cucumber or potatoes, can help.

Rats injected intraventricularly with anti-DBH-SAP (Cat. # IT-03) will lose considerable body weight and are slow to regain. They, too, may benefit from food supplements, including nuts and other high calorie appetizing treats. Otherwise, common sense care of any neurologic deficits is indicated depending on the target and toxin being used.



1. Saporin is conjugated to specific targeting agent.
2. Conjugate is bound to receptor/antigen and internalized.
3. Target cells are eliminated; other cells are left untouched.

Dermorphin-SAP Kills MOR-Positive Cells

(continued from page 1)

The conjugate is made with dermorphin, first characterized from the skin of *Phyllomedusa sauvagei* by Montecucchi *et al*. (2). This agonist has one of the best profiles of specificity for the MOR of any known molecule, with exquisite affinity for the MOR (Fig. 2), while much lower affinity for the delta receptor (3). It has been documented to be internalized upon receptor binding, and with saporin attached takes in the ribosome-inactivating agent, causing protein synthesis inhibition and subsequent cell death. This specific lesioning tool is exemplary of many of Advanced Targeting Systems' products.

Dermorphin-SAP was developed from a collaboration with Ron Wiley, and a glimpse of the activity of this cytotoxin was published in the journal *Neuropeptides* (4). MOR-expressing neurons have long been considered some of the most important cells in the nervous systems because of their participation in pain, pain control, addiction, gastrointestinal motility, and mast cell function, among others. This specific cytotoxin provides new methods for understanding these neurons and how they work.

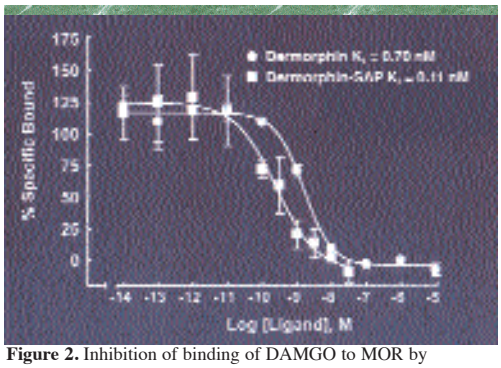


Figure 2. Inhibition of binding of DAMGO to MOR by dermorphin and dermorphin-SAP. Data demonstrate retention of binding after conjugation of dermorphin to saporin.

A product data sheet and ordering information for dermorphin-SAP (Cat. # IT-12) are available on the ATS website at: www.ATSbio.com.

MOR-expressing neurons play a role in pain, addiction, gastrointestinal motility and mast cell function.

References

1. Porreca F, Burgess SE, Gardell LR, Vanderah TW, Malan TP, Jr, Ossipov MH, Lappi DA, Lai J (2001) Inhibition of neuropathic pain by selective ablation of brainstem medullary cells expressing the μ-opioid receptor. *J Neurosci* 21(14):5281-5288.
2. Montecucchi PC, de Castiglione R, Piani S, Gozzini L, Erspamer V (1981) Amino acid composition and sequence of dermorphin, a novel opiate-like peptide from the skin of *Phyllomedusa sauvagei*. *Int J Pept Prot Res* 17(3):275-283.
3. Attila M, Salvadori S, Balboni G, Bryant SD, Lazarus LH (1993) Synthesis and receptor binding analysis of dermorphin hepta-, hexa- and pentapeptides. *Int J Pept Prot Res* 42:550-559.
4. Lappi DA, Wiley RG (2000) Entering through the doors of perception: characterization of a highly selective Substance P receptor-targeted toxin. *Neuropeptides* 34(5):323-328.

Targeting Ticklers

Warning Signs -- it may be time to vacate the lab when you see your labmate doing any of the following:

Is observed cradling a stir-bar while quietly muttering, "They'll never get you my dear, you're my special one, my one and only, and they can never take you away from me..."

Looks up dirty words in the Swiss protein data bank.

Requires sunglasses if the curtains are opened in the lab.

Starts an elbow fight with you because you're pipetting on her side of the lab.

Autoclaves articles of your clothing when you don't strictly adhere to the schedule of the sign-up sheet.

Scrawls the words "Lab Police" in magic marker on the back of his lab coat and starts using the butt of his pipetteman as a tool for safety enforcement.

Targeting Teaser Winners

Congratulations to the puzzle solvers from our last newsletter. Each winner receives $100 credit towards research product purchases from ATS.

The solution to the puzzle was:

Jumbles: SURGERY PROTOCOL HAMSTER DEPLETION HELIUM

Answer: How the scientist prepared the cold formula --- HE TURNED UP THE HEAT!

WINNERS: Nicole Sanders, *Washington State Univ* * Elena Yablonsky-Alter, *CUNY* * Bruce Pappas, *Carleton Univ* * Michael Levy, *Baylor College* * Wen Sheng, *Minnesota Research Foundation* * Christopher Herzog, *Ohio State Univ* * Brian Miller, *Univ of Texas* * Tim Saurer, *Ohio State Univ* * Ken Giuliano, *Cellomics Inc* * Lynn Young, *RW Johnson* * Bob Speth, *Washington Univ* * Peter Gillespie, *OHSU* * Charles Sevigny, *Univ of Virginia* * Christopher Flores, *Univ of Texas at San Antonio*

Targeting Tools: Featured Products

Saporin: a ribosome-inactivating protein

Saporin is a 30 kDa protein isolated from the seeds of the plant *Saponaria officinalis*. This pleasant resident of the banks of Southern European streams has long been known for medicinal properties, mainly due to its saponins, detergents that reside especially in the roots of the plant and which give the plant its name. It took the expertise of University of Bologna researcher Fiorenzo Stirpe to pull perhaps the most useful molecule from the plant, saporin.

Stirpe had screened several plants for ribosome-inactivating proteins. These proteins come in two different forms. One form is exemplified by the incredibly potent toxin ricin; it contains a cell-binding and internalization protein and an enzyme that upon entering the cell removes a single base from the ribosomal RNA of the large subunit of the ribosome. This enzyme action is a necessary characteristic of a ribosome-inactivating protein, cleverly termed a "RIP." Other examples of toxins with cell-binding chains are abrin (Brooke Shields used it for her suicide in the film *Blue Lagoon*) and volkensin, which has been used in neuroscience research as a suicide transport agent.

Most RIPs found in plants are in the second form, without cell-binding chains, and these plants are generally harmless (Fig. 1): RIPs from cucumber and asparagus are two examples. Stirpe could easily recognize the plants that had toxins, but had to screen the plants that weren't known to be toxic. Since *Saponaria* had a medicinal reputation, he included it in his screening. In 1983, he published on a RIP from *Saponaria*, SO-6, that was extremely active in cell-free protein synthesis inhibition, but also, in an observation that later would become very important, an unusual stability to denaturants, proteases and heat (1). This protein would become known as saporin. At that time, the RIP from ricin was widely used in targeted toxins. It had an unfortunate sensitivity to proteolytic attack when removed from its protective cell-binding chain, and this made it unable to function as a targeted toxin in cells that had high levels of proteases (2). This limited its use as a targeted toxin against, for instance, T lymphocytes, and caused considerable setbacks in the targeted toxin field. Stirpe demonstrated remarkable *in vivo* activities of the first targeted toxin made from saporin and, when targeted to T lymphocytes, it was devastating (3,4). Saporin was cloned by Doug Lappi and colleagues in Marco Soria's group (5);

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

Figure 1. The white arrow indicates *Saponaria officinalis* (saporin) growing in Dr. Douglas Lappi's garden. The yellow arrow points to Gangsta, Dr. Lappi's cat, who is perfectly safe playing around this plant.

this led to the use of recombinant saporin which Advanced Targeting Systems uses in the preclinical studies of SP-SAP. Presently, the highly active native saporin is sold by Advanced Targeting Systems in a sterile PBS solution ready for use *in vitro* and *in vivo*. Each lot is carefully assayed for full protein synthesis inhibition activity and not released unless it matches the highest levels (Fig. 2).

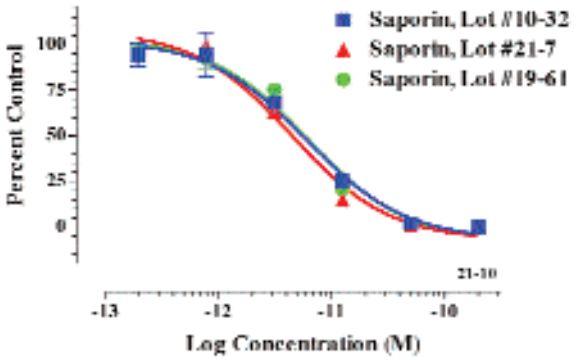


Figure 2. Protein synthesis inhibition by three different lots of saporin. No statistical difference is seen in these extremely active RIPs.

Coming Soon!

FITC- and HRP-labelled, affinity purified anti-saporin

References

1. Stirpe F, Gasper-Campani A, Barbieri L, Falasca A, Abbondanza A, Stevens WA(1983) Ribosome-inactivating proteins from the seeds of *Saponaria officinalis* L. (soapwort) of *Agrostemma githago* L. (corn cockle) and of *Asparagus officinalis* (asparagus) and from the latex of *Hura crepitans* L. (sandbox tree). *Biochem J* 216:617-625.
2. Bilge A, Howell-Clark J, Ramakrishnan S, Press OW (1994) Degradation of ricin A chain by endosomal and lysosomal enzymes - the protective role of ricin B chain. *Therapeutic Immunol* 1:197-204.
3. Thorpe PE, Brown ANF, Bremner JAG, Foxwell BMJ, Stirpe F (1985) An immunotoxin composed of monoclonal anti-thy 1.1 antibody and a ribosome-inactivating protein from *Saponaria officinalis*: potent antitumor effects *in vitro* and *in vivo*. *JNCI* 75:151-159.
4. Siena S, Lappi DA, Bregni M, Formosa A, Villa S, Soria M, Bonadonna G, Gianni AM (1988) Synthesis and characterization of an antihuman T-lymphocyte saporin immunotoxin (OKT1-SAP) with *in vivo* stability into nonhuman primates. *Blood* 72:756-765.
5. Benatti L, Saccardo MB, Dani M, Nitti GP, Sassano M, Lorenzetti R, Lappi DA, Soria M (1989) Nucleotide sequence of cDNA coding for saporin-6, a type-1 ribosome-inactivating protein from *Saponaria officinalis*. *Eur J Biochem* 183:465-470.

Targeting Technology

Advanced Targeting Systems' technology - Molecular Neurosurgery™ - is a modification of one of the most widely used techniques in the neurosciences: lesioning of a region by surgical means and observation of the effect.

Choose an ANTIBODY[§] specific to your cell type.

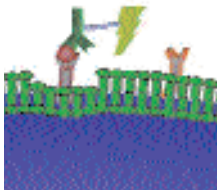
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

ATS binds SAPORIN with your ANTIBODY to make a powerful targeting agent.

[§]or growth factor, peptide, ligand, or cytokine

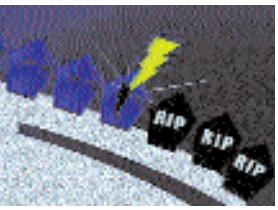
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.



Cells which do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these five Jumbles, one letter to each block, to form five words used in science.

MICCADAE

TULAQYI

EXMIR

TRILE

SNIRPYT

HOW THE TECHNICIAN FELT AFTER A FULL DAY OF STOCK SOLUTION PREPARATION

Arrange the circled letters to form the surprise answer, as suggested by the above cartoon.

Answer: LIKE A... ____ - "____"

Limit one entry per laboratory.

WIN $100.00

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by August 30, 2001.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

Please correct the address information below and provide the following:

Your Name:
Phone:
Fax:
Email:

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

11175-A Flintkote Avenue
San Diego, CA 92121

BULK RATE
UNITED STATES
POSTAGE PAID
PERMIT #1088
LA JOLLA, CA

October 2001
Volume 2, Issue 4

Targeting Trends

Reporting the latest news in Molecular Surgery

Inside this issue:

Newsletter Highlights

- Surfers with a Cause *(page 2)*
- Time Course of Action *(page 5)*
- Orexin-SAP *(page 7)*

Denise Higgins, Editor

ADVANCED TARGETING SYSTEMS

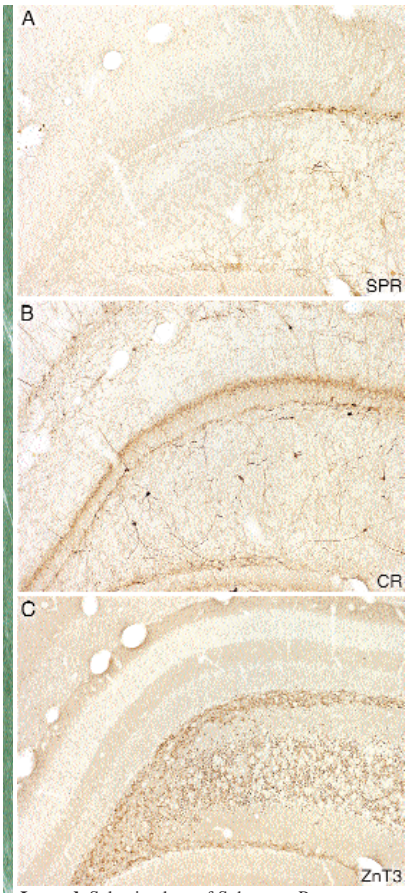
Immunolesioning Hippocampal Inhibitory Interneurons

Dr. Robert Sloviter, University of Arizona, contributes this issue's article from the laboratories of ATS customers. Dr. Sloviter summarizes his research with SSP-saporin, which he and his graduate student Jennifer Martin used to examine the role of inhibitory neurons in maintaining normal network excitability.

The mammalian hippocampus is perhaps the most intensely studied brain region for a variety of reasons. Hippocampal structure and function are highly conserved among mammalian species, and its highly laminar organization greatly facilitates experimental design and interpretation. However, its greatest attractions are its involvement in the normal functions of learning and memory, and in a variety of neurological disorders including stroke, Alzheimer's Disease, and epilepsy. One of the major issues of hippocampal research involves the structure and function of hippocampal inhibitory interneurons, and how they determine the behavior of excitatory hippocampal principal cells. We and others have sought to determine whether certain network behaviors might be the result of inhibitory neuron dysfunction or loss, but it has always been difficult to remove or disable inhibitory neurons selectively, without producing significant collateral damage.

After some failed attempts to lesion specific neuronal populations, we were excited to read the paper by Pat Mantyh and his colleagues,[1] in which they reported the efficacy of Substance P-saporin (SP-SAP) for removing SP receptor-positive cells in the spinal cord. Because some hippocampal inhibitory interneurons had been reported to express SP receptors (SPRs),[2] we purchased SP-SAP,

(continued on page 6)



Legend. Selective loss of Substance P receptor (SPR)-immunoreactive cells after intrahippocampal injection of SSP-SAP. (A) All SPR-positive cells and dendrites have been ablated on the left side of the photograph. (B) Calretinin (CR)-immunoreactive cells and fibers in an adjacent section survive in the SPR depletion zone. (C) In another adjacent section, zinc transporter-3 (ZnT3)-positive terminals are similarly unaffected in the SPR depletion zone.

ATS Receives $900,000 in NIH Funding

In September, Advanced Targeting Systems received two Small Business Innovation Research (SBIR) awards from the National Institutes of Health. The first is a Phase II grant from the National Institute of Neurological Disorders and Stroke. This project continues a collaboration with Drs. Joanne Berger-Sweeney (Wellesley College) and Mark Baxter (Harvard University) to further develop the mouse p75 immunotoxin. More than three-quarters of a million dollars will be invested in characterizing this lesioning agent for use in modeling and studying neurodegenerative diseases such as Alzheimer's disease (AD). Part of the project will include use of the immunotoxin in a transgenic mouse model of AD.

(continued on page 2)

Matthew Kohls, Sr. Research Associate, cooks up something interesting in the fermentor.

ATS Grants Funded

(continued from page 1)

The second award issued to ATS is a Phase I grant from the National Institute of Dental & Craniofacial Research. This $134,000 award will support research to develop an expression system using Substance P as the targeting agent. The purpose of this six-month study is to demonstrate that an expression plasmid can be introduced into Substance P receptor-bearing neurons and that the protein can be observed. If this is successful, then other expression systems will be tested for delivery of bioactive molecules that could diminish the transmission of the chronic pain signal.

Dr. Patrick Mantyh (University of Minnesota) is collaborating with ATS on this project. His laboratory will be testing the system on spinal cord neurons. Dr. Mantyh has been an important collaborator in the development of Substance P-Saporin (SP-SAP), a targeted toxin currently being tested in toxicology/safety studies as a possible therapeutic for chronic pain.

Since it's first SBIR grant was funded in 1994, ATS has received nearly three million dollars in support for research to develop innovative new products. The SBIR program is a valuable resource for small companies to be able to expand and enhance their in-house R&D efforts. ATS appreciates the ability to collaborate with some of the finest academic institutions and their scientists to meet the goals of these SBIR projects and meet the needs of research scientists throughout the world.

Upcoming Events

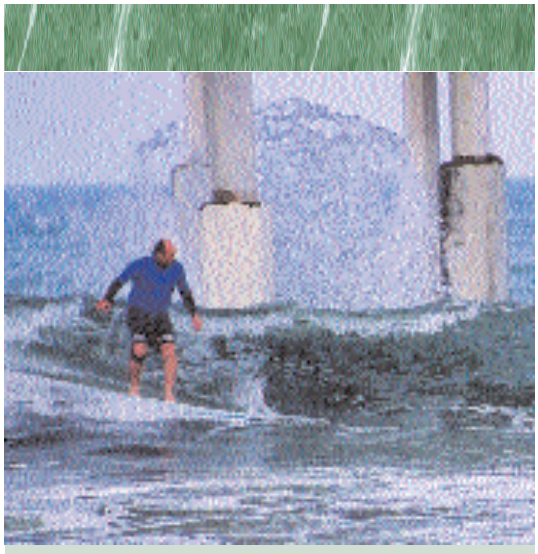
Society for Neuroscience (SFN)
San Diego CA • November 10 - 15, 2001
Booth #2247

3rd Forum of European Neuroscience (FENS)
Paris France • July 13 - 17, 2002

Surfing to Help Spinal Cord Injuries

by Doug Lappi

On Saturday, September 22, I attended the Tony Mezzadri Surf Contest in Ocean Beach, California. A number of years ago Tony, surfing at the Ocean Beach pier, was paralyzed with no movement in his legs and limited movement in his hands and arms. The Ocean Beach community rose to help Tony and this contest has been held yearly since 1994. The proceeds from entry fees, T-shirt sales, raffles, a spaghetti dinner, and donations from sponsor companies were originally to help Tony with this catastrophic event, but now enough money is collected that funds go to support spinal cord research. Dr. Mark Tuszynski's laboratory at UC San Diego has benefited from thousands of dollars of contributions. Advanced Targeting Systems has been a donor to this event for the past four years. The surf contest is a wonderful grass roots effort in which we are proud and happy to participate. Unlike last year's monstrous ten-foot surf that was striking the bottom of the pier, this year there was three- to four-foot surf that allowed the contestants to have fun and show their stuff. At the end, money goes to a great cause in supporting work by an excellent scientist.



Contestants at the Tony Mezzadri Surf Contest hang five (*left*) and shoot the Ocean Beach Pier (*above*) in support of spinal cord research.

Targeting Topics: Recent Scientific References

Summarized by Matthew Kohls

Focal Inhibitory Interneuron Loss and Principal Cell Hyperexcitability in the Rat Hippocampus After Microinjection of a Neurotoxic Conjugate of Saporin and a Peptidase-Resistant Analog of Substance P

Martin JL, Sloviter RS
Journal Comp Neurol 436:127-152, 2001.

The authors used SSP-SAP (0.4 ng/10 nl; Cat. #IT-11). See Cover Story.

Selective Cholinergic Denervation Inhibits Expression of Long-Term Potentiation in the Adult but not Infant Rat Hippocampus

Motooka Y, Kondoh T, Nomura T, Tamaki N, Tozaki H, Kanno T, Nishizaki T
Devel Brain Res 129:119-123, 2001.

The authors studied the possible role of cholinergic systems in long-term potentiation (LTP), which is one of the most intensively studied models of learning and memory. 192-Saporin (4.2 µg/5 µl, Cat. #IT-01) injections were made in both infant and adult rats and the probability of LTP development was studied in hippocampal slices from animals treated 2 weeks or 2 months before. Cholinergic denervation by 192-Saporin did not affect LTP expression in the infant brain, however, the results strongly suggest that cholinergic systems in the adult brain participate in an LTP pathway.

Effects of Hypocretin-Saporin Injections into the Medial Septum on Sleep and Hippocampal Theta

Gerashchenko D, Salin-Pascual R, Shiromani PJ
Brain Res 913:106-115, 2000.

Hypocretin, also known as orexin, neurons are located only in the lateral hypothalamus. Recently, the loss of these neurons was shown to be associated with narcolepsy. The authors used orexin-SAP (100 ng/0.5 µl; Cat. #IT-20) to eliminate parvalbumin and cholinergic neurons (orexin B receptor-expressing) in the rat medial septum. They used 192-Saporin (1 µg/ 1 µl; Cat. #IT-01) to contrast the effect and eliminate only cholinergic neurons (NGF/p75 receptor-expressing). Hippocampal theta activity was completely eliminated in orexin-SAP treated rats by day 12, suggesting that orexin neurons influence cognitive processes critical for survival.

Transneuronal Tracing from Sympathectomized Lumbar Epaxial Muscle in Female Rats

Daniels D, Miselis RR, Flanagan-Cato LM
J Neurobiol 48(4):278-290, 2001.

The authors use pseudorabies virus (PRV) to study central neural networks such as the one controlling the lordosis reflex (increased curvature of the spine). To aid in the separation of the sympathetic nervous system and higher order systems, rats were treated with lumbar injections of anti-DBH-SAP (156 ng to 5 µg; Cat. #IT-03), then labeled with PRV. PRV labeling in the brain was absent in areas associated with vasomotor tone, but persisted in areas implicated in control of the lordosis response.

Please visit our website (www.ATSbio.com) to see a complete list of references.

Hippocampal Sympathetic Ingrowth Occurs Following 192-IgG-Saporin Administration

Harrell LE, Parsons D, Kolasa K
Brain Res 911:158-162, 2001.

Electrolytic lesions of the medial septal region in rats cause peripheral sympathetic fibers from the superior cervical ganglia to grow into the cholinergically-denervated areas of the hippocampus. This lesioning method is non-specific and disrupts several other cell types in the area of the lesion. The authors infused 192-Saporin (1 µg/10 µl saline into medial septum; Cat. #IT-01) to eliminate only the cholinergic neurons, leaving other cell types intact. Hippocampal sympathetic ingrowth still occurs when only the cholinergic neurons are eliminated, indicating that this occurrence is in response to the loss of cholinergic projections from the medial septum.

Selective Antibody-Induced Cholinergic Cell and Synapse Loss Produce Sustained Hippocampal and Cortical Hypometabolism with Correlated Cognitive Deficits

Browne SE, Lin L, Mattsson A, Georgievska B, Isacson O
Exp Neurol 170:36-47, 2001.

The authors used 192-Saporin (two 2.5-µg bilateral injections of 1 µg/µl; Cat. #IT-01) to eliminate cholinergic neurons in the rat, then measured cerebral rates of glucose utilization. The findings show sustained reduction in glucose utilization in the brain regions showing loss of cholinergic neurons, specifically the frontal cortical and hippocampal regions. These same animals demonstrated impaired performance in a Morris water maze. The results reinforce the theory that cholinergic systems influence metabolism and cognition in the cortex and hippocampus.

(continued on page 4)

Targeting Topics: Recent Scientific References

(continued from page 3)

Selective Loss of Cholinergic Neurons Projecting to the Olfactory System Increases Perceptual Generalization Between Similar, but Not Dissimilar, Odorants

Linster C, Garcia PA, Hasselmo ME, Baxter MG
Behav Neurosci 115(4):826-833, 2001.

Selective cholinergic lesioning of the basal forebrain has been linked to attentional and cognitive deficits. 192-Saporin (Cat. #IT-01) was administered to the horizontal limb of the diagonal band of Broca (0.3 µl at 0.175 µg/µl in each hemisphere) destroying projections to the olfactory bulb and cortex. The results demonstrate cholinergic lesions affect the perceptual qualities of odors, and may possibly represent a general mechanism for cholinergic effects on information processing.

Contribution of the Cholinergic Basal Forebrain to Proactive Interference from Stored Odor Memories During Associative Learning in Rats

de Rosa E, Hasselmo ME, Baxter MG
Behav Neurosci 115(2):314-327, 2001.

Proactive interference (PI) is the damaging effect of previously learned information on the acquisition of new, related information. Human patients with basal forebrain (BF) damage due to aneurysms are sensitive to PI. The authors administered 192-Saporin (Cat. #IT-01) to the horizontal limb of the diagonal band of Broca (two 0.2-µl injections of 0.175 µg/µl in each hemisphere) in rats and evaluated performance in an olfactory discrimination task. The treated rats had more difficulty acquiring an overlapping odor pair when muscarinic receptors were blocked by scopalomine. These results indicate that cholinergic neurons have a role in the modulation of PI in associative learning.

It's enough to raise your blood pressure!

Deuchars J, Deuchars S
Trends Neurosci 24(4):200, 2001.

The authors review studies completed by Schreihofer and Guyenet using anti-DBH-SAP (Cat. #IT-03) to eliminate C1 adrenergic neurons. The results show that, although C1 neurons play a role in some sympathoexcitatory responses, they are probably not responsible for maintaining sympathetic tone.

Effects of selective immunotoxic lesions on learning and memory.

Baxter MG
Methods Mol Biol 166:249-265, 2001

Dr. Baxter presents a brief review of studies using immunotoxins to study learning and memory. In particular, this chapter (from the book entitled "Immunotoxin Methods and Protocols") focuses on the use of 192-Saporin (Cat. #IT-01) for elimination of basal forebrain cholinergic neurons and cerebellar Purkinje cells.

Please visit our website (www.ATSbio.com) to see a complete list of references.

Distribution and Co-Localization of Choline Acetyltransferase and p75 Neurotrophin Receptors in the Sheep Basal Forebrain: Implications for the Use of a Specific Cholinergic Immunotoxin

Ferreira G, Meurisse M, Tillet Y, Lévy F
Neurosci 104(2):419-439, 2001.

ME20.4 is a monoclonal antibody (Cat. #AB-N07) that has been shown to bind the p75 receptor in rabbit, sheep, dog, cat, raccoon, pig, and several primate species. Ferreira *et al*. investigate ME20.4-SAP (bilateral, 150 µl per ventricle, 50-150 µg total; Cat. #IT-15) use in sheep to assess distribution and localization of p75. The authors demonstrate 80-95% loss of basal forebrain cholinergic neurons and acetylcholinesterase-positive fibers in the hippocampus, olfactory bulb, and entorhinal cortex.

Hypocretin-2-Saporin Lesions of the Lateral Hypothalamus Produce Narcoleptic-Like Sleep Behavior in the Rat

Gerashchenko D, Kohls MD, Greco M, Waleh NS, Salin-Pascual R, Kilduff TS, Lappi DA, Shiromani PJ
J Neurosci 21(18):7273-7283, 2001.

Orexin (also knows as hypocretin) peptides are produced exclusively by neurons in the lateral hypothalamus, however non-specific lesioning in this region has not produced narcoleptic-like sleep. Gerashchenko *et al*. use orexin-SAP (490 ng/0.5 µl; Cat. # IT-20) to specifically eliminate orexin neurons in rats. The treated rats displayed several sleep disturbances found in narcolepsy, including increased slow-wave sleep, and sleep-onset REM sleep periods. The data suggest that orexin-SAP can be used to create a model for narcolepsy in rats (see page 7, Featured Products).

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

Immunolesioning Hippocampal Inhibitory Interneurons

(continued from page 1)

hoping to use this approach in the hippocampus. However, we discovered in pilot experiments that SP-SAP, when injected directly into the hippocampal parenchyma, did not diffuse sufficiently far from the injection site to destroy interneurons in an area large enough for our purposes. Fortunately, ATS had just developed a conjugate using a peptidase-resistant SP analog (SSP-SAP), which we obtained and tested while we conducted an anatomical study designed to determine exactly which hippocampal interneurons constitutively express SPRs, and should therefore be vulnerable to SSP-SAP. That study demonstrated that most inhibitory neurons of all hippocampal subregions expressed SPRs, and that no excitatory principal cells or glia were SPR-positive.[3]

We found that 10 nl of a solution containing less than 1 ng of SSP-SAP was capable of selectively eliminating all SPR-positive neurons within a 2-mm diameter sphere of tissue. The survival of SPR-negative elements within the SPR depletion zone was remarkable and included excitatory neurons, glia, myelinated fibers, and a number of afferent fiber systems originating outside the hippocampus. Selective loss of SPR-positive inhibitory interneurons was associated with a highly focal disinhibition and hyperexcitability[4] that was clearly not caused by a global neurological insult that invariably causes a myriad of non-specific pathologies.

SSP-SAP diffuses to target a larger area than SP-SAP

Our results indicate that epileptiform behavior is intrinsic to the hippocampal network and does not require the principal cell loss or synaptic reorganization that other models of network hyperexcitability exhibit as a result of less specific neurological injuries. At the least, our results clearly indicate that SSP-SAP will be an extremely useful tool for a wide variety of studies in the hippocampus and other SPR-positive brain regions.

References

1. 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.
2. Acsády L, Katona I, Gulyas AI, Shigemoto R, Freund TF (1997) Immunostaining for substance P receptor labels GABAergic cells with distinct termination patterns in the hippocampus. *J Comp Neurol* 378:320-336.
3. Sloviter RS, Ali-Akbarian L, Horvath KD, Menkens KA (2001) Substance P receptor expression by inhibitory interneurons of the rat hippocampus: enhanced detection using improved immunocytochemical methods for the preservation and colocalization of GABA and other neuronal markers. *J Comp Neurol* 430:283-305, 2001.
4. Martin JL, Sloviter RS (2001) Focal inhibitory interneuron loss and principal cell hyperexcitability in the rat hippocampus after microinjection of a neurotoxic conjugate of saporin and a peptidase-resistant analog of Substance P. *J Comp Neurol* 436:127-152.

Targeting Ticklers

Excerpts from "The Ultimate Scientific Dictionary"

Butyl: An unpleasant-sounding word denoting an unpleasant-smelling alcohol.

Chemical: A substance that: 1. An organic chemist turns into a foul odor; 2. an analytical chemist turns into a procedure; 3. a physical chemist turns into a straight line; 4. a biochemist turns into a helix; 5. a chemical engineer turns into a profit.

First Order Reaction: The reaction that occurs first, not always the one desired. For example, the formation of brown gunk in an organic prep.

Natural Product: A substance that earns organic chemists fame and glory when they manage to synthesize it with great difficulty, while Nature gets no credit for making it with great ease.

Research: (Irregular noun) That which I do for the benefit of humanity, you do for the money, he does to hog all the glory.

Scientific Method: The widely held philosophy that a theory can never be proved, only disproved, and that all attempts to explain anything are therefore futile.

Targeting Teaser Winners

Congratulations to the puzzle solvers from our last newsletter. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

The solution to the puzzle was:

Jumbles: ACADEMIC QUALITY MIXER LITER TRYPSIN

Answer: How the technician felt after a full day of stock solution preparation --- LIKE A “MULTI-MEDIA” EXPERT!

WINNERS: Dr. Chien Li, The Salk Institute * Bob Speth, WSU * Pelin Chen, OR Primate Res Ctr * Teri Milner, Weill Med College, Cornell University * Kristen Phend, Univ of North Carolina * Gina Broadnax, Ohio State University * Reema Shafi, Ohio State University * Christopher Nelsen/Michelle Rivera, Ohio State University

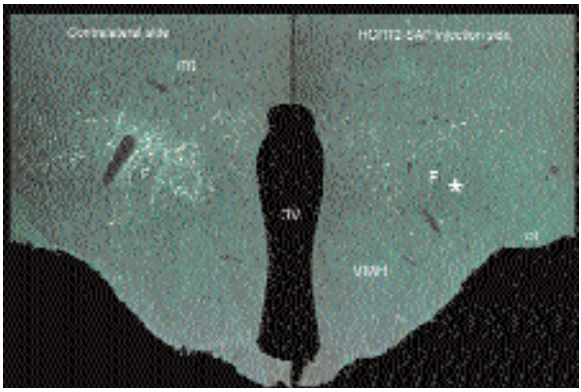
Targeting Tools: Featured Products

Orexin-SAP

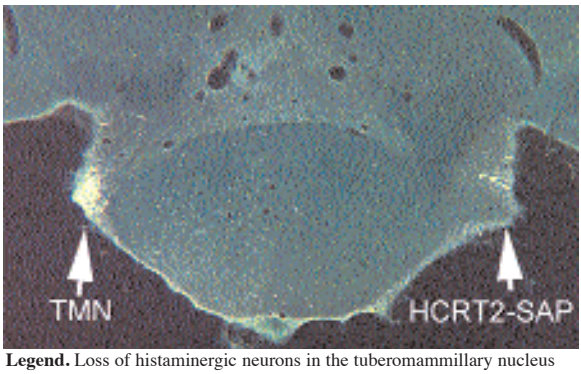
Work on hypocretin, or orexin, as it is also known, is some of the most interesting in all of biology these days. When first characterized a mere three years ago by two groups, it was thought to be involved in feeding (hence the name orexin from the Greek *orexis* meaning appetite). But two articles exploded onto the scene in 1999 and indicated a fundamental role in sleep for the hypocretin/orexin receptor axes. Chemelli *et al*. reported that knocking out the orexin gene produced mice that suffered from narcolepsy.[1] In a prominent model of narcolepsy, some dobermans are found to lapse into a cataplectic state while doing things like running after a ball thrown by their master. Lin *et al*. used positional cloning to determine that narcolepsy in this astounding model is due to a mutation in the hypocretin/orexin receptor gene.[2] These reports clearly put orexin/hypocretin into the narcolepsy mix. Thannickal *et al*. then reported that missing neurons were the problem in the human disease; that hypocretin/orexin neurons were depleted.[3] These new discoveries make the sleep field into something to watch for incredible new results.

Because of the new, exciting work on this system, Peter Shiromani approached Advanced Targeting Systems to request that we construct a molecule that would remove orexin/hypocretin receptor-expressing neurons. He reports on the properties of this targeted cytotoxin in recent issues of *Brain Research* and the *Journal of Neuroscience*.[4,5] It turns out that hypocretin-2/orexin B-SAP (Orexin-SAP, Cat. #IT-20) is able to eliminate specifically orexin receptor-expressing neurons. Interestingly, these neurons also contain orexin, probably for some sort of feedback loop mechanism, so that, like in the human disease, orexin neurons are lost also. The rats treated with this material injected in the lateral hypothalamus have narcoleptic symptoms and will fall directly into REM sleep while happily munching on rat chow (videos available at www.jneurosci.org).[5]

This new tool binds best to the hypocretin-2/orexin 2 receptor, but still has affinity for hypocretin-1/orexin 1 receptor. These are G protein-coupled receptors, and, as seen with the other peptide toxins from ATS that target these receptors—SP-SAP (Cat. #IT-07), SSP-SAP (Cat. #IT-11), dermorphin-SAP (Cat. #IT-12) and corticotropin releasing factor-SAP (Cat. #IT-13)—upon ligand binding, the complex is rapidly internalized. The internalized saporin then inhibits protein synthesis by ribosomal inactivation and the target cell dies. The figures provided here by Dr. Shiromani show the remarkable specificity and potency of orexin-SAP. It provides a simple model for narcolepsy and is destined to become an invaluable tool for the study of the role of hypocretin/orexin receptor-expressing neurons wherever they may occur.



Legend. Orexin-SAP (50 ng/0.5 µl) delivered to the lateral hypothalamus kills the orexin/hypocretin receptor-positive neurons. The asterisk marks the site of injection. VMH=ventromedial hypothalamus; F=fornix; 3v=third ventricle



Legend. Loss of histaminergic neurons in the tuberomammillary nucleus (TMN) after unilateral injection of orexin-SAP (50 ng/0.5 µl). The TMN neurons contain the orexin/hypocretin receptor and are heavily innervated by hypocretin fibers.[3] HCRT2-SAP (hypocretin 2-Saporin) = orexin-SAP

References

1. Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 98:437-451.
2. Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 98:365-376.
3. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM (2000) Reduced number of hypocretin neurons in human narcolepsy. *Neuron* 27(3):469-474.
4. Gerashchenko D, Salin-Pascual R, Shiromani PJ (2001) Effects of hypocretin-saporin injections into the medial septum on sleep and hippocampal theta. *Brain Res* 913:106-115.
5. Gerashchenko D, Kohls MD, Greco M, Waleh NS, Salin-Pascual R, Kilduff TS, Lappi DA, Shiromani PJ (2001) Hypocretin-2-saporin lesions of the lateral hypothalamus produce narcoleptic-like sleep behavior in the rat. *J Neurosci* 21(18):7273-7283.

Featured Antibodies: CRF and GHRF

Corticotropin Releasing Hormone (CRH/CRF)

Species Reactivity:	human, rat (AB-02)
Applications:	affinity chromatography; radioimmunoassay; immunohistochemistry (1:10,000)
Reference:	Widmaier EP *et al*. (1989) *Endocrinol* 124:583-590.

Growth Hormone Releasing Factor (GHRH/GHRF)

Species Reactivity:	entire human peptide sequence (AB-01) rat (AB-16) rat, affinity purified (AB-16AP)
Applications:	immunoblotting; immunohistochemistry; radioimmunoassay
Reference:	Bloch B *et al*. (1983) *Nature* 301:607-608.

Visit the ATS website for a complete list of antibodies.

Targeting Technology

Advanced Targeting Systems' technology - Molecular Neurosurgery™ - is a modification of one of the most widely used techniques in the neurosciences: lesioning of a region by surgical means and observation of the effect.

Choose an ANTIBODY[§] specific to your cell type.

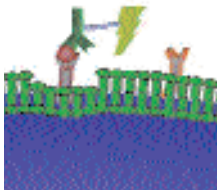
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

ATS binds SAPORIN with your ANTIBODY to make a powerful targeting agent.

[§]or growth factor, peptide, ligand, or cytokine

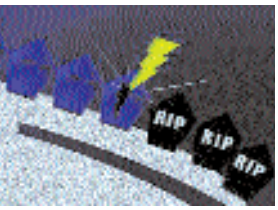
The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.



Cells which do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.



SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Targeting Teaser

Unscramble these four Jumbles, one letter to each block, to form four words used in science.

REAPHAN

LAFKS

GRINEYS

BRITCOSO

WHAT THE SCIENTIST FORGOT TO ADD TO THE FORMULA

Arrange the circled letters to form the surprise answer, as suggested by the above cartoon.

Answer: A...

WIN $100.00

Limit one entry per laboratory.

1. Solve the puzzle.
2. Fax in this entire page with the correct solution by November 30, 2001.
3. Win $100 credit toward your next purchase.

See last quarter's winners, page 6.

Please correct the address information below and provide the following:

Your Name:
Phone:
Fax:
Email:

"Molecular Surgery for Scientists"

www.ATSbio.com

Toll-Free: (877) 889-2288
Phone: (858) 642-1988
Fax: (858) 642-1989

ADVANCED TARGETING SYSTEMS

11175-A Flintkote Avenue
San Diego, CA 92121

BULK RATE
UNITED STATES
POSTAGE PAID
PERMIT #1088
LA JOLLA, CA